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PREPARATION OF UNHEATED SOY PROTEIN ISOLATES WITH LOW TRYPSIN INHIBITOR CONTENT

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ABSTRACT

The nutritional quality of soy protein products is affected by the processing conditions employed in their manufacture. Heat treatment during processing serves to inactivate the inhibitors of trypsin and chymotrypsin, enzymes which play a key role in the digestion of protein in animals. In the processing of defatted soy flour to prepare a soy protein isolate, generally no heat treatment is used. Instead, a high percentage of the trypsin inhibitors (TI) is physically removed in the whey fraction. However, depending on their mode of preparation, soy isolates may contain trypsin inhibitor activity as high as 40% of that found in raw soybeans. Using "salting in" techniques we found that a higher percentage (97.7%) of the TI was solubilized and removed with the whey fraction when the protein curd was precipitated from 0.1 N NaCl solution at pH 5.4. Using membrane techniques for the separation of TI from non-TI-protein, best results were obtained with 0.1 N. $MgCl_2$ where 79% of the TI was removed in the permeate.

INTRODUCTION

Edible soybean protein isolates were introduced commercially in 1957 and in the intervening years we have seen a steady increase in the use of these products as ingredients in a wide variety of fabricated or engineered foods. (Wolf, 1981) Widespread adoption of soybean proteins in foods has stimulated the need for more research on processing parameters that may be necessary to produce safe, nutritious soya foods (Wolf, 1983).

Osborne and Mendel (1917), reported that rats grew poorly when fed raw soybean meal. A number of biologically active substances have been identified in soybeans, but trypsin inhibitors (TI) have received the most attention. They not only inhibit trypsin and chymotrypsin, important enzymes involved in digestion of proteins, but indirectly, they stimulate secretion of pancreatic enzymes into the intestine which, in turn, cause pancreatic hypertrophy and loss of undigested pancreatic enzymes into the feces. Excessive loss of essential amino acids through the feces is believed responsible for inhibition of growth in young rats (Rackis, 1974).

Trypsin inhibitors are inactivated by moist-heat treatment which, at

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Table 1. Composition of Commercial Defatted Soy Flour Component

Moisture %	9.5
Ash %	6.6
Protein %	51.4
Nitrogen Solubility Index	85.8
Trypsin Inhibitor mg/g Protein	55

the same time, denatures the raw proteins causing modification in protein functionality (i.e., dispersibility, viscosity, emulsification, etc.). In previous studies at this Center, (Baker and Rackis, 1985) using ultrafiltration membranes with molecular weight cutoffs (MWCO) of 100,000 daltons or less, a significant portion of the TI (MW <21,500) was rejected by the membrane. This apparent contradiction might be explained if aggregation of the TI with non-TI proteins was occurring. Consequently for this study the more porous microfilter membrane was chosen, since it presents less of a barrier to the passage of TI into the permeate.

The earlier study at this Center had also shown that the separation of TI from the isoelectric precipitated protein curd was enhanced by the addition of 0.1 N sodium chloride. Other salts which are known to have a solubilizing effect on proteins in water, such as magnesium chloride, aluminum chloride and sodium lactate, were included in this study.

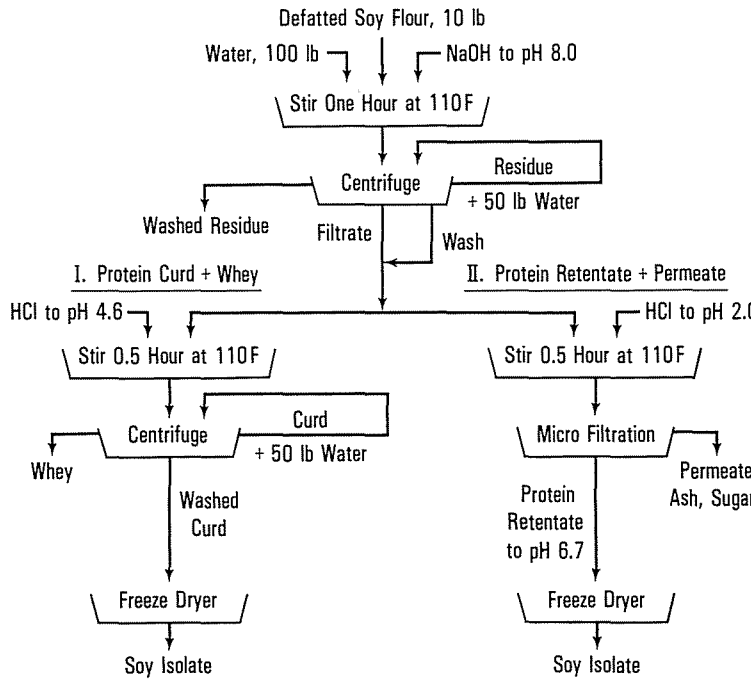

Fig. 1. Alternate procedures for preparation of soy isolates.

MATERIALS AND METHODS

Commercial defatted soy flour, Nutrisoy 7 B (Archer Daniels Midlands, Decatur, Illinois), was used as the starting material in all experiments. The commercial process for the preparation of defatted soy flour uses a mild heat treatment and results in minimal denaturation of protein as evidenced by the nitrogen solubility index of 85.8 (Table I). The TI activity of 55 mg/g protein approximates the value often observed with raw beans.

Trypsin inhibitor was determined by the method of Hammerstrand et al. (1981) which evolved primarily from the work of Kakade et al. (1974). Total solids were determined by evaporation.

Equipment and Procedure

Defatted soy flour (10 lbs) was suspended in 10 parts of water in a 30-gallon stainless steel agitated tank fitted with an internal coil for heating or cooling. Slurry pH was adjusted to 8.0 with sodium hydroxide and the temperature was regulated at 110 F. The insoluble residue, which consisted mainly of polysaccharides, was separated using a 4-inch solid bowl (17,000 rpm) centrifuge (Sharples, Pennwalt Corp., Warminister, Pennsylvania).

When the isoelectric precipitation procedure was followed (Scheme I, Figure 1), salts were added to the combined filtrate and wash and pH was adjusted with hydrochloric acid to the isoelectric region (pH 4.2 - 5.4). The precipitated curd was separated from the whey by centrifugation. The curd was resuspended with 5 parts of water and recentrifuged. The washed curd was freeze dried in a 5-shelf, 50-lb-capacity freeze dryer (Virtis Co., Gardiner, New York). Experiments were designed to test the effects on yield and TI removal of added salts, salt normality and pH.

In the second scheme (Scheme II, Figure 1) which utilizes membrane separation, salts were added to the combined filtrate and wash from the residue removal step and pH was adjusted to either 2 or 8 to achieve maximum solubility of the protein. The protein solution was then pumped to a spiral-wound microfiltration module with a molecular weight cutoff of >300,000 daltons. Feed volume was maintained at a constant level by the addition of fresh water at the same rate as permeate was being removed until permeate equal to 2 feed volumes was collected. The feed was next concentrated to <40 lb and freeze dried. In experiments run at pH 2, the retentate was adjusted to pH 6.5 before freeze drying. Experiments were designed to test the effect of added salts on yield and residual TI in the isolates.

Results and Discussion

Smith and Circle (1938) have shown that the extractability of proteins in defatted soybean meal is a function of pH (Figure 2). The region of minimum protein solubility encompasses a narrow region bounded by pH 3.8 to pH 5.2 with the isoelectric point situated near the mid point of the region at approximately pH 4.6. Precipitation of a protein curd in the isoelectric region and separation of the curd from the whey by centrifugation is one of the more common procedures used in the commercial preparation of soy protein isolates. The regions of maximum protein solubility (i.e. pH 1.5 - 2.5 and pH 7 and above) are of interest when membrane techniques are employed to separate the larger proteins from the other constituents of defatted soy flour. The membrane rejects the larger protein molecules while passing the smaller TI, sugars and ash. Wolf et. al. (1962) showed the TI (MW 8000-21,500) to be in the 2s ultracentrifuge fraction which

Table 2. Ultracentrifuge Fractions of Soybean Proteins

Fraction	Percent of Total	Component	Molecular Weight
2s	22	Trypsin inhibitors	8000-21,500
		Cytochrome C	12,000
7s	37	Hemagglutinins	110,000
		Lipoxygenases	102,000
		B-Amylase	61,700
		7s-Globulin	180,000-210,000
11s	31	11S-Globulin	350,000
15s	11	---	600,000

Source: Wolf et al. (1962)

contains the low M.W. proteins (Table 2).

Isolates Via Isoelectric Precipitation

With the control experiment (no added salts) a protein yield of 63% was obtained which was close to the expected yield in commercial processing

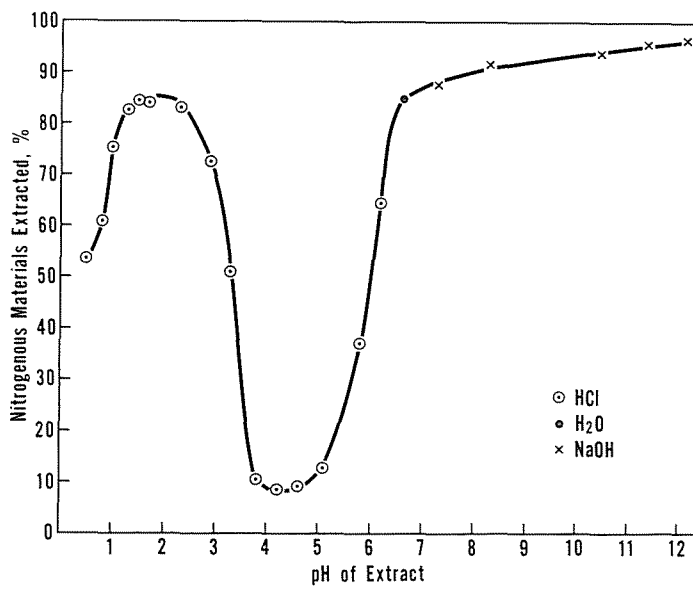

Fig. 2. Extractability of proteins in defatted soybean meal as a function of pH source, Smith and Circle (1938).

Table 3. Effect of Various Salts on Yield and Residual TI in Soy Protein Isolates Via Isoelectric Precipitation

		Soy Protein Isolates			
	Curd Precipitation pH	Protein Content %	Protein Yield %	Trypsin Inhibitor mg Per g Protein	Trypsin Inhibitor % of Original
Defatted Soy Flour	—	51.4	100	55	100
Soy Isolates					
Control	4.6	91.8	63	15.8	18.6
I 0.1N NaCl	5.0	91.8	52	4.6	3.9
0.1N $MgCl_2$	5.0	91.2	55	6.7	6.9
1.2% $AlCl_3$[a]	5.0	88.1	46	6.2	5.4
4.0% Na Lactate[b]	5.0	94.1	60	7.2	7.8
II 0.05N NaCl	5.0	92.5	59	13.6	14.7
0.10N NaCl	5.0	91.8	52	4.6	3.9
0.50N NaCl	5.0	81.9	14	7.8	2.3
III 0.10N NaCl	4.2	90.6	58	12.7	13.2
0.10N NaCl	4.6	91.2	60	8.2	8.9
0.10N NaCl	5.0	91.2	52	4.6	3.9
0.10N NaCl	5.4	92.5	51	2.6	2.3

[a]U.S. Patent 4,375,431, Bradford et al. 1983.
[b]Private communication, W. H. Martinez, USDA, NPS, Washington, D.C.

of soy isolates. (Meyer, 1966) The TI content of 15.8 mg/g protein (Table 3) approximated the values obtained when several commercial isolates were analyzed in our laboratory. When salts were added, residual TI content of the isolates was reduced in all cases; the best result (4.6 mg/g protein) was obtained with 0.1 N sodium chloride. When the normality of the sodium chloride was decreased to .05N, the beneficial effect of the salt was virtually eliminated. Increasing the normality to 0.50N solubilized so much of the protein that the curd yield was only 14% with most of the protein lost to the whey. Residual TI content of the isolate was shown to be a function of pH in the isoelectric region. Best results were obtained at the upper end of the region (pH 5.4) where the residual TI content was reduced to 2.6 mg/g protein with 97.7% of the TI removed in the whey. Protein yield was also a function of pH in the isoelectric region. Best yield was 60% obtained predictably at the isoelectric point, pH 4.6. At pH 5.4 the yield fell to 51% but this may be tolerable if the primary concern is low TI content in the isolate.

Isolate Via Membrane Processing

In the first series of experiments in which the feed to the microfilter was adjusted to pH 2, protein recovery was 67% of the original flour protein (no added salts) and residual TI was 39% of the original (Table 4). Both sodium and magnesium chlorides were found to be most effective at a concentration of 0.1 normal, while Bradford et. al. (1983) observed that the best results were attained with aluminum chloride at a level of 1.2 g per-100 g soy flour. Conversely, sodium lactate had little effect at concentrations below 4%. When salts were added, a greater quantity of TI was

Table 4. Effect of Various Salts on Yield and Residual TI in Soy Protein Isolates Via Membrane Processing

	Feed To Microfilter pH	Soy Protein Isolates			
		Protein		Trypsin Inhibitor	
		Content %	Yield %	mg Per g Protein	% of Original
Defatted Soy Flour	—	51.4	100	55	100
Soy Isolates					
Control	2.0	88.2	67	32	39
0.1N NaCl	2.0	86.3	69	24	29
0.1N $MgCl_2$	2.0	85.7	64	18	21
1.2% $AlCl_3$[a]	2.0	83.1	67	22	27
4.0% Na Lactate[b]	2.0	83.8	69	28	34
Control	8.0	88.2	50	28	29
0.1N NaCl	8.0	85.6	36	27	18
0.1N $MgCl_2$	8.0	85.3	38	18	19
1.2 % $AlCl_3$[a]	8.0	86.9	53	27	26
4.0% Na Lactate[b]	8.0	87.5	47	28	24

[a]U.S. Patent 4,375,431, Bradford et al. 1983.
[b]Unpublished data - W. H. Martinez, USDA, NPS, Washington, D.C.

removed with the permeate in all cases. If aggregation of TI with either itself or the non-TI-protein does, in fact, occur, the salts then appear to be partially responsible for dissociation of the aggregate. Best results were obtained with 0.1N $MgCl_2$ which gave a protein recovery of 64% and a residual TI content of 21% of the original.

In the second series of experiments in which the feed to the microfilter was adjusted to pH 8, protein recovery was only 50% with the control. This would indicate that mid-range MW proteins that were rejected when fed to the membrane at pH 2 were passing into the permeate at pH 8. With 0.1N NaCl or $MgCl_2$ the protein recovery was even less, (36-38%). The protein content of the isolates obtained via membrane processing ranged from 83.1 to 88.2% on a moisture-free basis (MFB). The Soybean Digest Blue Book (1966) defines isolated soy protein as containing not less than 90% protein (N X 6.25) on a moisture-free basis. The fact that the isolates produced were slightly lower in protein content indicates that the diafiltration washing procedure may have been inadequate and should probably have been extended beyond collecting permeate equal to two feed volumes, perhaps as many as three or four volumes.

SUMMARY

The isolates obtained by Scheme I and Scheme II were evaluated using two criteria as follows: first, the residual TI content of the isolate; second, the percent recovery of the original protein in the isolate.

When isolates were prepared via isoelectric precipitation (Scheme I) best results were obtained using 0.1N NaCl at pH 5.4 where 97.7% of the original TI was removed and 51% of the original protein was recovered in the isolate.

When isolates were prepared using membrane separation (Scheme II) best results were obtained using 0.1N $MgCl_2$ at pH 2.0 where 79% of the original TI was removed and 64% of the protein was recovered in the isolate.

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The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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