Phytic Acid Content of Flaxseed As Influenced by Cultivar, Growing Season, and Location

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Phytic acid content of meal from eight flaxseed cultivars grown in 1991, 1992, and 1993 at four locations in western Canada was determined to examine the effect of cultivar, location, and growing season. Flaxseed contained 23−33 g of phytic acid per kilogram of meal, and averages for years and locations were significantly different among all cultivars. Yearly differences in phytic acid were significant at most locations. Variation in phytic acid was mainly due to cultivar and the interaction of cultivar with location and year. The stability of phytic acid concentration in various environments appears to be genetically controlled. In flaxseed, phytic acid was independent of oil content and was not related to seed yield. A negative correlation was found between phytic acid and iodine value and phenolic acids of flaxseed.

Keywords: *Flaxseed; phytic acid; cultivar effects; seasonal variations; stability; Linum usitatissimum; environment; flaxseed meal*

INTRODUCTION

Phytic acid is a natural plant inositol hexaphosphate commonly found in seeds and represents the principal form of stored phosphate. Besides the well-known adverse effects of reduced mineral bioavailability in human and animal nutrition, phytic acid may also react directly with proteins and starch and reduce their solubility and digestibility (Thompson, 1993). Phytic acid has hypocholesterolemic, antioxidative, anticarcinogenic, and hypolipidemic effects and has been suggested to have a role in the prevention of caries and platelet aggregation in the treatment of hypecalciuria and kidney stones (Potter, 1995; Thompson, 1993). Phytic acid also has physiological effects similar to those of high-fiber diets and, as such, may be partly responsible for some of the health benefits (Thompson, 1994). The function of phytic acid is not only for energy storage and antioxidation of fats of seeds but also for protecting seed from fungal invasion (Dayi et al., 1995).

In oilseeds, phytic acid accounts for ∼1.5% of defatted and dehulled oilseed meal, such as soy, peanut, and sesame meal (Erdman, 1979). It is structurally integrated with the protein bodies as phytin, a mixed potassium, magnesium, and calcium salt of inositol (Zhou and Erdman, 1995). Phytin from defatted flaxseed flour, isolated by Bolley and McCormack (1952), contained 13% organic phosphorus and constituted about 6% of the flour (∼1.4% of organic phosphorus). Defatted flaxseed meal contained 1.8−3.0% phytic acid, representing ∼70% of the total phosphorus (Bhatty and Cherdkiatgumchai, 1990). The phytate in flaxseed was reported to have no effect on zinc status of rats (Ratnayake et al., 1992).

Phytate concentration in wheat is strongly influenced by environmental factors such as seasonal differences (Nahapetian and Bassiri, 1976). Dintzis et al. (1992) reported significant differences in phytate content among three locations but not between red and white wheat cultivars. Yearly differences in phytic acid of four oat cultivars grown at three locations for four years were significant only at some locations (Miller et al., 1980). However, cultivar ranking for phytate concentration was nearly constant at each location over the four years. According to Miller et al. (1980), seed phytic acid and phosphorus levels are largely a function of available soil phosphorus. Thus, 98% of the variation in phytic acid phosphorus of soybeans was attributable to a positive linear effect of available soil phosphorus (Raboy and Dickinson, 1993). In flaxseed, cultivar difference in phytic acid was attributed to low total phosphorus content of flaxseed grown at one particular location (Bhatty and Cherdkiatgumchai, 1990).

Seed forms a major component of animal feed, but because phytate can minimally be utilized by monogastric animals, supplementation of the feed with inorganic phosphate or fungal phytase becomes necessary. Transgenic plants expressing the fungal phytase gene have been reported to fully substitute for fungal phytase supplementation of basal diets (Whitelan, 1995). An alternative means of improving the nutritional quality of flaxseed for animal feed is via genetic reduction in phytic acid. These efforts often begin with surveys of cultivars and typically reveal substantial quantitative variation in seed phytic acid. Because phytic acid has two apparently conflicting roles, as an antinutrient with health benefits as well as physiological and neutraceutical functions, our investigation focused on determinants of the variability in flaxseed.

MATERIALS AND METHODS

Samples of eight oil-type flaxseed cultivars were obtained from standardized cooperative tests conducted at four locations (Brandon and Portage la Prairie in Manitoba, and Elrose and Melfort in Saskatchewan) during the 1991, 1992, and 1993 growing seasons. Experimental design was according to procedures established by the Western Expert Committee on Grain for official registration of flax in Canada (Anonymous, 1992).

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Table 1. Phytic Acid Content (g/kg) of Meal from Flaxseed Cultivars Grown at Four Locations for Three Years

cultivar	mean[a]	range	slope
AC Emerson	28.33[b]	23.04−31.16	0.27
AC Linora	22.75[g]	14.12−29.14	1.48
Flanders	24.23[f]	16.31−30.18	0.01
Linola 947	32.49[a]	25.26−38.05	1.43
McGregor	25.99[d]	17.65−30.42	1.42
NorLin	27.27[c]	19.31−34.15	0.83
Somme	23.99[f]	13.29−29.23	1.23
Vimy	25.27[e]	18.84−31.75	1.35

[a] Means followed by the same superscript are not significantly different by Duncan's multiple range test at 5% level.

Flaxseed was defatted by the multisequential method of Appelqvist (1967) with petroleum ether. The defatted meal was dried in a forced-air oven for at least 24 h at 50 °C. Phytic acid was determined essentially as described by Haug and Lantzsch (1983). Briefly, defatted meal (0.5 g) was extracted with 2.4% HCl (10 mL) for 1 h under continuous shaking (Wrist-Action Shaker, Burrell Corporation, Pittsburgh, PA). After centrifugation (3000*g*, 30 min; IEC Centra-8 Centrifuge, International Equipment Company, Needham Heights, MA), 0.5 mL of the supernatant was mixed with 1 mL of ferric ammonium sulfate solution (0.2 g/L) in 0.2 N HCl in a test tube and boiled in a water bath for 0.5 h. Afterward, the tube was cooled in ice water for 15 min and allowed to equilibrate to room temperature. The absorbance was read at 519 nm (DU-50, Beckman, Beckman Instruments, Inc., Irvine, CA), 30 s after the addition of 2 mL of 1% bipyridine−thioglycolic acid solution. The concentration of phytic acid was calculated from a similarly prepared standard curve obtained with sodium phytate (Sigma Chemical Company, St. Louis, MO).

Protein content (N × 5.41) of defatted meal was determined by the Kjeldahl method with a Tecator digester and a Kjeltec (System 1002) distillation unit (Tecator AB, Höganäs, Sweden). Oil content was determined on seed, oven dried to 1% or less moisture, by a nuclear magnetic resonance (NMR) analyzer (Robertson and Morrison, 1979). Iodine value was calculated from fatty acid composition (AOCS, 1993, Method Cd 1C-85).

At least three determinations were made for all assays. Analyses of variance by the general linear models (GLM) procedure, means comparison by Duncan's test, Pearson correlation, and variance components using PROC VARCOMP procedure were performed according to SAS methods (SAS Institute, Inc., 1990).

RESULTS AND DISCUSSION

The phytic acid content of flaxseed differed significantly among cultivars (Table 1). Phytic acid level ranged from 22.8 g/kg for AC Linora to 32.5 g/kg for the low linolenic acid yellow-seeded cultivar Linola 947. The range in phytic acid content was usually ∼12−16 g/kg of meal, except for AC Emerson and Somme where it was ∼29 and 66% of the mean, respectively. These values are comparable to those reported previously for flaxseed (Bhatty and Cherdkiatgumchai, 1990; Ratnayake et al., 1992) and are lower than published values for other oilseed meals except soybeans (Erdman, 1979).

Effects of cultivar, year, location and their interactions were all highly significant ($p < 0.0001$) in contributing to variation in phytic acid (Table 2). The variation had large components due to cultivar (C) and year (Y), both of which had similar effect, which was ∼13 times larger than that due to location (L). Similarly, the effect of L × Y interaction was >2.5 times as large as that of C × L or C × Y interaction. These interactions, however, did not contribute to the variability in phytic acid because their variance was smaller than that of the experimental error. The variation in phytic acid of flaxseed was mainly due to cultivar, year, and C × Y × L interaction which explained 37, 14, and 36% of the total variability, respectively. The high variance of the C × Y × L interaction regarding phytic acid indicates that cultivar responded differently to year for each location. Similar significant cultivar and C × L interaction effects in phytic acid content of flaxseed have been reported previously (Bhatty and Cherdkiatgumchai, 1990) on a limited set of data.

Table 2. Analysis of Variance for Phytic Acid of Flaxseed Grown at Four Locations for Three Years

source	df	mean squares[a]	*F* value	variance components (%)
cultivar (C)	7	458.17	242.40	37.32
year (Y)	2	490.16	259.32	13.96
location (L)	3	34.35	18.17	0
C × L	21	29.60	15.66	0
C × Y	14	32.27	17.07	0
L × Y	6	86.48	45.75	4.37
C × Y × L	42	40.68	21.52	36.33
error (CV = 5.23)	288	1.89		8.02
total	383	21.24		

[a] All mean squares significant at the 0.0001 probability level ($p > F = 0.0001$).

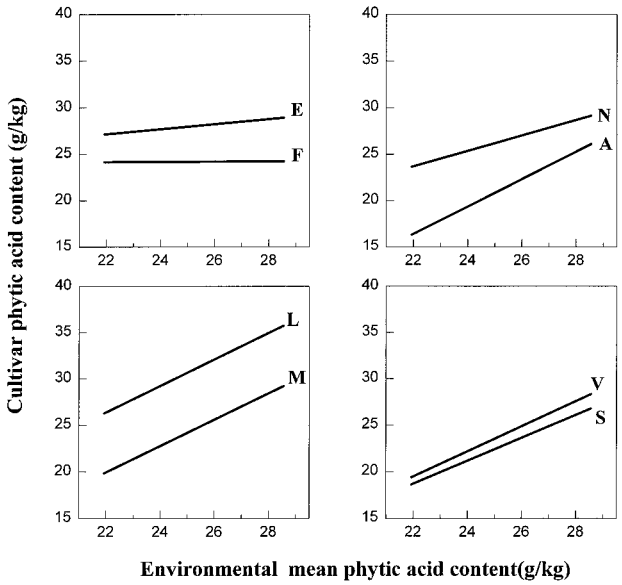

Figure 1. Influence of environment (L × Y) on cultivar performance (phytic acid g/kg). Cultivars: (E) AC Emerson; (F) Flanders; (N) NorLin; (A) AC Linora; (L) Linola 947; (M) McGregor; (V) Vimy; (S) Somme.

The complexity of cultivar and environment interaction is illustrated in Figure 1, where the performance of each cultivar, in regard to phytic acid, was regressed in each environment against the 12 environmental (L × Y) means. This method was used to assess the effects of environment (climate, growing sites, etc.) on the ability of the cultivars to accumulate phytic acid. The slopes (Table 1) varied between 0.01 (Flanders) and 1.48 (AC Linora). In terms of slopes, the cultivars can be divided into those with above average response (AC Linora, Linola 947, McGregor, Somme and Vimy), those with an average response (NorLin), and those with little response (AC Emerson and Flanders) to environment. Similarly, the magnitude of the slope reflects the stability of phytic acid content of a cultivar within a given environment. Thus, cultivars AC Emerson and Flanders with low slope values were highly stable, whereas cultivars AC Linora, Linola 947, and McGregor had phytic acid content that increased linearly with increasing environmental means ($r = 0.708$, 0.716, and

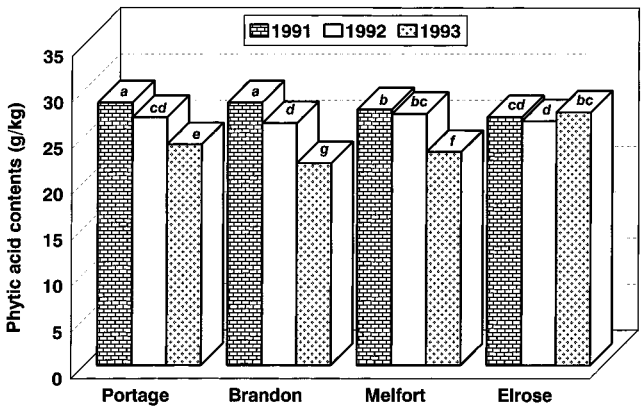

Figure 2. Phytic acid content of flaxseed grown in three different years at four locations. Bar graphs with the same letter are not significantly different ($p < 0.001$).

Table 3. Correlation Coefficients for Phytic Acid of Flaxseed

component	phytic acid correlation coefficient
oil	0.126
protein	0.272[a]
iodine value	−0.503[b]
total phenolic acids	−0.354[b]

[a] $p < 0.01$. [b] $p < 0.0001$ ($n = 190$).

0.751, respectively; $p = 0.01$). Interestingly, Linola 947 and one of its parents, McGregor, showed a parallel trend, suggesting that the stability of phytic acid may be genetically controlled.

Generally, flaxseed grown at Elrose had higher levels of phytic acid than that grown at Brandon (Figure 2), although only 1.41 g/kg separated the mean phytic acid concentrations at these locations. Seasonal effects had a greater impact on phytic acid of flaxseed than locations, except at Elrose. Flaxseed grown in 1991 had significantly higher phytic acid than seed grown in subsequent years 1992 and 1993. The mean phytic acid content of flaxseed grown in 1993 across all locations was 13.7% lower than that of flaxseed grown in 1991. Flaxseed grown in Manitoba (Brandon and Portage la Prairie) showed significant differences in phytic acid content within the three years, whereas that grown in Saskatchewan (Elrose and Melfort) was less affected by season despite large differences in locations. This variability in phytic acid content of flaxseed across locations and years further illustrates the strong cultivar × environment interaction (Table 2).

Comparison of phytic acid with oil and protein contents of flaxseed (data for oil and protein not presented) showed poor correlation. The Pearson correlation coefficients of phytic acid were 0.126 and 0.272 for oil and protein contents, respectively (Table 3). The weak association between phytic acid and both protein and oil suggests that phytic acid content of flaxseed should be unaffected by these characters. When phytic acid was compared with total phenolic acids of flaxseed from a previous study (Oomah et al., 1995), a negative correlation was obtained ($r = -0.355$). This negative correlation is perhaps a reflection of the yellow-seeded cultivar Linola 947 having the highest phytic acid content (32.5 g/kg, Table 1), but the lowest mean total phenolic acid content of 7.89 g/kg seed of all eight cultivars. The iodine value, an expression of the degree of unsaturation as well as the oxidative stability of an oil, was inversely correlated to phytic acid content ($r = -0.503$). The relatively strong inverse correlation of phytic acid with iodine value suggests that increases in phytic acid content in flaxseed could lead to a reduction in iodine value, thereby increasing the oxidative stability of the oil. Iodine value was calculated from fatty acid composition, so it would be reasonable to conclude that phytic acid content affects fatty acid composition.

Phytic acid content was unrelated to flaxseed yield ($r = 0.027$, $p = 0.80$; data for yield obtained from PRRCG 1992, 1993, 1994 and not presented), indicating that environmental and genetic factors responsible for yield (e.g., soil conditions and fertility) and plant growth habits did not influence phosphorus supply and uptake. Hence, differences in phytic acid content may be caused by phosphorus utilization efficiency of each cultivar, which then determines phytic acid content of the seed. Our assumption is supported by the suggestion of Bhatty and Cherdkiatgumchai (1990) that the difference in phytic acid is due to total phosphorus content of flaxseed cultivars. It is also possible that phytic acid concentration is differentially affected by environment among cultivars (Figure 1); that is, whereas an environment may affect yield or protein concentration similarly in all or most cultivars, it may be either favorable or unfavorable for phytic acid concentration depending on the cultivar.

The data presented indicate that flax cultivars differ in phytic acid content. The findings suggest the possibility of breeding flax cultivars low in phytic acid content for increased utilization of flaxseed for monogastric animals, especially for poultry and swine diets where phosphorous availability may be increased by >50%. On the other hand, cultivars with high levels of phytic acid content might be sought for their health benefits and could become raw materials for fractionation and use in the neutraceutical industry. The stability of phytic acid might be a concern for breeding improvement. In this regard, cultivars AC Emerson and Flanders indicate a high degree of stability for this trait, responding similarly to several environments. The results suggest that effective selection can occur for phytic acid even with widely differing environments of the type encountered in this study without any detrimental effect on flaxseed yield.

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