Technical

Phytic Acid Removal from Soybeans by a Lipid Protein Concentrate Process[1]

J.R. FORD[2], G.C. MUSTAKAS, and R.D. SCHMUTZ, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

ABSTRACT

Over 90% of phytic acid has been removed from full-fat soy flour by a lipid-protein concentrate process previously reported in 1974 by the Northern Regional Research Center. In the current study, parameters for optimizing phytic acid removal were evaluated. By changing both the molar concentration of the calcium chloride solution used and the pH of the initial acid slurry, various amounts of phytic acid and mineral elements were recovered in the acid-precipitated curd. A mathematical treatment of the data using multiple regression analusis showed phytic acid removal possible from 10 to 90%, zinc recovery from 10 to 90%, and calcium concentrations equalling twice that of the original starting flour. All variable conditions introduced into the process had no effect on protein (90-93%), fat (98-100%), and iron (94-96%) recoveries. The results presented in this report can be applied to a large number of processes now being used for making edible soy proteins, and thereby low phytin-containing products can be acheived.

INTRODUCTION

The principal source of phosphorus found in soybeans (approximately three-fourths) is in the form of phytin, the calcium-magnesium-potassium salt of phytic acid (inositol-[hexaphosphoric]). Phytic acid makes up about 1-1.5% of soybeans by weight (1). The presence of phytic acid in diets has shown many nutritional effects, some of which are due to its activities on the bioavailability of dietary mineral elements.

Only within the last 10-15 years has the significance of zinc in practical nutrition been recognized. In general, the zince in animal products is more readily absorbed than that in plant products, particularly those that arise from plant seeds. The phytates in soybeans, ions peculiar to plants, bind zinc and thus decrease its availability for nutritional purposes. A publication by O'Dell (2) discusses phytic acid's many effects on rat, pig, and chicken growth. In these studies, soybean protein caused a lower percentage absorption of zinc from the intestine; and phytic acid, when added to a control casein diet, decreased the availability of zinc. Generally, growth rate in animals declined as the ratio of phytic acid to zinc increased. It would appear that the manipulative control of the phytic acid content in soy products might help in regulating the zinc (and other mineral elements) to phytic acid ratio for use in various diets. Phytate phosphorus may not be nutritionally available as a phosphorus source due to the absence of the enzyme phytase in soybeans (3); therefore, phytate removal probably does not mean a depletion in phosphorus value.

Recent publications by the U.S. Department of Agriculture's Agricultural Research Service show that excessive quantities of zinc in the diet may cause an unfavorable zinc-to-copper ratio in rats, high enough to induce excessive cholesterol output (4). Another study showed that a low zinc intake by pregnant rats may cause learning disabilities of the offspring (5).

Studies of tofu, a fermented soy food product, by Saio et al. (6) showed that the use of calcium in protein precipitation, along with heat application, tended to increase the phosphorus solubility in the isoelectric region.

In the current study, several variables were included in experiments to find their possible effects on phytic acid reduction in the resultant soy lipid-protein concentrate (LPC), a new product previously developed at this Center to serve as a bland, inexpensive milk substitute for aiding world food problems (7). This LPC product was tested by the Human Nutrition Laboratory, Agricultural Research Service, Grand Forks, North Dakota (Evans, Gary, private communication, 1974), and results showed no marked effect on zinc absorption and availability when the zinc-labeled protein solutions were stomach tubed in rats. The

[1]Presented at the AOCS Meeting, New York, May 1977.

[2]Now a chemical engineering graduate student at Purdue University, W. Lafayette, IN.

TABLE I

Regression Coefficients for Polynomial Modeled Observations
Recovery = $AX_1 + BX_2 + CX_1X_2 + DX_1^2 + EX_2^2 + F$
Where X_1 is the pH and X_2 is the Molar Salt Concentration

	Coefficients						Standard error of estimate	Correlation coefficients R
	A	B	C	D	E	F		
Recovery of								
1 Phytic acid	-268.7	-13,827	3264	21.87	-21,143	811.6	9.8	0.937
2 Zinc	47.21		186.3			-179.8	17.3	0.886
3 Calcium		-9822	1942			49.6	16.7	0.723
Concentration ratio								
4 Phytic acid	-2.265	-157.41	36.99	0.1605	-221.41	7.606	0.17	0.867
5 Zinc	0.5809		3.379			-2.285	0.18	0.907
6 Calcium		-325.65	77.44			0.7146	0.18	0.967

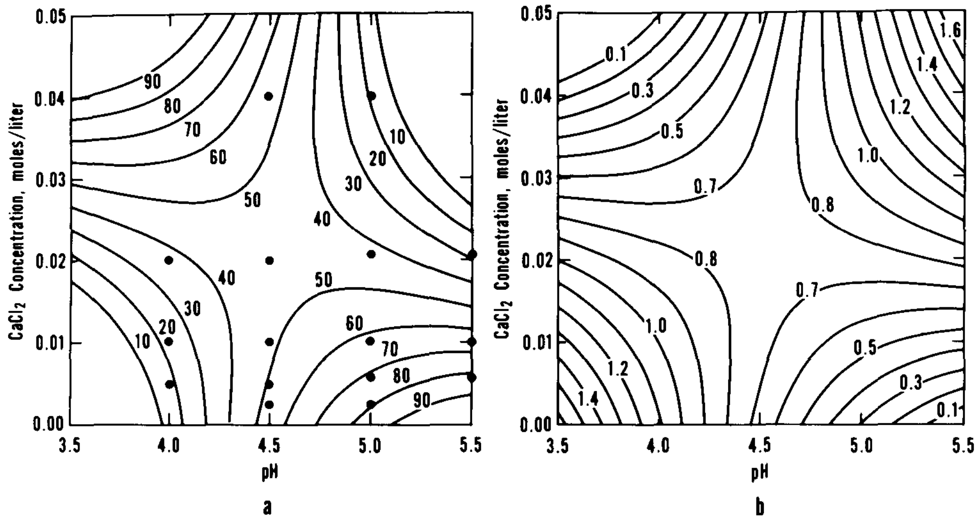

FIG. 1. (a) Percent reduction of phytic acid from the curd as a function of pH and $CaCl_2$ concentration. Percent reduction = 100-(% recovery in curd). (b) Concentration ratio of phytic acid as a function of pH and $CaCl_2$ concentration. Points shown in Figure 1 are actual condition points from which the curves in all the figures (1, 2, and 3) were plotted according to the polynomial equations. The data predictability is expressed by the standard error of estimate and the correlation coefficients in Table I.

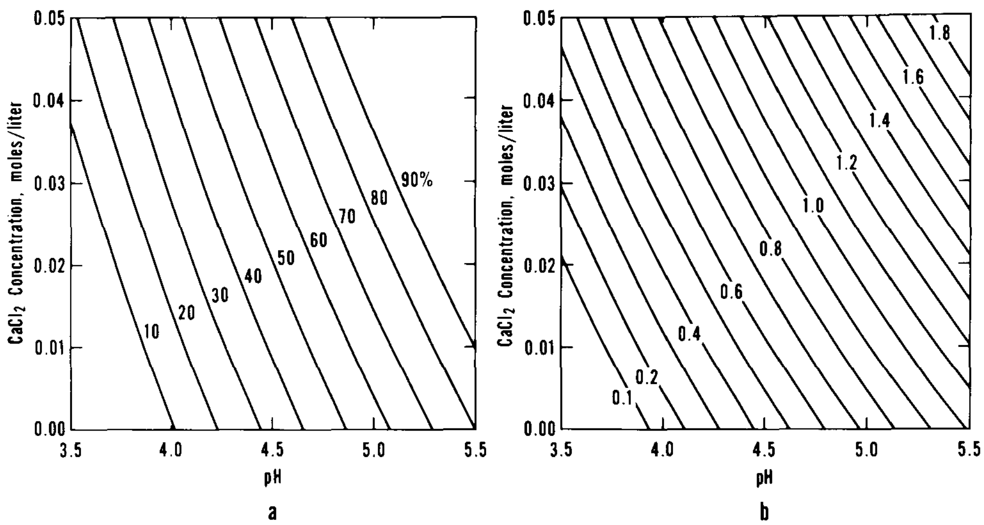

FIG. 2. (a) Percent recovery of zinc in the the curd as a function of pH and $CaCl_2$ concentration. (b) Concentration ratio of zinc as a function of pH and $CaCl_2$ concentration.

zinc retained by the rat was measured up to a period of 7 days. On the basis of this finding, it appeared that the LPC process had a high potential for reducing phytin content in soybeans and that the parameters in the process should be investigated in more detail.

EXPERIMENTAL PROCEDURES

Full-fat soy flour was prepared by the process of Mustakas (7). The starting full-fat flour contained 7.6% protein nitrogen, 25.4% fat, 0.20% calcium, 0.25% magnesium, 0.0064% zinc, 0.012% iron, and 0.40% phytic acid phosphorus on a dry-weight basis. One-half pound (227 g) of the flour was mixed for 20 min with 2 liter of a calcium chloride solution. The pH was adjusted to the desired level with HCl. The resultant acid slurry was then heated with sparge steam to 200 F for 2 min, held in a boiling water bath for 10 min, and subsequently cooled to 70 F in 2 to 3 min. The pH was again adjusted and the slurry further cooled 30 min in an ice bath until a temperature of 35 F was attained.

The cold acid slurry was filtered in an International SB, size 1 centrifuge through a cloth-lined perforated basket (5.25 in. radius x 3.75 in. height) at 3 to 20 x *g*. Following the collection of 1.2 liter of whey, a half liter of distilled wash water was added to the filter cake in the centrifuge. Three additional washes were made following the collection of a half liter of whey from the previous wash. Residual free water was then removed at 115 x *g*. Samples of curd and whey were neutralized with a solution of 20% sodium hydroxide and freeze dried for subsequent analyses. The curd fraction contains most of the protein, whereas the phytic acid is transferred into the whey fraction. A representative curd analyzed 9% protein nitrogen and 32% fat (dry basis), and a typical whey fraction analyzed about 2.5% protein nitrogen and 0.25% fat (dry basis). More complete analyses of these fractions are given in the original reference to the LPC process (7).

Moisture, crude fat, and nitrogen contents were determined by the standard AOCS methods (8). Residual oil was determined by gas liquid chromatography (GLC) (9).

Phytic acid phosphorus was isolated from the total phosphorus by precipitating with iron using the method of Oberleas (10). The phosphorus from the ferric phytate was then measured by the method of Truog and Meyer (11).

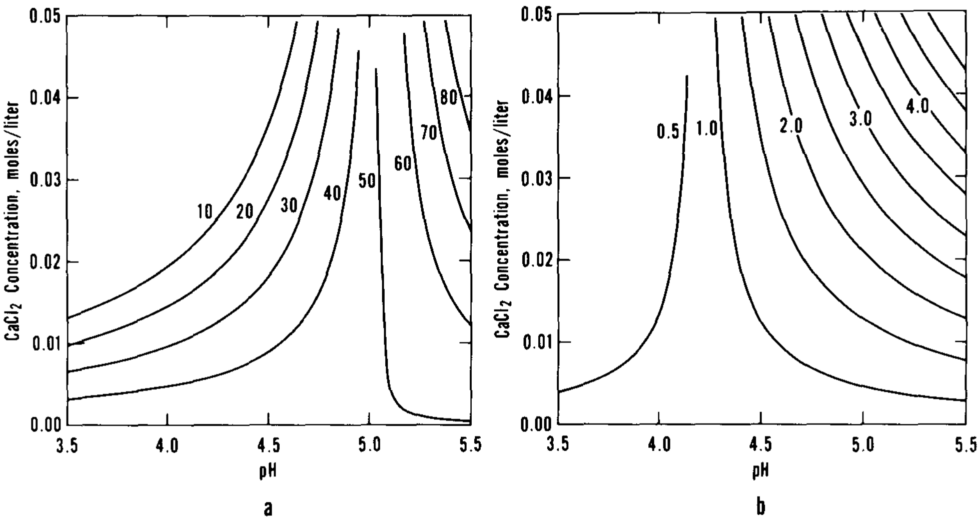

FIG. 3. (a) Percent calcium recovery in the curd as a function of pH and $CaCl_2$ concentration. (b0 Concentration ratio of calcium as a function of pH and $CaCl_2$ concentration.

TABLE II

Recovery in Curds of Protein, Fat, Iron, and Magnesium vs. Process Conditions

Process conditions[a]		% Recovery in curd of			
pH	Calcium chloride concentration, moles/liter	Fat	Protein	Iron	Magnesium
4.0	0.01	98.7	91.1	93	1
4.5	0.01	99.6	92.1	96	6
5.0	0.0025	99.6	92.3	94	13
5.0	0.005	99.5	92.7		14
5.0	0.01	99.5	91.3	94	15
5.0	0.02	98.9	91.4	94	20
5.0	0.04	99.1	90.9		11
5.5	0.01	98.6	89.5		29

[a]Experiments selected over a range of process conditions.

Metals were analyzed by the method of Garcia et al. (12).

The first experimental study checked five process variables for their effect on the removal of phytic acid from soy flour. Those variables studied at two levels were: (a) pH of the acid slurry (at 4.0 and 4.5), (b) calcium chloride concentration (at 0.0025M and 0.0225M), (c) the time held at 200 F (at 10 and 20 min), (d) the number of water washes (at 3 or 6), and (e) the two types of acids used (HCl or H_2SO_4). Of these five, only pH and calcium salt concentration demonstrated a significant desired effect. Sixteen of twenty possible runs were then conducted at various pH (4.0, 4.5, 5.0, 5.5) and calcium chloride concentrations (0.0025, 0.005, 0.01, 0.02, 0.04). After each run, the resultant curd and whey samples were analyzed for total protein, fat, calcium, magnesium, zinc, phytic acid, and in some cases, iron. Results were statistically treated by multiple regression analysis by fitting the following general second degree equation to the data:

$$\text{Recovery} = AX_1 + BX_2 + CX_1X_2 + DX_1^2 + EX_2^2 + F$$

(where X_1 is pH and X_2 is molar salt concentration)

The constants (A through F) were determined for various data sets. Values predicted by the equation after substituting for $X_1 + X_2$ were compared with the original data to determine correlation coefficient R. If there were no correlation between the dependent variable (constituent of recovery) and the two independent variables (pH, salt concentration), R would equal zero. The closer that R is to 1, the better are the predictions. Figures were extrapolated in accordance to equations as derived from multiple regression.

The recoveries of constituents (as plotted in the tables and figures) are described by quantitative (percent recovery) and qualitative (concentration ratio) results.

$$\%\ \text{recovery} = \frac{\text{(Total weight of constituent in curd)}}{\text{(Total weight of constituent in curd plus whey)}} \times 100$$

$$\text{Concentration ratio} = \frac{\text{(\% constituent in curd)}}{\text{(\% constituent in original flour)}}$$

RESULTS AND DISCUSSION

Initial Factorial Experiment

Statistical analysis of the factorial experiment for the first study showed that recoveries of protein, fat, and phytic acid were not affected by such variables as: cook time, acid type, or extended washes in the ranges tested. However, both pH level and calcium chloride concentration variables were related to the quantity of phytic acid removal into the whey fraction.

Phytic Acid Removal

Graphs were plotted from the equations derived by multiple regression analysis of the recoveries (Table I, Nos. 1, 4). The contours generated by these equations are plotted in Figures 1a and 1b.

From these experiments, it can be concluded that phytic acid removal from full-fat soy flour can be controlled in the lipid-protein concentrate process by adjusting the acid slurry pH and calcium concentration.

Phytic acid removal is best attained either at the combination of low pH levels (3.5-4.0) and high calcium con-

TABLE III

Mineral Nutrients Furnished by Soy Flour and LPC Curd Compared with RDA Requirements

	Phytic acid phosphorus (mg)	Zinc (mg)	Calcium (mg)	Iron (mg)	Magnesium (mg)
Full-fat soy flour 100 g starting material	400	6.4	200	12	250
RDA requirements[a]		15	800	10	350
LPC curd 100 g freeze-dried material (pH = 5.5, $CaCl_2$ concentration = 0.0025	40	6.4[b]	200[b]	16	90

[a]National Research Council recommended daily dietary allowances (1974) male age 23-50.
[b]Concentration ratio = 1.0.

centration (0.04M) or the combination of high pH levels (5.0-5.5) and low calcium levels. The latter combination also gives high recoveries of zinc in the curd as discussed in the paragraph below.

Zinc Recovery in the Curd

Zinc does not occur at high levels naturally in the soybean. Although soybeans contain an adequate amount of zinc, the binding properties of the associated phytic acid make this element unavailable for metabolism. Zinc recoveries may be calculated from equation coefficients from Nos. 2 and 5 (Table I) and are plotted in Figures 2a and 2b. The graph in Figure 2a shows that 70-90% recovery of zinc occurs near a region of 90% removal of phytic acid (pH = 5.5; $CaCl_2$ concentration = 0). Figure 2b indicates the concentration of zinc in the curd to be equivalent to that in the starting flour in this same region (concentration ratio = 1.0).

Calcium Recovery in the Curd

Generally, better recoveries of calcium in the curd were obtained at the high pH values (Fig. 3a). Concentration ratios of up to four times the calcium found inherently in the starting flour were achieved at high pH and high calcium chloride concentrations as seen in Figure 3b. However, these concentration levels occur under conditions of poor phytic acid removal (see Figs. 1a and 1b). In the range of 90% phytic acid removal, the concentration of calcium in the curd is the same as in the initial flour.

Recovery of Protein and Fat in the Curd Fraction

High and consistent recoveries were obtained over the variable ranges tested for protein (90-93%), fat (98-100%), and iron (94-96%). Magnesium was generally washed out into the whey. These results are shown in Table II.

Mineral Nutrients Furnish by LPC Curd

Residual zinc, calcium, iron, and magnesium metals found in the curd after reducing phytic acid from 400 mg to 40 mg by the LPC process are given in Table III. For comparison, the middle row of figures also gives the RDA requirements for male adults 23-50 years of age. While conducting the LPC process to remove phytates, no reduction of zinc, calcium, or iron concentration results from the values present in the original soy flour. As discussed above, less of the magnesium is recovered in the curd fraction. Based on RDA requirements, LPC curd is a good source of the mineral nutrients. Although calcium levels can be enhanced in the curd to as high as 800 mg (Fig. 3b concentration ratio = 4.0), the conditions needed to achieve this yield result in poor phytate removal.

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