# Environmental and Cultivar Effects on Oat Phytic Acid Concentration[1]

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**ABSTRACT**

Groat phytic acid concentration (GPA) was measured in four oat cultivars grown from 1974-1977 at three Wisconsin locations. GPA averages for years and locations were significantly different among all four cultivars. Yearly differences in GPA were significant at some locations. Locational differences in GPA for a single year were significant only in the record drought year of 1976. Overall differences were small relative to the average GPA, except for those occuring in 1976. Cultivars ranked almost the same in GPA for overall years and locations, suggesting that their phytic acid production is similarly affected by environment. In addition, groat phosphorus was highly correlated with GPA.

Serious mineral deficiencies in humans and monogastric animals can be caused by phytic acid, the hexaphosphate of myoinositol and the major storage form of phosphorus (P) in seeds (Roberts and Loewus 1968). Diets consisting mainly of whole grains and legumes can contain enough phytic acid to cause deficiencies of Ca (Anonymous 1967a, Taylor 1965), Mg (Davies and Nightingale 1975), and possibly Fe (Anonymous 1967b, Davies and Nightingale 1975, Mahloudji et al 1975, Morris and Ellis 1976, Walker et al 1948). Phytase, which is found in seeds, some monogastric and ruminant microflora, and other microorganisms (Taylor 1965), destroys the chelating ability of phytic acid by removing its phosphate groups. In the ruminant, phytic acid is a major P source and causes no mineral deficiencies because it is completely hydrolyzed by phytase (Punj et al 1969).

Little work has been done in examining phytic acid variation among grain cultivars within a species. Ashton and Williams (1958) found no differences among six oat cultivars. Lolas et al (1976) found a range of phytic acid concentration among oat, barley, wheat, and soybean cultivars but did not report whether or not cultivars were statistically different in groat phytic acid (GPA). Kleese et al (1968) found significantly different seed P among cultivars of the same species for oats, wheat, and soybeans. This study can be related to phytic acid because the ratio of seed P to phytic acid is quite constant in these crops, as it is in barley and beans (Ashton and Williams 1958, Lolas et al 1976).

In this study we investigated the effects of environment and cultivar on GPA concentration in oats.

## MATERIALS AND METHODS

### Field Design

The oat cultivars Otee, Wright, Holden, and Lodi were grown from 1974 through 1977 at three Wisconsin locations: Janesville, Manawa, and Racine. Plots were 5.5 m long and 1.5 m wide and contained four rows, from which only the center two were harvested. Four replications were grown, but in 1974 and 1975 replicates were bulked before phytic acid analysis. As a result, the analysis of variance (ANOVA) was conducted on treatment means. The interaction of location with year and cultivar was tested by the error mean square from an ANOVA conducted on the replicated data of 1976 and 1977. All factors were considered random. The ANOVAs and mean comparisons were conducted using the methods described by Snedecor and Cochran (1967).

### Phytic Acid Determination

Oat groats were ground in a Wiley Micromill (Arthur H. Thomas, Philadelphia, PA) to pass through a 20-mesh sieve. Moisture was determined using a moisture tester (C. W. Barbender Instruments, Inc., South Hackensack, NJ). The following procedure, which was derived by combining and modifying the methods of Wheeler and Ferrel (1971) and Makower (1969), was used in measuring phytic acid concentrations in duplicate samples. (All heating was done in a boiling water bath and all centrifugations were perfromed at 20° C.)

1) Extract 1.8 g of flour with 36 ml of 3% trichloroacetic acid (TCA) by shaking 30 min on a React-R-Shaker (Udy Analyses Co., Boulder, CO) or 60 min on a Wrist-Action shaker (Burre Corp., Pittsburg, PA) at the highest setting.

2) Centrifuge the suspension at 8,900 × *g* for 10 min.

3) Add 0.63 ml of iron solution (2 mg of ferric iron per ml of 3% TCA) to a 5-ml aliquot of supernatant in a 16-ml polypropylene centrifuge tube. Heat for 45 min.

4) Cool and centrifuge for 15 min at 12,000 × *g*.

5) Wash the precipitate three times with 3% TCA and once with water. Each time, vortex the precipitate, add 5 ml of wash, heat 10 min, and then centrifuge 10 min at 15,000 × *g*.

6) Vortex the precipitate. Add 6 ml of 0.5*M* NaOH. Heat 30 min.

7) Centrifuge for 15 min at 21,000 × *g*. Wash with 5 ml of water and repeat the centrifugation.

8) Dissolve the $Fe(OH)_3$ in 10 ml of 0.5*M* HCl with heating for 10 min.

9) Dilute the resulting $FeCl_3$ solution to 100 ml with water.

10) Remove a 3-ml aliquot. Add 5 ml each of 0.05%

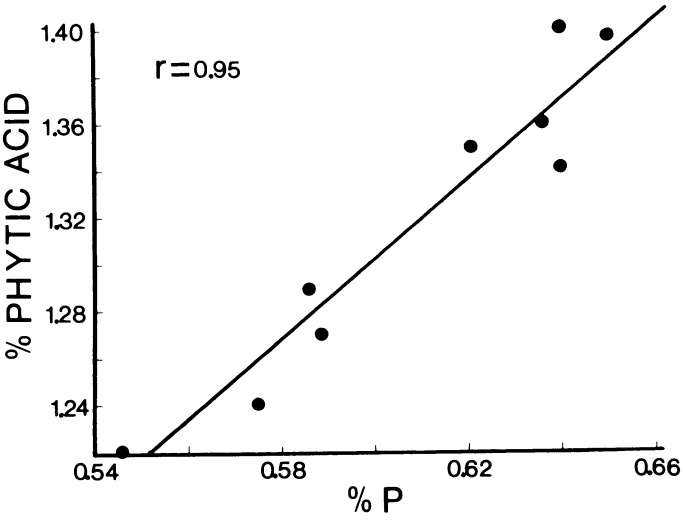


**Fig. 1.** Correlation of groat phosphorus and phytic acid concentration (% dry weight).

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*o*-phenanthroline and 0.2% *p*-hydroquinone. Mix, and add 0.18 ml of 25% sodium citrate solution. Mix, and read the absorbance at 510 nm after 5 min.

11) Prepare a standard curve with $FeCl_3.6H_2O$, diluting with 0.5*M* HCl to prevent $Fe(OH)_3$ formation. Calculations are based on the assumption that four ferric atoms combine with one molecule of phytic acid.

### Phosphorus Determination

Samples were ground as previously described and digested in a concentrated solution of $H_2SO_4$ and 30% $H_2O_2$ on a microKjeldahl unit. Molybdate and $SnCl_2$ were used for color development (Allen 1974).

## RESULTS AND DISCUSSION

### Accuracy of Phytic Acid Determination

This method gave 94–97% recovery of sodium phytate (Sigma, St. Louis, MO; $Na_{12}$ phytate, 97% pure) added to the aliquot described in Step 3. The amount of sodium phytate was estimated to be equal to the phytic acid in the aliquot, and twice the amount of iron solution (1.26 ml) was added.

Nine individual oat groat samples were also assayed for P as well as for phytic acid, with a resulting correlation of 0.95 (Fig. 1). This agrees with a 0.91 correlation by Lolas et al (1976).

### Environmental Study

In the following discussion, differences referred to are significant at the 5% level of probability and are between the means of percent GPA. All of the cultivars were different when averaged across years and locations (Tables I and II). This indicates that any one year (Y) or location (L) had the same relative effect in each cultivar (C), as evidenced by the nonsignificant differences in the C × L, C × Y, and C × L × Y interactions (Table I). The significant difference in the L × Y interaction indicates that the effect of any one year on phytic acid production was not the same at each location. Table II shows that this interaction is due primarily to the very low phytic acid concentration at Manawa in 1976, which was much lower than at the other two locations. A record drought occurred in 1976, and Racine, Manawa, and Janesville received rainfall that was 11.05, 22.53, and 18.54 cm below normal, respectively (Heiser 1978). This might have caused the low 1976 values. Furthermore, the very low phytic acid concentration at Manawa in 1976 may have been caused by the proportionately smaller amount of precipitation at this location (Heiser 1978).

Differences among locations within a year occurred only in 1976, when phytic acid concentrations were different among all three locations. Again, the drought might have been responsible for this, in that rainfall was quite different at each location.

Although the phytic acid concentration at some locations showed significant yearly changes and cultivars were significantly different, these differences were relatively small when compared to actual GPA concentration. Only 0.1% separated the highest and lowest mean phytic acid concentration for cultivars. This difference is only 7.9% of the mean phytic acid concentration found in this study. The coefficients of variation (CV) for each location (Table II) show that yearly changes at any one location were small relative to the corresponding phytic acid mean at that location. If the data for 1976, a year of extreme drought, are excluded, the CV for each location is decreased and therefore may be more representative of

**TABLE I**
**Analysis of Variance for the Phytic Acid Concentration (% dry weight) of Four Oat Cultivars Grown at Three Locations over Four Years**

| Source | Degrees of Freedom | Mean Square ($\times 10^{-4}$) | F[a] |
|---|---|---|---|
| Cultivar (C) | 3 | 227.72 | 41.95[b] |
| Location (L) | 2 | 74.57 | 1.12 |
| Year (Y) | 3 | 330.2 | 4.58[c] |
| C × L | 6 | 1.41 | 0.31 |
| C × Y | 9 | 4.31 | 0.94 |
| L × Y | 6 | 68.98 | 15.67[b] |
| C × L × Y | 18 | 4.58 | 0.66 |
| Error | 47 | 6.93 | ... |

[a] Main effects were tested by pooled variances, and the two-way interactions were tested by the three-way interaction mean square.
[b] $P = 0.01$.
[c] $P = 0.05$.

**TABLE II**
**Phytic Acid Concentration (% dry weight) of Four Oat Cultivars Grown at Three Locations over Four Years[a]**

| Location | Year | Cultivar | | | | Year Mean | Location Mean | CV[b] (%) |
|---|---|---|---|---|---|---|---|---|
| | | Otee | Wright | Holden | Lodi | | | |
| Racine | 1974 | 1.35 | 1.29 | 1.26 | 1.29 | 1.30 de[c] | | |
| | 1975 | 1.32 | 1.30 | 1.23 | 1.25 | 1.28 cd | 1.26 | 2.8 |
| | 1976 | 1.27 | 1.24 | 1.15 | 1.23 | 1.22 b | (1.28)[d] | (2.0)[d] |
| | 1977 | 1.34 | 1.24 | 1.21 | 1.20 | 1.25 c | | |
| Cultivar mean | | 1.32 | 1.27 | 1.21 | 1.24 | | | |
| Janesville | 1974 | 1.38 | 1.29 | 1.30 | 1.28 | 1.31 de | | |
| | 1975 | 1.37 | 1.31 | 1.24 | 1.29 | 1.30 de | 1.29 | 2.1 |
| | 1976 | 1.29 | 1.26 | 1.22 | 1.23 | 1.25 c | (1.30) | (1.2) |
| | 1977 | 1.32 | 1.24 | 1.24 | 1.29 | 1.28 cd | | |
| Cultivar mean | | 1.34 | 1.28 | 1.25 | 1.28 | | | |
| Manawa | 1974 | 1.37 | 1.30 | 1.30 | 1.31 | 1.32 e | | |
| | 1975 | 1.35 | 1.30 | 1.26 | 1.32 | 1.31 de | 1.25 | 8.2 |
| | 1976 | 1.17 | 1.10 | 1.05 | 1.09 | 1.10 a | (1.30) | (2.5) |
| | 1977 | 1.32 | 1.28 | 1.16 | 1.22 | 1.26 c | | |
| Cultivar mean | | 1.30 | 1.25 | 1.19 | 1.24 | | | |
| Overall cultivar mean | | 1.32 | 1.27 | 1.22 | 1.25 | Grand mean = 1.27 | | |

[a] Means of duplicate analyses.
[b] CV = coefficient of variation.
[c] Means followed by the same letter are not significantly different at the 5% level using Duncan's New Multiple Range Test.
[d] 1976 data are not included in mean or CV values enclosed in parentheses.

the average phytic acid change. Yearly changes in phytic acid concentration at a particular location can be expected to be small unless the weather changes appreciably. These data indicate that cultivar ranking for GPA concentration was nearly constant at each location over the four years. This implies that environmentally produced changes in phytic acid concentration will probably be similar for different oat cultivars.

## LITERATURE CITED


ALLEN, S. E. 1974. Chemical analysis of ecological materials (1st ed.). Blackwell Scientific Publications: Oxford.

ANONYMOUS. 1967a. Zinc, calcium, and phytate. Nutr. Rev. 25:215.

ANONYMOUS. 1967b. Effect of phytate on iron absorption. Nutr. Rev. 25:218.

ASHTON, W. M., and WILLIAMS, P. C. 1958. The phosphorus compounds of oats. I. The content of phytate phosphorus. J. Sci. Food Agric. 9:505.

DAVIES, N. T., and NIGHTINGALE, R. 1975. The effect of phytate on intestinal absorption and secretion of zinc, and whole body retention of Zn, copper, iron, and manganese in rats. Br. J. Nutr. 34:243.

HEISER, M. (ed.) 1978. Wisconsin Agricultural Statistics, p. 18. Wisc. Dept. Agric., Trade, and Consumer Prot.: Madison.

KLEESE, R. A., RASMUSSON, D. C., and SMITH, L. H. 1968. Genetic and environmental variation in mineral element accumulation in barley, wheat, and soybeans. Crop Sci. 8:591.

LOLAS, G. M., PALAMIDIS, N., and MARKAKIS, P. 1976. The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. Cereal Chem. 53:867.

MAHLOUDJI, M., REINHOLD, J. G., HAGHSHENASS, M., RONAGHY, W. A., SPIVEYFOX, M. R., and HALSTEAD, J. A. 1975. Combined zinc and iron compared with iron supplementation of diets of 6 to 12 year old village school children in Southern Iran. Am. J. Clin. Nutr. 28:721.

MAKOWER, R. U. 1969. Extraction and determination of phytic acid in beans (phaseolus vulgaris). Cereal Chem. 47:288.

MORRIS, E. R., and ELLIS, R. 1976. Isolation of monoferric phytate for wheat bran and its biological value as an iron source to the rat. J. Nutr. 106:753.

PUNJ, M. L., KOCHAR, A. S., and BHATIA, I. S. 1969. Utilization of phytin phosphorus by rumen microorganisms. Indian Vet. J. 46:88.

ROBERTS, R. M., and LOEWUS, F. 1968. Inositol metabolism in plants. VI. Conversion of myo-inositol to phytic acid in Wolffiella floridana. Plant Physiol. 43:1710.

SNEDECOR, G. W., and COCHRAN, W. C. 1967. Statistical Methods (6th ed.). Iowa State University Press: Ames.

TAYLOR, T. G. 1965. The availability of the calcium and phosphorus of plant materials for animals. Proc. Nutr. Soc. 24:105.

WALKER, A. P., FOX, F. W., and IRVING, J. T. 1948. The effect of bread rich in phytate phosphorus in the metabolism of certain mineral salts with special reference to calcium. Biochem. J. 42:452.

WHEELER, F. L., and FERREL, R. E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. 48:312.


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