

METHOD FOR FRACTIONATION OF VEGETABLE PROTEINS BY REDUCTION

This application is a continuation of now abandoned application Ser. No. 826,389 filed Feb. 5, 1986.

FIELD OF THE INVENTION

The present invention relates to a method for fractionation of proteins, particularly, edible vegetable proteins such as soybean protein.

BACKGROUND OF THE INVENTION

The production of a soybean protein isolate has been carried out heretofore by treating a soybean protein source in an aqueous system to fractionate it into a water soluble or dispersing fraction and a water insoluble or precipitate fraction (an insoluble residue called "okara" or "soy pulp"), subjecting the water soluble or dispersing fraction to isoelectric precipitation at pH 4 to 5, usually, at pH 4.2 to 4.6 to form a precipitate, and working up the precipitate by, for example, neutralization and drying to isolate a desired fraction of soybean protein.

Also, like other many vegetable proteins, soybean protein is composed of various protein fractions having complex higher-order structures. For example, in a method for fractionation of soybean protein by ultracentrifugation based on difference in sedimentation constant between protein fractions, soybean protein is fractionated into various protein fractions such as 2S, 7S, 11S, 15S and the like and these fractions have different physical properties. Further, each protein fraction is composed of several subunits. For example, 7S protein fraction has 3 subunits and 11S protein fraction has 12 subunits. Various studies have shown that the natures of these protein fractions and subunits (e.g., higher-order structure, interaction between subunits, etc.) are varied according to alterations in circumstances (e.g., ionic strength, pH, temperature, concentration, etc.), and their properties are also varied.

By utilizing these facts, various methods for fractionation of soybean protein and other vegetable proteins have been proposed. And, in many of these methods, physical, functional and chemical properties of the resultant fractionated and isolated soybean protein products are considerably varied, even if conditions employed (e.g., ionic strength, pH, presence of a certain salt, concentration, temperature, difference in order of operations, etc.) are only slightly changed. These variations result from not only the composition ratio of the above protein fractions such as 7S, 11S and the like, but also higher-order structural change, interaction between protein fractions and/or subunits, and the like.

Among these known methods for fractionation of vegetable proteins, Japanese Patent Laid Open Publication No. 48-56843 discloses fractionation of 11S fraction and 7S fraction of soybean protein by using a diluted calcium salt. Japanese Patent Laid Open Publication No. 49-31843 discloses preparation of 7S fraction of soybean protein by removing an insoluble fraction in the presence of sodium chloride or potassium chloride at pH 1.2 to 4.0. Japanese Patent Laid Open Publication No. 51-86149 discloses extraction of a thermal coagulative viscous protein from an aqueous slurry of a source of vegetable protein such as an oilseed material with water at pH about 5.1 to 5.9. Japanese Patent Laid Open Publication No. 55-124457 discloses preparation of 7S

protein fraction from soybeans by extraction at pH 5.40 to 5.85 and isoelectric precipitation at pH 4.5. Japanese Patent Laid Open Publication No. 55-153562 discloses preparation of a soybean protein product by fractionation of soybean protein into 1st fraction at pH 6.0 to 7.0, a 2nd fraction at pH 5.0 to 5.6 and a 3rd fraction at pH 4.0 to 4.8 and respective isolation of the 2nd and the 3rd fractions. Japanese Patent Laid Open Publication No. 56-64755 discloses preparation of a protein product by extraction of vegetable protein with an aqueous extracting medium at pH about 6.5, precipitation at isoelectric point, heating at 115° to 145° F. and concentration of solids content to 44% or more. Japanese Patent Laid Open Publication No. 57-132844 discloses preparation of a protein isolate by extraction of a fraction from an aqueous slurry of vegetable protein at pH 6.5 to 8.0 in the presence of sulfite ion and drying. Japanese Patent Laid Open Publication No. 58-36345 discloses isolation of 7S and 11S fractions by adjusting pH of an aqueous slurry of vegetable protein resulted from isoelectric precipitation to 5.0 to 5.6, and adjusting a salt concentration to 0.01 to 0.2M.

Further, experimental methods for fractionation of soybean protein have been reported by Roberts, R. C. and Briggs, D. R., *Cereal Chem.*, 42: 71-85 (1965); Eldrige, A. C. and Wolf, W. J., *Cereal Chem.*, 44: 645-652 (1967); Wolf, W. J. and Sly, D. A., *Cereal Chem.*, 44: 653-668 (1967); and Thanh, V. H. et al, *Plant Physiol.*, 56, 19-22(1975). Particularly, the report of Thanh, V. H. discloses fractionation of 7S globulin of soybean protein by extraction of soybean meal with a Tris-buffer solution containing beta-mercaptoethanol (pH 7.8), centrifugation at 10,000 r.p.m. to remove insoluble materials, adjustment of the pH of the supernatant to 6.6, dialysis, centrifugation at 10,000 r.p.m. to fractionate into a crude 11S fraction and a crude 7S fraction, isoelectric precipitation of the 7S fraction, washing, and then lyophilization. Yamauchi, F. et al, *Agric. Biol. Chem.*, 39, (1975) report the isolation of 11S fraction of soybean protein from the same crude 11S fraction as that in Thanh et al by washing, neutralization and dissolution in a buffer solution.

However, these known methods for fractionation of vegetable protein are insufficient for industrial purpose, particularly, for application in the industrial production of a soybean protein isolate.

In order to overcome such a drawback of the known methods, the present inventors have made intensive studies. According to the present inventors' study, various problems are involved in the known methods. For example, (1) the fractionation of a crude 7S fraction and a crude 11S fraction is hardly expected by mere substitution of an inorganic acid and the like for the Tris-buffer solution to adjust pH; (2) a chemical agent such as the Tris-buffer solution and beta-mercaptoethanol can not be used in the food industry; (3) particularly, beta-mercaptoethanol has a strong unpleasant odor and is impossible to use in foodstuffs; and (4) it is difficult to successfully separate a solution part (crude 7S fraction) from a precipitated slurry (crude 11S fraction) with an industrial continuous centrifuge of low centrifugal force (e.g., decanter) because the solution obtained by adjusting pH to 6.6 after separation of the insoluble material by centrifugation has a very high viscosity.

It has been found that these problems can be solved by (1) treating a vegetable protein source in an aqueous system with a sulfite compound, a glutathione compound or a cysteine compound at pH 6.5 or higher, and