

## 7S AND 11S VEGETABLE PROTEIN FRACTIONATION AND ISOLATION

### BACKGROUND OF THE INVENTION

Fractionation studies pertaining to isolation and separation of soy protein of varying molecular weight have been extensively reported. The 2S, 7S, 11S and 15S proteins are the most commonly reported soy protein fractions. Soybeans as a Food Source (CRC Press, Cleveland, Ohio, 1971) reports the 2S protein (8,000-21,500 M.W.) typically comprises approximately 22%, the 7S (110,000-210,000 M.W.) approximately 37%, the 11S (about 350,000 M.W.) about 31% and the 15S (about 600,000 M.W.) approximately 11% of the total weight of the protein composition of defatted soybean products. These protein fractions may be precipitated from solution at an isoelectric precipitating pH with the pH 4.0-5.0 range. In addition, soybeans contain a water-soluble protein fraction which is not precipitable at the isoelectric precipitating pH. These proteins remain soluble in water throughout the pH 4.0-5.0 range and are commonly referred to as the whey proteins.

U. S. Pat. No. 4,172,828 by Davidson et al. discloses a multiple-staged isolate separation recovery process. The initial soy protein extraction is conducted at pH 6.2-6.8 and isolated from the extract by cooling. Another fraction is then isolated from the extract by precipitation at a pH 4.5. Two other soy isolate fractions may be obtained by heating the extract and precipitating an isolate therefrom at a pH 5.3 with the remaining isolated fraction being obtained from the extract by a pH 4.5 curding and cooling step.

A patent by Calvert (U.S. Pat. No. 2,451,659) discloses extracting a soy protein at a pH 4.2-4.8 in the presence of an enzyme inhibiting agent and an oxygen excluding or blanketing agent. A patent issued to Eberl et al., U.S. Pat. No. 2,479,481 discloses a method for producing a substantially undenatured vegetable isolate. According to the Eberl et al. patent, the protein extraction may be suitably conducted at a pH 6.0-9.0. An isolate is curded and recovered from the extract by a pH 4.3-4.9 adjustment with sulfur dioxide. U.S. Pat. No. 3,303,182 discloses an isolation process in which the soy solubles are extracted at a temperature in excess of 80° C. The heated extract is then rapidly cooled to below 50° C. with curding of the isolate therefrom at a pH 4.2-5.0. U.S. Pat. No. 4,188,399 by Shemer discloses extracting the water-soluble protein and carbohydrate constituents at a pH 5.1-5.9 in the presence of an antioxidant followed by a pH 4.5 adjustment with phosphoric acid to provide a viscous proteinaceous solution containing more than 70% by weight 7S soy protein fraction. A U.S. Pat. No. by Melnychyn et al. U.S. Pat. No. (3,360,753) discloses a process for producing a freeze-dried isolate. The process is conducted in the presence of specific types of oxidizing or thiol bearing reagents which are capable of reacting with disulfide linkages at elevated temperatures with the extracted protein being precipitated at pH 4.5.

An early U.S. Pat. No. by John R. Turner (2,489,208) discloses the use of an alkaline material such as sodium sulfite, sodium carbonate or sodium hydroxide to extract glycinin at a pH 6.4-6.8. The glycinin is then precipitated from the extract by adjusting the extract to its isoelectric pH (e.g. pH 4.2-4.6) with sulfur dioxide.

Several publications also report the affects of saline solutions upon the extraction of soy proteins. A publication by A. K. Smith et al., (Jr. American Chemical Society, Vol. 60, June 1938, pages 1316-1320) mention "that the amount of protein extracted from seed by neutral salts depends upon the kind and concentration of salt used". Smith et al. reports that the extraction of soybean meal with pH 6.7 water alone yields more protein extract than an aqueous extraction in the presence of neutral salts. The aqueous extraction of defatted soy meals at a pH 4.0-7.0 in the presence of water-soluble sulfite, bisulfite or dithionate salts, preferably an alkali metal (including ammonium) salt and precipitation of an isolate at pH 4.8 has been reported by British Pat. No. 1,377,392.

U.S. Pat. No. 4,131,607 by Petit et al. discloses a two-stage alkaline extraction. The extraction is initially conducted in the presence of sodium sulphite and magnesium salt at a pH 7.0-8.5 which is then increased to a pH 10.0-10.5 to complete the extraction. The protein extracts are then precipitated or curded by adjusting the extract to a pH 4.5-5.5. A patent issued to Martinez et al. (U.S. Pat. No. 3,579,496) similarly discloses a double extraction process. A 0.008 M aqueous polyvalent cationic salt solution is initially employed to extract a protein fraction which is curded therefrom at pH 3.8-4.2. The second extraction is conducted at an alkaline pH in excess of pH 9.0 with the extracted protein being recovered at pH 6.8-7.1.

Thanh et al. (Jr. Agr. Food Chem., Vol. 24, No. 6, 1976, pages 1117-1121) disclose a preparative technique for precipitating 11S from a crude 7S and 11S soy globulin mixture by adjusting the protein extract buffered with dilute tris(hydroxymethyl)aminomethane to a pH 6.4. The acid-precipitated 11S protein is recovered by centrifugation and the 7S protein is separated from the supernatant by pH 4.8 acid-precipitation. The 7S precipitate was redissolved in water, buffered with tris and adjusted to pH 6.2 to solubilize the 7S therefrom. The pH, ionic strength, tris buffer and protein concentration reportedly affect the efficacy of the 7S and 11S fractionation.

Other articles reporting means for the separating of the 7S or 11S components include "Purification of the 11S Components of Soybean Protein" by Eldridge et al. (Cereal Chem. Vol. 44, Nov. 1967, pages 645-652), "An Electrophoretic Analysis of Soybean Protein" by Briggs et al., (Cereal Chem., Vol. 27, May 1950, pages 243-257) and "Purification and Characterization of the 11S Component of Soybean Proteins" by Wolf et al., (Archives of Biochemistry and Biophysics 85, pages 186-199 (1959)).

As evident from the above art, a host of 7S and 11S fractionation processes have been proposed. Although many of the proposed fractionation processes may be useful to laboratory study and characterize the different protein fractions of soy proteins, the proposals generally are unsatisfactory of adaptation to a commercial manufacture of 7S and 11S isolates.

### DESCRIPTION OF THE INVENTION

According to the present invention there is provided an improved method for separating and recovering at least one vegetable protein fraction from a crude vegetable protein extract mixture which contains 7S and 11S protein, said method comprising: