Removal of Phytate from Soy Protein

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ABSTRACT

Phytate, as a minor constituent of soybeans, has been reported to interfere with the dietary mineral absorption of mammals. Commercial soy protein isolate contains 2-3% tightly bound phytate. Practical methods have not been found to remove phytate from soy protein materials. However, in this reported process, the phytate content was reduced to extremely low levels using commercially feasible processing techniques. The first step of this process was the extraction of water soluble components from soy flakes using routine commercial procedures. In the second step, the extract was adjusted to pH 11.6 at 28 C to insolubilize the phytate. The phytate was removed by centrifugation or vacuum filtration. Neutralization followed the phytate removal. The temperature and pH are critical for phytate removal, but also must be carefully controlled to prevent protein degradation. Lysinoalanine was not detected nor was cysteine or other amino acids found to be degraded. The third step of this process was purification of the protein. In this case, the low phytate soy extract was purified by ultrafiltration until permeate equivalent to 1½ times the volume of the extract was collected. The phytate and phosphorus contents of the resulting soy protein isolate were 0.1 and 0.2%, respectively, compared to 2.6 and 0.8 for typical commercial soy protein isolate. The PER was significantly better than an acid isolate prepared from the same soy flakes or the commercial soy isolate tested. The retenate obtained after purification by ultrafiltration was a translucent liquid with a protein content of 5-6%. This protein was in a very soluble form and was stable to heat.

INTRODUCTION

The use of soy preparations in foods as an economical source of protein has increased tremendously in recent years. As the utilization of soy protein becomes more widespread in our food supply, modifications and improvements in the nutritional and functional characteristics become more important. Commercially available soy isolates contain up to 1% phosphorus, while the phytate content for a typical isolate may be between 1.9-2.5%. Phytate, myoinositol hexaphosphate, is commonly determined in protein materials as the ferric iron precipitable phosphorus. The phytic acid phosphorus represents ca. 70% of the total phosphorus found in soy protein isolate. About 90% of the phosphorus is extracted from soybean meal during the manufacture of soy protein isolate.

The chemical composition and structure of phytate as it exists in nature have not been clearly defined. Vohra et al. (1) suggested that sodium phytate corresponded to the formula $C_6H_6O_{24}Na_{12}\cdot 3H_2O$ (formula weight 977.8). It has also been suggested that phytate exists in seeds as phytin, the calcium-magnesium-potassium salt of phytic acid. The work of Lolas and Markakis (2) showed that 99.6% of the total phytic acid in mature dry beans was in a water soluble form.

The binding of phytate to protein can be demonstrated by solubility characteristics of nitrogen and phosphorus compounds in solvent-extracted soybean meal over a broad pH range. An example, shown in Figure 1, was taken from the work of Fontaine et al. (3). The solubility of the phosphorus compounds in the water extract of soybean meal varied with the pH of the solution, and was similar to the solubility of the major protein components. It is interesting to note, however, that as the pH was adjusted above pH 4, the phosphorus became soluble sooner than the protein. This difference in solubility could be used to reduce the phytate content of soy isolate. Phytate is extracted from defatted soybean flakes and is precipitated with the protein during the manufacture of soy protein isolate as suggested by the data in Figure 1 (3).

McKinney et al. (4) showed that as much as 80% of the phosphorus can be removed from basic solution by precipitation with calcium and barium ions and subsequent centrifugation of the phytate from soybean meal extract (Table I). They also showed that phosphorus-free soy protein, suitable for fundamental studies, could be prepared from wet acid curd by dialyzing the curd against 1 N sodium chloride solution. They noted that both phytic acid and sodium phytate readily passed through the cellophane casing used for dialysis, although calcium phytate did not. Phytin could also be removed from the wet protein curd obtained by alkali extraction and acid precipitation by slurrying with a saturated solution of sodium or ammonium sulfate. In this case the protein is not dispersed, yet the phytin is removed. Thus McKinney et al. (4) found that phytate could be precipitated by barium ions and separated from the protein by centrifugation. In addition, phytate bound to the protein at low pH could be dissociated from the protein with high ionic strength solutions.

The results shown in Table II demonstrate the removal of phosphorus from the soybean meal water extract by dialysis and anion exchange. In this work, Smith and Rackis (5) removed 78% of the phosphorus utilizing a combination of dialysis and treatment with the strong anionic exchange resin, Dowex-1-X10. This resin is capable of removing the phytate anion from the protein.

Table III shows phytate removal from soybean meal extract by equilibrium dialysis for 7 days at 25 C (6). The permeability of phytate at pH 5.5 follows closely the ideal behavior of a fully permeable substance. It was suggested

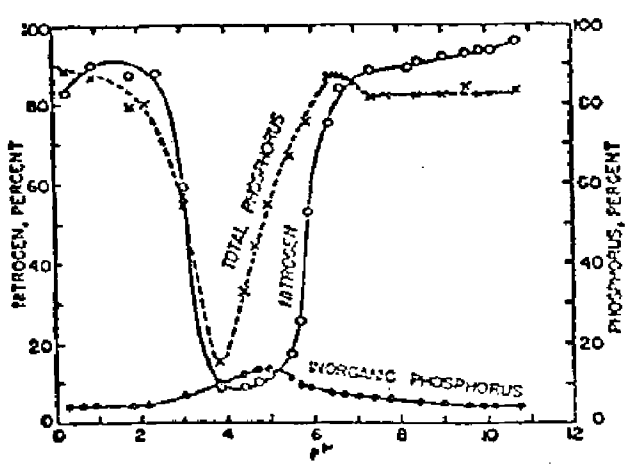

FIG. 1. The percentage of the total nitrogen and phosphorus of solvent-extracted soybean meal which is soluble in hydrochloric acid and sodium hydroxide solutions at different pH values. The inorganic phosphorus values are reported as percentage of the total meal phosphorus (3).

TABLE I

Precipitation of Phytate from Soybean Meal Extract with Calcium and Barium Ions (4)

Agent	pH	Treatment	P, % in protein
0.2% NaOH	9.7	0.2% $Ba(OH)_2$, pH 10.9, centrigued to remove ppt.	0.46
0.2% NaOH	9.7	0.2% $Ba(OH)_2$, pH 9.5 and heated to 80° C, centrifuged.	0.27
NaOH	10-11	Used meal after leaching with .5 N Cl_3CCOOH; .2% $Ba(OH)_2$ added to alkaline dispersion, centrifuged.	0.22
0.1% NaOH	9.5	5% $BaCl_2$, 17 hr at 5 C, centrifuged, Na_2SO_3 added to remove Ba, clarified and ppt.	0.18
0.1% NaOH	9.5	5% $BaCl_2$, 17 hr at 5 C, centrifuged, dialyzed.	0.32

TABLE II

Removal of Phosphorus and Nitrogen Compounds from Water Extract of the Meal by Dialysis and Anion Exchange (5)

	% P removed	% N removed
Dialysis 24 hr., pH 7.2	40.5	7.5
Dialysis 48 hr., pH 6.5	72.0	7.0
Dialysis 48 hr., pH 8.7	52.0	28.0 ppt.
Anion Exchange, pH 7.5	56.5	4.0
Anion Exchange, pH 6.5	82.3	24.0
Dialysis and anion exchange, pH 7.0	78.2	10.1
Dialysis and anion exchange, pH 7.0	78.5	9.2

TABLE III

Phytate Removal from Soybean Meal Extract by Equilibrium Dialysis for 7 Days at 25 C (6)

pH	Dialysis bath solvent	Presence of 0.01 M EDTA	Percent total phosphorus removed	Phosphorus content (g P/100 g protein)
8.5	0.03 M sodium borate buffer	No	74	0.38
8.5	do	Yes	84	0.22
8.5	0.05 M tris HCl buffer	No	61	0.58
8.5	do	Yes	75	0.34
5.5	Water	No	96	0.06
2.0	Water (HCl)	No	42	0.81

that phytase in the meal enhanced the removal of phytate by dialysis at this pH. Another explanation may be that the phytate is not strongly bound to the protein at this pH. Significant amounts of phytate can also be removed at pH 8.5 in the presence of 0.01 M EDTA. Removal of phytate from pH 2 to 4.5 is inefficient because of the strong association between protein and phytic acid unless excess divalent cations are used to displace the phytate from the protein.

Figure 2 shows the removal of phytic acid from soybean meal extracts by ultrafiltration at 65 C (6). If we compare extracts ultrafiltered without EDTA and with EDTA, we find that those extracts at pH 8.6 and .05 M EDTA added have reduced phosphorus content. The chelation of the divalent ions facilitates the membrane transfer of the phytate by ultrafiltration or dialysis.

Data in Figure 3 show the solubility of nitrogen-containing substances and phytic phosphorus as a function of pH. These results were obtained from the British patent of Gillberg and Tornell (7). Phytic acid contents from 2.2 to 3.9% have been found in defatted *Brassica*, commonly referred to as Rapeseed. From this figure, it can be seen that unlike soy phytic acid, the maximum phosphorus solubility was around pH 4.8. The solubility of the phytate, however, is at a minimum at pH 11 where the solubility of the protein is at a maximum.

Ford et al. (8) reported a process to remove phytic acid from soybeans. In this process, phytic acid was leached from soyflour slurry at pHs of 3.5 to 4.0 and high calcium content (.04 M) or the combination of high pH levels (5.0 to 5.5) and low calcium levels. With appropriate conditions, 90% of the phytate could be washed from the curd.

NEED FOR PHYTATE REMOVAL

What is the effect of phytate removal on the functional properties of the soy protein? Why should phytate be removed from soy preparations?

First of all, neutral dispersions of phytate-free protein have greater solubility, and alkaline dispersions exhibit greater clarity than soy protein isolates with phytate present. The removal of phytate reduces the tendency of

the protein to aggregate in water dispersions. The isoelectric point of the protein is increased, and the solubility on the acid side of the isoelectric point is improved. Phytate removal therefore results in proteins with improved functional properties.

Secondly, phytic acid can also affect the nutritional value of the product either by forming complexes with the protein, thus obstructing the enzymatic digestion of the protein, or by reacting with the calcium, magnesium, copper, zinc or iron in the food and thereby inhibiting the adsorption of these important minerals. At the pH values prevailing in the intestine, phytic acid forms insoluble complexes with the above mentioned metal ions. It has been established that consumption of foods rich in phytic acid results in a negative calcium balance.

Thirdly, it may be important in purified diets to reduce the unavailable phosphorus contributed by the soy protein. Perhaps a high Ca/P ratio is desired, but it cannot be achieved without having a low phosphorus protein.

Fourthly, the interesting aspect of phytate removal technology is the effect that the process of removal may have on new applications of that protein in food systems.

LOW PHYTATE PROCESS

The rest of this paper will describe a process for preparation of low phytate soy protein which was recently developed in our laboratories (9). The process includes precipitation of the phytate at high pH and subsequent centrifugation or filtration. Purification of the protein was accomplished in this example by ultrafiltration.

The process flow diagram for removal of phytic acid from soy protein is shown in Figure 4. The first step of this process was the extraction of water soluble components from soy flakes. The following conditions were used: 1) soy flakes with high protein dispersibility index, 2) water-to-flake ratio of 16:1, 3) pH of 9 to 10, 4) centrifugation at 5000 x g for 15 to 20 min. These extraction conditions were chosen for routine laboratory preparations but could be modified without affecting the subsequent removal of phytate. It should be noted, however, that the protein coefficient (protein ÷ protein + CHO) increased as the pH of extraction increased from 7 to 10. It was interesting to observe that more phytate was extracted at pH 7 than pH 10. Perhaps insoluble metal salts formed at the higher pH were not readily extracted from the soy flake.

In the second processing step, the phytate removal was achieved by first adjusting the pH of the soy extract from 9 or 10 to between 11 and 12 to insolubilize the phytate. The phytate was then removed by centrifugation or vacuum filtration. After the phytate was removed, the pH of the soy extract was rapidly adjusted to 7 with dilute HCl. The

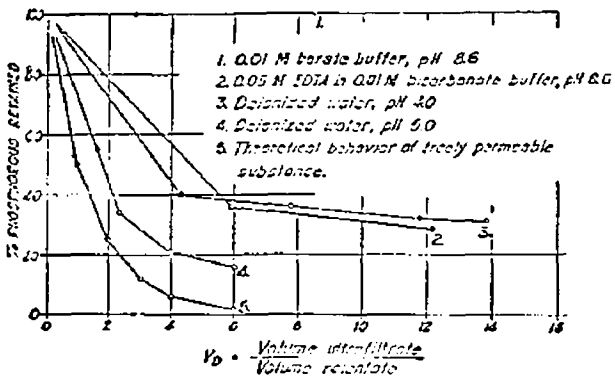

FIG. 2. Removal of phytic acid from soybean meal extracts by ultrafiltration at 65 C (6).

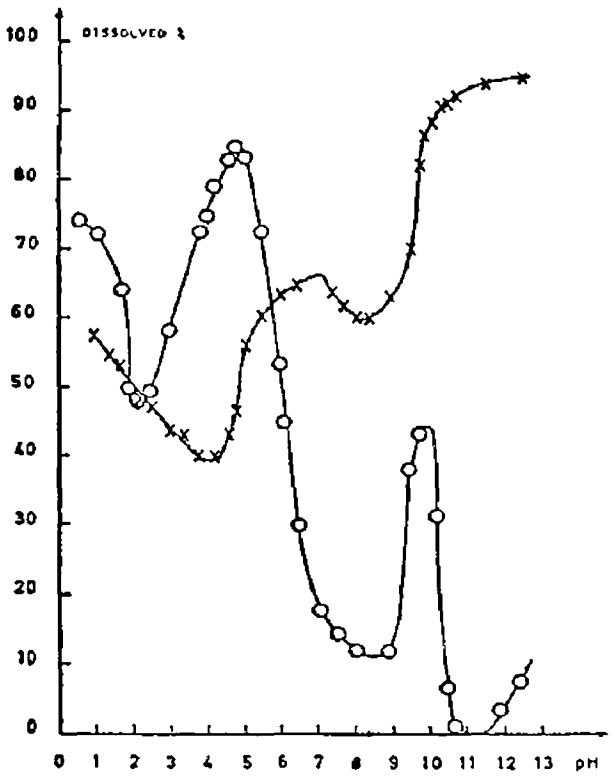

FIG. 3. Solubility of nitrogen-containing substances (x) and phytic phosphorus (o) as a function of pH (7).

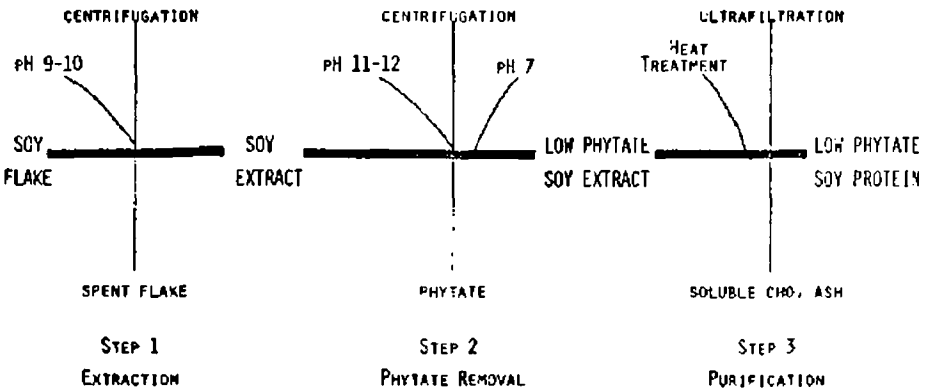

FIG. 4. Process outline for removal of phytic acid from soy protein.

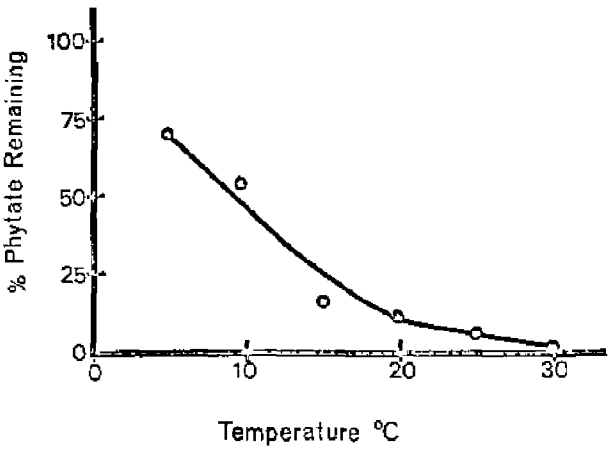

FIG. 5. Effect of temperature on removal of phytate (9).

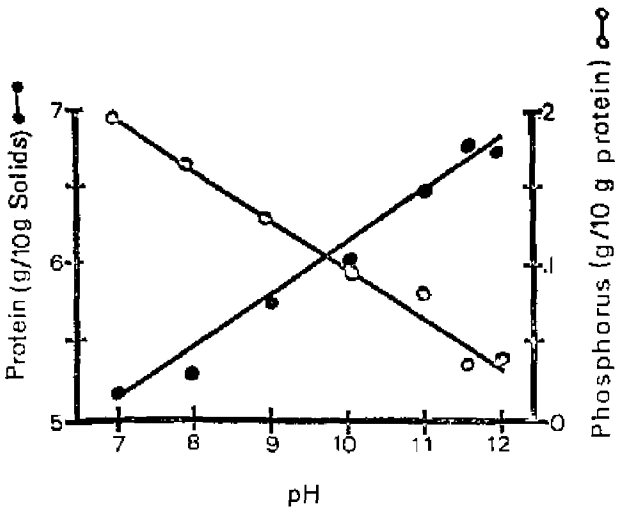

FIG. 6. pH Effect on extraction of protein and phosphorus.

third processing step was the purification step which was ultrafiltration.

Temperature control of the second processing step was critical. Figure 5 shows the effect of temperature on the removal of phytate from the soy extract. These results are expressed as the percent phytate removed from the soy extract. In this experiment, the extract was adjusted to pH 12 and centrifuged at each temperature tested. The samples were stored overnight at 4 C. The phytate precipitate was spun out at 70 x g for 30 min, and the phytate volume was compared to the phytate-containing control. These results showed that 90% of the phytate could be removed if the processing temperature was controlled between 20-30 C.

Phytate can also be removed from soy protein by adjusting the extraction pH between 11 and 12. In this case, some of the phytate was removed with the spent flake and the rest by a subsequent centrifugal polishing step. The effect of pH on the extraction of protein and phosphorus is shown in Figure 6. Protein extracted from the soy flakes increased as the pH of extraction increased, while the phosphorus level in the soy extract showed a decrease as the extraction pH was adjusted from 7 to 12. Thus, temperature and pH must be carefully controlled to optimize the removal of phytate from soy protein.

The conditions used to precipitate the phytate from the soy extract approach conditions that will degrade the

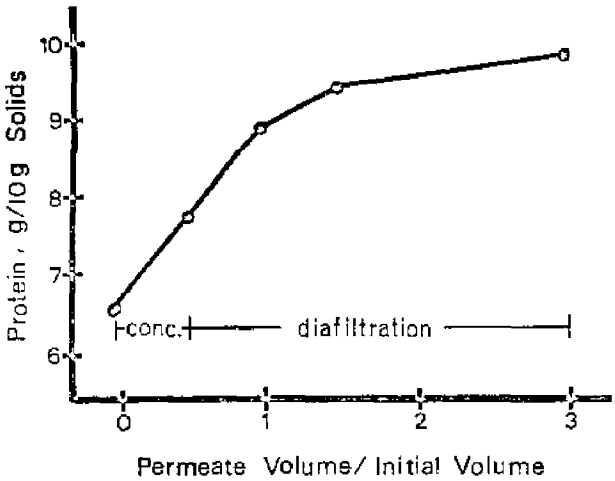

FIG. 7. Extract purification by ultrafiltration.

TABLE IV

Composition of Ultrafiltered Low Phytate Soy Protein (LPSP) Compared to a Commercial Soy Protein Isolate (CSPI)

	g/100 g solids		Amino acids	g/100 g protein	
	LPSP	CSPI		LPSP	CSPI
Protein	93	95	CYS	1.1	0.9
Fat	2.9	1.9	MET	1.2	1.0
Ash	3.4	4.0	LYS	6.8	7.4
Ca	0.1	0.1	TYR	3.7	3.5
P	0.2	0.8	TRY	1.4	1.2
Na	0.6	1.1	HIS	2.1	—
K	0.8	0.1	ARG	6.8	—
CHO	5.2	3.1	THR	3.8	3.9
Phytic acid	0.1	2.6	SER	5.2	4.8
LAL	—	—	PHE	5.6	4.7

protein. The temperature and pH are critical not only for the phytate removal, but also they must be carefully controlled to prevent protein degradation. To detect protein degradation, the level of cysteine and the presence of absence of lysinoalaine were monitored. Under the conditions of this process, pH 11.5 from 20 to 30 C for less than 2 hr, no protein degradation was detected. Extracts have been held at pH 11 and 20 to 30 C for as long as 6.75 hr with no reduction in cysteine observed and no lysinoalanine detected. In studies where the temperature was increased to 50 C for 2 hr at pH 12, cysteine was degraded and lysinoalanine was detected. It was apparent that temperature was more important for protein degradation than time at high pH.

A pasteurization heat treatment of 220-230 F for 1 min was given to the low phytate soy extract after it had been neutralized and before it was purified. The main purpose for this heat treatment was to reduce the microbiological load prior to ultrafiltration. In addition, the ultrafiltration flux rate improved as a result of the applied heat. Too much heat at this point in the process could be detrimental to protein quality as well as protein solubility. This heat treatment also served to reduce the trypsin inhibitor levels in the low phytate soy extract.

The third low phytate protein isolation step was the purification of the protein. This step can be accomplished by acid precipitation or ultrafiltration. Figure 7 shows the result of purification of the neutralized soy extract by concentration and diafiltration. For this experiment, a Romicon XM-50, 20 mil hollow fiber cartridge was used with a flow rate of 12 gallons/min with 25 psi at the inlet and 15 psi at the outlet with the temperature at 45 C. The

soy extract which started at 2.5-3.0% protein was first concentrated to one-half of its original volume. The protein was further purified by diafiltration where water was added continuously to the retentate to achieve constant volume. The results showed that 94% protein purification can be achieved by generating permeate equivalent to 1.5 times initial volume. The average flux rate for the process using the above stated conditions was 23 gallons per square foot of membrane surface per day. Even though the XM-50 membrane had a molecular weight cutoff of 50,000, only 5% of the nitrogen was lost during the ultrafiltration procedure.

The permeate produced had a solids level of 0.7% with carbohydrate and ash making up most of those solids.

Table IV shows the composition of ultrafiltered low phytate soy protein (LPSP). The first major different between commercial soy protein isolate (CSPI) and LPSP was the significant reduction in phosphorus and phytic acid. The phosphorus content was reduced from 0.8 to 0.2% and the phytic acid was reduced from 2.6 to 0.1%. Because of the ultrafiltration purification step, the soy whey proteins were included in this composition. It follows then that the sulfur-containing amino acids cysteine and methionine were significantly higher than commercially available soy isolate. The corresponding nutritional value as measured by PER was also higher than the PER of commercial soy isolate or the PER of an acid isolate prepared from the same soy flakes.

The retentate obtained after purification by ultrafiltration was a translucent liquid with a protein content of 5 to 6%. This protein was in a very soluble form and was stable to heat. Sterilized soy milk-type products have been formulated with this protein, producing products with good physical stability.

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[Received October 5, 1978]