Current Aspects of Soy Protein Fractionation and Nomenclature[1]

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ABSTRACT

Significant advances have been made recently in studying the basic physico-chemical properties of the major soy storage proteins and their subunits using such techniques as differential solubility, ion exchange chromatography, gel filtration chromatography, immunoelectrophoresis, isoelectric focusing and dissociating gel electrophoresis. This paper reviews and discusses the principal findings from this work and summarizes the current status of the nomenclature of the major soy storage proteins, e.g., 11S glycinin, 7S β-conglycinin and γ-conglycinin and their subunits.

INTRODUCTION

Storage proteins have been defined as those proteins which are laid down at one stage of the life cycle for future use at a metabolically more active stage (1). Because a problem exists in distinguishing among the many proteins found in seeds as to which have storage functions and which have structural or metabolic roles, an arbitrary method has been adopted whereby extracted proteins constituting 5% or more of the total protein fraction generally are suspected to be storage proteins (1).

Hughes and Murphy (2) reported that the total protein content of 10 varieties of soybeans ranged from 39.4-44.1%. The accumulation of storage proteins begins shortly after the cessation of cell division in the developing cotyledons (3). These storage proteins were first extracted and characterized by Osborne and Campbell (4), who believed that the soybean contained one major globulin component which they name glycinin. Later authors (5,6) demonstrated that this globular component was heterogeneous and reported four resolvable ultracentrifugal components at pH 7.6, 0.5 ionic strength (2S, 7S, 11S, 15S). The use of this ultracentrifugal nomenclature has been retained throughout the literature. Consequently, the two major soy protein components have come to be known simply as the 7S and 11S fractions (7).

7S Globulins

Currently, the 7S globulins (Table I) are classified into three major types (8). Additionally, types I and II can be distinguished electrophoretically (8,9), while types I and III can be distinguished by immunoelectrophoresis (10).

β-Conglycinin

β-Conglycinin is the major 7S globulin and is reported to constitute 16.8-20.9% of the soluble protein fraction when determined by rocket immunoelectrophoresis (11). It undergoes a typical isomerization reaction from the 7S form to the 9S form when the ionic strength of its solution at neutral pH is changed from 0.5 to 0.1 (5,7,10,12-14). Naismith (5) proposed that this isomerization was actually a dimerization. Differences in sedimentation values among different groups have been attributed to the fact that the actual sedimentation coefficients obtained for this fraction are very concentration dependent (7). In addition, it has been reported that at intermediate ionic strengths (between 0.5 and 0.1), it is possible to observe varying amounts of the two sedimenting species (7,12). Consequently, it has been commonly accepted that the 7S form constitutes the β-conglycinin monomer with the 9S form being the dimer.

More recently, however, Iibuchi and Imahori (15) reported that the true β-conglycinin monomer is a 5.6S form obtained when the ionic strength of a neutral solution is increased to 0.8-1.0, but agreed that its true dimer was obtained at an ionic strength of 0.1 as a 10.0S form. At intermediate ionic strengths, they observed a single peak with sedimentation coefficients approaching that of the dimer as the ionic strength was lowered from 1.0 to 0.1. These results are in direct disagreement with the dual peaks observed by earlier workers (7,12). Iibuchi and Imahori (16) rationalized their results based on the theory of Gilbert (17), which proposes that a mixture of monomers and dimers should give a single peak during sedimentation if they are in dynamic equilibrium, resulting in an increasing S value as the equilibrium shifts to the dimer form (15). An additional point of conflict is the statement by Koshiyama (12) that the 7S form remains stable at ionic strengths greater than 0.5, but no supporting evidence was provided.

TABLE I

Classification of 7S Soybean Globulins[a]

Type	Ionic strength, neutral pH 0.5	0.1	Name
I	7S	9S dimer	β-conglycinin (B_1-B_6)
II	7S	Insoluble aggregate[b]	β-conglycinin (B_0)
III	7S	7S monomer	γ-conglycinin

[a]Adapted from Yamauchi et al. (8).

[b]Sykes and Gayler (9) reported a 13.4S form.

β-Conglycinin Molecular Weight

Early reports by Roberts and Briggs (7) stated the molecular weight of the 7S fraction, as determined by ultracentrifugal data, to be 330,000. Later information, also based on ultracentrifugal data from various groups, disagrees markedly with this report but tends to be fairly consistent with one another, i.e. 180,000-210,000 (10,12) and 150,000-175,000 (18) for the 7S monomer, and 171,000-199,000 for the 5.6S monomer (16). Additional evidence supporting the validity of these latter molecular weight data, based on purified β-conglycinin subunit composition and molecular weight values, is as follows: 172,000-204,000 (16) and 141,000-171,000 (18,19).

β-Conglycinin Purification

Numerous experimental schemes have been devised for extracting soy proteins and purifying isolated fractions (3-7, 13-16 and 20-23). These schemes generally concentrate on separating and copurifying β-conglycinin and glycinin, although others have taken into account factors necessary for the recovery of the minor components of α-conglycinin (24) and γ-conglycinin (10,24). Essentially, these methods involve extracting the proteins in alkaline solution followed by centrifugation and isoelectric precipitation at pH 4.5-5.0. After resolubilization at alkaline pH, crude fractions are obtained by ammonium sulfate precipitation (7) or gel

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chromatography (15). Then additional purification can be performed by ion exchange (24) or concanavalin (Con) A chromatography (15,25). Hill and Breidenbach (13) described a relatively simple method for obtaining mg quantities of both 7S and 11S without precipitation. Crude alkaline soy extract was fractionated by preparative ultracentrifugation using a 10-30% linear sucrose gradient.

Perhaps the most popular method currently available for preparing working (g) quantities of both 7S and 11S fractions was reported by Thanh and coworkers (14,21). This method exploits the differing solubilities of these proteins in dilute tris buffers. The 11S proteins are first precipitated and recovered by adjusting an alkaline soy extract to pH 6.4 and centrifuging at 2-5 C. Next, the 7S proteins are precipitated and recovered by adjusting to pH 4.8. β-Conglycinin is further purified from the isolated 7S fraction by resolubilizing in alkaline tris buffer and passing through a DEAE Sephadex A-50 column followed by gel chromatography with Sepharose 6B (22).

Precisely how the fractions obtained by each of these various purification schemes differ has not been explored, but current methods indicate that all final 7S fractions are predominantly β-conglycinin and final 11S fractions are mainly glycinin. Evidence supporting this is commonly obtained by a variety of analytical methods including sedimentation, dissociating disc electrophoresis and immunoelectrophoresis (13,15,21,22,24,25).

β-Conglycinin Components

An additional issue of some conflict has been whether purified β-conglycinin consists of a single protein or a family of proteins containing different proportions of similar subunits. Catsimpoolas and Ekenstam (24) obtained three bands by non-dissociating gel electrophoresis for β-conglycinin, but because this protein formed only one long immunoprecipitin band by disc immunoelectrophoresis, they attributed these three bands to a single protein undergoing the association/dissociation reaction typical of β-conglycinin. Hill and Breidenbach (13) also obtained three bands from their β-conglycinin preparation by non-dissociating gel electrophoresis, but they attributed this to individual components which exhibited differential accumulation rates during seed development and maturation (26). Iibuchi and Imahori (16) also obtained three fractions from purified β-conglycinin; they were isolated by ion exchange chromatography and identified by non-dissociating gel electrophoresis. In addition, each of their isolated β-conglycinins was reported to undergo the monomer to dimer conversion (16). Thanh and Shibasaki (22) report the isolation of six components from their purified β-conglycinin fraction by ion exchange chromatography. These components were named B_1 through B_6 on the basis of the elution order from the ion exchange column. Based on the results of the Ouchterlony immunodiffusion technique, components B_1 through B_4 exhibited one precipitin band and were, therefore, antigenically identical with one another and with the total β-conglycinin. Similarly, components B_5 and B_6 were shown to be identical, but due to the lack of some unique antigenic determinants (the β subunit), only partially identical with the other β-conglycinin components and the total protein (22). In addition, each of the six isolated components exhibited single bands by non-dissociating gel electrophoresis (22), and were found to have unique subunit structure when subjected to both thin-layer gel electrofocusing with 6M urea (22) and dissociating gel electrophoresis in the presence of 0.1% SDS and 8M urea (18). Differences also were shown in the amino acid composition and carbohydrate content of these six components (18,22).

Assuming that each component consisted of three subunits, Thanh and Shibasaki (18,22) divided the above six β-conglycinins into three major groups. Group A proteins contained two β subunits, group B contained one β subunit and group C had no β subunits. Iibuchi and Imahori (16) also classified their three β-conglycinins according to their individual β subunit content, but in a manner opposite that of Thanh and Shibasaki (18,22). Thus, groups C, B and A of Iibuchi and Imahori (16) correspond to groups A, B and C of Thanh and Shibasaki (18,22). Examination of data for these groups (Table II) reveals that their order of elution by ion exchange chromatography, gross subunit composition and N-terminal amino acids are all similar. Whereas the molecular weights of like groups differ somewhat, the overall molecular weights increase with order of elution for both classification schemes. The amounts of glutamate and proline have been reported by both research groups to increase with elution order, but the hydrophobic amino acids (leucine, phenylalanine and valine) decrease. One major difference in the results from these two research groups is the carbohydrate content of groups containing no β subunit (Groups A^a and C^b in Table II).

A major problem arises from the fact that the components of β-conglycinin, isolated by ion exchange chromatography, are regarded as individual monomers by both Thanh and Shibasaki (22) and Iibuchi and Imahori (16), even though both research groups isolated these fractions using NaCl gradients at <0.5 ionic strength. These components would be expected to be eluted under these conditions as dimers and, if they are eluted as dimers, are they homogeneous, as the results imply, or heterogeneous? As stated earlier, ultracentrifugal work at these intermediate ionic strengths reveals a mixture of 7S and 9S forms (7,12) or some intermediate sedimentation coefficient, also indicative of a 7S ↔ 9S equilibrium (15,27). Thanh and Shibasaki (22) state that although increasing ionic strength buffers were used to fractionate the components on an ion exchange column, this probably is not responsible for generating the observed multiple forms, because no interconversion of the components was observed upon rechromatography of the isolated components. However, after recovery from the ion exchange column, the isolated components were immediately dialyzed against the low ionic strength start buffer and rechromatographed under the same gradient conditions. Thus, they were not exposed to a sufficiently high ionic strength to convert them entirely to their 7S form before being rechromatographed. If these components were eluted as heterogeneous dimers which do not undergo dissociation-reassociation at these low ionic strength conditions, then no conversion of one form to another would be expected. Since non-dissociating electrophoresis is performed at low ionic strengths, the existence of separate bands may not be conclusive proof of homogeneity, because these bands may be dimeric rather than monomeric. This concept is supported by Thanh and Shibasaki (27), who report that the multiple bands obtained from non-dissociating electrophoresis of total β-conglycin are associated B_1-B_6 forms of β-conglycinin. Furthermore, if one takes into account the ultracentrifugal results of Iibuchi and Imahori (15), indicating that the true monomer of β-conglycinin is not obtained until the ionic strength reaches 0.8-1.0, then the fractionation of β-conglycinin at 0.5 ionic strength may represent some dimer contamination. Citing differences attributed to variety of soybeans and sample preparations used, Thanh and Shibasaki (27) report a stable 7S form only between ionic strenghs 0.5-1.0.

These questions might be summed from the preceding as follows:

(i) Are the β-conglycinin components eluted from the ion exchange column in the monomer or dimer form?

TABLE II

Comparison of β-conglycinin Groups Fractionated by Ion Exchange Chromatography

Characteristic	β-conglycinin groups					
	C[a]	A[b]	B[a]	B[b]	A[a]	C[b]
Order of elution from ion exchange column	1st	1st	2nd	2nd	3rd	3rd
Subunit composition	$\alpha_1\beta_2$	$(\alpha/\alpha')_1\beta_2$	$\alpha_2\beta_1$	$(\alpha/\alpha')_2\beta_1$	α_3	$(\alpha/\alpha')_3$
Molecular weight from subunits, K	172	141	188	156	204	171
N-terminal amino acids	Val/ Leu(Ile)	Val+Tyr/ Leu	Val/ Leu(Ile)	Val+Tyr/ Leu	Val	Val+Tyr
Carbohydrate content, %	4.0	3.9	4.96	4.3-4.6	2.65	5.1-5.3

[a]From Iibuchi and Imahori (16).
[b]From Thanh and Shibasaki (18,22).

(ii) If these components elute as dimers, are they homogeneous, i.e., consisting of only one β-conglycinin component type, or heterogeneous? The information for the first question might be obtained by examining each component in the ultracentrifuge at the same ionic strength that it elutes during ion exchange chromatography. Regarding the second question, reports by Thanh and Shibasaki (18,22) and Iibuchi and Imahori (16) indicate that if the β-conglycinin components are eluted as dimers during ion exchange, those dimers are either homogeneous or, if heterogeneous, contain predominantly or exclusively monomers of the same general group.

β-Conglycinin Subunits

β-Conglycinin has been reported to have as many as 14 subunits (12) and as few as two subunits (16). As proposed by Thanh and Shibasaki (19,22), most current authors accept that β-conglycinin consists of at least three major subunits, designated α, α′ and β, which are separable by dissociating gel electrophoresis in the presence of SDS or SDS and urea (3,18,22,23,28-32). Thanh and Shibasaki (19,22) initially listed five subunits, including minor γ and δ subunits, for which very little characterization data have been reported. Further, these subunits were not evident in electrofocusing studies of isolated β-conglycinins, even though they reportedly comprised as much as 5-15% of the isolated B_1-B_6 components (22). These latter two subunits were omitted in later discussions regarding the subunit structure of the β-conglycinins and during β-conglycinin reconstitution studies (18,33).

Electrofocusing data of Thanh and Shibasaki (19) revealed that the β subunit split into four bands indicating charge heterogeneity, but the α and α′ bands each consisted essentially of a single band, although in an earlier publication (22) they indicated that minor bands also might be associated with α and α′ subunits. Results of Lei et al. (31), who studied purified β-conglycinin with two-dimensional electrophoresis (isoelectric focusing in the first dimension followed by SDS gel electrophoresis in the second dimension), agreed with the findings of Thanh and Shibasaki (19). Their β subunit also showed charge heterogeneity by splitting into four components, whereas the α and α′ subunits exhibited homogeneous spots. The major difference between these two groups was in their subunit isoelectric points. Thanh and Shibasaki (19) reported the isoelectric points to be between pH 4.9 and 6.0, whereas photographs published by Lei et al. (31) show the isoelectric points between pH 5.2 and 6.1. This information, however, disagrees with results of Beachy et al. (28), who report that when their purified β-conglycinin was subjected to two-dimensional electrophoresis, in a manner similar to that reported by Lei et al. (31), the α and α′ subunits were resolved into multiple components, but the β subunit remained as a single species. Beachy et al. (28) reported the existence of three minor β-conglycinin subunits, resolvable by SDS gel electrophoresis and designated γ_1-γ_3, each of which also showed multiple components when subjected to two-dimensional electrophoresis. These minor subunits migrated between the α and β subunits on SDS gel electrophoresis (28).

The charge heterogeneity displayed by individual subunits is reportedly best explained by the existence of closely related multigene families, which code for some or all of these subunits (34). Such multigene families have been reported for both the α and α′ subunits of β-conglycinin (34). Nucleotide differences within the groups of DNA molecules that code for the synthesis of α and α′ subunits give rise to a limited number of amino acid changes which lead to slight charge differences and result in the charge heterogeneity shown by these subunits (28,34).

There is some disagreement regarding subunit molecular weights. Thanh and Shibasaki (19), after determinations under four sets of conditions, reported the molecular weights of α′ and α subunits to be 57,000 and β subunit to be 42,000. In SDS gel electrophoresis, these numbers were 59,000 for α′ and α and 44,000 for β subunits. Also using SDS gel electrophoresis, Iibuchi and Imahori (16) obtained a molecular weight of 68,000 for the α subunit and 52,000 for the β-subunit, whereas Beachy et al. (35) recorded 83,000 for α′, 76,000 for α and 53,000 for β subunits.

An area of agreement is in subunits' amino acid content. Gayler and Sykes (23) state close similarities for α′, α and β amino acid profiles when compared to those of Thanh and Shibasaki (19); Meinke et al. (3) report amino acid compositions and immunological cross reactivities for the α′ and α subunits similar to those of Thanh and Shibasaki (19).

B_0-Conglycinin

Recently, an additional β-conglycinin component, designated B_0 isomer, has been isolated and characterized by two research groups (8,9). Yamauchi et al. (8) purified their B_0-conglycinin by coprecipitating it with the glycinin fraction at pH 6.4 according to the method of Thanh and Shibasaki (21). After resolubilization, most of the glycinin was precipitated with 75% saturated ammonium sulfate, and the β-conglycinin components were then precipitated with 90% saturated ammonium sulfate. This latter protein precipitate was resolubilized, bound to a concanavalin A column and eluted with α-methyl D-mannoside. The α′ and α subunit-containing β-conglycinins were separated from the B_0-conglycinin on a DEAE Sepharose CL-6B column using a NaCl gradient.

The approach used by Sykes and Gayler (9) to obtain B_0-conglycinin is somewhat simpler. B_0-Conglycinin was pre-

cipitated with the crude glycinin fraction according to a modified version of Thanh and Shibasaki (21), dialyzed against water and freeze-dried. After resolubilization, the B_0-conglycinin was separated from the glycinin by Sepharose 6B chromatography. The B_0-conglycinin, designated β_3 by the authors, eluted as a shoulder on the latter part of the main peak. Rechromatography of the B_0-conglycinin fraction on Sepharose 6B yielded a purified B_0-conglycinin. Both research groups agree that B_0-conglycinin consists of a single subunit which migrates in SDS electrophoresis at the same rate as the β subunit of the other β-conglycinins. Immunochemical determinations indicate that it is antigenically similar to the other β-conglycinins. For example, Yamauchi et al. (8) report that B_0-conglycinin fused with the band between the β subunit and the antisera raised to a β-conglycinin mixture. Sykes and Gayler (9) show strong antigenicity against antisera raised to a mixture of β-conglycinins but no cross reactivity to β-conglycinin fractions containing only α and α' hybrids. These results indicate only antigenic determinants of β subunit are present in B_0-conglycinin.

N-Terminal amino acid analysis performed upon B_0-conglycinin by both research groups yielded only leucine. This agrees with the N-terminal results for the β subunit reported by Thanh and Shibasaki (19). In addition, Sykes and Gayler (9) did an amino acid profile of the B_0 component and found it in relatively good agreement with that of the β subunit isolated from other β-conglycinins (19). In the ultracentrifuge, B_0-conglycinin sedimented at 7.5-7.8S at 0.5 ionic strength (8,9). Yamauchi et al. (8) recorded a change to 6.3S at 1.0 ionic strength, and precipitation of the component, due to excessive aggregation, at ionic strengths less than 0.2. Sykes and Gayler (9) did not observe the B_0 component at ionic strengths above 0.5, but reported a 13.4S aggregate at 0.1 ionic strength rather than a precipitate. In any event, both research groups showed that B_0-conglycinin does not undergo the typical 7S $\leftrightarrow$ 9S conversion as do the other known β-conglycinins between 0.5-0.1 ionic strength. Subunit molecular weights, as determined by SDS gel electrophoresis, were 42,000 (8) and 48,000 (9). The molecular weight of B_0-conglycinin was reported as 126,000 (8) and 134,000-137,000 (9). Yamauchi et al. based their determination on crosslinking results from SDS gel electrophoresis, whereas Sykes and Gayler (9) made their determinations from ultracentrifugal data using both the Archibald and sedimentation equilibrium methods. From these results, both research groups concluded that the protein was made up of three β subunits.

B_0-conglycinin comprises 8-10% of the crude glycinin fraction (8) and has an isoelectric point of approximately 5.66-6.00 (8,9). Further, this component, in its purified form, can readily be fractionated from other β-conglycinins by 7% non-dissociating gel electrophoresis. Reportedly, it doesn't move from the origin of the separating gel and can, therefore, be easily identified (8).

Traditionally, β-conglycinins have been defined as the 7S globulins of soybean seeds which are capable of dimerization at low ionic strength (10,33). In light of this recent evidence, Sykes and Gayler (9) suggest that this definition should be broadened to include proteins composed entirely of β or α' subunits, since neither of these 7S globulins dimerize to a 9S form at low ionic strength. Characterization by molecular weight and subunit structure was listed as a more comprehensive basis for classification than sedimentation behavior.

Yamauchi et al. (8) provided the rationale for naming this new component B_0-conglycinin. The β-conglycinin component numbers originally were established on the basis of their β subunit content, e.g., two β subunits for B_1 and B_2 (Group A), one β subunit for B_3 and B_4 (Group B) and no β subunits for B_5 and B_6 (Group C) (19,22). Therefore, following this nomenclature, a β-conglycinin component containing three β subunits should be designated B_0.

γ-Conglycinin

γ-Conglycinin was first isolated by Catsimpoolas and Ekenstam (24). This component subsequently was purified and partially characterized by Koshiyama and Fukushima (36).

Crude 7S globulin was first subjected to concanavalin A chromatography using α-methyl D-mannoside as eluant. The partially purified 7S fraction was passed over a Sepharose 6B column to fractionate γ-conglycinin from the main β-conglycinin peak. Next, this γ-conglycinin fraction was purified by preparative-scale, non-dissociating gel electrophoresis. The γ-conglycinin fraction was extracted from the gel and finally passed over a short affinity column containing β-conglycinin antibody bound to Sepharose 4B. This step removed any remaining β-conglycinin from the γ-conglycinin fraction (36).

γ-Conglycinin reportedly sediments at 6.5S in 0.5 ionic strength buffer and lacks the ability to dimerize to a 9S form at 0.1 ionic strength. Its molecular weight, based on sedimentation information, is between 102,000 and 104,000. Its carbohydrate content is 5.49%, and it possesses no agglutinin ability or lipoxidase activity. It is believed to comprise approximately 3% of the total soy globulin fraction (36). Catsimpoolas (37) reported that γ-conglycinin contained nine subunits on the basis of disc gel electrophoresis in a system containing phenol, acetic acid, mercaptoethanol and urea.

Glycinin

Glycinin is that soy protein component which sediments as a single, well-defined peak of approximately 11S in the ultracentrifuge at ionic strengths above 0.35 (38). It partially and reversibly associates to form unresolved material of 19S and higher S values at ionic strengths of 0.1-0.2. At very low ionic strengths (<0.01) and neutral pH, a small amount of 11S dissociates to a 7S and a 3S form (38). Equilibrium between these latter two forms and the 11S component is reached within three days at 4 C. Readjustment to a higher ionic strength results in the ready conversion of the 7S to the 11S form, but the 3S is not reconverted to 11S (38). Hughes and Murphy (2) initially reported that glycinin made up 31.4-38.3% of the total protein fraction and 13.5-17.8% of the total weight of 10 soybean varieties. Using rocket immunoelectrophoresis, Murphy and Resurreccion (11) more recently have reported glycinin contents of 38.1-50.8% of the total protein fraction, but these results are considerably higher than their earlier findings (2) and those obtained by others using ultracentrifugal analysis. They explain these differences as being due to the increased sensitivity and specificity of procedures used in the latter study (11). The molecular weight of glycinin has been investigated by a variety of techniques and is most often reported to be 320,000-360,000 (1,39).

Glycinin Purification

Numerous methods have been reported in the literature for purifying the glycinin fraction. Nearly all the current methods involve separating an initial crude glycinin fraction from an alkaline soy protein extract by precipitation in the cold at pH 6.3-7.0 (39-43). The glycinin fraction may then be purified by chromatography on a hydroxyapatite column (39,40) followed by Sepharose 6B chromatography (40), or simply chromatographed on Sepharose 6B only (42). Another method involved purification of the crude glycinin fraction by ion exchange chromatography (43). A more de-

tailed procedure (41) involves the use of ammonium sulfate to fractionate the crude glycinin component followed by concanavalin A and Sepharose 6B chromatography. The glycinin peak was then subjected to ion exchange chromatography to remove most of the final contaminating proteins (98-99% pure) (41).

Although each of the above research groups claims to have isolated a highly purified glycinin fraction, fundamental differences in these glycinin fractions still remain. The formation of a 7S component, the so-called half-molecule or monomer of glycinin, derived from the dissociation of the 11S fraction in low (<0.1) ionic strength buffers (38), has been regarded as a basic physico-chemical property of glycinin. Badley et al. (40) claim that chromatographing glycinin on a Sepharose 6B column with pH 7.6 (1.0M) potassium phosphate (KH_2PO_4) buffer removes the glycinin fraction that dissociates into its half-molecule at low ionic strength, thereby yielding purified, non-dissociating glycinin. The monomerizing glycinin component reportedly aggregates in this buffer and was removed in the void peak along with any glycinin polymers (40). These results were confirmed by examination of the nondissociating glycinin in the ultracentrifuge where a single component was observed in both 0.5 ionic strength buffer and water, and by the presence of a single band in non-dissociating PAGE (pH 8.9) at both low and high ionic strength. Badley et al. (40) further stated that resolubilization of freeze-dried glycinin always yielded polymerized glycinin, so, for critical experiments, fresh material was required. Kitamura et al. (41) subjected their purified glycinin fraction to Sepharose 6B chromatography in the presence of the 1.0M phosphate buffer used by Badley et al. (40), but failed to remove the monomerizing fraction. Instead, they obtained the typical two-band pattern indicating both monomer and dimer on non-dissociating PAGE (pH 8.9).

There is also disagreement regarding the ionic strength required for the elution of glycinin from DEAE Sephadex A-50 ion exchanger during purification. Utsumi et al. (43) rationalized the effectiveness of their ion exchange purification of glycinin by stating that contaminating β-conglycinin was not bound to the column at 0.25M NaCl and above. This result, however, contradicts the published information of Thanh and coworkers (14,22) showing β-conglycinins fractionated on DEAE Sephadex A-50 using a 0.2-4.0M NaCl gradient. Therefore, the use of ion exchange chromatography to effectively separate glycinin from β-conglycinin is in question.

Glycinin Heterogeneity

Since glycinin generally has been considered to be a single homogeneous protein, little work has been done to determine whether glycinin is, indeed, a singular protein or a heterogeneous mixture of proteins comprised of different proportions of similar subunits, as is the case for β-conglycinin (22). Utsumi et al. (43) purified a glycinin fraction by ion exchange and subjected it to non-dissociating, 4-30% gradient gel electrophoresis. They reported the resolution of two major zones which consisted of glycinins (dimers) and the corresponding half-molecules of glycinins (monomers). The dimer region consisted of three major bands, with molecular weights of 340,000, 345,000 and 360,000, and a minor band with a molecular weight of 375,000. Their glycinin fraction eluted from the ion exchange column in a single broad peak, and they claimed that differing proportions of these major and minor bands were obtained by examining different parts of the major fraction. Utsumi et al. (43) also reported that, while the ratio of acidic subunits was fairly similar among these latter samples, the ratio of basic subunits differed, with samples eluting from the latter part of the ion exchange peak having a higher content of 19,000 to 18,300 molecular weight subunits.

Glycinin Subunit Purification

Glycinins consist of two major classes of subunits, the acidics and the basics (Table III). Badley et al. (40) separated these two classes simply by heating 1% glycinin solutions to 100 C for 30 min to almost quantitatively precipitate the basic subunits. Other researchers have fractionated the acidic subunits by DEAE Sephadex A-50 anion exchange chromatography in the presence of urea and a 0.0-0.4M NaCl gradient (39,41,42). Basic subunits, which elute in the void peak of DEAE Sephadex A-50 chromatography, are fractionated by CM Sephadex C-50 cation exchange chromatography, also in the presence of urea and using a 0.0-0.3M NaCl gradient (41,42). The advantage of the ion exchange procedures is that, in addition to separating the acidic from the basic subunits, individual acidic and basic subunits can be resolved. The subunits are named according to their elution order from the respective ion exchangers

TABLE III

Chemical and Physico-Chemical Properties of Glycinin Subunits

Reference	Subunit									
	A_1	A_2	A_3	A_4	$F_{2(1)}$[a]	$F_{2(2)}$[a]	B_1	B_2	B_3	B_4
	Molecular weight by SDS PAGE (K)									
Catsimpoolas et al., 1971	37	37	37	–	–	–	22	22	22	–
Kitamura et al., 1976	37	37	37	45	–	–	22.5	22.5	22.5	22.5
Moreira et al., 1979	37	37	42	37	37	10	19	19	≤19	≤19
Iyengar and Ravestein, 1981	38	38	45	38	–	–	--------	20.2-21.5		--------
Mori et al, 1981	-------	34.8-38	------				--------	17.9-19		----------
	N-Terminal amino acid									
Badley et al., 1975	---	1 Leu; 1 Ile; 1 Phe	--				----------	Gly	----------	
Moreira et al., 1979	Phe	Leu	Ile	Arg	Phe	Ile	----------	Gly	----------	
Iyengar and Ravestein, 1981	Phe	Leu	Ile	Ile or Arg?						
	Molar ratios									
Kitamura et al., 1976	1	1	2	2	–	–	1	1	2	2

[a] A_1 was renamed A_{1b} and $F_{2(1)}$ and $F_{2(2)}$ were later renamed A_{1a} and A_5, respectively (44).

and, thus, their relative degree of acidity or basicity. For example, A_1 becomes the first acidic subunit eluted from the anion exchanger, while A_4 is the last eluted acidic subunit, and an analogous nomenclature is used for the basic subunits eluted from the cation exchanger (41,42). Moreira et al. (42) isolated two additional acidic components of differing molecular weights which coeluted as a single peak from the anion exchange column following the void peak, but before the NaCl gradient was started. These slightly acidic components were originally termed $F_{2(1)}$ and $F_{2(2)}$ (42), but later designated A_{1a} and A_5, and A_1 was redesignated A_{1b} (44).

Glycinin Subunit Composition

The total number of individual subunit types comprising glycinin has been another area of conflict. Catsimpoolas (45) isolated three acidic and three basic subunits with molecular weights of 37,000 and 22,000 for the acidics and basics, respectively, on SDS-PAGE (46). Kitamura et al. (41) isolated four acidic subunits and four basic subunits, while Moreira et al. (42) reported six acidics and four basics.

It can be seen from Table III that the major discrepancy between the molecular weights reported by Kitamura et al. (41) and Moreira et al. (42) lies in which acidic subunit has the greatest molecular weight. Kitamura et al. (41) record A_4 as having the greatest molecular mass, whereas Moreira et al. (42) record A_3 as the greatest. Recently, Mori et al. (47) examined the acidic and basic subunits of glycinin from 18 different cultivars of soybeans and found each group to break into two general molecular weight classes on SDS-PAGE, i.e., 34,800 to 38,000 for the acidic subunits and 17,900 to 19,000 for the basic subunits.

Good agreement exists regarding N-terminal amino acid content (see Table III). Badley et al. (40) and Iyengar and Ravestein (39) report leucine, isoleucine and phenylalanine as the N-terminals for the acidic subunits, although Iyengar and Ravestein (39) admit that their N-terminal result of isoleucine for A_4 is unclear and could, indeed, be arginine. Moreira et al. (42) also report leucine, isoleucine and phenylalanine as the acidic N-terminal amino acids along with arginine, which is the N-terminal of A_4. All three groups record glycine as the only N-terminal amino acid of the basic subunits.

Badley et al. (40) suggested that glycinin consisted of two hexagonal rings stacked one on top of the other. This model consisted of two identical half-molecules of glycinin with each consisting of three acidic and three basic subunits. Kitamura et al. (41) isolated four acidic and four basic subunits and concluded that glycinin consisted of two similar, but not identical, glycinin monomers. In addition, they reported that each monomer consisted of three intermediary subunits. These intermediary subunits contained an acidic and a basic subunit linked by a disulfide bond (41); this concept was further supported by the work of Iyengar and Ravestein (39), who also showed the presence of intermediary subunits. Thus, according to this model, glycinin contains 12 subunits with, in addition to hydrophobic, electrostatic and hydrogen bonding, at least six disulfide bonds participating in the maintenance of a stable molecule (41).

Iyengar and Ravestein (39) presented a glycinin model slightly different from that reported by Kitamura et al. (41) and Badley et al. (40). During the preparation of their intermediary subunits, they consistently obtained the A_4 subunit as a "free" subunit and not as a part of an intermediary subunit in the structure of glycinin. The other three acidic subunits were involved as parts of intermediary subunits. Based on this evidence and a reinterpretation of the electron micrographs of Badley et al. (40), Iyengar and Ravestein (39) presented a glycinin model comprising two identical half-molecules, each consisting of three intermediary subunits, and two additional acidic subunits thought to occupy the central hole of each half-molecule so that each glycinin monomer consists of three basic and four acidic subunits. However, Staswick et al. (48) suggest that the A_4 subunit may be linked non-covalently to the B_3 subunit. This concept is supported by later work (49).

Glycinin Polymorphism

The degree of heterogeneity present in the individual acidic and basic subunits of various soybean cultivars has been the subject of much investigation. Work performed by Kitamura et al. (50) and Mori et al. (47) suggests polymorphism exists with respect to glycinin composition. Mori et al. (47) compared 18 different soybean cultivars and, through the use of isoelectric focusing (IEF) in the presence of urea and mercaptoethanol (ME), were able to divide their subunits into five different groups based on charge difference: group I contained seven acidics and eight basics; group II, seven acidics and seven basics; group III, six acidics and seven basics; group IV, six acidics and five basics, and group V, six acidics and three basics. Kitamura et al. (50) looked at differences on gel electrophoresis of the total glycinin fraction and noted differences only in the acidic subunit fraction. However, both groups examined only an isolated total glycinin fraction and did not clearly identify which of the individual glycinin subunits were present or exhibited heterogeneity in the various cultivars.

Recently, Staswick and Nielsen (49) compared the glycinin acidic and basic subunit composition of two different soybean cultivars. Within these cultivars, they found as many as seven acidic subunits, designated A_{1a}+A_5, A_{1b}, A_2, A_3, A_4 and A_6, and five basic subunits, designated B_{1a}, B_2, B_{1b}, B_3 and B_4. Additional work by Moreira et al. (42,44) and Staswick et al. (48) identified heterogeneity withspect to size and charge for most of these acidic and basic subunits. Staswick et al. (48) did not rule out an artifactual origin of this heterogeneity even though steps were taken to limit proteolysis during their isolation and characterization by the addition of diisopropylphosphofluoridate and phenanthroline. Explanations considered more plausible include heterogeneity due to products from alternate coding sequences and variability in the post-translational modification of precursors to the intermediary subunits (48). More recent work (51) supports the latter two considerations. Thus, based on sequence heterogeneity (51) and the charge heterogeneity found by IEF (44,48), it appears that the acidic and basic subunits, isolated by ion exchange chromatography, are actually a family of similar proteins which differ slightly at the primary level.

Tumer et al. (52,53) showed that a specific acidic and basic subunit are synthesized from a single messenger RNA in which there is a NH_2-terminal leader sequence followed by the acidic peptide component, a 4-amino acid linker peptide, and ending with the basic peptide component. Recent work by Marco et al. (54) confirms this conclusion. Thus, since all of the components are covalently linked, these precursor subunits of glycinin are unaffected by reducing agents. During final processing, this single precursor molecule (M_r=60,000) is cleaved to form the acidic and basic subunits, and these subunits are linked via disulfide bonding (52,53). Staswick et al. (48) provided supporting evidence for this idea by showing that pairing between subunits is nonrandom. They reported the subunit pairings to be $A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}, A_3B_4 and A_5B_3. Subunit A_4 is either not linked to a corresponding basic subunit via a disulfide bond or its disulfide bond linkage to a basic subunit is particularly sensitive to cleavage.

The individual acidic and basic components traditionally

SOY PROTEIN NOMENCLATURE

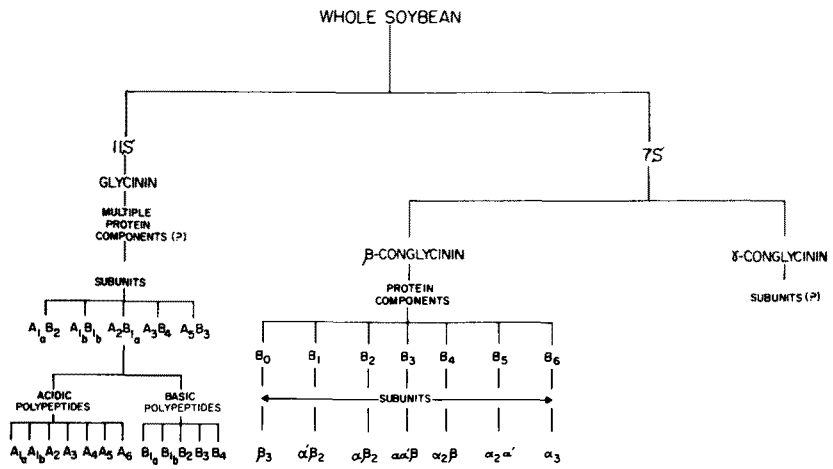

FIG. 1. Fractionation scheme for major soybean storage proteins and their subunits.

have been defined as the subunits of glycinin with the acidic-basic pairs being referred to as "intermediary" subunits. However, Staswick et al. (55) suggest that this model should be changed because each pair of acidic and basic components is encoded by a single gene, synthesized as a single precursor protein, and remains covalently linked through a disulfide bond in the final glycinin molecule (54). Based on this evidence, Staswick et al. (55) argue that the fundamental units which comprise glycinin are the acidic-basic pairs and not the individual acidic and basic components as previously thought. Thus, the individual acidic and basic components should now be referred to as the acidic and basic polypeptides, while the acidic-basic pairs become glycinin subunits. This concept and the arguments which support it have been exhaustively summarized by Nielsen (56).

The information presented in the present paper is summarized in Figure 1. The nomenclature used for glycinin is consistent with that suggested by Nielsen (56). The roles of acidic polypeptides A_4 and A_6 have not been described completely, although Nielsen (56) has speculated that A_4 may participate in a glycinin subunit of $A_4A_5B_3$ composition.

Soybean proteins are limiting in methionine and cystine. Staswick et al. (48) report an eight-fold difference between glycinin subunits having the highest and lowest methionine content. More importantly, the high methionine-containing acidic and basic polypeptides pair together, while those low in methionine also are paired (48). Glycinin is the most prevalent storage protein in soybeans, and if the methionine deficient subunits can be replaced by high methionine-containing subunits, this would be expected to exert a marked improvement on the nutritional quality of soybean storage proteins (48). What effects, if any, this would have on the functionality of the proteins in food applications would have to be explored.

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Effects of Hydrogenation and Additives on Cooking Oil Performance of Soybean Oil

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ABSTRACT

Soybean oil was continuously hydrogenated in a slurry system to investigate the effects of linolenate content and additives on cooking oil performance. Room odor evaluations carried out on oils heated to 190 C after frying bread cubes showed that the oils hydrogenated with Cu catalyst to 2.4% linolenate (Cu-2.4) and with Ni catalyst to 4.6 linolenate (Ni-4.6) had a significantly lower odor intensity score than the unhydrogenated soybean oil (SBO). Other hydrogenated oils (Cu-0.5 and Ni-2.7) were not significantly better than SBO. Oil hydrogenated with Ni (Ni-0.4) scored poorly because of its strong "hydrogenated-paraffin" odor. The performance of all partially hydrogenated oils (2.4, 2.7 and 4.6% linolenate) was improved by adding methyl silicone (MS), but the most hydrogenated oils (0.5 and 0.4% linolenate) were not improved. Although with tertiary butyl hydroquinone (TBHQ) no improvement was obtained, with the combination of TBHQ + MS all odor scores were lower, indicating a synergistic effect. Evaluations of bread cubes after intermittent heating and frying showed that the breads fried in most hydrogenated oils (Ni-0.4, Cu-2.4 and Ni-2.7) were rated significantly better in flavor quality than breads fried in SBO. The bread cubes fried in MS-treated oils had significantly higher flavor quality scores than breads fried in SBO or SBO containing TBHQ. Dimer analyses by gel permeation chromatography and color development after heat treatments also did not correlate with sensory analyses.

INTRODUCTION

Much work has been reported on thermal oxidation and deterioration of unsaturated fats when heated in air under deep fat frying conditions (1-12). However, little information is available on the effect of partial hydrogenation on the performance of soybean oil as a cooking oil and on the flavor and oxidative stability of foods fried in such oil.

Direct sensory evaluation of the effect of deep fat frying on oil quality is difficult because used heated fats have flavors and odors that are too intense to be measured reliably. To overcome this problem, Evans et al. (13) developed a useful test based on evaluation of the room odor produced by oils heated under standardized conditions. Recent studies based on this room odor test with samples heated statically at 190 C showed a significantly lower odor intensity from soybean oil partially hydrogenated with copper or nickel catalysts than from unhydrogenated oil (14). The use of combinations of citric acid (CA), methyl silicone (MS) and tertiary butyl hydroquinone (TBHQ) also lowered the odor intensity of the heated oils. These results are in contrast to those obtained in storage stability studies of similar samples evaluated as salad oils by tasting at 50 C after accelerated storage at 60 C (15,16). Salad oil evaluations showed no significant improvement in the flavor stability during storage by either partial hydrogenation or by the use of antioxidants such as BHA, BHT or TBHQ. However, the oxidative stability based on peroxide values showed an improvement by partial hydrogenation of the oils as well as by the use of these antioxidants.

In this paper we report the effect of hydrogenation to different linolenate contents, and of stabilizers such as MS and TBHQ, on cooking performance of soybean oil under deep fat frying conditions. A continuous slurry hydrogenation system (17,18) was used, and copper-chromite and nickel catalysts were compared. Methods were developed to evaluate the performance of hydrogenated oils under deep fat frying conditions by both sensory and instrumental techniques. The room odor test was modified to include evaluation of oils that had been used to fry bread cubes. The fried bread cubes also were evaluated after storage under different conditions.

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EXPERIMENTAL

Continuous Hydrogenation

The continuous slurry system used to hydrogenate soybean oil with copper-chromite (Cu) and nickel (Ni) catalysts was described previously (17). The hydrogenated oils were bleached, deodorized and stabilized with CA in combination with either MS or TBHQ. Analyses of the bleached and deodorized oils and their evaluations for flavor and oxidative stability are reported in another paper (19). The fatty acid compositions of these oils are summarized in Table I. Hydrogenated oils are designated by the catalyst used and % linolenate (e.g. Cu-0.5 = hydrogenated with Cu catalyst to 0.5% linolenate).

Frying Procedures

The following schedule was designed to test the performance of oils for frying bread cubes repeatedly and to evaluate room odor intensity, flavor quality and stability of the fried bread.

Day 1. Oil sample (600 g) weighed in a 2-qt deep fat fryer ("Electric Multi-Fry," Northland Aluminum Prods., Minneapolis, Minnesota) was heated to 190 C in 15 min. One-hundred g of white bread ("Butternut" sandwich style),