The Fractinations and Characterizations of Soy Isolate, 7S and 11S PRF

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大豆蛋白質의 分劃과 分劃蛋白質의 特性

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Introduction

Ultracentrifugal investigations have classically shown that soybean storage proteins consist of four components with sedimentation constants equal to about 2, 7, 11, and 15S. Among these components, 7S and 11S proteins are the principal components of soy proteins. Thus many studies have been reported on the 7S (conglycinin) and 11S (glycinin) proteins, and much information has been accumulated concerning their molecular structure, subunits and mechanisms of the dissociation and association of these proteins (Wolf, 1978). Also, physicochemical properties of the 7S and 11S proteins were summerized by Kinsella (1979).

Several methods based on such different properties and physical behavior of these soy proteins have been used to prepare 7S and 11S soy proteins (Wolf *et al*, 1962; Catsimpoolas and Ekenstam, 1969; Saio and Watanabe, 1973). A cryoprecipitation and isoelectric precipitation may easily prepare 7S and 11S proteins from an aqueous extract of soy flour, respectively. However, 7S and 11S PRF (protein-rich fration) may sometimes have more practical significance in food aplications than purified 7S and 11S soy proteins. Thus Saio *et al* (1973, 1974) reported a simple method, based on differential solubilities of 7S and 11S PRF in calcium chloride solution. However, none of these methods presented a straightforward continuous preparation of the two proteins; neither afforeded a large-scale fractionation of the two proteins with as little cross-contamination as possible. Thanh and Shibasaki (1976) simultaneously isolated 7S and 11S proteins by a simple method, based on differential solubilities in dilute Tris- HCl buffer.

Therefore, this paper was undertaken to fractinate isolate, 7S and 11S PRF by the simultaneous method, modified for commercial use, and characterize the fractionated proteins by the determination of extract yield, amino acide content, PAGE(polyacrylamide gel electrophoresis) and the determination of molecular weight of the protein subunits by SDS-PAGE

Meterials and Methods

Materials

Sound, mature soybeans of the White-hilum variety were obtained from a local market.

Protein Fractionation

Defatted meal soybean meal was prepared by the

reported method by Kang (1984). Isolate, 7S and 11S PRF were fractionated from the defatted soybean meal as shown in Fig.1. The extract yields were expressed by extractable nitrogen contents (%) measured by the micro-kjeldhal method and compared with the nitrogen content of defatted meal (100%).

Amino Acid Content

Amino acid analysises were performed with a Beckman analyzer (model 121-C). The lyophilized and ground proteins were hydrolyzed in constant boiling 6 N HCl for 24 hrs, under nitrogen. No corrections were made for amino acid losses due to hydrolysis.

Electrophoresis and Determinations of Molecular Weight.

The PAGE (polyacrylamide gel electrophoresis, 7% gel) was carried out by the method described by

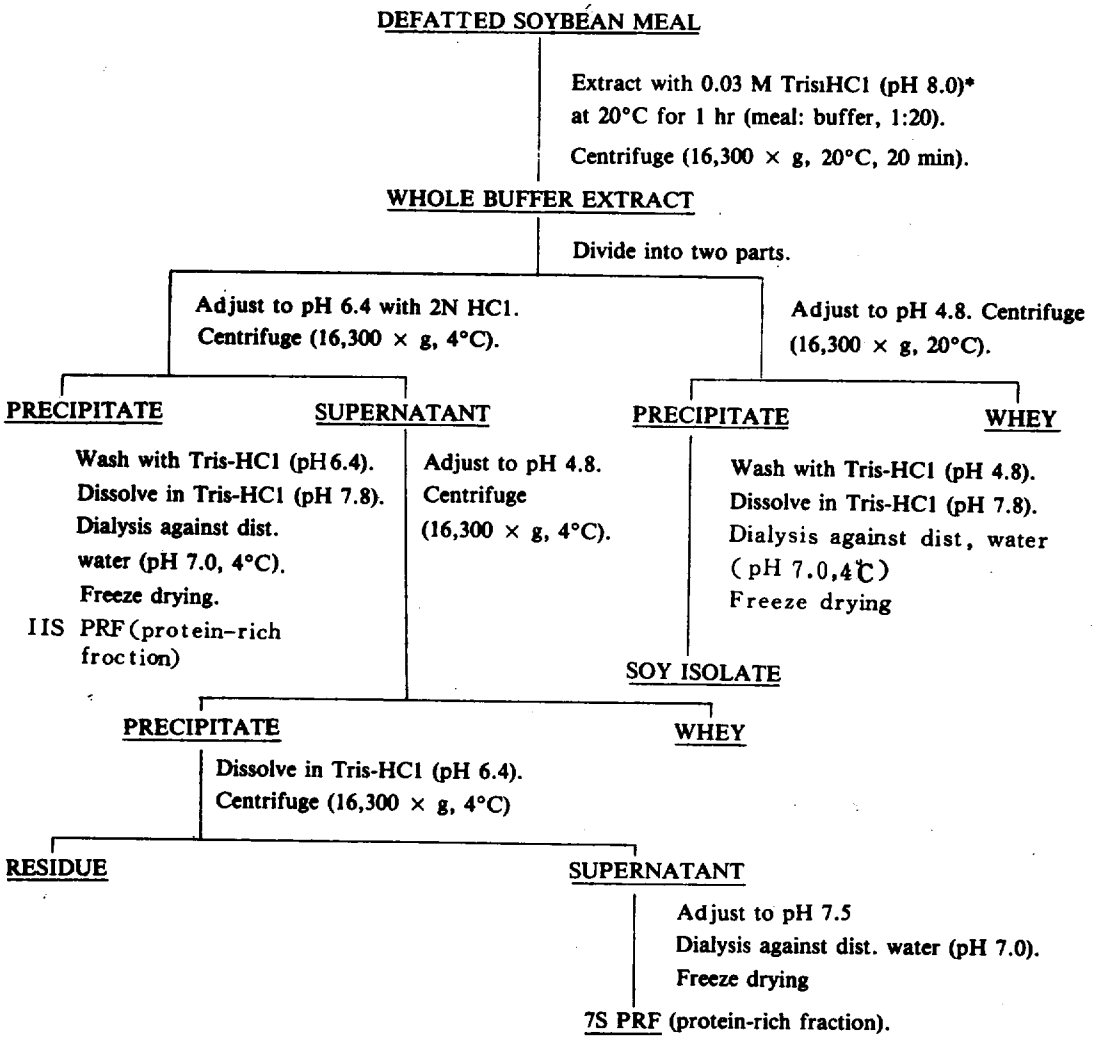

Fig. 1. Method for continuous fractionation of isolate, 7S PRF and 11S PRF.
*Tris-HCl (Tris hydroxymethyl amino methane-HCl) buffer containing 2-ME (2-Mercaptoethanol).

Green and Moore (1981). 60 μl of 1% sample solution was applied to a gel. Gels were run at 6 mA/tube for 1.8 hr, and then the gels were stained with Coomassie Brilliant Blue and destained by diffusion in methanol-water-acetic acid (2:2:1, v/v/v). The SDS-PAGE (sodium dodecyl sulfate-PAGE) was carried out by the method of Swank and Monkres (1971) at 8 mA/tube for 5hrs. The staining and destaining methods were the same as described above in PAGE.

Molecular weights were calculated from standard curve (Fig. 2) obtained by marker proteins, as demonstrated by Cunningham et al (1978); Bovine Serum Albumin (68,000), Ovalbumin (45,000), Chymotrypsin A (25,000) and Ribonuclease (13,000).

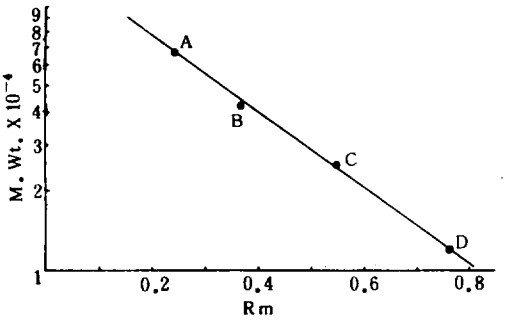

Fig 2. Standard curve for molecular weight determination on SDS-PAGE gels.
A ; albumin (Mw 67 × 10^3)
B ; ovalbumin (Mw 43 × 10^3)
C ; chymotrypsin (Mw 25 × 10^3)
D ; ribonuclease A (Mw 13.7 × 10^3).

Results and Discussion

Protein Fractionation

Soy isolate, 7S and 11S PRF were fractionated with 0.03 M Tris-HCl buffer (pH 8.0) as shown in Fig. 1, based on the continuous isolation process of 7S and 11S soy proteins described by Thanh and Shibasaki (1976). Although the Thanh and Shibasaki process included a cryoprecipitating step for 11S protein, the step was omitted from this study because it was ineffective on a large-scale and continuous fractionation. Also, dialysis in the previous process was carried out against Tris-HCl buffer, while the step in this study was carried out against distilled water (pH 7.0), for excluding the effects of Tris-HCl and 2-ME in the prepared proteins. The changed process might permitted mutually cross-contamination between 7S and 11S proteins to a certain degree. Thus the fractionated 7S and 11S proteins in this study were termed 7S PRF and 11S PRF (protein-rich fraction), respectively. In fact, the results of PAGE and SDS-PAGE analysis, which will be discussed in next paragraph, showed some cross-contamination between the 7S and 11S PRF.

The yields of the fractionated proteins were slightly increased using 2-ME with Tris-HCl buffer for isolate, 7S PRF and 11S PRF, especially in whey protein, as shown in Table 1. Generally, the inclusion of thiol reagent (mainly mercaptoethanol) in the ex-

Table 1. Yields of the soy proteins fractionated with Tris-HCl[a,b]

Treatments	Defatted flour	Isolate	7S PRF	11S PRF	Residue	Whey
Buffer with 2-ME, no dialysis	100	27.4	16.9	10.9	18.2	21.9
Buffer with 2-Me, dialysis	100	26.0	16.7	10.3	18.2	21.9
Buffer only, dialysis	100	21.0	12.5	7.0	43.3	8.7

a Extractable nitrogen % measured by micro-Kjeldhal method. b Dry basis.

tractant significantly increases yield of protein, since the thiols apperantly cause depolymerization of disulfide-linked storage proteins, rendering them more soluble in the solvent (Kinsella, 1979). However, the thiols in the extractant can badly affect the protein product since their flavor is very unpleasant. Accordingly, the thiols must be removed from the protein products if they are used for increasing yield. Dialysis or ultrafiltration have been usually used for removing the thiols. Dialysis in this study was affected no more than 1% on the decreasing yields of prepared fractions. Ultrafiltration method with thiols should be prepared for the continuous fractionation of soy proteins in the future since the method is more rapid and continuous than dialysis.

On the other hand, the relative amounts of 7S PRF and 11S PRF in this study indicated that 7S PRF accounted for usually a higher amount than 11S PRF, in contrast with general estimates that 11S (glycinin) accounted for 50-60% of soy globulins (Kinsella, 1979). The result may be responsible for omitting a cryoprecipitating step from the fractionation process in this study.

Anino Acid Content

The amino acid contents of the fractionated proteins are shown in Table 2. In general, the contents of glutamic, aspartic acid, leucine, arginine, and lysine gave relatively higher values, while those of tryptophan, cysteic acid and methionine gave lower values in the three fractionated proteins, as about 1% or lower. There wer significant differences in the contents of cysteic acid, methionine and tryptophan between 7S PRF and 11S PRF. The different contents were 25 to 30% higher in 11S PRF than in 7S PRF. However, the differences were very low when compared with the 5 to 6 fold differences reported by Wolf (1978). The result was probablly caused by low purities in the proteins used in this experiment. The content of lysine was higher in 7S PRF than in 11S PRF.

Electrophoresis and Determination of Molecular Weight.

The electrophoretic patterns of soy proteins fractionated are shown in Fig. 3. In PAGE, the isolate

Table 2. Amino acid contents and protein contents of soy proteins fractionated[a]

	Isolate	7S PRF	11S PRF
Amino acid %			
Lysine	5.26	5.77	4.74
Histidine	2.13	2.15	2.45
Ammonia	1.90	1.73	2.93
Arginine	7.03	7.07	9.59
Aspartic acid	10.56	10.18	11.17
Threonine	3.09	2.79	3.11
Serine	4.57	4.47	4.68
Glutamic acid	18.50	18.01	20.27
Proline	4.58	4.45	5.15
Glycine	3.62	3.25	3.95
Alanine	3.50	3.44	3.30
Valine	4.18	4.03	4.27
Methionine	1.11	0.86	1.27
Isoleucine	4.22	4.21	4.09
Leucine	7.27	7.17	7.13
Tyrosine	3.28	3.25	3.27
Phenylalanine	4.65	4.86	4.65
Cysteic acid	0.96	0.73	1.14
Tryptophan	1.17	1.01	1.32
Total	91.58	89.43	98.57
Nitrogen %[b]	16.06	14.69	16.82
Protein %[c]	100.38	91.81	105.13

a Moisture-free basis.

b Measured by micro-Kjedhal method.

c N % × 6.25.

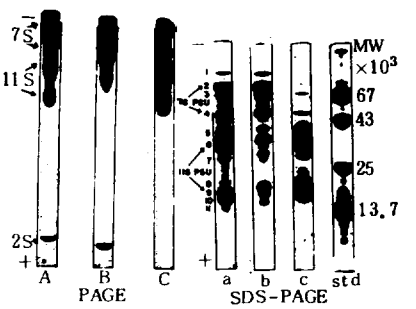

Fig 3. PAGE and SDS-PAGE of soy proteins.

was separated into several bands: 7S, 11S and 2S were separated into at least four, two and one bands, respectively. Also, 2S protein band appeared in 7S PRF with the highest relative mobility, but it completely disappeared in 11S PRF. Although the qualitative analysis of the cross-contamination between 2S PRF and 11S PRF was not carried out, a little mutual cross-contamination was occured with higher in 11S PRF as shown by PAGE analysis (Fig. 3). The PAGE patterns in this study were similar to those obtained from previous reports (Thanh and Shibasaki, 1976; HIll and Breidenbach, 1974). The results in PAGE indicated that the modified fractionation process in this study had scarcely affected the electrophoretic patterns of soy proteins except the scarcely affected the electrophoretic patterns of soy proteins previous process (Thanh and Shibasaki, 1976).

The soy proteins fractionated were further characterized by SDS-PAGE. In the SDS-PAGE analysis, 11 distinguishable bands detected were numbered consecutively in order of increasing relative mobility (Fig. 3). Thus the estimated molecular weights of the 11 bands which were calculated from standard curve (Fig. 2) are presented in Table 3. The linear regression coefficient (r) for the standard curve was greater than 0.98 on SDS-acrylamide gels. Amongst the 11 bands (A of SDS-PAGE in Fig. 3), numbers 2, 4, and 6, 9 PSU (protein subunit) referred to main PSU of 7S and 11S proteins (Rhee et al, 1981). Mean molecular weights of the PSU varied from about 97,000 to 14,000 as presented in Table 3. The molecular weights 33,891, 17,660, respectively. Thus numbers 6, 9 PSU of 11S protein were equal to basic and acidic subunits previously repoted by Catsimpoolas et al (1969).

Table 3. Mean molecular weights of protein subunits present in soy proteins

No. of bands[b]	Mean Rm[b]	Mol. Wt.	S[c]
1	0.138	87,579	±2,437
2	0.2	72,828	±4,007
3	0.238	65,043	±1,816
4	0.328	49,751	±2,075
5	0.4095	39,039	±1,628
6	0.457	33,892	±2,768
7	0.5238	27,778	± 769
8	0.628	20,370	± 564
9	0.676	17,660	± 972
10	0.705	16,199	± 675
11	0.743	14,464	± 599

a Labeled on protein bands in Fig. 3.
b Calculated from standard curve (Fig. 2).
c Standard deviation.

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國 文 抄 錄

大豆蛋白質을 Tris-HCl緩衝液을 利用한 連續的인 分劃法에 의하여 分離大豆 蛋白質 및 7S와 11S 蛋白多劃分으로 나누고 抽出 條件의 變化에 따른 收率變化와 分劃된 蛋白質에 대하여 아미노酸 組成, 電氣泳動 및 各 蛋白質 subunit에 對한 分子量을 測定하였다.

2-ME使用에 의하여 收率이 增加하였으며 透析에 의하여 收率 減少는 1%이내였다.

얻어진 蛋白質 劃分에 對한 아미노酸 組成은 報告된 것과 類似하였으나 7S 및 11S蛋白多劃分間에 cysteic acid, mcrhionine 및 tryptophan 含量 差異는 報告된 7S 및 11S 蛋白質의 差異보다 상당히 적었다.

電氣泳動 結果에 따르면 7S 및 11S 蛋白多劃分間에 약간의 서로 混入을 볼 수 있었으며 2S 蛋白質은 7S 蛋白多劃分에 包合되어 나타났다.

大豆蛋白質 subunit는 11개가 나타났으며 이들 分子量은 約 97,000에서 14,000사이에 걸쳐 分布하였다.