Technical

The Preparation of Soy Products with Different Levels of Native Phytate for Zinc Bioavailability Studies

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ABSTRACT

Soy products with low, intermediate and normal phytate levels were prepared in the pilot plant for subsequent rat-feeding experiments to evaluate zinc bioavailability. The low level (0.29%) phytate product was made by precipitation of the protein curd at pH 5.5, whereas the normal level (1.05%) phytate product was produced by a similar process except that the phytate previously isolated from the whey fraction was added back to the original curd as native phytate. The intermediate level (0.73%) phytate product was also produced by acid precipitation, but at pH 4.5. The pH 5.5 precipitation process yielded a large quantity of whey in which the ratio of water content to phytate was over 1,000 parts to 1. However, ca. 75% of the water was subsequently removed by reverse osmosis (RO), which increased the concentration of phytate in the whey fraction and facilitated its isolation. Protein was first removed from the whey by precipitation with trichloracetic acid, then phytate was precipitated in the supernatant with ferric chloride. Another series of experiments was run to find optimal conditions to convert ferric phytate to the more soluble sodium phytate form, using a minimal amount of sodium hydroxide so that the phytate could be recycled back to the curd without causing a large increase in sodium content of the product. There were only minor differences in the protein, lipid and mineral contents of the three products.

INTRODUCTION

The soybean is an excellent alternative source of protein and, when properly processed, its products have good nutritional quality. However, soybeans contain 1–1.5% phytic acid by weight (1) and perhaps other metal-binding agents that can reduce mineral bioavailability. The presence of phytic acid in animal diets has been shown to have adverse nutritional effects, some of which undoubtedly are due to its effect on the bioavailability of dietary mineral elements. Published data from numerous animal feeding trials indicate lower bioavailability of zinc, calcium, magnesium, phosphorus and possibly iron from diets containing high phytate foods (2–4).

The purpose of this study was to produce soy products at several phytate levels while still maintaining adequate levels of minerals and protein. This was accomplished by a process for soy lipid-protein concentrate (LPC), which utilizes precipitation of protein curd at different pH values (5). A low-phytate soy product was produced by acid precipitation of the protein curd at pH 5.5 according to the study of Ford et al. (6). At this pH, the phytic acid was removed in the whey fraction. A subsequent procedure was developed to (a) concentrate the phytate removed in the whey fraction by reverse osmosis, (b) deproteinize the whey, and (c) isolate the native phytate. After conversion of the removed phytate to a more soluble sodium form, it was added back to the original curd to produce a normal phytate soy product. This normal phytate product, together with an intermediate one and the low-level phytate product, were used in rat-feeding trials at the University of Illinois, Urbana, Illinois, for zinc bioavailability studies.

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EXPERIMENTAL PROCEDURES

Materials

Full-fat soybean flour was produced by cracking and dehulling whole soybeans, heat treating for 5 min at 150 F, flaking, cooling and grinding in an Alpine Contraplex pin mill. The composition of the full-fat flour is shown in Table I. Trichloracetic acid and ferric chloride were reagent grade. Other chemicals used in the procedures were also of analytical quality.

Analytical

Moisture, crude fat, ash, crude fiber, protein, urease and nitrogen solubility index were run according to official AOCS methods (7-13). Trypsin inhibitor was determined by a modification of the standard analytical procedure (14). Metals were analyzed by the method of Garcia et al. (15). Lipoxygenase was run by the method of Smith (16). Phytic acid was measured by Earley's procedure (17).

Procedure

Procedures for production of the lipid protein concentrate (LPC) were those described by Mustakas (5). Using these procedures, Ford et al. predicted removal of ca. 50% of the phytic acid at pH 4.5 and more than 90% at pH 5.5 (6). An expected contribution of the present study was to develop a procedure for isolating phytate from the whey fraction and adding the separated phytate back to the LPC to yield a normal phytate product. The process developed was varied to produce three products having low, intermediate and normal phytate content.

The general procedure depicted stepwise in Figure 1 was: tap water (55 kg) was heated to a boil in a 113-ℓ jacketed kettle. Full-fat soy flour (4.5 kg) was then added and

TABLE I

Analysis of Full-Fat Soy Flour

Moisture (%)	3.0
Protein (N x 6.25) (%)	39.0
Crude fat (%)	23.1
Crude fiber (%)	2.7
Ash (%)	4.7
Phosphorus (%)	0.9
Phytic acid (%)	1.3
Trypsin inhibitor (mg/g)	31

the slurry formed was stirred for 5 min at 93–97 C. Sulfuric acid (20%) was added to attain the desired experimental pH (products I and III, pH 4.5; product II, pH 5.5). The slurry was cooked for 10 min at 97 C without stirring, then cooled to room temperature. The procedure was repeated with two additional 4.5-kg batches of soy flour, and the slurries from the three experimental cooks were combined and stored overnight at 1 C. The following day, the curd was separated from the whey in a Tolhurst centrifuge (1,400 x g) fitted with a 61-cm diameter solid bowl. Washing of the curd was accomplished by reslurrying the curd in tap water and recentrifuging.

The curd fraction from each process was resuspended in water and colloid milled, cooked, homogenized and spray-dried as previously described (6). In the product III process, the native sodium phytate isolated from the whey of product II was added back at the colloid milling step.

The whey from the pH 4.5 procedure was discarded. However, the combined whey and washes from the pH 5.5 procedure were processed as shown in Figure 1. The whey was dewatered on an Osmonics spiral wound reverse osmosis (RO) module (3.2 m^2). The module was rated for 97% rejection of sodium chloride. Protein was precipitated from the whey by the addition of 3% trichloracetic acid, and the precipitate was removed in a Sharples centrifuge (13,000 x g) fitted with a 10-cm diameter bowl. Ferric chloride was added to the deproteinized whey, and the solution was boiled for 30 min to precipitate the native phytate as the ferric salt. Earley (17) had shown that phytate recovery was best when the ratio of iron to phosphorous in the precipitating mixture was in the range of ca. 4 times the theoretical ratio (i.e., 4 x 4 Fe/6P); this established procedure was, therefore, followed in these experiments. Ferric phytate crude isolate was separated from the whey in a Sharples centrifuge (35,000 x g) fitted with a 2.5-cm diameter solid bowl.

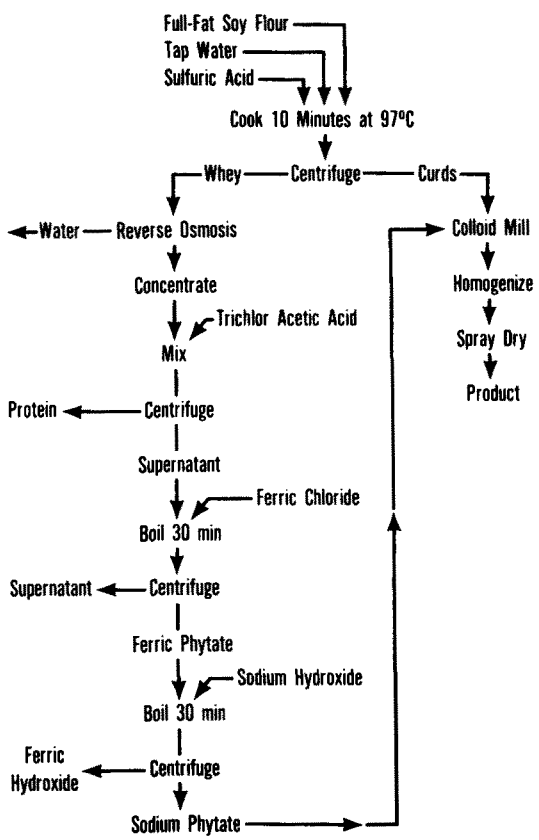

FIG. 1. Isolation of native phytate from soy whey.

In order to establish optimal conditions for the conversion of ferric phytate to sodium phytate, a series of experiments was run using incremental quantities of sodium hydroxide. These experiments were conducted as described next.

One g of the crude isolate of native ferric phytate was suspended in 100 ml of distilled water. The specified increment of sodium hydroxide (1.5 N) was added, adjustment to the final volume was made with distilled water and the pH was measured. The sample was boiled for 30 min and then cooled, and the pH was measured again.

RESULTS AND DISCUSSION

Curd Characteristics and Whey Treatment

The procedure conducted at pH 4.5 produced a hard curd with good filtration characteristics. This curd was both filtered and washed on an Ametek continuous rotary filter. Conversely, when the pH 5.5 procedure was used, a slimy curd resulted with poor filtration characteristics. Because of this, the curd was separated from the whey on a Tolhurst centrifuge fitted with a solid bowl. Washing was accomplished by reslurrying the curd in tap water and recentrifuging.

The process yielded a large quantity of whey (270 kg) in which the ratio of water to phytate was well over 1,000 parts to 1. Approximately 75% of the water was removed by reverse osmosis which increased the concentration of phytate in the whey and facilitated its isolation. This procedure also reduced the quantity of trichloracetic acid required in the process by over 70% of the quantity normally used.

Solubilization of Ferric Phytate

The procedures found in the literature for the conversion of ferric phytate to sodium phytate called for large excesses of sodium hydroxide. Since the excess sodium would also be recycled back to the process together with the sodium phytate, the sodium level in the normal phytate product would be increased to an intolerably high level, especially for feeding trials. Thus, it was important to establish conditions for the conversion that required minimal sodium hydroxide. In the series of experiments to reduce the amount of sodium hydroxide, the first limit was observed when the ferric hydroxide precipitate, which is necessary to remove the iron, was no longer formed and instead a soluble sodium-iron complex of phytate was formed (test 5, Table II). Further reductions in sodium hydroxide quantities were realized as the phytate concentration was gradually increased. Again, the limit was reached when no ferric hydroxide precipitate was formed (tests 10,11). The conditions in test 9 were chosen to be used in the final process.

A second concern was whether the phytate would precipitate along with the ferric hydroxide, since the pH limit was in excess of 11.3 shown by other investigators (18,19) to cause precipitation of phytate. However, no evidence of phosphorus was found in the ferric hydroxide precipitate, indicating that the phytate was staying in solution. It was pointed out by de Rham and Jost (18) that it was sometimes necessary to add 0.01 M calcium to cause precipitation. Apparently, sufficient calcium was lost in the mother liquors from the ferric phytate precipitation so that precipitation of phytate was not a problem at pH 12.2.

The native ferric phytate crude isolate contained 13.0% protein, 51.9% ash, 16.9% iron, and 50.4% phytic acid on a dry-weight basis. The first run of the series (Table II) consumed 150 meq of sodium hydroxide/g of ferric phytate and approximates the quantities in the laboratory pro-

TABLE II

Conversion of Native Ferric Phytate Crude Isolate into Sodium Phytate Using Decreasing Amounts of Sodium Hydroxide

Test no.	Water (g) / Ferric phytate (g)	NaOH / Ferric phytate (g)	Millequivalents pH: Before boil	Millequivalents pH: After boil	Precipitate	Supernatant
1	1000:1	150	12.6	12.6	Dark brown	Pale yellow
2	1000:1	75	12.2	12.2	Dark brown	Pale yellow
3	1000:1	50	12.1	12.0	Dark brown	Yellow
4	1000:1	40	11.8	11.8	Dark brown	Orange
5	1000:1	20	11.6	11.3	None	Red
6	500:1	30	12.0	12.0	Dark brown	Pale Yellow
7	200:1	30	12.6	12.6	Dark brown	Yellow
8	100:1	21	12.6	12.6	Dark brown	Yellow
9	100:1	15	12.4	12.2	Dark brown	Yellow
10	100:1	10	12.1	11.4	None	Red
11	100:1	6	11.5	8.5	None	Red

cedure of Wheeler and Ferrel (20). In test 9, the condition used in the established process, only 15 meq of sodium hydroxide was required.

Product Assays

The phytic acid levels of the three products gave a range of values that were significantly different. Product II (low) had a phytic acid content of 0.29%; product I (intermediate) had 0.73%; and product III (normal) had 1.05% (Table III). Product III had a higher ash value than the other two, reflecting the recycling of the native phytate. The sodium level in product III is also higher because of the recycled sodium phytate and excess sodium hydroxide. Because recycled sodium hydroxide was substituted for calcium hydroxide in the pH adjustment of product III at the colloid milling step, calcium content is lower than in the other two products. It can be concluded from this study that the native phytate content can be sufficiently varied in the soy products produced by the procedures described to provide a suitable range of phytate values for rat-feeding trials on zinc bioavailability.

TABLE III

Composition of Soy Products

	Product phytate levels[a] I Intermediate	II Low	III Normal
Moisture, %	5.7	5.8	3.8
Protein (N x 6.25), %	48.8	48.5	43.6
Fat, %	23.8	24.4	21.6
Fiber, %	3.7	3.1	3.3
Ash, %	3.3	3.3	5.0
Total phosphorus, %	0.52	0.46	1.25
Phytate phosphorus, %	0.20	0.08	0.29
Phytic acid, %	0.73	0.29	1.05
Zn, ppm	23	13	28
Fe, ppm	84	93	112
Na, %	0.06	0.10	0.68
Ca, %	0.49	0.55	0.12

[a]Product I-Precipitation of protein curd at pH 4.5. Product II-Precipitation of protein curd at pH 5.5. Product III-Precipitation of protein curd at pH 5.5 plus recycled native phytate.

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