Low-Phytate, Full-Fat Soy Protein Product by Ultrafiltration of Aqueous Extracts of Whole Soybeans[1]

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ABSTRACT

The feasibility of using ultrafiltration (UF) to eliminate phytic acid from aqueous extracts of soybeans was studied. Soybeans were cleaned, soaked, blanched, and ground, and the slurry was filtered through a plate-and-frame filter press to obtain the water extract. After adjustment to the appropriate pH and temperature, the extract containing 3.5–3.9% total solids was processed in a pilot-scale hollow-fiber UF unit by discontinuous and continuous diafiltration techniques. Elimination of phytic acid from the soy concentrate was governed by the environment and state of binding of phytate to rejected species and did not follow expected behavior for a nonrejected solute that is freely permeable through the membrane. UF to a volume concentration ratio of 5 resulted in 65, 43, and 27% elimination of phytic acid at pH 6.7, 8, and 10, respectively. Dilution at the same pH and reultrafiltration eliminated about 92% of phytic acid at pH 6.7 and more than 80% at pH 8 and 10. The final product assayed 60% protein, 35% fat, and 0.64 mg phytic acid per gram of solids (dry basis).

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Phytic acid is thought to play a significant role in decreasing the bioavailability of minerals by complexing with or binding cations and forming phytate-mineral or protein-mineral-phytate complexes (O'Dell 1969, O'Dell and Savage 1960). These complexes may be insoluble or otherwise unavailable under physiologic conditions, thus making the complexed minerals also unavailable (Smith and Rackis 1957), although Churella (1976) claimed that the effect of phytic acid on mineral availability also is influenced by other minerals in the diet, heat treatment, nature of dietary protein, pH, and possibly other factors.

Because of high content and quality of protein, soybeans will play a major role in alleviating protein shortages, both for nutritional and economic reasons. Soybeans, however, contain antinutritional and off-flavor factors that must be reduced or eliminated to improve nutritional and functional properties. Proper heat treatment can prevent lipoxygenase-induced off-flavors and inactivate trypsin inhibitors, but it is relatively ineffective against oligosaccharides and phytic acid. Commercial methods of preparing protein isolates and concentrates reduce oligosaccharide concentration to negligible levels but still contain about 1–2% phytic acid (dry basis), ie, as much as 70% of the phytate in the original soybean (Churella 1976, Okubo et al 1975, Omosaiye 1978).

Ultrafiltration (UF) is a viable commercial process for producing purified protein concentrates from milk or cheese whey (Horton 1974) with excellent functional properties. Its use in effectively lowering oligosaccharide concentration of soy protein extracts was recently documented (Omosaiye et al 1978). The chief virtues of UF are its nonthermal character and relatively high selectivity, which can be controlled by proper choice of membrane characteristics and operating conditions. Our purpose was to evaluate the feasibility of using UF for producing soy protein isolates or full-fat concentrates low in undesirable components, particularly phytic acid, and to gain a deeper understanding of the nature of protein-phytate interactions, such as those influenced by the chemical environment.

MATERIALS AND METHODS

Water Extracts of Soybeans

The procedure outlined by Omosaiye (1978) and Omosaiye et al (1978) was used. Essentially it consisted of blanching presoaked soybeans briefly to inactivate lipoxygenase, grinding hot in a 1:10 bean/water ratio, filtering in a plate-and-frame filter press to remove coarse insolubles, and washing the filter cake with an additional 10 parts water. For pH studies, NaOH or HCl of sufficient strength was used to result in a 2–5% dilution of the extracts. Reagent was added with vigorous stirring after the extract was cooled below 10° C. The extract was then stored overnight for equilibration and the pH readjusted again, if necessary, before UF processing. Batches of 250–330 lb of the extract were prepared for each run.

Ultrafiltration

A pilot-size hollow fiber UF unit (Romicon, Inc., Mass. Model HF1SS) was used to process the soybean water extracts. Operating conditions were 25 psig inlet pressure, 10–15 psig outlet pressure, and 50° C temperature. The membrane used (HF15-45-XM50) was in the form of 660 noncellulosic hollow fibers, 15 ft^2 in UF area with a nominal molecular weight exclusion limit of 50,000. Details of the operation and performance characteristics of this system are given elsewhere (Cheryan and Schlesser 1978, Omosaiye 1978).

Analytical Methods

Moisture, ash, and Kjeldahl nitrogen were analyzed by AOAC standard methods (1970). Fat was analyzed by a soxhlet extraction procedure using methanol/chloroform 1:2 v/v as solvent. Total solids were measured by a gravimetric procedure. Phytic acid was determined by the method of Wheeler and Ferrel (1971) with one modification: *o*-Phenanthroline instead of potassium thiocyanate was used for the colorimetric determination of iron (AOAC, 1970). *o*-Phenanthroline is preferred because of its high sensitivity and color stability. The standard curve for iron vs. absorbance gave typical correlation coefficients of 0.999 or better between iron concentrations of 8×10^{-5} and 1.8×10^{-3} mg/ml. Phytate phosphorus was calculated assuming a 4:6 Fe/P molar ratio and data reported are an average of at least two determinations. Details of the reproducibility of the method and calculations involved are given elsewhere (Omosaiye 1978).

Presentation of Data

Protein content is expressed as total Kjeldahl nitrogen multiplied by 6.25. UF data are presented in terms of volume concentration ratio (VCR) where VCR = initial feed volume (V_o)/ retentate volume (V_R). The percent water or solution removed as permeate can be expressed as $(1-1/VCR) \times 100$. Continuous diafiltration data are presented in terms of volumes diluted (V_D) defined as the ratio of volume of liquid permeated to initial V_o.

The rates of removal of phytic acid is most conveniently expressed as

$$\text{Percent remaining in retentate} = \frac{C_R V_R}{C_o V_o} \times 100$$

where C_o = concentration of phytic acid in original extract (VCR 1) and C_R is concentration of phytate in the retentate after the initial

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V_o had been reduced by UF to V_R. (Data prefixed with R in Tables II, III, and IV indicate samples taken during reultrafiltration [re-UF], ie, the second stage of a two-stage process.)

RESULTS AND DISCUSSION

The extractability of phytate from a biological system containing proteins depends on its chemical environment, ie, the type of cations and ionic strength, nature of protein, and pH of the solvent. Soybeans in this experiment (Bonus 1975) had a phytic acid content of 1.24% (±0.012% standard error) (range 1.21–1.28%), dry basis. Table I shows a typical analysis of the products obtained during a particular run. About 73% of the total solids, 83% of the protein, ash, and fat, and 95–99% of the phytic acid of the original soybean were recovered in the extract, which is used as feed for the UF unit (ie, VCR 1). The major loss of solids was due to fiber and other coarse particles that had to be filtered out prior to processing in hollow-fiber units. Details of the extraction process and UF are available (Cheryan and Schlesser 1978, Omosaiye 1978, Omosaiye et al 1978). Unlike other reports (deBoland et al 1975, Lolas and Markakis 1975, O'Dell and deBoland 1976), very little phytic acid was leached out during the soaking of soybeans before grinding, perhaps due to the low soak temperature (4° C) and low bean/water ratio (1:3). Particle size also could have been a factor since we used whole soybeans and other workers used ground soy meal or soy flakes. Conditions also were apparently unfavorable for any significant in situ action by endogenous phytase.

Removal of Phytate by Ultrafiltration

Results of UF experiments reveal the importance of phytate-protein interactions and the chemical environment. Ideally, a freely permeable solute (ie, 0% rejection) should follow the theoretical (broken) line shown in Fig. 1 during UF. A 50% elimination of the solution (VCR 2) should result in 50% elimination of the solute and 80% elimination (VCR 5) should result in reduction of solute amount by 80%. Similarly, rediluting the retentate to the original volume and repeating UF a second time to VCR 5 (re-UF) should eliminate another 80% of the remaining 20% (96% overall removal). This was not the case with the phytic acid-soybean system where the maximum removal during the first stage UF was 65% at pH 6.7, with less removal at other pH values (Fig. 1). Phytic acid and its known salts have molecular weights less than 1,000 and should freely permeate the XM-50 membrane used in this study, which has a 50,000 molecular weight exclusion limit. The fact that it does not readily go through the membrane indicates that either phytate is insoluble under UF processing conditions or that interactions occur between phytic acid and some rejected components of the system, thus retarding passage through the membrane.

The general concensus appears to be that phytate exists in the soluble form (Crean and Haismann 1963, deBoland et al 1975, Lolas and Markakis 1975, Smith and Rackis 1957), although higher pH values of 8–10 favor formation of calcium and magnesium salts of phytic acid, compounds with limited solubility (Saio et al 1967, Smith and Rackis 1957). Above pH 10.3, the phytate is almost completely insoluble (Goodnight et al 1976). At low pH, phytate exists as soluble, un-ionized salts in combination with various cations (Hill and Tyler 1954, Okubo et al 1976). Hence, solubility characteristics of phytic acid apparently are not the reason for its nonremoval during UF, except possibly at high pH.

Protein-Phytate Interactions

The more likely explanation for the phenomena in Fig. 1 is a specific interaction between phytic acid and a rejected species such as proteins, the nature of which is influenced by pH and other environmental factors. UF data suggest that this interaction is strongest at the two pH extremes studied. During UF, less than 27% of the phytic acid was removed at pH 2 and pH 10, even though VCR 5 implies removal of 80% of the solvent (Fig. 1). Upon re-UF, however, the behavior of phytic acid was quite different. At acidic pH, phytate removal rates followed the previous pattern, indicating little or no change in the nature of the phytate-protein interaction. At alkaline pH, however, phytate removal rates were significantly greater upon repeated UF, following theoretical behavior quite closely, suggesting that the phytate-protein complex was dissociated or substantially reduced under re-UF conditions.

Data in Table II show that the phytate/protein ratio decreased only slightly during the first stage UF at the two pH extremes. In contrast, the oligosaccharides were reduced fivefold at all pH values as expected for a noninteracting freely permeable solute (Omosaiye 1978, Omosaiye et al 1978). The proteins, but not phytate, are expected to be almost totally retained due to molecular size. That phytate was retained to almost the same extent indicates that protein-phytate interactions occurred at pH 2 and pH 10, which retarded the removal of phytate with the solvent. The data suggest that two different mechanisms may occur, controlled essentially by pH.

Low pH. Indications are that there may be a strong charge effect. Of the 12 replaceable protons in the phytic acid molecule, six are strongly dissociated with a pK of 1.8 (Crean and Haismann 1963). Hence, the phytate molecule will be strongly negatively charged under all processing conditions in this study. Proteins, on the other hand, are positively charged at pH 2 and a protein-phytate complex will be formed as a result of this strong electrostatic interaction (Hill and Tyler 1954; Okubo et al 1975, 1976; Saio et al 1967). Smith and Rackis (1957) suggested that this interaction is rapid and followed by a nonionic, irreversible reaction. Hence, since the protein is almost completely rejected by the membrane (Cheryan and Schlesser 1978, Omosaiye 1978), the phytate also will not pass through the membrane. The small loss during UF (Fig. 1) is probably partly due to removal of phytate-calcium or other phytate salt (which exists at low pH in a soluble, un-ionized form) and partly to absorption of material on the membrane (Omosaiye 1978).

This strong phytate-protein interaction at acidic pH is the reason why protein isolates prepared by isoelectric precipitation contain as much as 60–70% of the original phytic acid of the raw soybean

TABLE I
Proximate Analysis of Soybeans, Its Water Extract and Ultrafiltration Processed Product (pH 6.7) (%)

	Soybeans	Extract	UF Product[a]
Total solids	90.0	3.56	12.6
Protein (N × 6.25)	39.0	1.72	7.48
Fat	21.6	0.94	4.30
Phytic acid	1.14	0.06	0.008
Ash	4.20	0.19	0.357
Other[b]	24.06	0.65	0.455

[a]Two-stage ultrafiltration.
[b]Carbohydrate, fiber, etc.

TABLE II
Phytate/Protein Ratio (mg Phytic Acid per g Protein) During Ultrafiltration

Volume Concentration Ratio	pH 2	pH 6.7	pH 8	pH 10
1	37.7	35.0	35.8	33.7
2	36.3	28.0	31.4	34.3
3	35.3	25.8	31.6	33.3
5	32.3	14.5	25.4	31.3
R1	32.3	14.5	25.4	30.7
R2	31.8	4.13	...	19.8
R5	24.1	1.07	3.88	5.55

(Churella 1976, Okubo et al 1975, Smith and Rackis 1957). Attempts have been made to dissociate this complex by displacement of the phytate using multivalent cations such as calcium (Hill and Tyler 1954; Okubo et al 1975, 1976). Ford et al (1978) used this principle to remove a substantial amount of phytate from a lipid protein concentrate. Combinations of high pH and low calcium concentrations or low pH/high calcium levels during acid precipitation reduced phytate concentrations by 90%. No such attempts were made in this study because low pH operation was undesirable from a product point of view. Partial hydrolysis of the oligosaccharides occurred and some off-flavors were produced during processing (Omosaiye 1978). There is also the possibility that soybean proteins may be denatured at this pH.

High pH. At alkaline pH, the nature of the phytate-protein complex is apparently quite different. A fairly strong protein-phytate interaction occurs (O'Dell and deBoland 1976), but electrostatic effects are minor at high pH. There is experimental evidence that multivalent cations such as calcium mediate the binding between phytate and protein, and a certain minimum concentration of such cations is necessary for maintaining this interaction (Okubo et al 1976, Saio et al 1967). Hence phytic acid probably exists as ionized salts or ternary complexes, as postulated by the following mechanisms:

$$\text{Cation} + \text{Phytic Acid} \rightleftharpoons (\text{Cation-Phytic Acid}) \quad (1)$$

$$\text{Protein} + \text{Cation} + \text{Phytic Acid} \rightleftharpoons (\text{Protein-Cation-Phytic Acid}) \quad (2)$$

During UF, only free phytic acid, cations, or soluble cation-phytic acid compounds can permeate the membrane. The amount of phytate removed from the system will depend on the relative magnitudes of the association constants governing the above equations. Data in Fig. 1 and Table II reveal that during the first stage of UF, the amount of phytate removed decreased with increasing pH. This indicates either increasing strength of the salt linkage, ie, higher association constant with increasing pH, or decreasing permeability of the phytate-cation salt (equation 1) due possibly to its decreasing solubility at higher pH (Okubo et al 1976, Saio et al 1967, Smith and Rackis 1957).

The ternary complex appears to be fairly labile in alkaline solution (Saio et al 1967) and, analogous to the low pH conditions mentioned, successful removal of phytate from the system depends on the ability to dissociate the protein-salt-phytate complex, perhaps by removing cations from the system or otherwise rendering them unavailable for complexation. This is probably the mechanism by which re-UF improved the rate of removal of phytate from the soybean water extracts. During first stage UF, the equilibrium partitioning nature of ideal UF implies that the *concentration* of the freely permeable compounds enumerated will remain essentially unchanged on either side of the membrane. The ash concentration data in Table III can be used as an indicator of cation concentration. An increase was observed during UF since some of the salts were bound to (impermeable) proteins; but more than 50% of the salts originally present at VCR 1 were removed by UF to VCR 5. Hence, when the retentate at VCR 5 was diluted back to the original volume for re-UF, the cation concentration was considerably less at comparable VCRs. This will cause the

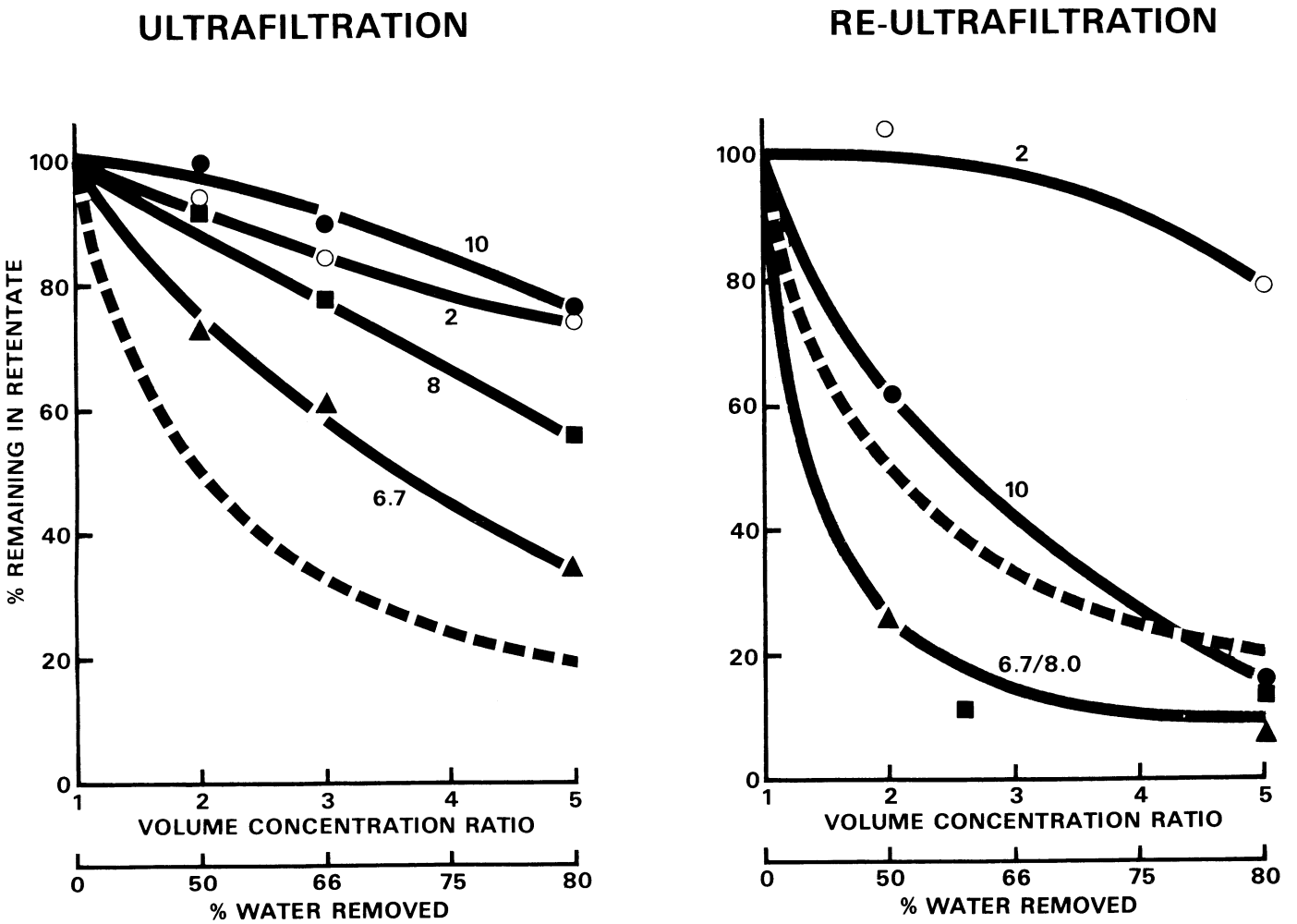

Fig. 1. Removal of phytic acid from soybean water extracts by ultrafiltration. Variable is processing pH. Broken lines denote theoretical or ideal behavior.

reactions in equations 1 and 2 to shift to the left, thus causing a greater dissociation of the ternary complex. Apparently the concentration of basic ions during re-UF was below a critical value, ie, insufficient to cause any significant interaction. The phytate removal then followed theoretical behavior[3], resulting in a net removal of 80–92% depending on the pH.

Continuous Diafiltration

This method involves adding water at the appropriate pH and temperature to the feed tank at the same rate as permeate flux, thus keeping V_0 constant during processing. This technique is suggested as one way to overcome problems of concentration polarization, which can significantly lower flux, especially at high VCR (Cheryan and Schlesser 1978, Omosaiye 1978, Omosaiye et al 1978). A typical continuous diafiltration (CD) experiment is shown in Fig. 2. For this particular case, the extract at pH 6.7 (3.78% total solids) was first ultrafiltered to VCR 2 (5.84% total solids) and then CD was started. From this point the concentration of the rejected species was held constant, while the concentration of the nonrejected species decreased in proportion to V_D. The decrease in total solids is due primarily to elimination of oligosaccharides, ash, and phytic acid.

Theoretically, a completely nonrejected solute requires about 3.22 volumes permeated through to achieve a 96% removal of solute (Omosaiye et al 1978). This implies that a much larger permeate volume has to be processed by CD than by repeated UF/re-UF to achieve the same purification. For example, comparison of Figs. 1 and 2 indicates UF/re-UF required $2 \times V_0$ and CD required $4 \times V_0$ to achieve a 96% removal of phytic acid. Flux during CD did not remain high enough, however, to offset the larger volumes processed; consequently a longer processing time was necessary and may limit the usefulness of this method. In addition, final total solids are lower during CD (compare Table I and Fig. 2), thus increasing the costs of subsequent concentration/dehydration. Preliminary trials indicate that a sequence of UF-CD-UF is necessary to optimize removal of phytate vs. processing rates.

Ultrafiltered Product

The most efficient removal of phytate from soybean water extracts occurred at pH 6.7. At this intermediate pH, no strong electrostatic attraction is evident and the strength of the salt linkage that stabilizes the ternary complex is apparently quite weak. In addition, phytic acid is almost completely water-soluble. All these factors combine to facilitate removal of phytate from the system at this pH. UF at a pH much lower than this is not recommended, at least with hollow-fibers, because preliminary trials at pH 5.5 revealed poor UF characteristics due to protein instability and consequent plugging of hollow fibers.

No endogenous phytase action is envisaged in this operation; the heat treatments during blanching (90°C/3 min) and grinding (~80°C/5 min) are probably enough to inactivate it. In addition, the pH values studied are far removed from the pH optimum of known phytases. In any event, phytase activity in soybeans, if it exists, is too low to be of practical significance.

Table IV shows the composition of the products obtained using the processes described. A two-stage UF process resulted in a 24-fold decrease in phytic acid, with the phytate/protein ratio decreased by 95%. The final level of 0.064% (corresponding to about 30 mg phytate P/100 gm protein) compares with the CD method of Okubo et al (1975) that required $V_D = 6$, 65°C, pH 5.0, and the presence of phytase to achieve 230 mg P/100 gm protein, with 0.13% using the pH 12 basification-centrifugation method of Goodnight et al (1976), and with 0.14% using the calcium/pH adjustment method of Ford et al (1978). It is fortuitous that the optimum pH for removing phytate in our process is that of the normal pH of soybean water extracts; hence no chemical treatment or pH adjustment is necessary. Temperature requirements are dictated more by microbial and UF flux considerations than by specific phytate requirements. The final level of phytate in the system or the phytate/protein ratio can be controlled simply by controlling the volume of solvent permeated or expelled (ie, VCR or V_D). A companion study revealed that overall protein losses during UF processing are lower than during conventional protein isolate manufacturing methods. In addition, the low temperatures and mild operating conditions result in functional properties of the UF products that appear to be superior in some respects. Research in these areas will be reported.

[3]Data showing anomalous behavior on re-UF can be attributed partly to the greater difficulty of phytate analysis at low concentrations and partly to absorption by the membrane.

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TABLE III
Ash Concentration (% w/w) in Rententate During Ultrafiltration Processing

Volume Concentration Ratio	pH 6.7	pH 8.0	pH 10
1	0.194	0.249	0.302
2	0.266	0.377	0.452
3	0.317	0.433	0.536
5	0.437	0.542	0.712
R1	0.087	0.108	0.142
R2	0.158	...	0.281
R5	0.357	0.417	0.528

TABLE IV
Composition of Soy Protein Concentrates (% Dry Basis) Produced by Ultrafiltration at pH 6.7

Concentrate	Protein	Fat	Phytate	Ash	Other[a]
Original soybean	43.3	24.0	1.27	4.71	26.72
Water extract (VCR 1)	48.3	26.4	1.68	5.34	18.28
Ultrafiltered (VCR 5)	56.7	32.5	0.823	3.43	6.55
Reultrafiltered (R 5)	59.7	34.2	0.064	2.85	3.19

[a]By difference. Includes fiber, carbohydrate, etc.

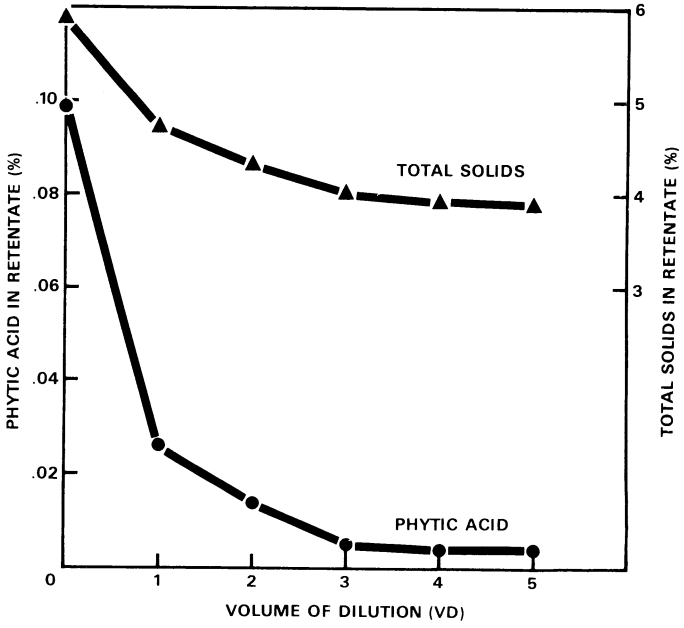

Fig. 2. Removal of phytic acid by continuous diafiltration. pH = 6.7.

LITERATURE CITED

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1970. Official Methods of Analysis (11th ed.). The Association: Washington, DC.

CHERYAN, M., and SCHLESSER, J. E. 1978. Performance of a hollow fiber system for ultrafiltration of aqueous extracts of soybeans. Lebensm. Wiss. Technol. 11:65.

CHURELLA, H. 1976. Phytic acid level in infant soy protein isolate formulas and its effect on mineral availability. PhD Thesis, Ohio State University.

CREAN, D. E. C., and HAISMANN, D. R. 1963. The interaction between phytic acid and divalent cations during the cooking of dried peas. J. Sci. Food Agric. 14:824.

deBOLAND, A. R., GARNER, G. B., and O'DELL, B. L. 1975. Identification and properties of phytates in cereal grains and oilseed products. J. Agric. Food Chem. 23:1186.

FORD, J. R., MUSTAKAS, G. C., and SCHMUTZ, R. D. 1978. Phytic acid removal from soybeans by a lipid protein concentrate process. J. Am. Oil Chem. Soc. 55:371.

GOODNIGHT, K. C., HARTMAN, C. H., Jr., and MARQUARDT, R. F. 1976. Aqueous purified soy protein and beverage. US Patent 3,995,071.

HILL, R., and TYLER, C. 1954. The reaction between phytate and protein. J. Agric. Sci. 44:324.

HORTON, B. S. 1974. Ultrafiltration of whey and milk for protein recovery. Proc. Fourth Int. Congr. Food Sci. Technol., Inst. de Agro, Valencia, Spain. Vol. 4, pp.322–331.

LOLAS, G. M. and MARKAKIS, P. 1975. Phytic acid and other phosphorus compounds of beans (Phaseolus vulgaris L.) J. Agric. Food Chem. 23:13.

O'DELL, B. L. 1969. Effect of dietary components on zinc availability. Am. J. Clin. Nutr. 22:1315.

O'DELL, B. L., and deBOLAND, A. 1976. Complexation of phytate with proteins and cations in corn germ and oilseed meals. J. Agric. Food Chem. 24:804.

O'DELL, B. L., and SAVAGE, J. E. 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. Med. 103:304.

OKUBO, K., MYERS, D. V., and IACOBUCCI, G. A. 1976. Binding of phytic acid to glycinin. Cereal Chem. 53:513.

OKUBO, K., WALDROP, A. B., IACOBUCCI, G. A., and MYERS, D. V. 1975. Preparation of low-phytate soybean protein isolate and concentrate by ultrafiltration. Cereal Chem. 52:263.

OMOSAIYE, O. 1978. Hollow fiber ultrafiltration of water extracts of whole soybeans. PhD Thesis, University of Illinois.

OMOSAIYE, O., CHERYAN, M. and MATTHEWS, M. E. 1978. Removal of oligosaccharides from soybean water extracts by ultrafiltration. J. Food Sci. 43:354.

SAIO, K., KOYAMA, E., and WATANABE, T. 1967. Protein-calcium-phytic acid relationships in soybean. I. Effects of calcium and phosphorus on solubility characteristics of soybean meal protein. Agric. Biol. Chem. 31:1195.

SMITH, A. K. and RACKIS, J. J. 1957. Phytin elimination in soybean protein isolation. J. Am. Chem. Soc. 79:633.

WHEELER, E. L., and FERREL, R. E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. 48:312.

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