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Phenolic Acids in Flaxseed

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Ninety-six samples of flaxseed from eight cultivars grown at four locations in western Canada for three years were used to study the effect of cultivar, location, and growing season on the phenolic acid contents of the seed. Flaxseed contained 8–10 g/kg of total phenolic acids, about 5 g/kg of esterified phenolic acids, and 3–5 g/kg of etherified phenolic acids. The esterified phenolic acids represented 48–66% of the total phenolic acids and were not dependent on cultivar. Variation in phenolic acids was mainly due to seasonal effect. While growing location had significant effects on phenolic acid contents of individual cultivars, no overall significant location effect was observed. The yellow-seeded flaxseed cultivar Linola 947 had lower levels of total and esterified phenolic acids compared to the traditional brown-seeded type. Phenolic acid contents of flaxseed were independent of protein and oil contents. A strong positive correlation was found between the constitutive concentrations of total and etherified phenolic acids in flaxseed and resistance to fusarium wilt.

Keywords: *Flaxseed; phenolic acids; fusarium wilt; seasonal variations; cultivar effects; Linum usitatissimum*

INTRODUCTION

Phenolic compounds are widely distributed in plants, and several functions have been attributed to them. Plant phenolics have antipathogen, antiherbivore, and allelopathic properties (Brice and Morrison, 1982; Nazeem et al., 1984; Goldstein and Spencer, 1985; Akin and Chesson, 1989; Jung, 1989; Ray and Hastings, 1992; Arnason et al., 1994). Although genetic variation in tolerance to allelopathic stress has been identified in flax, genetic variation in allelochemical production has not been investigated (Ray and Hastings, 1992). Qualitative and quantitative changes in phenolic acids have been associated with loss of embryogenic potential of *Medicago sativa* L. (Cvikrová et al., 1991) and interference with growth of flax seedlings (Hradilik et al., 1986). Volynets and Kornelyuk (1974) found changes in phenolic acids of flaxseed after treatment with herbicides such as 2,4-D and suggested that herbicide-resistant cultivars are slow accumulators of phenolic compounds, while cultivars sensitive to herbicides show rapid increase in concentration of these compounds.

In oilseed products, phenolic compounds occur as the hydroxylated derivatives of benzoic and cinnamic acids, coumarins, flavonoid compounds, and lignins (Ribereau-Gayon, 1972). Dabrowski and Sosulski (1984) reported the levels of total and esterified phenolic acids to be 81 and 73.9 mg/100 g, respectively, for dehulled defatted flaxseed meal. The major phenolic acids for dehulled defatted flaxseed meal were *trans*-ferulic (46%), *trans*-sinapic (36%), *p*-coumaric (7.5%), and *trans*-caffeic (6.5%) for both total and esterified phenolic acids. In comparison to other oilseeds, flaxseed has very low levels of bound phenolic acids at 7.2 mg/100 g and is also a rich source of ferulic acid (Dabrowski and Sosulski, 1984). In fact, ferulic acid constituted almost 50%, while chlorogenic acid comprised only 34.2%, of the total phenolics in the methanolic extract of defatted flaxseed meal (Harris and Haggerty, 1993).

Phenolic constituents may contribute to the dark color, bitter taste, and objectionable flavor of some oilseeds (Arai et al., 1966) and their products and as such are considered to be undesirable components. In an attempt to remove antinutritional compounds from flaxseed, Varga and Diosady (1994) found that extraction of the meal with methanol–ammonia reduced both soluble phenolic acid esters and insoluble (bound) phenolic acids by 20 and 29%, respectively, although the free phenolic acid remained unchanged. Varga and Diosady (1994) reported total phenolic acid contents of 442 and 355 mg/100 g for hexane-extracted and methanol–ammonia extracted flaxseed meals, respectively. Esterified (soluble phenolic acid esters) phenolic acids constituted 54%, while insoluble bound phenolics comprised only 26–29% of the total phenolics (Varga and Diosady, 1994).

Flax is among many plant materials studied for beneficial effects on health and as a neutraceutical (Haumann, 1993). It is a natural source of major plant food phytochemicals such as flavonoids, coumarins, lignans, and phenolic acids (Caragay, 1992). The demonstration of clinical activity associated with the consumption of flaxseed has stimulated interest in exploring phytochemicals present in the seed (Caragay, 1992; Harris and Haggerty, 1993; Thompson et al., 1991). Antioxidative properties of a number of fractions from ethanolic extracts of flaxseed have been suggested (Amarowicz et al., 1993; Amarowicz and Shahidi, 1994). Since phenolic compounds play such diverse and important roles in plant physiology and food chemistry, our investigation focused on determinants of variability of these compounds 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, Elrose and Melfort in Saskatchewan) during

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Table 1. Total, Esterified, and Etherified Phenolic Acids (Grams per Kilogram) in Flaxseed Cultivars[a]

	phenolic acids			
cultivar	total	esterified (1 M NaOH)	etherified (calcd)	Wilt reaction[b]
AC Emerson	9.92[b]	4.79[c]	5.13[a]	2.2
AC Linora	10.34[a]	5.38[a]	4.97[a]	2.3
Flanders	8.46[e]	5.12[b]	3.34[c]	3.4
Linola 947	7.89[g]	5.19[ab]	2.67[d]	3.7
McGregor	8.13[f]	5.42[a]	2.72[d]	3.2
NorLin	9.48[c]	5.21[ab]	4.27[b]	3.1
Somme	8.85[d]	5.42[a]	3.44[c]	3.3
Vimy	9.52[c]	5.30[ab]	4.22[b]	2.6

[a] Means within the same column followed by the same superscript are not significantly different ($P < 0.001$). [b] Wilt reaction based on a scale of 1–9 (1 = most vigorous, 9 = severely wilted) (*lsd = 0.3, P = 0.05*) (Prairie Registration Recommending Committee for Grain, 1994).

1991–1993 growing seasons according to procedures established by the Prairie Registration Recommending Committee for Grain (1992).

Whole flaxseed was ground in a coffee grinder to pass a 1 mm screen. The Prussian blue assay for the determination of total phenolics was carried out essentially as described by Price and Butler (1977). Briefly, ground flaxseed (100 mg) was extracted with absolute methanol (5 mL) for 30 min. An aliquot was microfuged at 13000 rpm for 10 min (Biofuge, Baxter Diagnostic Corp., Ontario, Canada). The supernatant was diluted 100 times with distilled water mixed with 0.1 M $FeCl_3$ in 0.1 N HCl (3 mL) for 3 min, followed by the timed addition of 0.008 M $K_3Fe(CN)_6$ (3 mL). The absorption was read after 10 min at 720 nm on a Spectronic 601 (Milton Roy Co., Rochester, NY) spectrophotometer. The ester-bound phenolic acids were released after alkaline hydrolysis (1 M NaOH) for 30 min at room temperature prior to the determination of phenolics as described above. The values obtained for the ester-bound phenolics were subtracted from the values for total phenolics to provide a measure of ether-bound phenolics (Provan et al., 1994). Phenolics obtained by this treatment were assumed to have been ether linked. In all cases, chlorogenic acid was used as a reference standard.

Protein content (N × 5.41) of defatted meal was determined according to 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).

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, and variance components were performed according to SAS methods (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

The relative amounts of the three classes of phenolic acids present in flaxseed differed significantly among cultivars (Table 1). Total phenolic acids ranged from 7.9 g/kg for the low linolenic yellow-seeded Linola 947 to 10.3 g/kg for the cultivar AC Linora. Esterified phenolic acid accounted for 48–66% of the total phenolic acids. Its concentration in the eight cultivars analyzed varied between 4.8 and 5.4 g/kg. Etherified phenolic acids have been suggested to be predominantly ferulic acids (Provan et al., 1994). Our data are comparable

Table 2. Analysis of Variance for Phenolic Acids of Flaxseed Grown at Four Locations for 3 Years

		mean squares[a] of phenolic acids		
source	df	total	esterified	etherified
location (L)	3	16.63	24.52	73.56
		(0)	(0.12)	(0.23)
cultivar (C)	7	17.72	1.10	22.15
		(0.20)	(0)	(0)
year (Y)	2	191.13	69.34	44.55
		(2.52)	(0.84)	(0)
L × C	21	10.14	4.91	15.91
		(0.29)	(0)	(0.41)
L × Y	6	23.79	17.22	65.62
		(1.07)	(0.79)	(2.94)
C × Y	14	11.92	6.11	20.53
		(0.44)	(0.02)	(0.83)
L × C × Y	41	8.51	3.56	13.69
		(4.17)	(1.95)	(6.68)
error	95	0.11	0.13	0.26
CV %		3.65	6.96	13.27

[a] All mean squares are significant at 0.0001 probability levels. Values in parentheses are variance components.

to those reported previously (Dabrowski and Sosulski, 1984; Varga and Diosady, 1994) for flaxseed. Varga and Diosady (1994) also reported that esterified phenolics of hexane-extracted and methanol–ammonia-extracted flaxseed meal constituted 54% of the total phenolic acid content.

To further elucidate the variability in flaxseed phenolics, environmental effects (year and location) were studied in combination with cultivars. The results of analysis of variance for phenolic acid contents of flaxseed grown at four locations and three years (Table 2) showed that the phenolics were dependent on cultivar, location, and year and their interactions. Seasonal effect (year) and cultivar × location × year interaction had a much larger relative contribution to the variation in total phenolic acids than cultivar or location. The variance components (Snedecor and Cochran, 1967) for year, cultivar, and location and location × cultivar × year interaction for total phenolic acids were 2.5, 0.2, 0, and 4.2, respectively. The variation in total phenolic acids was mainly due to seasonal effect and not to location effect. Location was also reported (Mueller et al., 1978) to have no significant effect on the sinapine content of eight rapeseed cultivars grown at four locations in western Canada.

The variation in esterified phenolics was mainly due to year, location, and their interactions and not to cultivar effect. The location × year and location × year × cultivar interactions play an important part in the overall variability of the esterified phenolics. Although location, year, cultivar, and their interactions were significant sources of variation for etherified phenolic acids (Table 2), only interaction effects contributed to the overall variability. Location does not play a significant role in the variability of the etherified phenolics since the variance (0.23) was generally smaller than that of the experimental error (with a variance component of 0.26).

Generally, flaxseed grown in Manitoba (Portage la Prairie and Brandon) had higher levels of total and etherified phenolic acids than that grown in Saskatchewan (Melfort and Elrose) (Figure 1). The amount esterified phenolics of flaxseed grown in Saskatchewan was 14% higher than that of flaxseed grown in Manitoba. The differences in phenolic acids at various locations could partly be due to differences in soil conditions. Portage la Prairie, Brandon, and Melfort

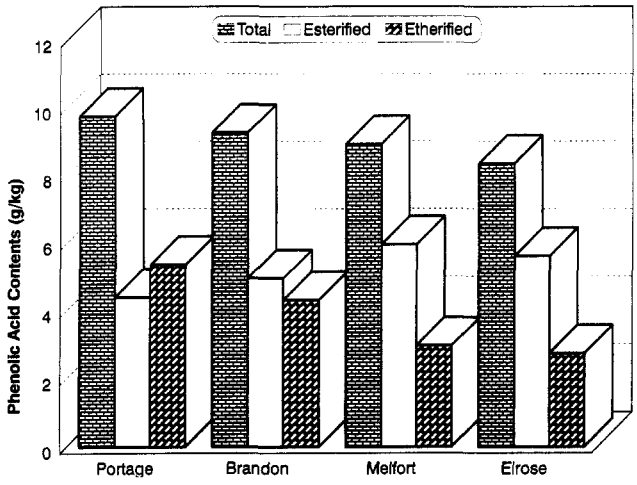

Figure 1. Effect of location on the phenolic acid contents of flaxseed.

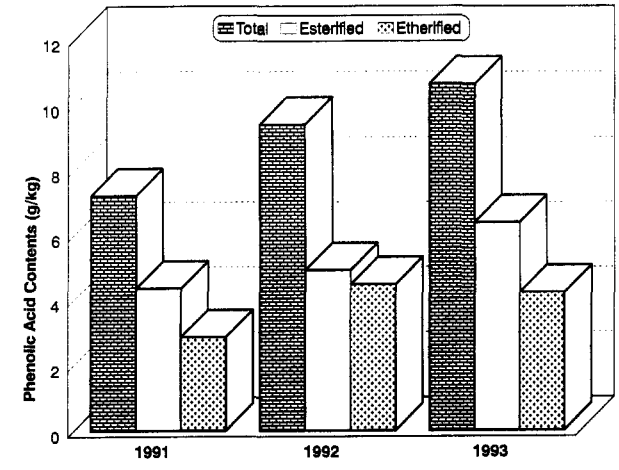

Figure 2. Phenolic acid contents of flaxseed grown in three different years.

are located in the black soil zone I, while Elrose is located in the brown soil zone II (Prairie Registration Recommending Committee for Grain, 1992).

Seasonal effects had a greater impact on phenolic acids of flaxseed than location (Figure 2). Flaxseed grown in 1993 had higher total and esterified phenolic acids than that grown in previous years. Flaxseed grown in 1991 accumulated the lowest concentration of total, esterified, and etherified phenolic acids compared to that grown in subsequent years, 1992 and 1993. Although the total phenolic acids differed significantly from year to year, the contribution of the esterified phenolics was fairly constant at 53–60% of the total.

The major differences between a traditional brown-seeded flax cultivar NorLin and a yellow-seeded cultivar Linola 947 (Table 1) were reflected in the concentrations of total and etherified phenolic acids, while those of the esterified phenolic acids were similar for both seed types. The low level of etherified phenolic acids in Linola 947 suggests that its hull characteristics have some influence on the simple phenolic compounds in seed. A similar observation was made in canola (Krygier et al., 1982): the yellow-seeded cultivar Sarson had lower levels of free and esterified phenolic acids than the regular brown-seeded types, cv. Tower and Candle. Thus, a relationship between the pigmented polyphenols of cv. NorLin and its phenolic acids is not unreasonable.

Comparison of total, esterified, and etherified phenolic acids with oil and protein contents of flaxseed (data for oil and protein not shown) showed poor correlation. The Pearson correlation coefficients of the total, esterified, and etherified phenolic acids were 0.083, 0.001, and 0.069 for oil and −0.270, −0.227, and −0.096 for protein contents, respectively (Table 3). The weak association between the phenolic acids and both protein and oil suggests that in flaxseed changes in phenolic acids should have very little effect on protein and oil contents. Nonsignificant correlation coefficients between esterified and total phenolic acids suggest that the concentrations of these phenolic acids are independent of one another. Esterified phenolic acids were negatively correlated with etherified phenolic acids; i.e., an increase in one leads to a decrease in the other. This analysis also showed a highly significant positive correlation ($r = 0.83$) between etherified and total phenolic acids content such that the cultivar with the highest total phenolic acid content showed the highest concentration of etherified phenolic acids.

Table 3. Correlation Coefficients for Esterified, Etherified, and Total Phenolic Acids of Flaxseed

	phenolic acids		
	esterified	etherified	total
oil	0.001	0.069	0.083
protein	−0.227[a]	−0.096	−0.270[b]
esterified phenolic acids		−0.547[c]	0.022
etherified phenolic acids			0.825[c]

[a] $P < 0.002$. [b] $P < 0.0002$ [c] $P < 0.0001$ ($n = 190$).

Cultivars with high concentrations of total and etherified phenolic acids were invariably those associated with high resistance to flax wilt, caused by *Fusarium oxysporum* f. sp. *lini* (Bolley) Snyd. & Hans. (Table 1). A multicomparison analysis of the data showed that resistance in flax to fusarium wilt is strongly correlated ($r = -0.91$, $P = 0.002$) with the concentrations of total and etherified phenolic acids in the seed. The poor correlation ($r = 0.30$, $P = 0.5$) between wilt reaction and the static level of esterified phenolic acids suggests that the latter is not involved in the expression of resistance. The high total and etherified phenolic acids in resistant flax cultivars may possibly be acting as antifungal agents or may be functioning as part of an integrated defense mechanism against wilt by providing phenolic structural barriers leading to the actual process of lignification (Friend, 1985; Bennett and Wallsgrove, 1994). A similar observation was made by Nazeem et al. (1984), who found that cultivars resistant to flax rust caused by *Melampsora lini* (Pers.) Lev. had higher concentrations of phenolic compounds than susceptible ones. Susceptibility to maize weevil (Arnason et al., 1994) was also found to be negatively correlated with phenolic acid content.

The data presented indicate that flax cultivars differ in their content of phenolic acids. The findings suggest that it might be possible to breed flax cultivars low in phenolic acids content for the increased utilization of flaxseed in food and feed markets. On the other hand, cultivars with high levels of phenolic acids content might be sought for (a) their increased production of allelochemicals and hence increased tolerance to weeds, thereby decreasing the demand for use of often toxic herbicides, and (b) for their resistance to fusarium wilt and possibly other diseases. Cultivars with high levels of phenolic acids may be needed for their greater mechanical resistance to stored products insects (i.e. increased storability of the seed) and for fractionation of such compounds for the neutraceutical industry.

Further, it appears that modifications to the phenolic content of flaxseed could be accomplished without detrimental effects on the levels of oil and protein in the seed.

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