

# Variability in Soy Flour Composition

Michael A. Porter<sup>a,\*</sup> and Alison M. Jones<sup>b</sup>

<sup>a</sup>Cargill Soy Protein Products, Minneapolis, Minnesota 55440, and <sup>b</sup>Cargill Sweeteners North America, Wayzata, Minnesota 55343

**ABSTRACT:** Soy flour is commonly used as a protein source in industrial fermentations. As an essentially natural product, the composition of soy flour can vary from year to year as a result of changes in growing conditions. The composition of soy flour from a single manufacturing plant was determined over an 8-yr period. Protein concentration varied from 51.2% in the 1997 crop to 53.2% in the 1998 crop, but overall amino acid composition was relatively constant. The concentrations of valine, isoleucine, tyrosine, and alanine showed significant variation between years. Crude fat levels were steady, but the fat by acid hydrolysis (FAH) levels varied, suggesting changes in the level of phospholipids. The difference between the crude fat and FAH analyses (a proxy for phospholipids) had a strong positive correlation ( $P < 0.001$ ) with the total phosphorus concentration, consistent with this interpretation. FA distribution varied year to year with linoleic acid providing the most dramatic effect. Fe and P concentrations showed significant variation, but Al, Ca, Cu, K, Mg, Mn, Mo, Ni, and Zn did not vary significantly. Although P concentration had a significant correlation with proxy phospholipid levels, there was not a significant correlation between P concentration and phytic acid concentrations.

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**KEY WORDS:** Amino acid composition, consistency, lipid, minerals, protein, soy flour.

Soybeans are an important raw protein source. They are widely grown geographically, are relatively high in protein, and are processed to a relatively constant set of commodity standards. Soybean flours and meals find use in standard and specialty animal feeds, many foods, and numerous industrial products.

One common use of soy flour, grits, and soybean meal is as a protein-rich raw material for enzyme and antibiotic fermentations. The quality of the soy protein feedstock depends on a number of factors, including the type of processing used, the consistency of process control, and the quality of the incoming raw soybeans. Many suppliers around the world provide defatted soy flours and meals to fermenters. A major concern expressed by fermentation scientists is the variability in the raw material from year to year and from supplier to supplier. As soy flour produced from one crop is displaced by soy flour produced from another crop, it is not uncommon for yields in fermentation processes to change by 10 or even 25%. Similarly, soy flours and meals from different suppliers often result in very different yields. Although often expressed as a sup-

plier-to-supplier difference, this is usually dependent on the origin of the soybeans. Soybeans from different soils and climates differ in composition (1). By comparison, processing causes relatively little variation in general chemical composition. As one of the crudest raw materials in many fermentation media, much of the variability in fermentation productivity is attributed to changes in the soy flour. When the variation decreases fermenter productivity, fermenters become very interested in understanding and reversing the cause of the decline.

Variability in the composition of soybean meals and flours has been assessed in the past, but typically in the context of geographical differences (1,2) or varietal differences (3–6). Traina and Breene (7) compared the compositions of full-fat flours processed by differing methods and different soybean originations. Although the studies of Cromwell *et al.* (1) and Harmon *et al.* (2) collected a large number of soybean meal samples from two consecutive years, we are not aware of any studies that report changes in composition over a longer time period.

Flour, grits, and meal are produced from soybeans by mechanically removing the hull, followed by extraction of the oil with hexane. Residual hexane is removed either by indirect heating followed by steam sparging in a desolventizer-toaster (to make meal) or by direct contact with superheated hexane in a flash desolventizer. The desolventized soy is then heat-processed, ground, and segregated to the desired particle-size distribution according to product specifications. The distinction between flour, grits, and meal is due, primarily, to the particle size range of the product. Some suppliers produce their flour and grits only from flash desolventizers, whereas others may use either desolventizer method; meals are almost always produced with desolventizer-toasters. Different suppliers and the processing facilities of each supplier manufacture using variations on the theme described in this paragraph. However, at a gross level, the desolventizing method of particle-size distribution does not change the composition. This report will focus on soy flours produced with a flash desolventizer.

The stimulus to begin collecting these data was a year in which a few major industrial fermenters experienced a major decrease in yield (confidential personal accounts). We began looking for compositional differences that might explain the yield loss. This effort was expanded to look at year-to-year variation in a range of compositional factors. We have now collected data on one type of soy flour from a single plant from eight soybean crops. In this paper, we report on the variations in protein, fat, ash, mineral, amino acid, and FA composition over this period.

\*To whom correspondence should be addressed at Cargill Soy Protein Solutions, P.O. Box 5699, Minneapolis, MN 55440-5699.  
E-mail: michael\_porter@cargill.com

## MATERIALS AND METHODS

All soy flour samples were obtained from Cargill Incorporated's Cedar Rapids, Iowa, "West" soy processing plant. The raw materials consumed by this plant were all grown within a 90-km radius and delivered by growers directly to the plant by truck. Although soybeans were rejected if too highly contaminated (by dirt, sticks, etc.) or damaged (broken or spoiled beans), no other selection criteria were used to discriminate between acceptable and unacceptable beans. The soy flour produced at this plant came from the flash desolventizer system; the particular product analyzed in this study was 200-mesh, 70 PDI (protein dispersibility index, Ref. 8) soy flour. PDI is a standard analytical measure of the extent of heat treatment received by the flour (8). Soy flour with a PDI of 70 indicates that it is partially toasted. As indicated above, the chemical composition of this product would be the same as other flours or soybean meal produced at the same plant.

Samples were collected irregularly early in the study, but after 1995, samples were collected during each quarter of the year. Samples were divided and sent to various commercial laboratories for analysis. Different commercial laboratories were used at different times during this study, but the methods used were highly standardized within the industry (Table 1). Additional components were added to the analysis during the course of the study, and some data are available only from more recent crops.

**Statistical analyses.** Data were compiled and statistically analyzed using SPSS 10.1 software (SPSS Inc., Chicago, IL). The primary methods used were general linear model univariate ANOVA to detect year-to-year differences, mean calculation to generate means and 95% confidence intervals, and bivariate correlation and linear regression to detect significant relationships between measures, then generate equations for the relationships between factors. In all the graphs shown in this report, the error bars represent 95% confidence intervals.

## RESULTS AND DISCUSSION

Altogether, 27 soy flour samples were analyzed over an 8-yr period. Only one sample was collected during 1994, but three or four samples were collected in all other years. Some analy-

**TABLE 1**  
Analytical Methods Used in the Collection of Data

Analysis	Method	Reference
Al, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, K, Zn	AOAC 985.01	
Amino acid composition	AOAC 984.27	10
Ash	USDA 6.011 (1986)	11
Crude fat	AACC 8-16	12
Fat by acid hydrolysis	AOAC 920.39	10
FA profile	AOAC 922.06	10
Moisture	AOAC 969.33	10
Protein dispersibility index	AOAC 925.10	10
Phosphorus	AOCS Ba 10-65	8
Phytic acid	AOAC 984.27	10
Protein	<i>Anal. Biochem.</i> 77:536 (1977)	12
Free amino nitrogen	AOAC 991.20E	10
	Eur. Brew. Commission	9

ses did not begin the first year; deviations from the full analyses are noted where appropriate.

Given the relatively small geographic area from which these soybeans originated, these results should be considered to represent the better end of product consistency. If the soybeans were to originate from a wider area, composition would vary more widely due to greater differences in soil, weather, and range of cultivars. As the following results show, composition does vary from year to year even within this small geographical area.

Soy flour is much more complex than the analyses reported here would suggest. We made no effort to track changes in the concentrations of "minor" components such as vitamins, isoflavones, saponins, or organic acids. We also made no attempt to assess variations that might arise from the production process itself. Although the process itself is relatively mild in terms of inducing chemical reactions, there are nevertheless reactions that occur during the extraction and heating of the soy. The products of these reactions are beyond the scope of our analysis.

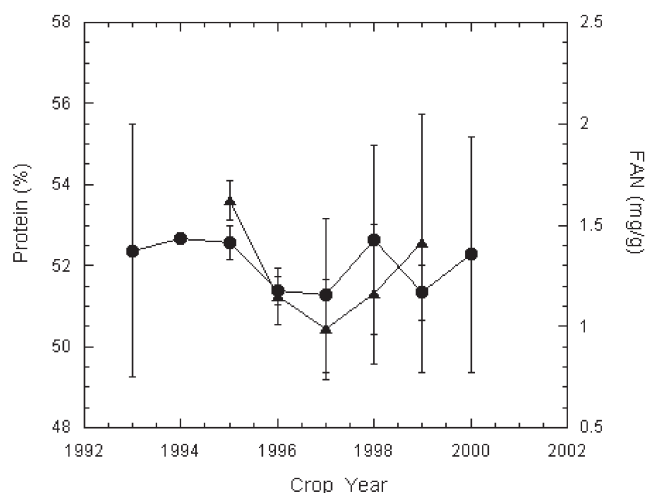
**Protein and amino acids.** Protein is one of the prime attributes of soy flour. Soy flour is typically greater than 50% protein (as is) and can approach 56% on a moisture-free basis (Table 2). As Figure 1 shows, the protein content of soy flour fell within the range of 51 to 53% (as is) over the 8-yr study period. Protein content varied significantly from year to year. From 1995 to 1996, protein concentration dropped approximately 1%. A similar drop in mean protein occurred between 1998 and 1999, but the variability between samples within the two crop years suggested that the difference was not statistically significant. Although a significant drop could arise from changes in processing, such as lower efficiency in hull removal or greater residual moisture, this decline was not due to such processing differences (data not shown).

Amino acid compositions were not determined before 1995. Table 3 shows the average amino acid composition of soy flour from the 1995 crop to the 2000 crop (24 samples total). There were no significant differences detected in the amino acid compositions between any of the years, except for the amino acids alanine, valine, isoleucine, and tyrosine. These four amino acids showed a sharp rise in concentration between 1997 and 1998 (Fig. 2). The increase in the concentration of these amino acids coincides with the widespread adoption of glyphosate-tolerant soybean cultivars. Analysis of some glyphosate-tolerant soybean cultivars and their parent lines did not reveal any similar selective increase in these

**TABLE 2**  
Mean Proximate Analyses of Soy Flours Collected at Cedar Rapids, Iowa, Processed from Crops Grown Between 1993 and 2001<sup>a</sup>

Analyte	Mean value	SD (n)	Prob.
Protein (% as is)	52.05	0.957 (30)	0.019
Moisture (%)	5.20	0.673 (29)	0.117
Ash (% as is)	6.31	0.192 (30)	0.012
Fat (by extraction)	0.80	0.140 (30)	0.435
Fat (by acid hydrolysis)	2.39	0.719 (30)	<0.001

<sup>a</sup>The mean and SD are expressed in percentage (as is). "n" is the number of samples included in the analysis. The probability of there being no differences between years on that measure is shown in the column labeled "Prob."



**FIG. 1.** The mean protein (●) and free amino nitrogen (FAN) (▲) concentrations (as is) in soy flour collected at Cedar Rapids, Iowa, processed from crops grown between 1993 and 2000. Error bars indicate the 95% confidence interval around the mean. Only a single sample was collected in 1994.

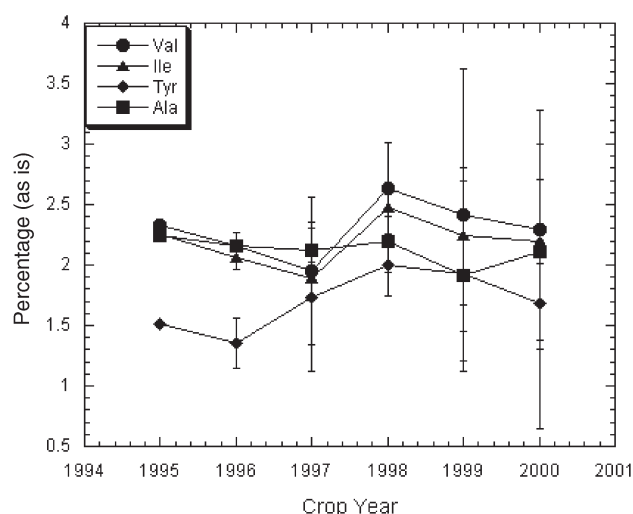
amino acids (4) whether or not the plants had been treated with glyphosate (6). Glyphosate tolerance itself may be an unlikely explanation for this observation, but introduction of other new cultivars, with or without glyphosate tolerance, may be part of the explanation. Cromwell *et al.* (1) observed significant differences in the amino acid compositions of soybean meals collected in 1989 and 1990, well before the widespread adoption of glyphosate-tolerant cultivars.

The principal reason soy flour is included in fermentation media is as a source of organic nitrogen. Consistency in protein concentration is desirable for fermenters, but perhaps less

**TABLE 3**  
Mean Concentrations of Amino Acids in Soy Flours  
Collected at Cedar Rapids, Iowa, Processed from Crops  
Grown Between 1995 and 2000 (23 samples)<sup>a</sup>

	Mean	SD	Prob.
Glx	8.97	1.778	0.587
Asx	6.02	0.487	0.308
Leu	3.94	0.341	0.307
Arg	3.71	0.237	0.057
Lys	3.30	0.223	0.452
Ser	2.76	0.307	0.269
Pro	2.90	0.865	0.232
Phe	2.51	0.208	0.779
Val	2.30	0.324	0.011
Gly	2.17	0.183	0.297
Ile	2.20	0.267	<0.001
Ala	2.12	0.151	0.015
Thr	2.05	0.252	0.089
Tyr	1.68	0.340	0.002
His	1.37	0.142	0.179
Cys	0.74	0.133	0.881
Met	0.71	0.091	0.966
Trp	0.62	0.199	0.094

<sup>a</sup>The mean and SD are expressed in percentage (as is). The probability of there being no differences between years on that measure is shown in the column labeled "Prob."



**FIG. 2.** The change in concentrations of alanine (■), valine (●), isoleucine (▲), and tyrosine (◆) in soy flour collected at Cedar Rapids, Iowa, processed from crops grown between 1995 and 2000. Error bars indicate the 95% confidence interval around the mean. Only a single sample was analyzed in 1995.

crucial than consistency in amino acid composition. The concentration of soy flour in a fermentation medium can be decreased or increased in the event of deviations from "normal" protein concentration. Deviations in the amino acid composition would be much more difficult to monitor and correct. Consequently, consistency in the concentration of amino acids is a virtue for fermenters.

Historically, soy flours and meals are used in fermentations with relatively robust organisms—organisms that excrete proteases to degrade the proteins in their media to amino acids and peptides. Soy protein is rarely used in systems where specific amino acid requirements must be met or where the organism is not proteolytic. For example, *Saccharomyces* yeast, some lactobacilli, and animal cell cultures depend on defined mixtures of free amino acids and/or peptides for their growth. Most of the organisms able to live on soy can synthesize the full range of amino acids from a common nitrogen supply.

One measure of readily available nitrogen is the so-called free amino nitrogen (FAN). Analytically, this represents the portion of the soy that reacts with ninhydrin and is readily soluble (9). FAN concentrations were analyzed on 22 soy flour samples collected between 1995 and 1999. The mean FAN content over this period was  $1.22 \pm 0.11$  mg/g (as is). The FAN concentration showed a significant variation from year to year (probability of no effect = 0.001; Fig. 1). Possible sources of variation could include the maturity of the soybeans at harvest and the moisture of the beans during preprocessing storage. In general, higher levels of FAN would favor more rapid initial microbial growth because the requirement for protease excretion would be delayed.

**Lipid.** There are a number of ways to look at the residual lipid content of soy flour. The most commonly used specification, commercially, is crude fat, which measures the lipid that can be extracted by an organic solvent (such as petroleum

ether or hexane) then recovered after evaporation of the solvent. Crude fat is a good measure of residual neutral TG content. As Figure 3 shows, the residual crude fat did not vary between the years studied.

Another method for analyzing residual fat involves acid hydrolysis of the lipid components to release the FA from solvent-insoluble compounds. This procedure is known as fat by acid hydrolysis (FAH). FFA and phospholipids comprise a good portion of this fraction, which also contains the hydrolyzed FA from the neutral TG. At present, there is no good direct measure of phospholipids available in commercial labs, so no direct results on phospholipid content are shown here.

In contrast to the stability of crude fat, the measured content of FAH varied over the study period (Fig. 3). Critically, the solvent-insoluble fraction (FAH – crude fat) is a large portion of the total lipid content, sometimes threefold more than the content indicated by crude fat. The phospholipids can be a source of nutritional phosphorus, and it may have direct metabolic effects. This lipid is capable of providing surface activity in the media (14).

If the change in FAH content can be attributed to a change in phospholipids remaining in the flour, the cause could be changes in the extraction process, cultivar distribution, or weather and could have implications for the phosphorus content in the flour.

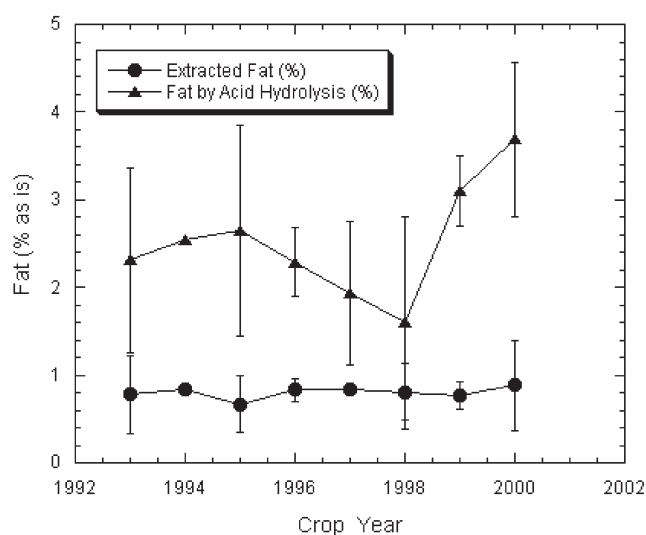
We did not anticipate a change in the ratio of phospholipid-to-crude lipid and remain unsure of its potential impact on fermentation. On the one hand, this suggests a shift in the supply of available phosphorus (assuming that phosphorus in phospholipids is readily biologically available), and an increase in the supply of “natural” surfactant in the media. On the other hand, this is a small change in the amount of phosphorus overall (see following section); inorganic phosphate is inexpensive and can be added without much difficulty.

In addition to process improvements, new cultivars are introduced regularly that could have an influence on the minor component composition of the soy oil. For example, this period

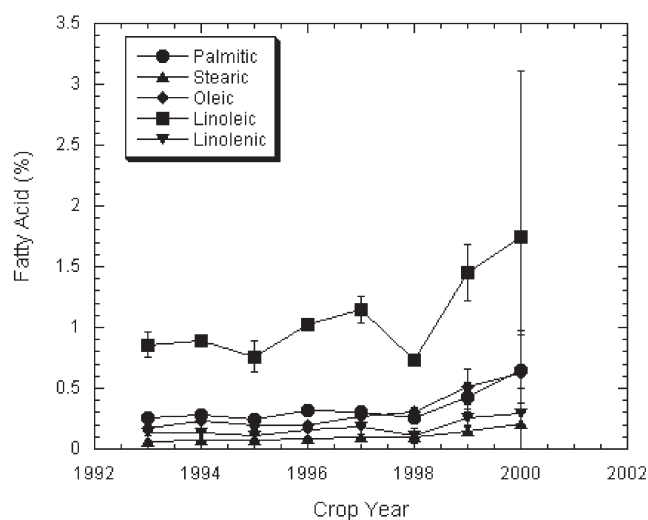
is also approximately when glyphosate-resistant soybean cultivars were introduced commercially. There is no indication that the composition of these soybeans differs from traditional soybeans with respect to phospholipids (3). Finally, the weather may be an important influence on oil content and composition. Good growing conditions favor high yields and high oil content in the soybeans. The period of declining FAH was also one of generally high soybean yields in east central Iowa. We are not aware of information showing an effect of weather on minor oil components.

Five FA comprised the majority of the lipid content in soy flour. The identification of the FA was done on independently prepared hydrolyzates of extracted oil. Of the five FA indicated in Figure 4, linoleic acid was not only the most prevalent but also the most variable in its presence in the soy flour. In 1999 and 2000, the linoleic acid content was significantly higher than that observed in the previous years or the following year. Notably, the linoleic acid content increased during the period when the overall FAH content was decreasing. Despite this apparent increase in linoleic acid, the proportion of the total oil comprising linoleic acid barely changed in these 2 yr (data not shown).

Defatted soy flour is not typically used in media for its lipid content. In fact, the lipid content of soy is generally completely disregarded. As a carbon source, the lipid is almost irrelevant, and as a source of antifoaming materials, it is ineffective. However, the residual oil is a possible source of oxidation breakdown products. Possibly the most important of these is the class of hydroperoxide compounds. These compounds are formed as one of the first reaction products of the oxidation of unsaturated carbons in FA. Formation of these hydroperoxides is catalyzed by metals, promoted by high temperature, and requires the presence of oxygen. These compounds are formed at a slow rate under standard storage conditions. Typically, the oxidation pathway continues and the compounds dissipate, but if they were introduced into a complete media, the hydroperoxides



**FIG. 3.** The mean fat concentration (as is) in soy flour collected at Cedar Rapids, Iowa, processed from crops grown between 1993 and 2000. Results from two methods are shown: fat by extraction (●) and fat by acid hydrolysis (▲). Error bars indicate the 95% confidence interval around the mean. Only a single sample was collected in 1994.



**FIG. 4.** The mean concentration of FA in soy flour collected at Cedar Rapids, Iowa, processed from crops grown between 1993 and 2000. Error bars indicate the 95% confidence interval around the mean. Only a single sample was analyzed in 1994. Individual FA analyzed were: palmitic (●), stearic (▲), oleic (◆), linoleic (■), and linolenic (▼).



could react with proteins, amino acids, other media components, and possibly the microbes themselves.

**Mineral elements.** Soy flour contains the majority of mineral components present in the original seed. Biologically, these minerals support the early growth of the germinating seedling. In a fermentation context, these minerals can contribute to the growth of the microbes by supplying necessary nutrients or interfere with production by stimulating microbial metabolism in undesirable ways. A number of microbially based processes are stimulated by the absence of particular minerals. Media components or formulations may be processed to remove or control the levels of the specified mineral (private communication). Variability in the levels of minerals in the raw ingredients cause these fermenters real trouble. Conversely, some fermentations are stimulated by high levels of mineral nutrients (private communication). These treatments can be interfered with by the variable presence of potential chelators. Finally, plants are capable of accumulating mineral nutrients beyond the concentrations observed in the soil. If heavy metals, as one example, were to accumulate to high levels, this could be toxic in the fermentation itself or subsequently, if consumed by humans or animals.

One of the major goals of this effort was to track year-to-year variation in a range of biologically important minerals. Not all minerals were analyzed throughout the study. For example, potassium was measured only in the 1998–2000 crops. Other minerals were analyzed for but not detected. Analysis for cobalt was first done in the 1996 crop but exceeded the detection limit in only 1 out of 19 samples. Similarly, chromium and aluminum were not always detected in soy flour samples submitted for analysis. Where this occurred, we have reported the mean of the results exceeding the detection limit, and have accepted that the true mean is a lower, but unknown, value.

The mean concentrations for a number of mineral elements are shown in Table 4. In contrast to many summaries of composition (1), this represents the average composition of soy material from a relatively small geographic area over a longer period of time. Soil chemistry is known to have a significant effect on plant chemical composition. For example, beans from the southern United States or some parts of Brazil are grown on clay soils with a high content of iron and aluminum. Soybeans from these areas also are high in iron and aluminum, and these elements are found in high concentrations in soybean meal made from those beans.

There was no detectable variation (at a 5% significance level) in the total content of most minerals over this 8-yr period at this location (Table 4). The chromium content apparently varied during this period (Table 4). However, we believe this conclusion is doubtful, as 19 of the 27 samples tested had no detectable chromium, and the eight samples with detectable levels were just above the detection limit. In contrast, phosphorus levels in soy are relatively high. Over the 8 crop years, mean phosphorus levels dropped about 7%, before rising sharply in the 1999 and 2000 crops (Fig. 5). Phosphorus in soy flour is found in a few prominent forms, with phytic acid and phospholipids predominating. Phytic acid (inositol hexaphosphate) is the principal storage compound for phos-

**TABLE 4**  
Mean Concentrations of Mineral Elements in Soy Flours Collected at Cedar Rapids, Iowa, Processed from Crops Grown Between 1993 and 2000<sup>a</sup>

Element	Years (samples)	Concentration (mg/100 g)	SD	P value
Aluminum <sup>b</sup>	6 (22)	1.03	0.424	0.577
Calcium	6 (24)	321	52.619	0.133
Chromium <sup>c</sup>	7 (8)	0.04	0.012	—
Copper	8 (29)	1.63	0.949	0.425
Iron	8 (28)	7.44	1.629	0.008
Magnesium	8 (30)	320	46.901	0.181
Manganese	8 (29)	3.11	2.378	0.679
Molybdenum <sup>d</sup>	5 (18)	0.14	0.040	0.052
Nickel	8 (29)	0.66	0.180	0.987
Phosphorus	8 (30)	7.72	0.546	<0.001
Potassium	3 (8)	2333	71.66	0.615
Zinc	8 (30)	5.15	3.390	0.460

<sup>a</sup>The mean and SD are expressed in percentage (as is). The probability of there being no differences between years on that measure is shown in the column labeled "Prob." Not all elements were measured in all years.

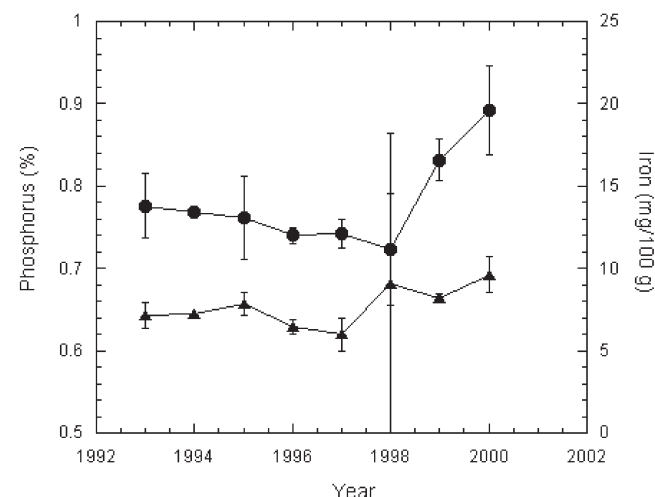
<sup>b</sup>Eight of 29 samples analyzed had results greater than the detection limit. No ANOVA was calculated.

<sup>c</sup>Twenty-two of 24 samples analyzed had results greater than the detection limit.

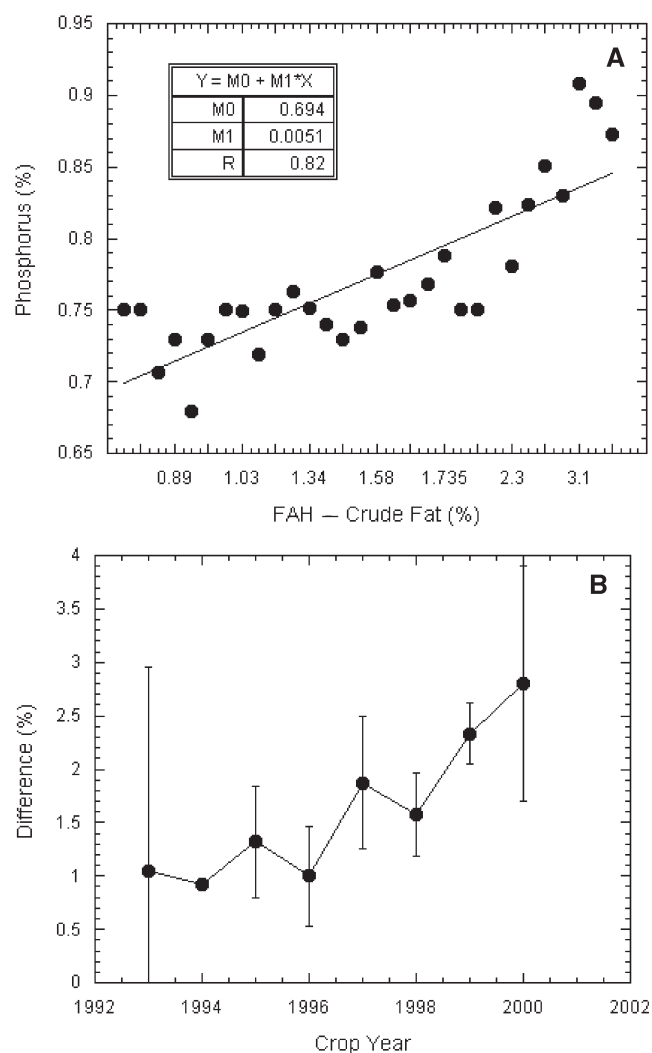
<sup>d</sup>Eighteen of 24 samples analyzed had results greater than the detection limit.

phorus in many seeds. Although easily hydrolyzed by the plant during germination, phytic acid is poorly hydrolyzed by many species. This would not be much of a direct problem in fermentation, but phytic acid is a very strong chelator of metal ions. Iron and zinc can be particularly strongly affected. Twenty soy flour samples from the 1996–1999 crops were analyzed for phytic acid. The mean concentration was found to be  $1.78 \pm 0.090\%$  (as is). The phytic acid concentration did not vary significantly (probability = 0.128) from year to year over this time period nor was it correlated with the phosphorus concentration ( $P = 0.898$ ).

In contrast, phosphorus concentration in the flour was strongly correlated ( $P < 0.001$ ) with the difference between



**FIG. 5.** The mean phosphorus (●) and iron (▲) concentrations in soy flour collected at Cedar Rapids, Iowa, processed from crops grown between 1993 and 2000. Error bars indicate the 95% confidence interval around the mean. Only a single sample was analyzed in 1994.



**FIG. 6.** Estimation of phospholipid content. The top panel (A) shows the comparison of phosphorus concentration (% as is) in soy flour with the calculated difference across the entire data set. The line is the calculated regression line for this comparison and is significantly different from zero ( $P < 0.001$ ). The bottom panel (B) shows the change in the mean difference computed by subtracting crude fat from fat by acid hydrolysis (FAH) from year to year. Only a single sample was analyzed in 1994.

the FAH and crude fat analyses. This relationship is shown in Figure 6A. Crude fat minus FAH was used as a proxy for phospholipids based on the observed marginal solubility of phospholipids in hexane. The correlation observed here ( $r = 0.827$ ) suggests that this proxy is a reasonable one. Figure 6B shows that the amount of phospholipids in the flour was apparently increasing over the study period.

Iron concentration also varied significantly over the study period from a low of 5.98 mg/100 g in 1997 to a high of 9.6 mg/100 g in 2000 (Fig. 5). The iron concentration was significantly correlated to the FAH ( $P < 0.001$ ) and phosphorus concentration ( $P = 0.008$ ), suggesting that this iron is the counterion to the phospholipids present.

The results reported here show that many components of soy flour are at relatively constant concentrations. Although

the protein concentration is measurably different from year to year, for example, the range of concentration is not high. In contrast, Fe and P levels showed significant changes in both statistical and absolute terms. Variation in the concentration of most of the variable components appeared to be random in nature, probably due to weather variation during cultivation. In contrast, the difference between fat by acid hydrolysis and crude fat analyses increased during the study period, a difference that may be due to changing phospholipid concentrations. Although the practical significance of the change in apparent phospholipids is unclear, over the time period studied this was the single most striking trend.

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