

# Stability of Phytate in Barley and Beans during Storage

Irene Ockenden,<sup>\*,†</sup> Duane E. Falk,<sup>‡</sup> and John N. A. Lott<sup>†</sup>

Biology Department, McMaster University, Hamilton, Ontario L8S 4K1, Canada, and  
Crop Science Department, University of Guelph, Guelph, Ontario N1G 2W1, Canada

The purpose of this study was to measure the stability of phytate in barley grains (*Hordeum vulgare*) and beans (*Phaseolus vulgaris*) during storage. Grains of four barley cultivars stored for 8–10 years varied in their phytate content and were either the same as the current value or lower by up to 17.7%. With accelerated dry aging of barley at 41 °C for 3 months there was less than 2% decrease in phytate and a slight drop in percent germination. When aged at 41 °C and 75% relative humidity (RH), phytate levels decreased 5 to 10%, depending on the cultivar, and no kernels germinated. Beans stored dry at room temperature and ambient humidity for 14 months had no decrease in phytate, but phytate levels in dry beans stored for 4 months at 41 °C dropped by 23%. At 41 °C and 75% RH the levels of phytate in beans dropped by 27%. Phytate was more stable in barley kernels than in beans.

**Keywords:** *Phytate; storage; aging; Hordeum vulgare; Phaseolus vulgaris*

## INTRODUCTION

Phytic acid or *myo*-inositol 1,2,3,4,5,6-hexakis[dihydrogen phosphate] (IUPAC-IUB, 1968) is an important component of all seeds and provides mineral nutrients during germination and seedling growth (Lott and Ockenden, 1986). Phytic acid is a chelating agent and has been called an antinutrient in food because it can decrease the bioavailability of essential elements such as calcium, iron, magnesium, and zinc (Morris, 1986). Considerable research has been aimed at decreasing the amount of residual phytate in foods prepared from grains and legumes to counteract the antinutrient effects (Fretzdorff and Brümmer, 1992). There is less information, however, on the stability of phytate within intact stored seeds. Most studies on storage of crop seeds deal mainly with losses in viability and increases in pathogen damage during long-term storage.

Glass and Geddes (1959) studied wheat stored under high temperature and moisture and found that the inorganic phosphorus level increased in the seeds with increased storage time. They concluded that phytate was being degraded by the seed phytase. Although phytase is not believed to be active in dry seeds (Maga, 1982), the possibility that it might function in seeds stored at high moisture and temperature has not been ruled out.

Many studies on beans in relation to phytate deal with the effect of phytate on bean hardening and subsequent prolongation of cooking times. Several of these studies have also indicated that phytic acid content of beans decreases during storage, with some varieties of beans showing very marked drops in phytate contents compared to other varieties (Hernandez-Unzon and Ortega-Delgado, 1989). Bernal-Lugo et al. (1990) studied two varieties of common beans stored at 41 °C and 75% relative humidity (RH). They found that one variety lost 20.7% and the other 66.1% of the phytate

during 33 days of storage. Unfortunately, there were no data given for storage at room temperature.

Such rapid losses of phytate from intact seeds are disturbing to those studying mineral storage in seeds. If some seeds can lose considerable amounts of their phytate in less than a year, comparisons of phytate levels in such seeds are invalid unless the age and storage conditions are also compared. In the warmer regions of the world, harvested seeds can be exposed to temperatures and humidities that could be classified as adverse storage conditions. Such seeds might suffer a reduction in phytate long before they reached the laboratories where their mineral composition was to be studied.

We have studied the storage stability of phytate in two species, the common bean (*Phaseolus vulgaris* cv. Gryphon) and four cultivars of winter barley (*Hordeum vulgare*). Winter barley and beans were stored at ambient temperature and humidity in the laboratory and in some cases also stored frozen. In addition, some of the samples were stored at elevated temperature and humidity.

## MATERIALS AND METHODS

**Source, Preparation, and Storage of Beans and Barley.** Four cultivars of winter barley (*Hordeum vulgare*), namely, Huron, OAC Elmira, OAC Halton, and OAC Acton, were grown in field plots of the University of Guelph. The site was fertilized yearly with 75 kg/hectare of nitrogen (34-0-0). No phosphorus was added, since soil testing showed that phosphorus was present in high to very high range. Rainfall and temperature were monitored at the site. The mature seeds were dried at 35 °C for 3 days and stored in cloth or paper bags at room temperature and humidity. One hundred grams of hulled barley kernels were ground roughly in a blender and then ground in an UDY Cyclone sample mill (Udy Analyzer Co., Boulder, CO). The flours passed through a 30 mesh (600  $\mu$ m) screen. Grains from 1984 to 1993 harvests were ground in 1993 and stored frozen (–23 °C) in polyethylene bags. Grains from 1994 harvest were ground shortly after harvest.

Beans (*Phaseolus vulgaris* cv. Gryphon) were grown in field plots of the University of Guelph in 1994. The harvested beans were stored at four conditions: whole, at room temperature; whole, frozen (–23 °C); ground, at room temperature; ground, frozen. The beans were ground using a Moulinex coffee

\* Author to whom correspondence should be addressed (e-mail ockenden@mcmaster.cis.mcmaster.ca; fax (905) 522–6066).

<sup>†</sup> McMaster University.

<sup>‡</sup> University of Guelph.

**Table 1. Mean Weight ( $\pm$ SD) of Intact Kernels and Phosphorus (P) and Phytic Acid (PA) Concentrations (means  $\pm$  SD) in Ground Kernels of Four Cultivars of Winter Barley (*Hordeum vulgare* L.) Grown over a 10 Year Period**

year of growth	1984	1986	1989	1990	1991	1992	1993	1994	mean
cv. Elmira									
kernel wt (g)	0.029 $\pm$ 0.000	0.025 $\pm$ 0.000	0.025 $\pm$ 0.001	0.033 $\pm$ 0.000	0.025 $\pm$ 0.001	0.034 $\pm$ 0.001	0.025 $\pm$ 0.002	0.030 $\pm$ 0.001	0.028 $\pm$ 0.004
total P (%)	0.428 $\pm$ 0.018	0.385 $\pm$ 0.014	0.413 $\pm$ 0.017	0.430 $\pm$ 0.014	0.484 $\pm$ 0.005	0.389 $\pm$ 0.019	0.476 $\pm$ 0.011	0.416 $\pm$ 0.015	0.428 $\pm$ 0.039
PA (%)	1.037 $\pm$ 0.025 <sup>ab</sup>	0.943 $\pm$ 0.025 <sup>c</sup>	1.030 $\pm$ 0.011 <sup>a</sup>	0.973 $\pm$ 0.014 <sup>bc</sup>	1.179 $\pm$ 0.032 <sup>d</sup>	1.047 $\pm$ 0.004 <sup>a</sup>	1.200 $\pm$ 0.075 <sup>d</sup>	1.047 $\pm$ 0.007 <sup>a</sup>	1.056 $\pm$ 0.092
cv. Huron									
kernel wt (g) <i>b</i>		0.031 $\pm$ 0.001	0.030 $\pm$ 0.001	0.039 $\pm$ 0.001	0.028 $\pm$ 0.001	0.036 $\pm$ 0.001	0.032 $\pm$ 0.000	0.034 $\pm$ 0.001	0.033 $\pm$ 0.004
total P (%)		0.394 $\pm$ 0.006	0.404 $\pm$ 0.007	0.425 $\pm$ 0.012	0.479 $\pm$ 0.009	0.405 $\pm$ 0.006	0.472 $\pm$ 0.016	0.429 $\pm$ 0.019	0.430 $\pm$ 0.034
PA (%)		1.001 $\pm$ 0.039 <sup>ab</sup>	0.976 $\pm$ 0.039 <sup>b</sup>	1.079 $\pm$ 0.028 <sup>ac</sup>	1.167 $\pm$ 0.057 <sup>d</sup>	1.104 $\pm$ 0.032 <sup>cd</sup>	1.328 $\pm$ 0.087	1.122 $\pm$ 0.028 <sup>cd</sup>	1.111 $\pm$ 0.117
cv. Halton									
kernel wt (g)		0.037 $\pm$ 0.000	0.027 $\pm$ 0.000	0.036 $\pm$ 0.001	0.028 $\pm$ 0.001	0.035 $\pm$ 0.002	0.031 $\pm$ 0.001	0.033 $\pm$ 0.001	0.032 $\pm$ 0.004
total P (%)		0.353 $\pm$ 0.009	0.365 $\pm$ 0.009	0.384 $\pm$ 0.013	0.443 $\pm$ 0.008	0.366 $\pm$ 0.010	0.396 $\pm$ 0.015	0.434 $\pm$ 0.016	0.392 $\pm$ 0.035
PA (%)		0.873 $\pm$ 0.014 <sup>abc</sup>	0.848 $\pm$ 0.043 <sup>a</sup>	0.923 $\pm$ 0.014 <sup>bc</sup>	1.136 $\pm$ 0.014	0.937 $\pm$ 0.057 <sup>c</sup>	1.030 $\pm$ 0.043 <sup>d</sup>	1.061 $\pm$ 0.014 <sup>d</sup>	0.973 $\pm$ 0.106
cv. Acton									
kernel wt (g)	0.037 $\pm$ 0.001	0.036 $\pm$ 0.001	0.028 $\pm$ 0.001	0.037 $\pm$ 0.001	0.025 $\pm$ 0.001	0.036 $\pm$ 0.001	0.034 $\pm$ 0.001	0.039 $\pm$ 0.000	0.034 $\pm$ 0.005
total P (%)	0.386 $\pm$ 0.004	0.392 $\pm$ 0.008	0.368 $\pm$ 0.004	0.406 $\pm$ 0.013	0.442 $\pm$ 0.008	0.391 $\pm$ 0.019	0.418 $\pm$ 0.004	0.427 $\pm$ 0.012	0.406 $\pm$ 0.024
PA (%)	0.966 $\pm$ 0.004 <sup>a</sup>	0.959 $\pm$ 0.028 <sup>a</sup>	0.820 $\pm$ 0.057	0.983 $\pm$ 0.039 <sup>ab</sup>	1.129 $\pm$ 0.021	0.983 $\pm$ 0.028 <sup>ab</sup>	1.051 $\pm$ 0.014 <sup>b</sup>	1.015 $\pm$ 0.028 <sup>ab</sup>	0.988 $\pm$ 0.088
mean PA (%) for 4 cvs		0.942 $\pm$ 0.054	0.919 $\pm$ 0.101	0.990 $\pm$ 0.065	1.153 $\pm$ 0.024	1.018 $\pm$ 0.073	1.177 $\pm$ 0.123	1.061 $\pm$ 0.045	

<sup>a</sup> Values within a row that are followed by the same letter are not significantly different at  $P = 0.05$ . <sup>b</sup> Cultivars Huron and Halton were not grown in 1984 and 1984 values are omitted from the analysis of the pooled means of PA.

grinder (flour passed through a 30 mesh screen) either prior to storage or at the time of analysis. The frozen beans or bean flours were stored in small lots equivalent to 100 g.

Beans and barley grains were also artificially aged by storage in a desiccator in an incubator at 41 °C. The humidity within the desiccator was maintained at 75% RH by using a saturated NaCl solution as described by Winston and Bates (1960). Samples of both barley and beans were also exposed to 41 °C in a dry state in sealed glass jars.

**Germination of Barley and Beans.** By use of germination as an indicator of seed deterioration (Delouche and Baskin, 1973), two lots of 100 aged and unaged seeds of each type were germinated in Petri dishes lined with moistened filter paper and stored in the dark at room temperature.

**Determination of Total Phosphorus Content of Barley and Beans.** Percent moisture in all the flours was determined at the time of each analysis by heating three lots of about 1 g of flour in an oven for 2 h at 130 °C (Roberts and Roberts, 1972). All results are expressed on a dry weight basis. Four lots of 1 g of barley flour in 15 mL porcelain crucibles were charred on a hot plate until smoking ceased and were placed in a muffle furnace for 4 h at 500 °C. Each crucible was treated first with 1.5 mL of 1:2 v/v HNO<sub>3</sub>/H<sub>2</sub>O (deionized) and then with 1.5 mL of 1:1 v/v HCl:H<sub>2</sub>O (Gorsuch, 1970; Ockenden and Lott, 1986). The ash residue was made to 100 mL with 1% HCl, and 1 mL was used for analysis. Since bean flour gave erratic results with dry ashing, three lots of 100 mg of bean flour were wet ashed with 3 mL of concentrated nitric acid and 0.5 mL of concentrated sulfuric acid until dense fumes filled the tubes (Ellis and Morris, 1983). About five drops of hydrogen peroxide were added, and the tubes were heated again to fuming. This addition was repeated if necessary to produce colorless solutions (Organ et al., 1988). The digests were made up to 25 mL with water, and 1 mL was used for phosphorus analysis. Total phosphorus was determined by the molybdenum blue method (AOAC, 1990).

**Extraction and Determination of Phytic Acid.** Duplicate samples of about 1 g of barley or bean flours were extracted with 50 mL of 2.4% HCl (AOAC, 1990) for 2½ h on a shaker. The extract was centrifuged (15 min at 10 000 rpm at 5 °C), filtered (Whatman no. 42) and stored at 4 °C. Econo-columns (Bio-Rad, 0.7 cm  $\times$  15 cm) were prepared with 0.5 g of resin (Bio-Rad anion exchange resin AG 1-X8 200–400 mesh, chloride form). The solutions applied to the column were derived from AOAC (1990) for phytate except that no EDTA was used and the extract was applied as 2 mL diluted to 25 mL with water. Phytate was eluted with 60 mL of 2 N HCl (Graf and Dintzis, 1982), and the column rate was set to 10 drops/min (Plaami and Kumpulainen, 1991). The HCl eluants were evaporated to 2–3 mL and digested with nitric and sulfuric acids as described for wet ashing except that no

hydrogen peroxide was used. The digests were diluted to 25 mL, and 5 mL was used for phosphorus analysis as described in the previous section. The values for phytic acid phosphorus were converted to phytic acid concentrations by multiplying by 3.55 as suggested by Raboy and Dickinson (1984). Total phosphorus in the extracts was determined by digesting the extracts and analyzing the digests for phosphorus to ensure the extractions were similar for all analyses.

**Statistics.** The results were analyzed using Minitab (Minitab Inc., State College, PA). Following analysis of variance the differences between means were determined using the Tukey test (Zar, 1984).

## RESULTS AND DISCUSSION

**Barley.** The percent of total kernel phosphorus extracted into acid was 83.87  $\pm$  3.46% ( $N = 30$ ). Cultivars Elmira, Halton, and Acton had a mean percent extraction in the 82–84% range but cv. Huron consistently had 87% extraction. Of the extracted phosphorus 83.91  $\pm$  3.19% ( $N = 30$ , mean for all extracts) was present as phytic acid. The mean percent of total seed phosphorus present as phytic acid was 70.04  $\pm$  3.48% ( $N = 30$ ).

The mean percent moisture for all the years from 1984 to 1993 was the lowest for cv. Acton at 7.86  $\pm$  0.44% ( $N = 17$ ) and the highest for cv. Elmira at 8.27  $\pm$  0.38% ( $N = 30$ ). The lowest moisture content for all four cultivars was in 1989 at 8.09  $\pm$  0.57% ( $N = 9$ ) and the highest in 1990 at 8.32  $\pm$  0.31% ( $N = 8$ ). Thus, the moisture contents were very similar year to year and cultivar to cultivar. The most recently harvested crop of 1994 had somewhat higher percent moisture with the lowest percent moisture for cv. Acton at 8.71  $\pm$  0.09% ( $N = 5$ ) the highest for cv. Elmira at 9.16  $\pm$  0.55% ( $N = 5$ ). After accelerated aging at 41 °C and 75% RH the percent moisture rose to about 12%.

Table 1 shows that there was year to year variation in kernel size and total phosphorus and phytic acid levels for each of the cultivars. Seed size was negatively but not significantly correlated with total phosphorus ( $r = -0.39$ ), while total phosphorus was positively and significantly correlated with phytate ( $r = 0.89$ ,  $P = 0.01$ ). Although there were cultivar to cultivar differences in the amount of phytate accumulated, there were much greater differences among the years of growth. The mean phytate levels over all the years for each of the

**Table 2. Percent Phytic Acid (mean  $\pm$  SD) in the 1994 Barley Kernels (*Hordeum vulgare* L.) Stored under Different Conditions**

cultivar	I <sup>a</sup>	II <sup>b</sup>	% change	III <sup>c</sup>	% change
cv. Elmira	1.090 $\pm$ 0.021	1.012 $\pm$ 0.018	-7.16	1.079 $\pm$ 0.021	-1.01
cv. Huron	1.154 $\pm$ 0.004	1.040 <sup>d</sup>	-9.88	1.132 $\pm$ 0.021	-1.19
cv. Halton	1.058 $\pm$ 0.028	0.969 $\pm$ 0.011	-8.41	1.051 $\pm$ 0.007	-0.66
cv. Acton	1.030 $\pm$ 0.032	0.987 $\pm$ 0.014	-4.17	1.051 $\pm$ 0.004	+2.04

<sup>a</sup> Control, kernels stored at ambient temperature and humidity. <sup>b</sup> Wet aged for 3 months at 41 °C and 75% RH. <sup>c</sup> Dry aged for 3 months at 41 °C. <sup>d</sup> Based on  $N = 1$ .

four cultivars were not significantly different at  $P = 0.05$ , but among the years, the crops of 1991 and 1993 had higher amounts of phytate than crops from the other years. The 1991 levels were significantly higher at  $P = 0.05$  than those of 1986 and 1989, while the 1993 levels were significantly higher ( $P = 0.05$ ) than those of 1986, 1989, and 1990.

The marked year to year variations in phytate were present despite the fact that the plants were grown in the same area and the growth conditions were kept as similar as possible. These variations were found to be correlated with differences in environmental conditions, particularly the yearly differences in temperature and precipitation. Accumulation of phytate was positively and significantly correlated ( $r = 0.91$ ,  $P = 0.01$ ) with the amount of precipitation during the whole (April–July) growing season. High temperature increased the accumulation of phosphorus but had less effect on the accumulation of phytate. The years 1991 and 1993 had abundant rainfall and high temperatures, and this produced lower yields and smaller kernel sizes but higher phosphorus and phytate levels (Table 1). Some of the increase in phytate may only be an apparent increase resulting from a lower starch content in the smaller kernels.

When the variations in phytate levels of individual cultivars were analyzed (Table 1), there were significant differences among the years for each of the cultivars. For cultivars Elmira and Acton there were no significant differences in phytate levels of 1984 and 1994. Phytate levels were significantly lower by 10.7% in 1986 than in 1994 for cv. Elmira but not for cv. Acton. The 1986 phytate levels of cv. Huron were 10.8% and those of cv. Halton were 17.7% lower than the respective levels in 1994, and these differences were significant at  $P = 0.05$ . Thus, the cultivars varied in the stability of their phytate during the years of storage at ambient temperature and humidity.

There were also differences in the percent germination of older and younger seeds, suggesting seed deterioration had occurred. Percent germinations for kernels of cv. Elmira were 42% for 1984, 68% for 1986, and 91% for 1994. For cv. Huron the percent germination in 1986 was 86% and in 1994 was 99%. These two cultivars had the highest and lowest differences in germination between the oldest and youngest crops with the other two cultivars having intermediate values. There was no direct correlation between the decreases in phytate levels and decreases in percent germination. For example, cv. Acton had the lowest decrease in phytate but the second lowest percent germination for the early years of growth.

When barley kernels were exposed to accelerated aging (Table 2), those exposed to both elevated temperature and elevated humidity had a higher loss of phytate than those exposed only to elevated tempera-

**Table 3. Percent Phytic Acid (mean  $\pm$  SD) in Whole Beans and Bean Flours Stored at Room Temperature or Frozen**

days from harvest	whole <sup>a</sup> seeds stored RT	whole seeds stored fr	ground <sup>b</sup> seeds stored RT	ground seeds stored fr
10	1.721 $\pm$ 0.021			
30	1.673 $\pm$ 0.010	1.706 $\pm$ 0.019	1.652 $\pm$ 0.021	1.821 $\pm$ 0.005
60	1.764 $\pm$ 0.003	1.739 $\pm$ 0.047	1.780 $\pm$ 0.009	1.778 $\pm$ 0.009
90	1.733 $\pm$ 0.004	1.769 $\pm$ 0.006	1.775 $\pm$ 0.033	1.806 $\pm$ 0.021
120	1.751 $\pm$ 0.033	1.754 $\pm$ 0.010	1.742 $\pm$ 0.014	1.819 $\pm$ 0.021
150	1.696 $\pm$ 0.012	1.741 $\pm$ 0.047	1.675 $\pm$ 0.050	1.757 $\pm$ 0.014
180	1.672 $\pm$ 0.020	1.749 $\pm$ 0.014	1.700 $\pm$ 0.018	1.689 $\pm$ 0.050
300	1.674 <sup>c</sup>	1.716 $\pm$ 0.016	1.688 $\pm$ 0.004	1.706 $\pm$ 0.020
360	1.714 $\pm$ 0.078	1.774 $\pm$ 0.025	1.711 <sup>c</sup>	1.754 $\pm$ 0.021
420	1.626 $\pm$ 0.004	1.744 <sup>c</sup>		

<sup>a</sup> Seeds stored whole were ground just prior to analysis. <sup>b</sup> Seeds were ground at 10 days postharvest and stored either at room temperature (RT) or frozen (fr.). <sup>c</sup> Based on  $N = 1$ .

ture. After 3 months at 41 °C and 75% RH the cultivars Elmira, Huron, and Halton had significantly lower phytate levels (at  $P = 0.05$ ) than the corresponding unaged kernels. Cultivar Acton had less of a decrease in phytate, and the mean was not significantly different from the control values. Thus, the phytate within cv. Acton was again more resistant to aging than that of the other three cultivars. That the aging regime was effective was shown by the total destruction of the ability to germinate in all four cultivars. The overall decrease in phytate was low, not exceeding 10%. Aging at only elevated temperature in the dry state did not significantly reduce the phytate levels in all four cultivars, and the decrease in germination was very slight (2–5%).

Since the phytate levels in this study were determined following column separation, it is possible that the phytate degradation in our aged seeds is greater than the results indicate, since phytate partially degraded to lower phosphates would also be recovered from the column. In unaged seeds the phytate is believed to be mainly in the hexaphosphate state (Lehrfeld and Morris, 1992) with only traces of the lower phosphates.

In this study, the winter barley cultivars, grown in Ontario, did not have a large reduction in phytate with either natural aging with time or accelerated aging. This indicates that the barley phytate had a higher stability than that reported for bean phytate (Hernandez-Unzon and Ortega-Delgado, 1989; Bernal-Lugo et al., 1990). Perhaps the higher stability of phytate was due to lower levels of phytase in barley than in beans (Bernal-Lugo et al., 1990).

**Beans.** The white beans had higher total phosphorus levels of 0.629  $\pm$  0.014% ( $N = 10$ ) than those of the barley cultivars (0.353–0.484%). The phytate levels in the beans were also higher at about 1.7% versus about 1.0% for barley. Percent total phosphorus extracted into acid was also generally higher for beans (89.48%  $\pm$  2.58%,  $N = 36$ ) than for barley (83.87  $\pm$  3.46%,  $N = 30$ ) for all analyses at the four storage conditions. Percent phytate in the extract was 86.64  $\pm$  3.07% ( $N = 36$ ) and in the flour was 77.50  $\pm$  1.96% ( $N = 36$ ). This represented a higher percent of the total phosphorus present as phytate in beans compared to 70.04  $\pm$  3.48% ( $N = 30$ ) in barley. The percent moisture in the beans was in the range 13.5–14.5% and was essentially the same for the four storage conditions. After aging at 41 °C and 75% RH the moisture content rose to about 15.5%.

Table 3 shows the phytate levels in ground and whole beans stored at room temperature or frozen. Analysis

**Table 4. Percent Phytic Acid (PA) in Whole Beans and Bean Flours Stored under Three Accelerated Aging Conditions (AAC)**

AAC	no. days aged	stored whole at RT	% decrease in PA	stored whole frozen	% decrease in PA	stored ground at RT	% decrease in PA	stored ground frozen	% decrease in PA
A <sup>a</sup>	30	1.583 ± 0.014	6.89	1.636 ± 0.019	6.11	1.620 ± 0.010	5.19	1.649*	6.64
	60	1.442 ± 0.156	15.24	1.544 ± 0.016	11.41	1.476 ± 0.005	13.69	1.481 ± 0.014	16.19
	120	1.250 ± 0.014	26.51	1.353 ± 0.008	22.40	1.410 ± 0.100	17.63	1.278 ± 0.125	27.57
B <sup>b</sup>	120	1.243 ± 0.012	26.72	1.344 ± 0.061	21.81				
C <sup>c</sup>	120	1.311 ± 0.087	22.76	1.368 ± 0.047	21.38				

<sup>a</sup> Ground and whole seeds (7 months postharvest) were aged at 41 °C and 75% RH for 1 month. The whole seeds were then ground for analysis and subsequent aging was on ground seed mixtures. Values represent means ± SD, N = 2. Value with asterisk is based on N=1.

<sup>b</sup> Whole seeds (11 months postharvest) were aged at 41 °C and 75% RH for 4 months and then were ground for analysis. <sup>c</sup> Whole seeds (11 months postharvest) were aged at 41 °C in sealed containers for 4 months and then were ground for analysis.

of variance showed that there were no statistically significant differences among all the means ( $P = 0.05$ ). Thus, there was no detectable degradation in phytate over the year of storage in the four states. Although the differences were too small to be significant, the mean phytate levels in the room temperature stored whole beans and flours were 2–5% lower than those of the frozen samples, which may indicate some degradation of phytate taking place.

Phytate levels in artificially aged beans are shown in Table 4. In accelerated aging A, after 30 days of wet aging at 41 °C and 75% RH, the phytate levels fell by 5–7%. For the second month of aging (60 days) all the samples were in the ground state, and during this period the decrease in phytic acid levels reached 11–16%. After the fourth month (120 days) of accelerated aging the loss varied from 17 to 27%. Samples that had been stored in the ground state prior to the aging process had a higher loss of phytate if they had been frozen than if they had been stored at room temperature. Seeds kept whole at room temperature until aging lost higher amounts of phytate than did seeds kept whole in the freezer prior to artificial aging. In accelerated aging B, whole seeds were aged for 120 days at 41 °C and 75% RH and the decrease in phytate level was very similar to that obtained in accelerated aging A. The dry accelerated aging C produced a somewhat lower reduction in phytate than the wet accelerated aging procedures, but the decrease in phytate was still over 20%. Beans, which in the freshly harvested state and at 1 year had 98–100% germination, lost all ability to germinate after 120 days of either wet or dry accelerated aging.

The higher losses in phytate with aging in the beans indicate that the phytate within beans was more susceptible to deterioration than that within barley. Stability of phytate in the white beans of cv. Gryphon was greater than that reported by Bernal-Lugo et al. (1990) even when compared to their most stable cultivar. They found that their most stable cultivar had less of the phytate than the cultivar that lost the most phytate during the 33 day period of aging at 41 °C and 75% RH. On the basis of their observations, one can postulate that the seeds of cv. Gryphon may be low in phytase.

This study has shown that barley, stored under relatively good storage conditions of moderate temperature and low humidity, lost little of its phytate and that the phytate was reduced by less than 10% even after 3 months of accelerated aging. Although there was a difference in phytate loss among the four cultivars, the differences were small. The small white beans studied also had relatively stable phytate, but the

overall loss with accelerated aging was greater than for barley. Kon and Sanshuck (1981), studying deterioration of phytate in relation to cooking times for several kinds of legumes, pointed out that climates with 25 °C and 65% RH, which produced some phytate degradation, were common in the tropics and subtropics. Given the tendency of beans to lose phytate, it becomes imperative to know the early storage history of beans as well as the time from harvest when reporting phytate levels for beans. It is conceivable that the very wide variation in phytate levels reported for beans may in part be related to the age of the beans studied and the stability of the phytate within the beans.

#### ACKNOWLEDGMENT

The beans were kindly provided by Professor T. E. Michaels, Crop Science Department, University of Guelph.

#### LITERATURE CITED

- AOAC *Official Methods of Analysis*, 15th ed; Association of Official Analytical Chemists: Arlington, VA, 1990; pp 56, 800–801.
- Bernal-Lugo, I.; Prado, G.; Parra, C.; Moreno, E.; Ramirez, J.; Velazco, O. Phytic acid hydrolysis and bean susceptibility to storage induced hardening. *J. Food Biochem.* **1990**, *14*, 253–261.
- Delouche, J. C.; Baskin, C. C. Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Sci. Technol.* **1973**, *1*, 427–452.
- Ellis, R.; Morris, E. R. Improved ion-exchange phytate method. *Cereal Chem.* **1983**, *60*, 121–124.
- Fretzdorff, B.; Brümmer, J.-M. Reduction of phytic acid during breadmaking of whole-meal breads. *Cereal Chem.* **1992**, *69*, 266–270.
- Glass, R. L.; Geddes, W. F. Grain storage studies XXVII. The inorganic phosphorus content of deteriorating wheat. *Cereal Chem.* **1959**, *36*, 186–190.
- Graf, E.; Dintzis, F. R. Determination of phytic acid in foods by high-performance liquid chromatography. *J. Agric. Food Chem.* **1982**, *30*, 1094–1097.
- Gorsuch, T. T. *The Destruction of Organic Matter*; Pergamon Press Ltd.: Oxford, 1970; pp 1–144.
- Hernandez-Unzon, H. Y.; Ortega-Delgado, M. L. Phytic acid in stored common bean seeds (*Phaseolus vulgaris* L.). *Plant Foods Hum. Nutr. (Dordrecht, Neth.)* **1989**, *39*, 209–221.
- IUPAC-IUB. The nomenclature of cyclitols. *Eur. J. Biochem.* **1968**, *5*, 1–12.
- Kon, S.; Sanshuck, D. W. Phytate content and its effect on cooking quality of beans. *J. Food Process. Preserv.* **1981**, *5*, 169–178.

- Lehrfeld, J.; Morris, E. R. Overestimation of phytic acid in foods by the AOAC anion-exchange method. *J. Agric. Food Chem.* **1992**, *40*, 2208–2210.
- Lott, J. N. A.; Ockenden, I. The fine structure of phytate-rich particles in plants. In *Phytic Acid: Chemistry and Applications*; Graf, E., Ed.; Pilatus Press: Minneapolis, 1986; pp 43–55.
- Maga, J. A. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *J. Agric. Food Chem.* **1982**, *30*, 1–9.
- Morris, E. R. Phytate and dietary mineral bioavailability. In *Phytic Acid: Chemistry and Applications*; Graf, E., Ed.; Pilatus Press: Minneapolis, 1986; pp 57–76.
- Ockenden, I.; Lott, J. N. A. Ease of extraction of calcium from ash of *Cucurbita maxima* and *Cucurbita andreana* embryos following dry ashing at different temperatures. *Commun. Soil Sci. Plant Anal.* **1986**, *17*, 645–666.
- Organ, M. G.; Greenwood, J. S.; Bewley, J. D. Phytin is synthesized in the cotyledons of germinated castor-bean seeds in response to exogenously supplied phosphate. *Planta* **1988**, *174*, 513–517.
- Plaami, S.; Kumpulainen, J. Determination of phytic acid in cereals using ICP-AES to determine phosphorus. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 32–36.
- Raboy, V.; Dickinson, D. B. Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. *Plant Physiol.* **1984**, *75*, 1094–1098.
- Roberts, E. H.; Roberts, D. L. Moisture content of seeds. In *Viability of Seeds*; Roberts, E. H., Ed.; Syracuse University Press: Syracuse, New York, 1972; pp 424–429.
- Winston, P. W.; Bates, D. H. Saturated solutions for the control of humidity in biological research. *Ecology* **1960**, *41*, 232–237.
- Zar, J. H. *Biostatistical Analysis*; Prentice Hall, Inc.: Englewood Cliffs, NJ, 1984; pp 186–190.

Received for review June 12, 1996. Revised manuscript received December 2, 1996. Accepted February 6, 1997.<sup>®</sup> Funding for field plots of barley and beans was provided by OMAFRA. Financial support for this research was provided by a grant awarded to John N. A. Lott by the Natural Sciences and Engineering Research Council of Canada.

JF960418+

---

<sup>®</sup> Abstract published in *Advance ACS Abstracts*, March 15, 1997.