Preparation of Soy Protein Concentrate by Ultrafiltration

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ABSTRACT: Defatted soy flour was enzymatically treated with commercial pectinases and diafiltered using a porous stainless steel ultrafiltration membrane system. The membrane soy concentrate (MSC) consisted of 78.5% protein and had reduced levels of phytic acid. Gel filtration profiles indicated that proteins with molecular weights greater than 6.5 kDa were rejected by the membrane and retained in the soy concentrate. A 10% (w/w) initial solids concentration was optimal in terms of processing time and yields, with marginal decreases in flux and permeability. The production of MSC by this process was highly reproducible with 17 to 26% higher protein recovery yields than current commercial processes.

Keywords: soy protein concentrate, ultrafiltration, membrane separation

Introduction

SOY PROTEIN CONCENTRATES AND ISOLATES ARE VALUABLE INgredients in foods due to their high nutritional value and possible health benefits. Their use has been limited due to undesirable flavors (Mattick and Hand 1969; Kalbrener and others 1971; Macleod and Ames 1988), reduced bioavailability due to the presence of high levels of phytic and nucleic acids (Wolf 1978; Maga 1982; Harland and Morris 1995) and functional problems such as insolubility (Anno and others 1985).

Edible soy proteins may be in the form of flours, concentrates, or isolates made from defatted soybean flakes. Flours and grits contain at least 50% protein. Soy protein concentrates contain at least 70% protein on a dry weight basis and are made by extracting the proteins in aqueous alcohol or with a dilute acid solution in the pH range of 4.0 to 4.8. Soy protein isolates contain a minimum of 90% protein on a dry weight basis and are produced by extracting the soy flour with a dilute alkali (pH $<$ 9) followed by centrifugation (Mounts and others 1987). The extract is adjusted to pH 4.5 with a food grade acid such as sulfuric, hydrochloric, phosphoric, or acetic to enable precipitation of proteins. The acid-precipitated protein curd is then centrifuged, washed, neutralized, and spray-dried to produce the soy protein isolate (Circle and Smith 1978). The yields of soy isolates and concentrates vary between 60 to 70% of the protein in the flour (Mounts and others 1987).

Phytic acid (inositol hexaphosphoric acid) is part of a large class of compounds that influence the functional and nutritional properties of foods. The phytic acid content in soy flour has been reported to be as high as 2.24% (Wolf 1978). Phytate forms insoluble or sparingly soluble complexes with proteins and mono- and divalent cations. Therefore, phytate in food components may cause the proteins and minerals to have limited bioavailability (Harland and Morris 1995). Several efforts have been made by numerous researchers to remove phytic acid. This includes, but is not limited to, phytase enzymes (Okubo and others 1975; Anno and others 1985), membrane ultrafiltration (Omosaiye and Cheryan 1979a), and anion exchange resins (Kumagai and others 1998).

Studies applying membrane filtration systems to soy protein separation were first conducted in the early- to mid-1970s. Omasaiye and others (1978) made a full-fat soy protein concentrate by ultrafiltration, using continuous diafiltration from soybean water extracts. This diafiltration method was found to be effective in removing the oligosaccharides from the full-fat soybean extract. The use of membrane filtration processing to produce soy protein products seemed to offer promise owing to its ability to separate the large protein fractions from the smaller unwanted phytate and oligosaccharide molecules (Omasaiye and Cheryan 1979b).

Membrane separation processes consume less energy when compared to other concentrating techniques, such as freeze-drying or evaporation. Lower consumption of energy is related to the absence of change in phase or state of the solvent during the ultrafiltration process. Another advantage of ultrafiltration is its operation at low, ambient, and high temperatures depending on the nature of the application and the solids to be concentrated. It is believed that products of ultrafiltration should have improved properties over conventionally produced soy protein isolates because heat and chemical treatments are not used (Cheryan 1983).

However, one important limitation to the process is that the solutes cannot be taken to dryness. Membrane processes are also known to be limited by higher solids. It is not the osmotic pressure of the retained macromolecules, but low mass transfer rates obtained with concentrated macromolecules and the high viscosity that makes the pumping of the retentate difficult. Skim milk has been thought to be economically concentrated to 38% solids (Cheryan 1986).

Our objectives were to utilize an ultrafiltration membrane system and enzymatic treatment as an alternative to acid precipitation to produce a soy protein isolate or concentrate with reduced phytic acid. The effect of initial solids concentration on processing time, flux, permeability, and yield were determined. The reproducibility of the ultrafiltration process for concentrating soy proteins from defatted soy flour was also established.

Materials and Methods

Materials

Ross Products Division of Abbott Laboratories (Colum-

bus, Ohio, U.S.A.) supplied defatted soy flour and acid-precipitated soy isolate. The two pectinase enzymes used were Sigma Pectinase supplied by Sigma Chemical Co. (St. Louis, Mo., U.S.A.) and Crystalzyme 100 XL supplied by Valley Research, Inc. (South Bend, Ind., U.S.A.).

Determination of phytase activity

The two pectinase enzymes were analyzed for phytase activity by the method of Chen and others (1956) in terms of phytase units (PU). The method was based on the measurement of the color formed by the release of inorganic phosphate formed by the reduction of a phosphomolybdate complex. One phytase unit was described as the amount of enzyme that liberated under standard conditions 1nmol of inorganic phosphorus from sodium phytate in 1 min.

Enzymatic treatment and ultrafiltration

A solution of 5% defatted soy flour (w/v) was enzyme treated for 3 h in a steam-jacketed kettle (Fig. 1). The temperature was maintained at 37 to 42 °C. Two different enzyme treatments, 0.3% (v/v) Pectinase, 0.9% (v/v) Crystalzyme 100XL, and a control without enzyme were used to process the defatted soy flour. The solutions were pumped through a membrane system (MWCO 300 kDa) using 3 porous stainless-steel tubular ultrafiltration membranes (60 cm $\times$ 1.57 cm i.d. per membrane) supplied by Graver Separations, Inc. (Glasgow, Del., U.S.A.). After concentration to 1x, the retentate was diafiltered with 1 vol of water. All retentate samples were adjusted to pH 9.0 with NaOH to increase protein solubility, and were then centrifuged for 20 min at 2000 $\times$ g. Both the supernatant and the pellet were collected and freeze-dried for analysis.

HPLC gel filtration separation

A Superose 6 HR 10/30 gel filtration column (Pharmacia, Uppsala, Sweden) with the UV absorbance detector set at 220 nm was used to determine the molecular weight profile of the soy flour, retentate, and permeate samples. The eluent was 0.1 M Tris, 0.1 M NaCl pH 8.0 buffer, at a flow rate of 0.5 mL min^{-1}. Soy flour and retentate samples were dissolved in 0.1 M Tris, 0.1 M NaCl pH 9.0 buffer. The samples were filtered through a 0.45-mm Nylaflo membrane (Pall Gelman Laboratory, Ann Arbor, Mich., U.S.A.) before sample injection. Standard proteins for molecular weight comparison included: apoferritin (MW 443,000), β-amylase (MW 200,000), bovine serum albumin (MW 66,000), ovalbumin (MW 45,000), a-lactalbumin (MW 14,200) and tryptophan (MW 204).

Determination of phytic acid

Samples of 5% (w/v) soy flour were treated with the 2 enzymes at 37 °C for 3 h and analyzed for phytate content by the method of McChance and Widdewson (1935).

Compositional analysis

The compositional analysis of soy flour, commercially available acid-precipitated soy isolate, and the membrane soy concentrate produced, included protein (AOAC 1975), carbohydrate (Brooks and others 1986), ash, and moisture (AOAC 1990).

Protein recovery and yields

The protein content of soy flour, membrane soy concentrate, permeate, and pellet were determined by standard AOAC procedure (1975) to calculate yields.

Process optimization and reproducibility

Defatted soy flour solutions of varying solids concentrations, namely 5%, 10%, and 12.5% (w/w), were used in the production of membrane soy concentrate to study the effect of solids concentration ratios on processing time, flux, permeability, and yields. Based on the results observed for process optimization, the appropriate solids concentration was used to produce membrane soy concentrate in 6 separate batches to establish reproducibility.

Results and Discussion

Membrane ultrafiltration

Flux (permeability), an important indicator of membrane functionality, is expressed as a volume flow of liquid through a unit area of membrane at some defined transmembrane pressure, and is measured in units of velocity; that is, liters $meter^{-2}$ h^{-1} (LMH) per unit of pressure (kPa) (Cheryan 1998).

The permeability of the membrane dropped rapidly with both enzyme treatments and the control at the beginning of the ultrafiltration process (Figure 2). This was due to the im-

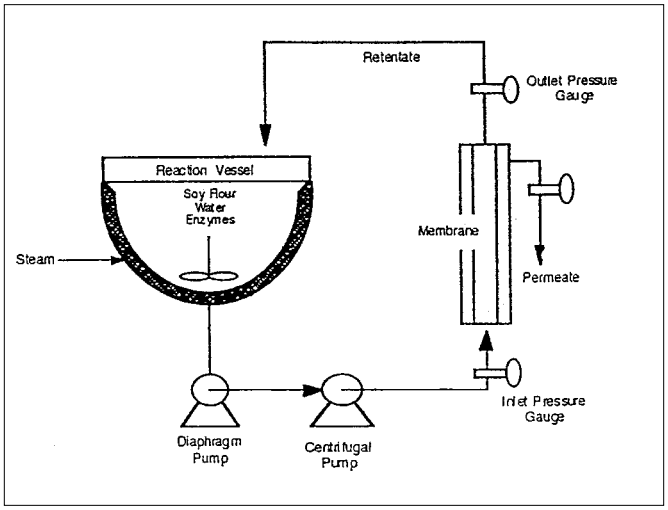

Figure 1—Schematic illustration of the ultrafiltration membrane system

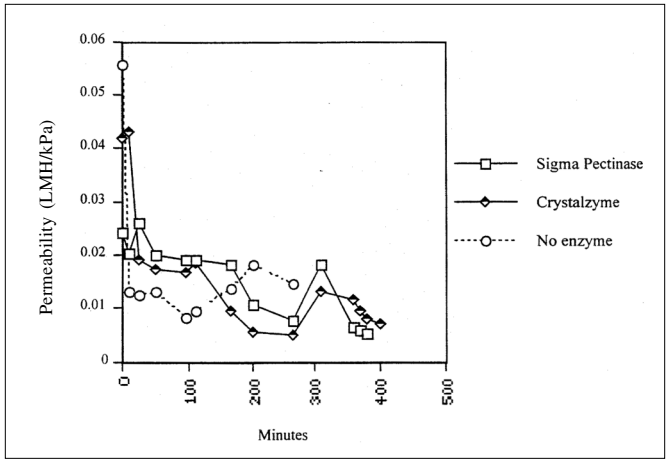

Figure 2—Comparison of membrane permeability with and without enzyme treatment

Table 1—Phytate content (%) of soy flour

Sample	Phytate content
Soy flour (control)	2.11
Soy flour (no enzyme/ultrafiltration/diafiltration)	1.18
Soy flour (pectinase enzyme/no filtration)	0.03
Soy flour (pectinase enzyme/ultrafiltration)	0.02
Soy Flour (pectinase enzyme/ultrafiltration/diafiltration)	0.02

Table 2—Protein content of soy flour, diafiltered retentate and permeate

Sample	Protein content (%)
Soy flour	51.2
Retentate (Sigma Pectinase-treated)	76.7
Retentate (Crystalzyme-treated)	78.5
Permeate	<0.5

Table 3—Compositional analysis of membrane soy concentrate (MSC) produced in 3 batches of varying initial solids concentration

Solids concentration (% w/w)	Protein (%)	Carbohydrate (%)	Ash (%)
5	78.3[a]	4.01	4.73
10	78.2[a]	4.77	4.89
12.5	76.1[a]	3.20	7.89

Means with the same letter are not significantly different ($P < 0.05$).

mediate formation of a secondary layer on the membrane surface. The permeability of the membrane increased slightly at the beginning of diafiltration, but then declined at a slow rate throughout the diafiltration process. No differences in membrane performance, as reflected by permeability, were noted between the 2 pectinase enzyme treatments.

While no differences in flux were observed, the permeability of the membrane decreased less rapidly when the soy flour was treated with either Sigma Pectinase or Crystalzyme. A slight increase in permeability was seen after diafiltration. The enzyme treatments increased membrane permeability during the initial stages of the process and during diafiltration, thus decreasing the overall process time for an equivalent volume (Figure 2).

Concentration of soy proteins

The HPLC profiles of proteins from soy flour, diafiltered retentate, and permeate are shown in Figure 3. The retention times for peaks in the soy flour and retentate were the same, but some changes in peak areas were observed. This indicated that most of the soy flour proteins were rejected by the membrane and were collected in the retentate. The 1st peaks in the permeate samples had a retention time around 40 min, which corresponds to a molecular weight of 6.5 kDa. Diafiltration with one-half the original volume of water was found to remove low molecular weight (6.5 kDa and below) proteins. Diafiltration with 1 or 2 vols of water has been known to be effective in the removal of low-molecular-weight peptides from soy protein hydrolysates (Deeslie and Cheryan 1991; De la Barca and others 2000). Based on the above observations, it is clear that proteins with molecular weights greater than 6.5 kDa were retained by the membrane, whereas proteins with molecular weights less than 6.5 kDa passed through the membrane into the permeate.

Reduction of phytic acid

Phytic acid content of soybeans has been reported to be 10 to 14.7 mg/g, and soy flour is known to contain 22.4 mg/g (Maga 1982). Okubo and others (1975), using ultrafiltration, produced a low-phytate soybean protein isolate. Omosaiye and Cheryan (1979a) found that ultrafiltration alone resulted in a 65% reduction of phytate. The phytate removal from proteins depended on the type of cations and ionic strength, the nature of the protein, and the pH of the solution. A pH of 6.7 was found to be optimum at which the phytate appeared to be water-soluble. This solubility was believed to be due to the presence of weak salt linkages complemented by the absence of strong electrostatic attraction. In this study too, membrane ultrafiltration alone, without the use of the pectinase enzyme, was found to reduce the phytic acid content (Table 1).

However, the use of commercial pectinase enzyme prior to ultrafiltration resulted in a much higher (90%) reduction of phytate (Table 1). The successful use of phytase enzymes for the reduction of phytic acid has already been investigated. Anno and others (1985) with the use of enzyme phytase reduced the phytic acid content in soybean milk to 0.52 to 1.11 mg/g.

Based on the results and reduction in phytate observed after the enzymatic treatment, the 2 pectinase enzymes were analyzed for phytase activity. While both pectinases were found to contain phytase activity, Sigma Pectinase was determined to have a 3-fold higher phytase activity (728 PU/mL) than Crystalzyme 100 XL (222 PU/mL). The enzyme Crystalzyme 100 XL was selected for subsequent production of membrane soy concentrate due to its lower cost price.

Compositional analysis

The protein, carbohydrate, ash, and moisture contents of soy flour, commercial acid-precipitated soy isolate (APSI),

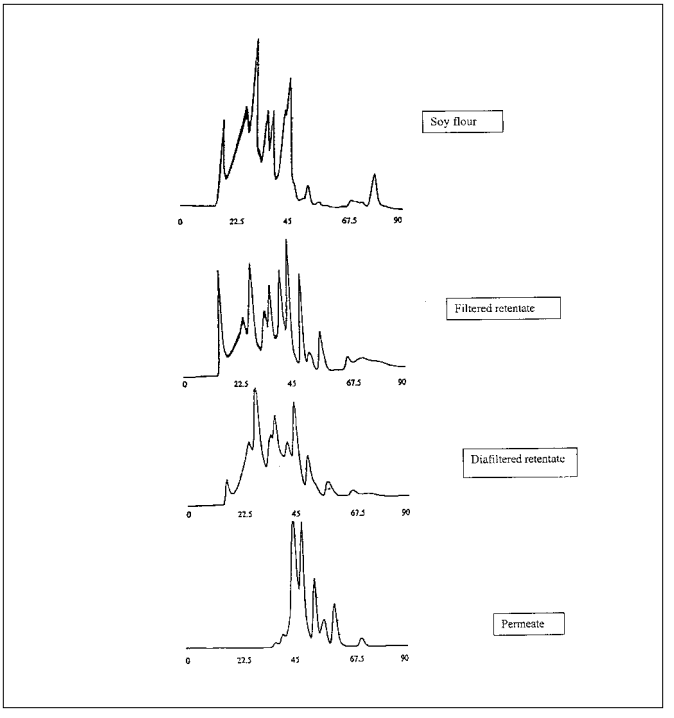

Figure 3—HLPC gel filtration profiles for soy flour, diafiltered retentate and permeate

Table 4—Per cent composition of the membrane soy concentrate and processing yields

Batches *	Protein (%)	Carbohydrate (%)	Ash (%)	Yield (%)
One	78.21[a]	5.22[a]	4.73	82.89
Two	78.92[a]	3.67[a,b]	4.86	77.75
Three	78.21[a]	3.28[b]	4.92	80.64
Four	79.53[a]	5.84[c]	5.34	84.71
Five	80.56[a]	5.78[c]	5.67	81.51
Six	81.08[a]	5.69[c]	5.60	87.32

* All batches were processed with an initial solids concentration of 10% (w/w). Means with the same letter are not significantly different ($P < 0.05$).

and membrane soy concentrates (MSC) produced by the method described herein, are shown in Figure 4. It was apparent that processing with pectinase enzymes and ultrafiltration, without the use of acid precipitation, can produce a soy protein concentrate approaching the composition of acid-precipitated soy isolate.

Protein recovery and processing yields

The protein content of soy flour was 51.2%. The retentate from the Sigma Pectinase enzyme treatment and ultrafiltration that was adjusted to pH 9.0 and centrifuged had a protein content of 76.7%. The retentate from the Crystalzyme 100XL enzyme treatment that had been adjusted for pH and centrifuged had a protein content of 78.5% (Table 2). The permeate contained less than 0.5% protein on a wet basis. It is possible that the nitrogen in the permeate is nonprotein nitrogen that was released from the nucleic acids. Omosaiye and others (1978) found that 5% of soybean nitrogen was nonprotein nitrogen. Thus, the rejection of the soybean proteins was estimated to be nearly 100%.

The yield of the protein fraction was 79%. Ten percent was lost in the permeate, and this loss probably cannot be reduced. Another 11% of the protein was lost in the pellet after centrifugation. Reduction of this loss may be possible in order to maximize the protein yield. The yields reported in the literature for soy protein isolates were 30% of the total soy flour and 60% of the protein (Mounts and others 1987). Therefore, the process described here would significantly increase protein yields over current commercial processes.

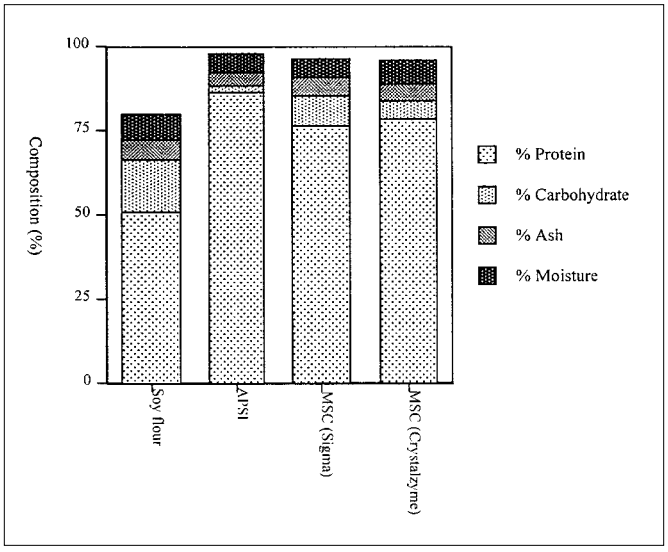

Figure 4—Compositional analysis of soy flour, acid precipitated soy isolate (APSI) and membrane soy concentrate (MSC

Effect of solids concentration on processing parameters and nutrient composition

Proteins from defatted soy flour at 5% (w/v) solids concentration were successfully concentrated using membrane separation technology. Optimization of the process with respect to higher solids concentration is advantageous when making commercial recommendations. Therefore, 3 different initial solids concentrations of 5%, 10%, and 12.5% (w/w) were processed in 3 different batches for the production of soy protein concentrate.

An increase in solids concentration was associated with a decrease in flux (Figure 5). A similar phenomenon has been observed when membranes were used to concentrate solids from alkaline protein extracts of soy flour (Lawhon and others 1977, 1978). Concentration polarization and fouling effects were thought to be responsible for the decrease in flux observed with an increase in solids ratio (Jayarajah and Lee 1999).

Doubling the solids concentration from 5% to 10% did not decrease the permeability remarkably, but a further increase in solids concentration to 12.5% altered the permeability such that the processing time greatly increased (Figure 6). Increasing the average transmembrane pressures from 400.75 kPa at 5% solids concentration to 468.62 kPa at 12.5% solids concentration was not sufficient to overcome the decrease in permeability of the ultrafiltration membranes. Hensley and others (1977) explained this to be a point of diminishing return when increased pumping energy is not always concomitant with a corresponding increase in flux. The protein content of the membrane soy concentrate was similar ($P < 0.05$) as shown in Table 3, thereby suggesting that the solids concentration did not affect the composition.

Reproducibility of the membrane separation process

Based on the results of process optimization, a 10% solids concentration was used to produce membrane soy concentrate in 6 separate batches. The nutrient composition of the membrane soy concentrate and the yields from each batch are summarized in Table 4. The protein composition of the membrane soy concentrate produced was consistent with the coefficient of variation being less than 1.5%, thereby establishing the reproducibility of the membrane separation process used in the concentration of soy proteins. Protein recovery based on yields was calculated to be in the range of 77 to 86%. Membrane processing used to concentrate soy

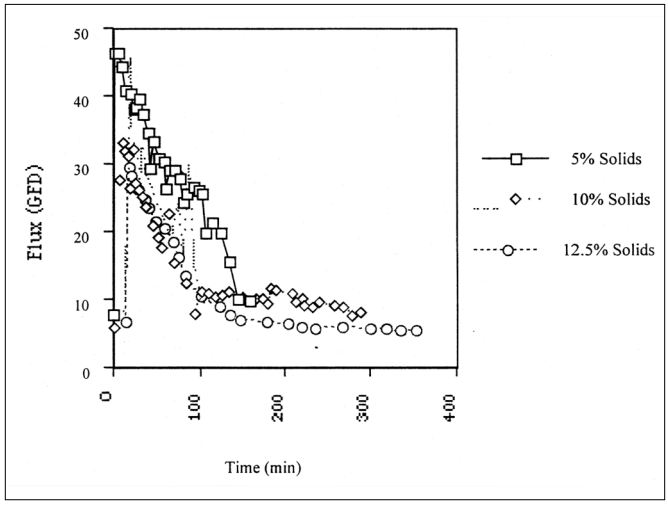

Figure 5—Effect of initial solids concentration on flux

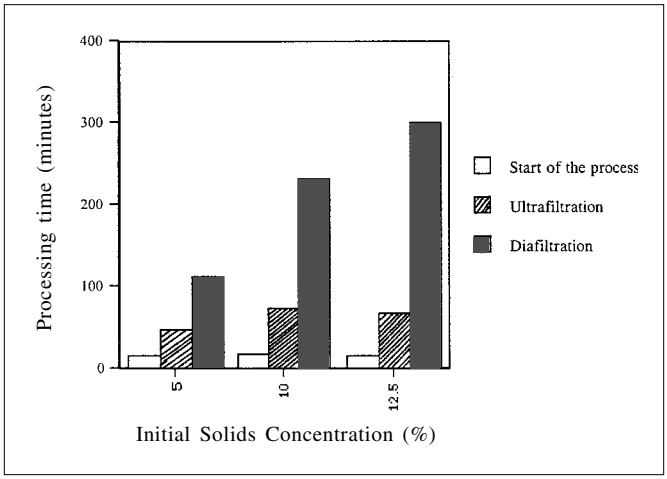

Figure 6—Effect of initial solids concentration on processing time

proteins increased protein recovery by 17 to 26% when compared to current commercial processes.

Conclusions

TREATMENT OF SOY FLOUR WITH COMMERCIAL PECTINASES followed by ultrafiltration resulted in a soy concentrate (78.5% protein) with a very low concentration of phytic acid. This is due to contaminant phytase activity found in these relatively crude commercial enzyme preparations. An increase in initial solids concentration was associated with a decrease in flux. Concentration polarization and fouling effects are thought to be responsible for the decrease in flux. Increasing the initial solids concentration from 5% to 10% did not substantially decrease the permeability. However, a further increase from 10% to 12.5% altered the permeability such that the processing time was greatly increased, even with an increase in pumping energy. The protein composition of the membrane soy concentrate produced in 6 different batches was consistent. Protein recovery based on calculated yields for the membrane separation technology was 17 to 26% higher than commercial processes currently in use for separation and isolation of soy proteins.

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