Semiautomated Determination of Phytate in Sorghum and Sorghum Products

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ABSTRACT

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Thirty sorghum varieties were analyzed for phytate phosphorus (phytate-P) using a semiautomated method. Phytic acid was isolated by extraction with dilute $HCl-Na_2SO_4$ and precipitated as Fe(III)-phytate through addition of $FeCl_3$. Following precipitation, Fe(III)-phytate was digested utilizing the micro-Kjeldahl procedure to release phytate-P. Phytate-P was determined colorimetrically using a modified Technicon method. Whole sorghums analyzed contained phytate-P levels of 0.17–0.38% (dry wt), accounting for 80–87% of the total phosphorus in the kernel. These levels were comparable to those in other cereals. Highest levels of phytate-P were found in the bran fraction, with lesser amounts in the whole grain and dehulled grain, respectively. The method was employed to quantify phytate-P in wheat, corn, and oilseeds (cottonseed and soybean flours). Processing effectively concentrated phytate-P levels in both sorghum and corn tortillas, whereas total phosphorus levels (total-P) remained unchanged. Tô, a thick African porridge prepared under acid conditions, had slightly less phytate-P than its parent flour. Overall error for the method was less than 5%.

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth largest cereal food crop in the world and is grown throughout Asia, Africa, India, and North America (Deyoe and Robinson 1979). In sorghum, as in other cereals and oilseeds, phytic acid is the major storage form of phosphorus (de Boland et al 1975, McCance and Widdowson 1935). Current interest in phytic acid is due to recent nutritional studies, which have shown phytic acid to chelate di and trivalent cations, particularly Fe, Ca, Zn, and Mg, rendering them unavailable for use by the body (Davies and Nightingale 1975, O'Dell 1969, Radhakrishnan and Sivaprasad 1980). Phytic acid also binds strongly with proteins at pHs below their isoelectric points (Cosgrove 1966). Thus, the presence of phytic acid is considered detrimental to the nutritional quality of the grain.

Phytic acid analysis in cereals was previously analyzed through quantification of phosphorus or iron in the Fe(III)-phytate precipitate isolated from the grain through precipitation and extraction techniques (Early 1944, Makower 1970, O'Dell et al 1972, Tangkongchitr et al 1981). Phytic acid levels were also determined indirectly through measurement of residual iron left in solution after the precipitation of Fe(III)-phytate (Wheeler and Ferrel 1971, Young 1936). Although results were generally accurate, the procedure was tedious and time-consuming and often resulted in erroneously high phytic acid levels for some samples (Samotus and Schwimmer 1962).

The purpose of this study was threefold: to develop an optimum sample preparation technique for the isolation of phytate-P from other phosphorus-containing compounds in sorghum; to adapt existing methods for use in a semiautomated system; and to compare phytate-P and total-P levels in sorghum varieties, sorghum-milling fractions, and sorghum foods. The ability to assay for the location and levels of phytate-P in sorghums with diverse kernel characteristics and the ability to trace the fate of phytic acid during processing may aid in selecting cultivars with improved nutritional quality for food.

MATERIALS AND METHODS

Samples

A white sorghum cultivar, CS 3541 (no testa), was used in preliminary studies designed to optimize the method. This cultivar was grown in 1980 at Halfway, TX.

Samples chosen for phytate-P and total phosphorus (total-P) analysis included the following.

Sorghum Cultivars Grown at ICRISAT in Patancheru, near Hyderabad, India. Twenty-four varieties were represented by 16 white cultivars (no testa), three white (with testa), two brown (with testa), two lemon yellow (no testa), and two red (no testa) were grown during 1979. Four white cultivars, one lemon yellow, and one yellow endosperm (all lacking a testa layer) were grown during 1980. The 1980 samples were milled in India using traditional methods of tempering and decortication with a carborundum mortar and a wooden pestle.

Ground Whole Wheat Samples. These included a commercially milled whole wheat flour and a freeze-dried, unfermented dough made from the same whole wheat flour and yeast (2%).

Processed Sorghum Products (tô and tortillas). Tô, a thick, African porridge product, was prepared from dehulled CS 3541 grain (grown at Halfway, TX in 1980) according to the laboratory procedure of Johnson (1981). Three preparation methods, acid, neutral, and alkali, produced tô with pHs of 5.0, 6.6, and 8.9, respectively. Fresh tô samples were freeze-dried, ground, and analyzed.

Tortillas were prepared according to the laboratory procedure of Bedolla et al (1981) from CS 3541 (grown at Halfway, TX in 1980) and a white food grade corn hybrid. Samples were collected at major steps in the tortilla-making process (*nixtamal, masa,* and tortilla) and then dried to 10% moisture.

Defatted Cottonseed and Soybean Flours. These samples were obtained from the Food Protein Research and Development Center, Texas A&M University, College Station, TX.

All samples not obtained in a flour form for analysis were ground in a Udy Cyclone Mill (Udy Corp., Boulder, CO) through a 1-mm screen mesh. Ground samples were stored at −4°C until analyzed.

Standards

A purified calcium phytate standard (98.6% phytate phosphorus) supplied by A. E. Staley Co., derived from corn through extraction, precipitation, and recrystallization, was used as an internal standard for all studies.

Phosphorus Analysis

All phosphorus determinations were performed on a Technicon Auto-Analyzer II System (Technicon Industries, Tarrytown, NY 19591) using a technique modified from Technicon method 334-74 W/B[+]. Reagent and sample flow-through system is depicted in Fig. 1. A blue complex is formed by the reaction of orthophosphate, molybdate, and antimony ion, which is reduced by ascorbic acid at an acid pH. The phospho-molybdenum complex is quantitated by absorption at 660 nm. Analytical results were reported by the Auto-Analyzer IIC Data Handler in parts per million phosphorus. A sample-to-wash ratio of 6:1 enabled 50 phosphorus determinations per hour.

Total-P Sample Preparation

Figure 2 outlines the sample preparation procedure for total-P. Samples were placed on a digestion block at room temperature and heated to 400°C over a 45-min period. Samples were digested an

additional 45 min at 400° C. Due to large sample size, digestion on a preheated block resulted in excessive foaming, rendering a nonquantitative digestion.

Phytate-P Sample Preparation

The sample preparation procedure for phytate-P outlined in Fig. 3 is an adaptation of the methods of Early (1944) and Ellis et al (1977). The resulting Fe(III)-phytate precipitate was dissolved in 7.0 ml of digestion mix and poured into a volumetric Kjeldahl tube for digestion. Five milliliters of deionized H_2O (used to wash each centrifuge tube) was also added to the Kjeldahl tube. Then, the samples were digested in the same manner employed for total-P (Fig. 2). Again, samples could not be digested on a preheated block due to the violent reaction resulting from rapid vaporization of the H_2O added to the sample tubes.

RESULTS

Sample Preparation

Phytic acid levels have been studied extensively in cereals such as wheat (Wheeler and Ferrel 1971), triticale (Singh and Reddy 1977), rice, corn (O'Dell et al 1972), and oats (Miller et al 1980). In contrast, comparatively little work has been done relating to the measurement of phytic acid in sorghum. Therefore, researchers were uncertain whether existing methods of phytic acid analysis could be applied to sorghum. Extraction media of 3% trichloroacetic acid (TCA, Wheeler and Ferrel 1971), 3% HCl (Pons et al 1953), and 10% Na_2SO_4 in 1.2% HCl (Early 1944) were compared to determine optimum extraction time and extraction medium.

Analysis of the supernatant recovered after Fe(III)-phytate precipitation showed significant phosphorus levels. This suggested that phosphorus in a non Fe(III)-phytate (nonprecipitatible) form was present and could be trapped in or adhere to the Fe(III)-phytate precipitate. To determine if this phenomenon affected phytate-P as analyzed, samples were analyzed with and without washing the Fe(III)-phytate precipitate. Significant differences ($\alpha = 0.05$) were observed between the two treatments, and washing of the ferric phytate precipitate was incorporated into the sample preparation scheme (Fig. 3). The sample preparation scheme for phytate-P depicted in Fig. 3 gave the most accurate and repeatable results for sorghum.

Automated Phosphorus Analysis

A 50-ppm stock phosphorus standard was prepared to approximate the acidity present after a digested sample was diluted to volume. No color development occurred when this standard was analyzed using the system and reagents as specified in the Technicon method. To optimize the system, color reagent (molybdate/antimony, ascorbic acid) concentrations were doubled. Triton X-100, a nonionic wetting agent known to precipitate the phosphomolybdate complex (McLaughlin and Meerovitch 1976, Roufogalis 1971), was removed from all reagents requiring it and was replaced with Levor IV, an ionic wetting agent, at low levels (four drops per liter). Daily cleaning of the system by sequentially pumping 10% NaOH, 10% H_2O_2, and deionized H_2O through the color reagent lines reduced system stabilization time to 15 min. After proper adjustment of these features, average recovery of standards prepared by dilution of the 50-ppm stock standard was $98.0 \pm 1.4\%$.

At $P = 0.05$, the method was not subject to day effects when either dehulled CS 3541 flour or calcium phytate was analyzed. Coefficients of variation were less than 0.05 for both samples (Table I). Recoveries of standard additions accomplished by adding calcium phytate to dehulled CS 3541 flour are reported in Table II. Recoveries were excellent. Slight elevations in P levels on days 2 and 3 may have been caused by residual phosphates from dish soap left in glassware or inadequate washing of the Fe(III)-phytate precipitate.

Sorghum Phytate-P

Phytate-P levels in 30 whole sorghum samples ranged from 1.72 to 4.07 mg/g (dry wt) with IS 5758 containing the greatest amount and M35-1 the least (Table III, Fig. 4). The overall mean for phytate and total-P in 30 whole sorghums was 3.2 ± 0.47 and 3.94 ± 0.55 mg/g (dry wt), respectively. These levels are typical for
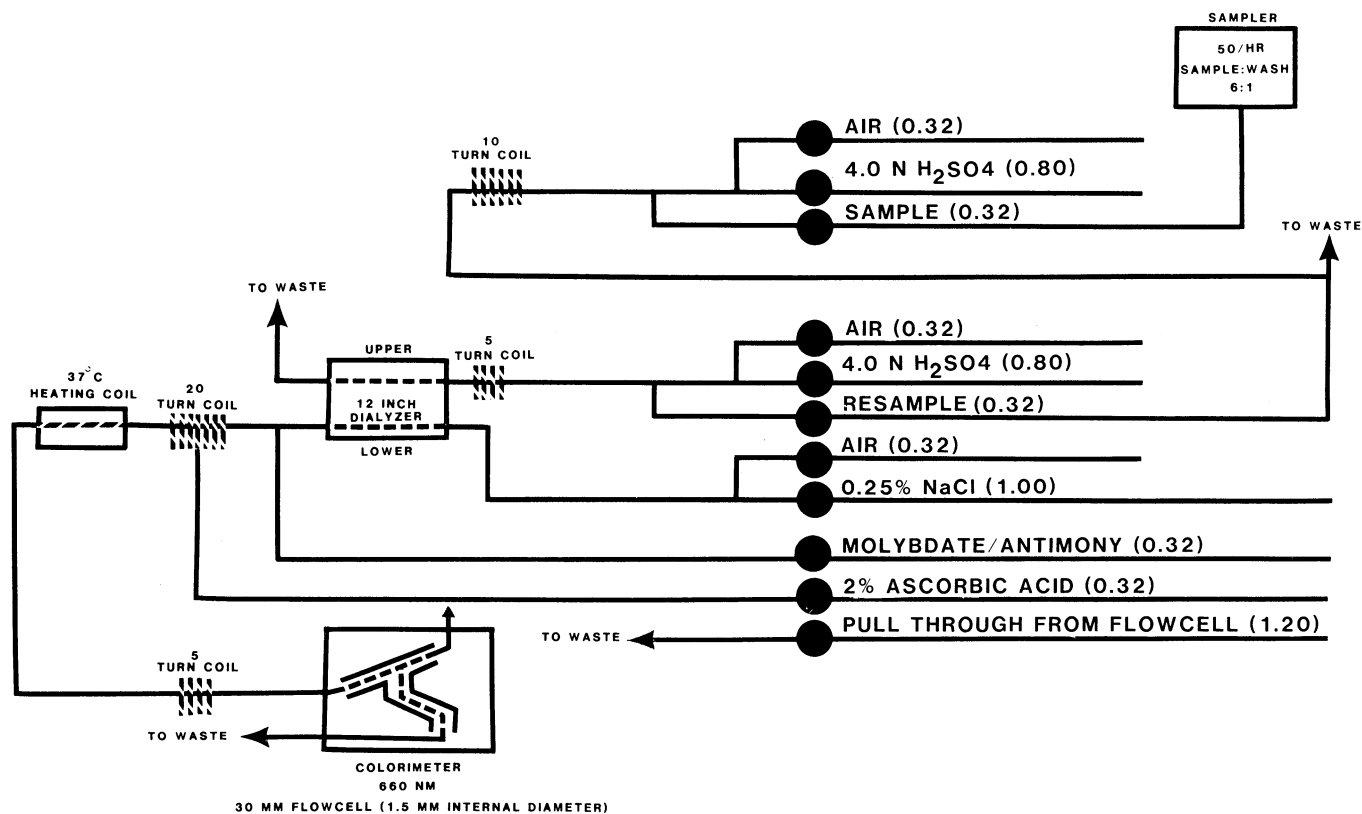

Fig. 1. Automated reagent and flow-through system for phosphorus determination. Figures in parentheses signify flow rates in milliliters per minute.

sorghum (Hulse et al 1980). Levels of 1.6–4.3 mg/g (dry wt) were reported for whole grain varieties (Wang et al 1959). Phytate-P levels in sorghum are no higher than those in wheat (3.35 mg/g, dry wt), barley (3.04 mg/g, dry wt), and corn (2.80 mg/g dry wt) but are significantly lower than soybean and other oilseeds (de Boland et al 1975, Lolas et al 1976).

Forty to fifty percent of both phytate and total-P can be removed by dehulling (Fig. 4). Phytate-P constituted 82–91, 56–84, and 85–95% of the total-P in whole grain, dehulled grain, and bran, respectively (Table IV). Differences between phytate-P levels in these fractions were dependent upon varietal effects, extent of milling, and initial level of total-P in the grain. Duncan's multiple range test showed statistical differences ($P = 0.05$) in phytate and total-P levels between cultivars and their subsequent milling fractions. Phytate-P content in the fractions obtained through traditional milling methods was greatest in bran, less in whole grain, and least in dehulled grain (Fig. 4). This suggests that the bran-aleurone area is a significant reservoir of phytate and total-P in sorghum.

In a similar study with bran and germ fractions obtained from a commercial dry-milling procedure, highest levels of phytate-P were found in the germ followed by the bran. The grits (endosperm) contained the least (Wang et al 1959). The ease and completeness of milling plays a major role in the level of phytate-P in the grain fraction. For example, the soft floury endosperm of IS 7788 was rapidly broken down into various kernel fractions, allowing only a small amount of pericarp to be removed. This resulted in an increase in the percent phytate-P of total-P in the dehulled grain as
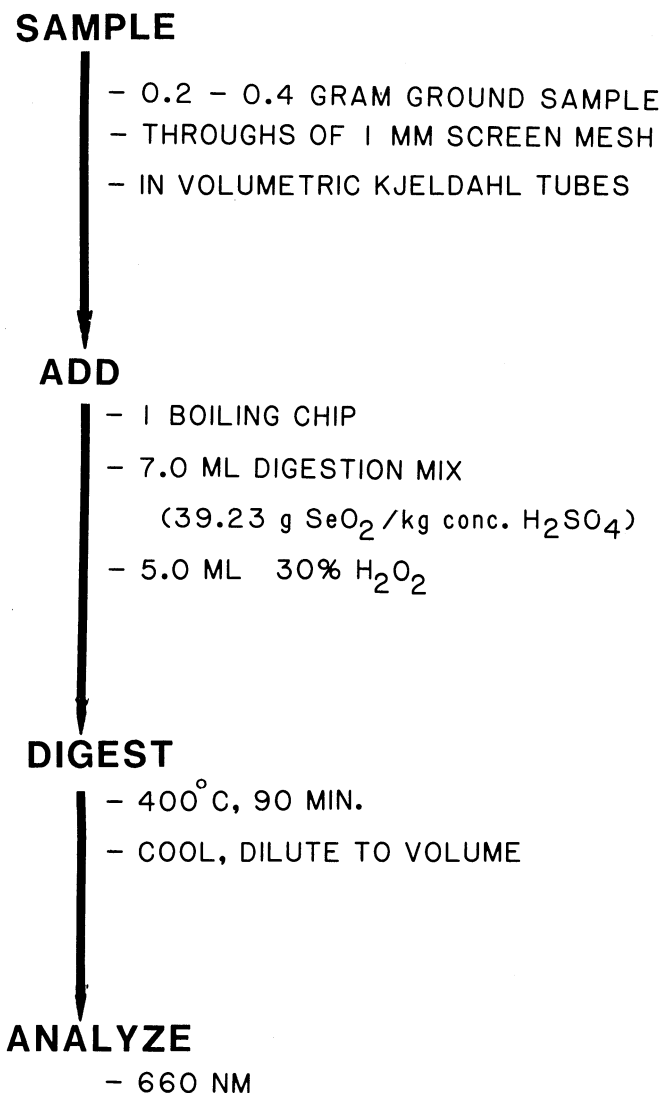
compared to the whole grain (Table IV). Because the pericarp contains little phytate-P, less pericarp and more aleurone tissue in the dehulled grain explains a consistant or slightly higher percentage of phytate-P of total-P observed in the dehulled grain. In general, bran fractions obtained by traditional manual milling techniques have a greater percentage of germ. Commercial dry-milling techniques would yield a more "pure" bran fraction. Differences in the content of the milling fractions obtained for this study would account for bran fractions containing 30–200% higher phytate-P levels than those reported by Wang et al 1959.

A linear relationship was established between phytate-P and total-P ($r = 0.995$, c.v. $= 6.30$, d.f. $= 81$) when data from all sorghum samples analyzed was compiled. A linear equation ($y = 0.943$
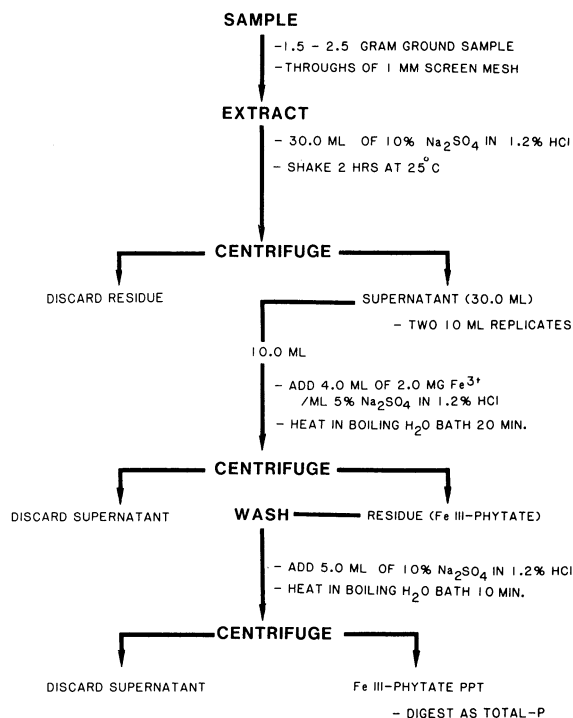
SAMPLE

- 0.2 – 0.4 GRAM GROUND SAMPLE
- THROUGHS OF I MM SCREEN MESH
- IN VOLUMETRIC KJELDAHL TUBES

ADD

- I BOILING CHIP
- 7.0 ML DIGESTION MIX
  (39.23 g SeO_2/kg conc. H_2SO_4)
- 5.0 ML 30% H_2O_2

DIGEST

- 400°C, 90 MIN.
- COOL, DILUTE TO VOLUME

ANALYZE

- 660 NM

Fig. 2. Sample preparation scheme for total-P.

Fig. 3. Sample preparation scheme for phytate-P.

TABLE I
Analysis of Variance for Phytate-P Levels
in Calcium Phytate and Dehulled CS 3541

	Calcium Phytate[a]		CS	3541[b]
Source of Variation	d.f.	ss	d.f.	ss
Day	3	73.64481667	4	0.09554792
Observation	5	163.1443000	5	0.11869137
Error	6	140.4001750	11	0.07898188
Total	14	332.8085733	20	0.28524581

[a]$\overline{x}$ = 148.54 Mg of phosphorus per gram. Standard deviation = 4.84.
[b]$\overline{x}$ = 2.49 Mg of phosphorus per gram (dry wt). Standard deviation = 0.08, coefficient of variation = 3.41, $\alpha = 0.05$.

TABLE II
Average Recoveries (%) for Standard Additions of Calcium Phytate
to Dehulled CS 3541 Flour on Four Different Days

Day	$\overline{x}$	Standard Deviation	Observations per Day
1	101.2	4.7	11
2	103.4	2.0	12
3	103.7	2.5	12
4	101.3	1.5	3

x −0.465) was calculated, suggesting the possibility of predicting phytate-P (y) from total-P (x) levels. This has, in fact, been done for wheat, oats, barley, and soybeans (Lolas et al 1976). Linear regression analysis also suggests that increasing the level of total-P in sorghum does not significantly increase other phosphorus sources in the kernel (eg, phospholipids, nucleic acids, etc). The greater the level of total-P, the larger the percentage is in the phytate form. Similar results were shown in oats (Miller et al 1980).

Processed Products

Tortillas. Sorghum and white corn tortillas were prepared on two different days. No statistical differences ($P = 0.05$) in total-P were observed between production days or tortilla production steps for either grain. The overall mean for total-P in whole grain and products derived from it was 4.41 ± 0.33 mg/g, dry wt (c.v. = 7.49) and 3.24 ± 0.24 mg/g, dry wt (c.v. = 7.50) for sorghum and white corn, respectively. Phytate-P levels in corn were comparable to those reported by O'Dell et al (1972). Statistical differences ($P = 0.05$) in phytate-P levels between days and processing steps were observed in both grains (Table V). In corn and sorghum, mean phytate-P levels in the tortilla were statistically higher than those in the whole grain, suggesting that the processing involved in tortilla production effectively concentrates phytate-P. This is reasonable because nonphytate phosphorus-containing components (starches, sugars, protein) as well as structural components of the kernel (germ, pericarp) are lost during the cooking/steeping process. Unavoidable differences in solubles lost in this step accounted for observed day effects. Analysis of the suspended solids obtained from the cooking/steeping H_2O showed phytate and total-P levels of 3.29 ± 0.5 and 3.69 ± 0.08 mg/g (dry wt), respectively for sorghum and 0.87 ± 0.07 and 3.46 ± 0.09 mg/g (dry wt), respectively for corn. Only trace amounts of phosphorus (<5 ppm) could be detected in the cooking/steeping water for both grains.

Tortillas were prepared from whole grain that was boiled and steeped in an alkaline solution with a high Ca^{++} concentration. Metal chelates of phytic acid are generally insoluble at an alkaline pH (Cheryan 1980), accounting for low levels of phosphorus in the cooking/steeping H_2O. Relatively equal amounts of total-P were lost from corn and sorghum in the cooking/steeping process. However, three times more phytate-P was lost from the kernel in the steeping and cooking water solids for sorghum. Thus, differences in the relative amounts and types of structural components of the kernel lost would account for higher phytate-P levels in the solids obtained from the cooking/steeping process in sorghum tortilla production.

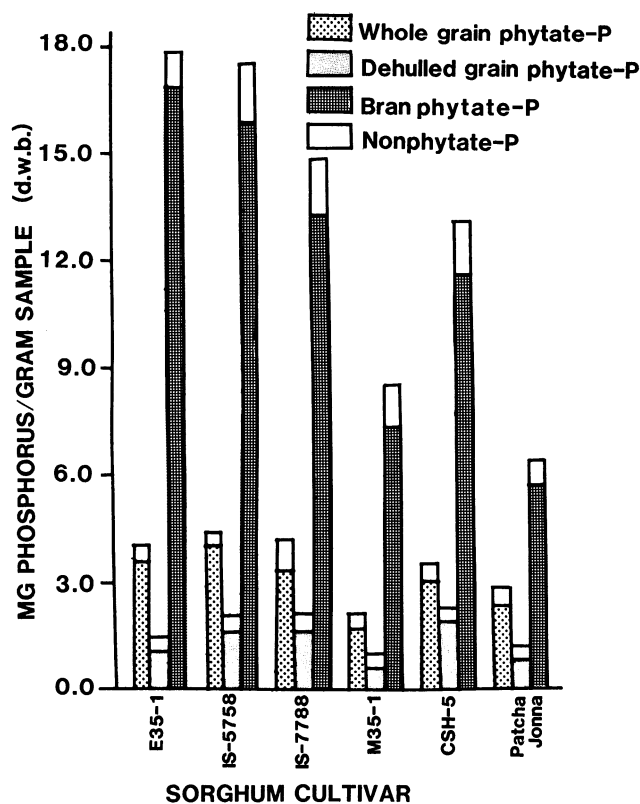Reasons for a gradual increase in phytate-P from *nixtamal* to

Fig. 4. Effects of hand milling upon phytate and total-P levels in six sorghum cultivars grown in India during 1980.

TABLE III
Total-P and Phytate-P Levels in 24 Whole Grain Indian Samples Grown in 1979

Variety	Total-P (mg/g sample[a])		Phytate-P		Percent Phytate-P of Total-P	Endosperm Type	Testa	Pericarp Color
	$\bar{x}$	Standard Deviation	$\bar{x}$	Standard Deviation				
P-721	4.49	0.10	3.52	0.03	78.4	Nonwaxy	None	White
Mothi	4.21	···	3.12	0.13	74.1	Nonwaxy	None	White
Patcha Jonna	4.34	···	3.42	0.02	78.4	Nonwaxy	None	Lemon Yellow
Dobbs	4.33	0.09	3.31	0.07	76.4	Nonwaxy	Present	Brown
E35-1	3.92	0.06	3.04	0.13	77.6	Nonwaxy	None	White
M-50009	3.62	0.07	3.07	3.09	84.8	Nonwaxy	None	White
M-50013	4.32	0.04	3.17	0.05	73.4	Nonwaxy	None	White
M35-1	3.88	0.12	3.36	0.04	86.6	Nonwaxy	None	White
IS-2317	4.01	0.11	3.26	0.13	81.3	Nonwaxy	Present	White
IS-8743	4.39	0.18	3.53	0.09	80.4	Nonwaxy	None	Red
CSH-5	3.34	0.11	2.57	0.02	76.9	Nonwaxy	None	White
Swarna	3.58	0.08	2.85	0.02	79.6	Nonwaxy	None	White
Segaolane	4.28	0.09	3.48	0.13	81.3	Nonwaxy	None	White
S-13	4.54	0.21	3.77	0.05	83.0	Nonwaxy	None	White
CO4	3.79	0.02	3.00	0.04	79.1	Nonwaxy	None	Red
WS-1297	3.87	0.13	3.28	0.09	84.8	Nonwaxy	Present	White
IS-158 OP	3.59	0.14	3.25	0.05	90.5	Waxy	None	White
M-35052	3.20	0.17	2.62	0.04	81.9	Nonwaxy	None	White
IS-7055	4.50	0.11	3.80	0.02	84.8	Nonwaxy	Present	Brown
S-29	4.56	0.10	3.57	0.04	78.3	Nonwaxy	None	White
M-50297	4.07	0.03	3.54	0.07	87.0	Nonwaxy	None	White
CS 3541	4.25	0.06	3.34	0.01	78.6	Nonwaxy	None	White
IS-9985	3.93	0.01	3.32	0.11	84.5	Nonwaxy	None	Red
IS-7035	3.71	0.01	3.03	0.06	81.7	Nonwaxy	Present	White

[a] Dry weight basis.

TABLE IV
Percent Phytate Phosphorus of Total Phosphorus in Sorghum Cultivars Grown in India During 1980

Sample	Whole Grain	Dehulled Grain	Bran
		Fraction	
E35-1	88.7	73.6	94.6
IS-5758	90.9	76.2	90.3
IS-7788	79.4	79.7	89.1
M35-1	80.5	56.9	85.6
CSH-5	88.4	83.9	88.9
Patcha Jonna	82.0	62.4	87.7

TABLE V
Mean Phytate-P Levels During Corn and Sorghum Tortilla Production

	Sorghum[a,b]	Corn[b]
Whole grain	3.34 a	2.27 b
Nixtamal	3.66 c	2.76 a
Masa	3.65 c	2.87 d
Tortilla	3.78 d	3.02 c
$\bar{x}$	3.69	2.81
Coefficient of variation	4.35	5.38
Standard deviation	0.16	0.15

[a] Numbers with the same superscript letter within a grain are not statistically different at $P = 0.05$.
[b] Milligrams per gram of sample, dry wt basis.

tortilla in corn tortilla production are unclear (Table V). The formation of phosphorylated inositols that precipitate upon addition of Fe^{3+} appears to be occurring. A lower nonphytate phosphorus level logically accompanied this formation. These same general trends appear in sorghum tortilla production (Table V); however, statistical differences ($P = 0.05$) were not observed between *nixtamal* and *masa*. A larger number of samples prepared over a longer period of time need to be analyzed to determine if these general trends exist.

Thick Porridges

Tô was prepared from a white sorghum, CS 3541. No statistical differences ($P = 0.05$) were observed in total or phytate-P levels between the dehulled grain or *tô* produced at any pH level. However, phytate-P levels in acid *tô* were slightly lower compared to levels found in the dehulled grain and *tô* produced under neutral and base conditions. In a study designed to evaluate the nutritional value of *tô* (Johnson 1981), a significant ($P = 0.05$) decrease in phytate-P was observed for *tô* prepared from a yellow sorghum (TAM 680), a brown sorghum (NK 300), and a Malian *tô* under acid conditions. Decreases in phytate-P levels were accompanied by increases in nonphytate-P, as in other studies (de Rham and Jost 1979). This seems reasonable because phytic acid has a slight tendency to hydrolyze under acid conditions (Maddaiah et al 1964).

The overall means for phytate and total-P in *tô* derived from CS 3541 were 2.50 ± 0.04 and 2.86 ± 0.07 mg/g (dry wt), respectively. In general, phytate-P levels in the whole grain are higher than the flour or *tô* produced from them. This can be attributed to the dehulling of the grain in preparation of the flour for *tô*.

Other Cereals and Oilseeds

Whole wheat samples were analyzed to compare results of the present method with recent data from another laboratory (Tangkongchitr et al 1981). Prior analysis of these samples resulted in phytate-P levels of 3.70 and 3.30 mg/g sample (as-is basis) for whole wheat and a yeasted whole wheat dough, respectively. Results from the method described here were 2.94 ± 0.15 and 3.18 ± 0.21 mg/g sample (as-is basis) for whole wheat and the yeasted dough, respectively. Both samples would be expected to show lower phytate-P levels, as analyzed by this method. Washing of the ferric-phytate residue to remove trapped nonphytate-P was omitted in the previous analysis. Also, the original analysis of the whole wheat sample (phytate-P = 3.7 mg/g) was completed 13 months before analysis in this laboratory. Wheat is known to contain phytases capable of hydrolyzing phytate-P (Lim and Tate 1973, Ranhotra 1974). Phytase activity and washing of the Fe(III)-phytate precipitate accounts for lower levels of phytate-P reported by this method.

Phytate-P levels in defatted soy flour and glandless cottonseed flour were 6.7 ± 0.06 and 13.14 ± 0.25 mg/g (as-is basis), respectively. These levels are comparable to results reported by others (Cheryan 1980, de Rham and Jost 1979, Wozenski and Woodburn 1975) and suggest that the method's applicability extends to oilseed flours.

CONCLUSIONS

The described method for phytate and total-P determinations was accurate, repeatable, and applicable to several cereals and

oilseeds. Coefficients of variation were 0.05 or less for all samples analyzed. Conditions in this work allowed analysis of 40 samples (three replications per sample) plus standards in three working days. Limitations of the method were the availability of a cool digestion block and the number of samples that could be centrifuged at one time. After digestion, samples could be stored indefinitely at 4° C before phosphorus analysis. Quantification of the phosphorus component of the Fe(III)-phytate precipitate accounted for phosphorus in the phytate form as well as that present in lower phosphorylated inositols, which would also precipitate upon addition of Fe^{3+}. The presence or absence of these lower phosphorylated inositols in sorghum as well as their nutritional significance is not known.

Sixty to ninety percent of the phosphorus in all the samples analyzed was in the phytate form. Phytate-P levels in sorghum were comparable to those found in other cereals. In sorghum, the bran fraction contained the highest levels of phytate-P (5.67–16.90 mg/g, dry wt) and dehulled grain the lowest (0.56–1.92 mg/g, dry wt).

Varietal effects appeared to be the most critical factor in selecting a sorghum variety for human consumption that would contain optimum available phosphorus. Extent of milling and type of processing would be of lesser concern because they do not selectively lower the nondigestible phytate-P levels. The strong linear relationship between phytate and total-P levels makes it unlikely that any attempt to increase nonphytate phosphorus by increasing total-P levels would meet with success.

Phytate-P was effectively concentrated through loss of kernel components in tortilla production. Comparison of sorghum tortillas to those made from corn showed that sorghum tortillas in this study had three times more nonphytate-P available (0.63 mg/g, dry wt) than did corn tortillas (0.22 mg/g, dry wt). Alkaline conditions did not affect the breakdown of phytates in tortillas or *tô*. However, acid conditions involved in the *tô*-making process were conducive to the hydrolysis of phytates.

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