# Variation in the Composition of Water-Soluble Polysaccharides in Flaxseed

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One hundred and nine accessions of flaxseed (*Linum usitatissimum* L.) from twelve geographical regions and including oil, fiber, and brown- and yellow-seeded types were used to determine the composition of water-soluble polysaccharides, total carbohydrates, protein, and oil. The content of water-soluble polysaccharides ranged from 3.6 to 8.0%. Neutral monosaccharides of the water-soluble polysaccharide fraction consisted mainly of glucose, xylose, galactose, and rhamnose. The latter varied 3-fold from 8 to 24% of total monosaccharides, although 92% of the accessions had rhamnose values between 10 and 20%. Fucose was a minor component of the water-soluble polysaccharide fraction and displayed the highest coefficient of variation. Xylose was the second largest carbohydrate component, and its mean values were highly correlated with cumulative frequency. Variation of galactose content of the water-soluble polysaccharide fraction of flaxseed from all accessions was normally distributed. Glucose concentration ranged from 21 to 40% and represented the major neutral monosaccharide in flaxseed. The ratio of rhamnose to xylose, an indication of the ratio of acidic to neutral polysaccharides, ranged from 0.3 to 2.2. Carbohydrate yield and sugar contents of the water-soluble polysaccharide fraction of flaxseed accessions were independent of protein and oil content. Flaxseed accessions, when grouped by geographical region or type, can be differentiated by principal component analysis.

**Keywords:** *Flaxseed; carbohydrates; gums; principal components analysis; variability; linseed; Linum usitatissimum*

## INTRODUCTION

Flaxseed gum comprises about 8% of the seed weight (BeMiller, 1973); however, its yield is dependent on extraction regimes (Cui et al., 1994; Fedeniuk and Biliaderis, 1994; Mazza and Biliaderis, 1989) and can range from 3.5 to 9.4%. The gum content in flaxseed also showed varietal and regional differences (Schuster et al., 1978). Differences in monosaccharide composition of flaxseed have been attributed to differences in cultivars (Wannerberger et al., 1991; Mazza and Biliaderis, 1989). Thus, an in-depth analysis of five cultivars by Wannerberger et al. (1991) showed that flaxseed polysaccharides are composed mainly of galacturonic acid (21–36%), xylose (19–38%), rhamnose plus fucose (11–16%), galactose (12–16%), arabinose (8–13%), and only small amounts of glucose (4–6%). The ratio of arabinose to xylose, according to these authors, ranged from 0.32 to 0.57. The presence of xylose and fucose in the ratio of 12.5:1 in flaxseed mucilage was reported by Heinze and Amelunxen (1985). According to these authors, xylose was the major neutral monosaccharide (≈41%), followed by arabinose (18.8%), rhamnose and galactose at 11%, and glucose and fucose levels of 3.9 and 3.5%, respectively. Although the relative monosaccharide composition varies with extracting conditions, galacturonic acid, galactose, xylose, and rhamnose are major monosaccharides, while fucose, arabinose, and glucose are minor constituents (Fedeniuk and Biliaderis, 1994; Muralikrishna et al., 1987).

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Flax is one of the crops targeted for genetic manipulation for seed oil (Green, 1986; Rowland and Bhatty, 1990) and nutritional therapeutics (Haumann, 1993). Two recent patents (O'Mullane and Hayter, 1993; Attström et al., 1993) have been issued for the use of flaxseed gum for cosmetic, therapeutic, and medical preparations. One method of increasing the concentration of gum and phytochemicals in flaxseed is through plant breeding. A requirement for targeted breeding, however, is quantitative and qualitative information on the distribution and variation of component phytochemicals in existing flax accessions or cultivars. This paper aims at providing new information on the carbohydrate composition of a large set of flaxseed accessions.

## EXPERIMENTAL PROCEDURES

One hundred and nine accessions of *Linum usitatissimum* seed samples were selected at random from the world collection comprising 3600 accessions maintained at the Agriculture and Agri-Food Canada Research Centre, Morden, MB. These accessions originated from the following geographical regions: Argentina (19), Australia (4), Canada (16), China (1), eastern Europe (9), Europe (17), India (1), Middle East (2), United States (4), and the former Soviet Union (5). Seventeen fiber and 14 yellow-seeded types were included in the analysis.

Water-soluble polysaccharides were extracted by mixing the whole seed with potassium phosphate buffer (pH 7.0) (1:13 w/v) for 2 h at 85 °C. Carbohydrate yields were those obtained using the phenol–sulfuric acid method (Dubois et al., 1956) with rhamnose as standard and expressed as percentage of seed weight. For analysis of component sugars, polysaccharides were hydrolyzed in a boiling water bath for 2 h in 2.0 M $H_2SO_4$. The neutral sugars in the hydrolysate were converted into the corresponding alditol acetates and then analyzed by gas–liquid chromatography following the procedure of Englyst et al. (1982) on a glass capillary column (DB-225, 30 × 0.25

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**Table 1. Composition of Water-Soluble Polysaccharides (Relative Percent) in Flaxseed**

| component | minimum | maximum | mean | SD[a] | CV[b] |
|---|---|---|---|---|---|
| carbohydrate content | 3.6 | 8.0 | 6.2 | 0.8 | 12.4 |
| L-rhamnose[c] | 8.2 | 23.8 | 14.2 | 3.1 | 21.4 |
| L-fucose[c] | 1.9 | 9.1 | 3.5 | 0.9 | 25.2 |
| L-arabinose[c] | 3.2 | 15.4 | 10.6 | 2.0 | 18.6 |
| D-xylose[c] | 10.9 | 32.0 | 23.2 | 4.4 | 18.8 |
| D-galactose[c] | 12.7 | 26.9 | 19.1 | 2.7 | 14.0 |
| D-glucose[c] | 21.3 | 40.0 | 28.9 | 3.6 | 12.5 |
| rhamnose/xylose ratio | 0.3 | 2.2 | 0.7 | 0.3 | 44.9 |
| arabinose/xylose ratio | 0.1 | 0.9 | 0.5 | 0.1 | 22.5 |

[a] Standard deviation. [b] Coefficient of variation in percent. [c] Neutral sugars are those from nonpectic polysaccharides.

mm i.d.) with allose as internal standard. Separation of the sugars was performed in a Model 3500 Varian gas chromatograph equipped with a flame ionization detector (injector and detector temperatures of 250 °C, column temperature 185–215 °C at 5 °C/min). Response factors were calculated for each sugar with authentic samples. Constituent sugars obtained under the above conditions are only relative values and are reported as such.

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 analyzer (Robertson and Morrison, 1979). The SAS system (SAS Institute, 1990) was used to calculate Pearson's correlation coefficients between carbohydrate yield, carbohydrate ratios, monosaccharide concentration, and protein and oil contents. A data matrix consisting of the 109 accessions and nine carbohydrate parameters (carbohydrate yield, arabinose/xylose ratio, rhamnose/xylose ratio, and monosaccharide contents) for each accession was analyzed using the principal component analysis (PRINCOMP) procedure of SAS (SAS Institute, 1990).

## RESULTS AND DISCUSSION

Mean content of water-soluble polysaccharides in 109 accessions ranged from 3.6 to 8% with an overall mean of 6.2% (Table 1). The distribution of carbohydrate showed a negatively skewed population (Figure 1b) with 73.4% ($\mu + \sigma = 45.9\%$ and $\mu - \sigma = 27.5\%$, where $\mu$ and $\sigma$ are mean and standard deviation, respectively) of the total included within one standard deviation of the mean. Only six accessions (427-2, ACC410, and Rarcagu from Argentina, Ungavin and CI-2785 from Australia, and Natasja, a fiber type) had water-soluble carbohydrate content equal to or above 7.5%. Accessions 2308 and 92-5103 from eastern Europe and China, respectively, had the lowest carbohydrate yields of 3.6 and 4.4%, respectively, among the 109 accessions. Three European accessions (Nivale, Ocean, and Pro 3243) and Andro, a Canadian cultivar, had carbohydrate yields below 5%. A carbohydrate yield of 4% was reported for 23 flaxseed cultivars (including both oil and fiber types) grown at one location, Svalov, Sweden, in 1988 (Wannerberger et al., 1991). The carbohydrate yield from flaxseed reported previously (Muralikrishna et al., 1987) falls within the values observed in this study, although variation in carbohydrate yield depends on extraction procedures (Mazza and Biliaderis, 1989).

Neutral sugar fraction in flaxseed was composed mainly of glucose, xylose, galactose, and rhamnose

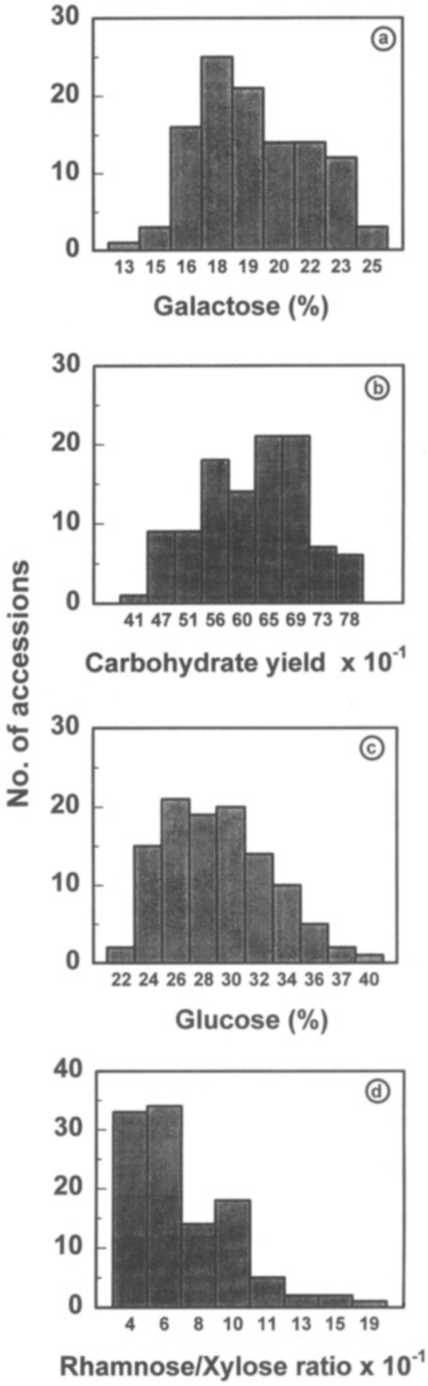


**Figure 1.** Frequency distributions of (a) galactose, (b) carbohydrate yield, (c) glucose, and (d) rhamnose/xylose ratio of 109 flaxseed accessions.

(Table 1). Rhamnose means varied almost 3-fold from 8 to 24% with an overall mean of 14.2%, resulting in a high coefficient of variation (Table 1). This sugar exhibited an asymmetric distribution opposite to that of the carbohydrate yield with 73.4% ($\mu + \sigma = 27.5\%$ and $\mu - \sigma = 45.9\%$) included within one standard deviation of the mean (Figure 2a). Almost two-thirds (62.5%) of the accessions had rhamnose contents within the $\mu - \sigma$ (11.1–14.2%). Only three accessions (Royale, Nike, and Reina, the last two are fiber types) had rhamnose contents lower than 10%. Five cultivars (Andro, Bison, Foster, Linott, and Omega) had rhamnose contents higher than 20%. A majority of the accessions (92%) had rhamnose values between 10 and 20%.

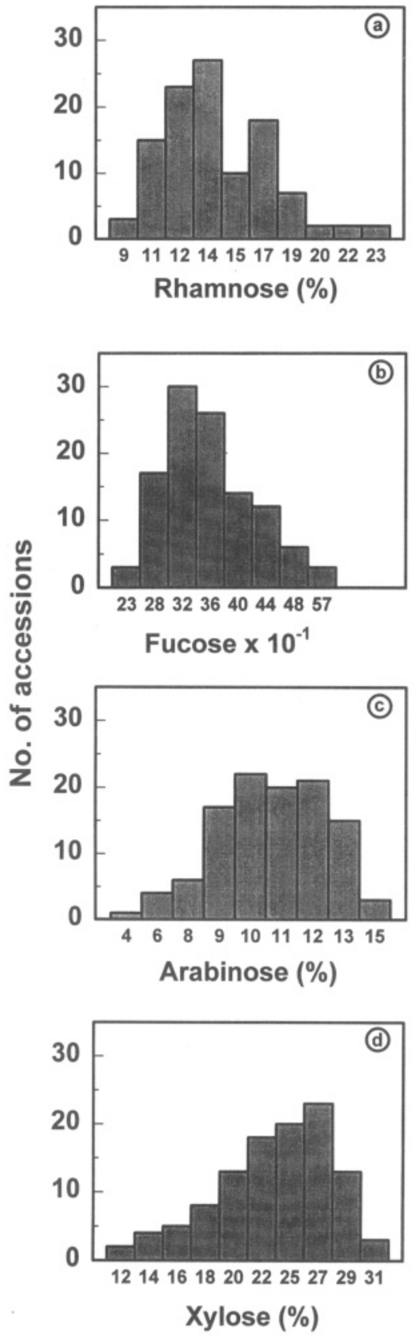


**Figure 2.** Frequency distributions of (a) rhamnose, (b) fucose, (c) arabinose, and (d) xylose of 109 flaxseed accessions.

Fucose was a minor carbohydrate component with an overall mean of 3.5% (Table 1). However, it had the highest coefficient of variation (25%) of the monosaccharides due to its larger than 4-fold variation between the minima and maxima. The mean fucose content of flaxseed is consistent with those reported by Fedeniuk and Biliaderis (1994) for flaxseed meal. A similar variation (CV = 25%) was reported by Bhatty and Cherdkiatgumchai (1990) for flaxseed meals. The distribution of fucose in flaxseed was positively skewed (Figure 2b), with 92 accessions (84.4% of the population) ($\mu + \sigma = 28.4\%$ and $\mu - \sigma = 56\%$) within one standard deviation of the mean. Two-thirds of the population was in the $\mu - \sigma$ region. Three accessions (Barbara, Foster, and Marine) had fucose contents below 2.5%, while three Argentinian accessions (427-2, ACC 409-2, and ACC 412-2) had the highest fucose levels above 6%. One hundred and two of the 109 accessions (i.e. 93.6% of the population) had fucose values between 2.5 and 6.0% (Figure 2b).

Arabinose content of flaxseed accessions varied more than 4-fold, similar to fucose content, with an overall mean value of 10.6% (Table 1). The mean value for arabinose is consistent with those reported in the literature (Wannerberger et al., 1991; Fedeniuk and Biliaderis, 1994). The distribution of arabinose (Figure 2c) showed four accessions at a mean value of 10.6% and 81 accessions (74.3%) within one standard deviation of the mean ($\mu + \sigma = 35.8\%$ and $\mu - \sigma = 34.9\%$). Szegedi 62, a Hungarian accession, had the lowest arabinose content (3.2%), contrary to the high (12%) level reported previously (Wannerberger et al., 1991). Three accessions (92-5103, Consea, and Nivale) had arabinose levels above 14%. Ninety-eight of the 109 accessions (90%) had arabinose contents between 7.5 and 13%.

The second largest carbohydrate component in flaxseed, xylose, ranged from 10.9 to 32% with an overall mean value of 23.2% (Table 1). These values are similar to those reported previously (Wannerberger et al., 1991; Fedeniuk and Biliaderis, 1994). Seventy-six accessions (69.7%) had mean values between $\mu + \sigma$ (41.3%) and $\mu - \sigma$ (28.4%), thereby skewing the distribution of xylose (Figure 2d). The cumulative frequency was correlated ($r = 0.97$) with the mean xylose values; i.e. the number of accessions increased linearly with increase in mean xylose values, producing the stepwise increase depicted in Figure 2d. An equal number of accessions had values between $\mu + 2\sigma$ (14.7%) and $\mu - 2\sigma$ (13.8%). Six accessions (22-87-2159, 2562, Atlante, Foster, K6189, and Pro 3243), mostly of European origin, had xylose levels below 15%, while only two accessions (Szegedi 62 and Tam 201) had xylose contents above 30%. Wannerberger et al. (1991) reported a xylose content of 38% for Szegedi 62.

Galactose content ranged from 13 to 27% with an overall mean of 19.1% (Table 1) and was comparable to values obtained by Fedeniuk and Biliaderis (1994), Wannerberger et al. (1991), and Muralikrishna et al. (1987). Variation of the galactose content of flaxseed from all accessions showed normal distribution (Figure 1a). Among 109 accessions, 70.6% had a mean galactose content of 19.1% ($\mu + \sigma = 33.9\%$ and $\mu - \sigma = 36.7\%$) with almost equal distribution on both sides of the mean value. Four European accessions (Linusit Gold, Pro 9515, Royal, and Szegedi 62) had galactose levels below 15%, while three accessions (22-87-2159, 2562, and Foster) had galactose contents above 25%. One hundred accessions (91.7%) had galactose contents between 16 and 25%.

Glucose was the major monosaccharide in flaxseed. Its concentration ranged from 21 to 40% with an overall mean of 28.9% (Table 1). The high concentration of glucose is attributed to the use of the phosphate buffer (pH 7.0) for the extraction of the carbohydrates from flaxseed. Extraction with water resulted in glucose levels comparable to those reported by Wannerberger et al. (1991), Fedeniuk and Biliaderis (1994), and Muralikrishna et al. (1987). This suggests the use of the buffer led to preferential extraction of another glucose-containing component and, as a result, to the release of larger amounts of glucose after hydrolysis. The distribution of glucose was asymmetric (Figure 1c) with 68 accessions (62.4%) within one deviation of the mean ($\mu + \sigma = 27.5\%$ and $\mu - \sigma = 34.9\%$). Eighteen accessions (16.5%) were in the range $\mu - 2\sigma$, the sample

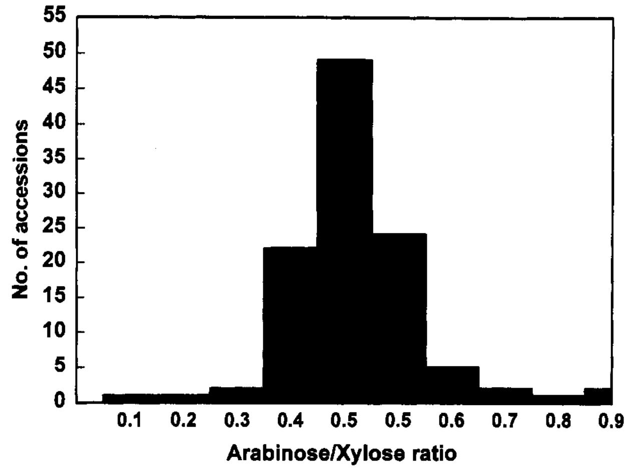


**Figure 3.** Variation of arabinose/xylose ratio in 109 flaxseed accessions.

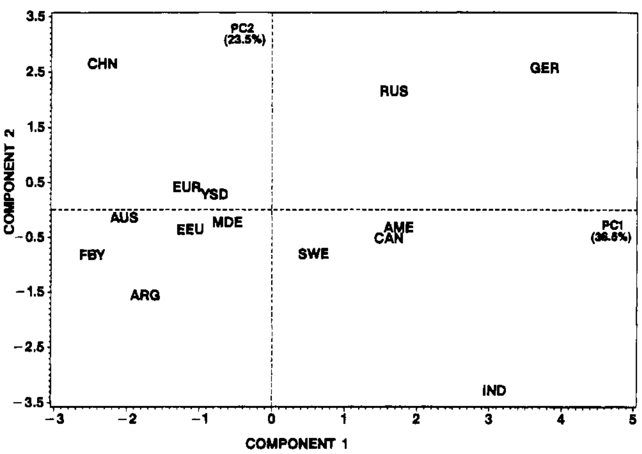


**Figure 4.** Classification of accessions grouped by geographical origin and type according to principal components 1 and 2. The codes for the origin of the accessions are as follows: AME, America; ARG, Argentina; AUS, Australia; CAN, Canada; CHN, China; EEU, eastern Europe; EUR, Europe; FBY, fiber-type flax; GER, Germany; IND, India; RUS, former Soviet Union; SWE, Sweden; YSD, yellow-seeded type.

mean minus twice the standard deviation, while only eight accessions (7.3%) were in the $\mu + 2\sigma$ range. Thus, the distribution of glucose in flaxseed accessions seems to be positively skewed. Two accessions (2308 and Consea) had the minimum glucose content of less than 23%, while four accessions (Pro 3243 and Pro 9515, both from Europe, and 22-87-2159 and Atlante) had glucose levels above 36%. Eighty-eight of the 109 accessions (80.7%) had glucose levels between 25 and 35%.

The ratio of rhamnose to xylose, indicative of the ratio of acidic to neutral polysaccharides (Fedeniuk and Biliaderis, 1994), reflects the viscous behavior of flaxseed gum. The rhamnose to xylose ratio ranged from 0.3 to 2.2 with an overall mean of 0.7 (Table 1). It had the highest variation (CV = 45%) between the minima and maxima (>7-fold). The high degree of variation suggests that the gum from flaxseed accessions has a very diverse characteristic covering the spectrum from acidic to neutral polysaccharide types. A similarly high coefficient of variation (39.9%) was obtained from the data of the five cultivars reported by Wannerberger et al. (1991). The rhamnose to xylose ratio of the two water-soluble fractions from Linott (designated CP and CS; Fedeniuk and Biliaderis, 1994) was comparable to the minimum and maximum ratios (Table 1) of the 109 accessions. The variation in the rhamnose to xylose

**Table 2. Loading Scores for Principal Component Analysis for Flax Population**

| | component | | | |
|---|---|---|---|---|
| trait | 1 | 2 | 3 | 4 |
| L-rhamnose | 0.822 | 0.169 | 0.184 | −0.371 |
| L-fucose | 0.590 | −0.647 | −0.181 | −0.160 |
| L-arabinose | −0.843 | 0.476 | 0.076 | −0.163 |
| D-xylose | −0.835 | −0.459 | −0.212 | −0.106 |
| D-galactose | 0.931 | −0.070 | 0.095 | 0.089 |
| D-glucose | 0.206 | 0.394 | 0.043 | 0.765 |
| (arabinose/xylose) ratio | −0.288 | 0.907 | 0.277 | −0.047 |
| (rhamnose/xylose) ratio | 0.869 | 0.378 | 0.255 | −0.042 |
| carbohydrate yield | −0.039 | −0.288 | −0.061 | 0.779 |
| eigenvalue | 4.29 | 2.37 | 1.87 | 1.40 |
| cumulative % of variance | 38.96 | 60.48 | 77.45 | 90.24 |

ratio (Figure 1d) did not show a normal distribution, with 84.4% of the accessions ($\mu + \sigma$ = 23.9% and $\mu - \sigma$ = 60.6%) within one standard deviation of the mean. Two cultivars (Reina and Royal) had the lowest rhamnose to xylose ratios. Accessions with a ratio of 1.0 or close to it were 2562, ACC 409, Dufferin, K6189, K6201, Kubensk, Ocean, and Pro 3243. The accessions with the highest rhamnose to xylose ratios of 2.2, 1.6, and 1.5 were Foster, 22-87-2159, and Omega, respectively.

The ratio of arabinose to xylose ranged from 0.1 to 0.9 with a mean of 0.5 (Table 1). A variation of 0.32–0.57 for five cultivars was reported by Wannerberger et al. (1991), which led them to suggest that a minor decrease in branching is encountered with increasing xylose content. The distribution of arabinose to xylose ratio (Figure 3) showed four accessions at the mean value of 0.47 and 90 accessions (82.6%) within one standard deviation of the mean ($\mu + \sigma$ = 27.5% and $\mu - \sigma$ = 51.4%). Szegedi 62, the accession with the lowest arabinose content, also had the lowest arabinose/xylose ratio of 0.10. Accessions Tam 201 and Raipur also had low arabinose/xylose ratios of 0.18 and 0.27, respectively. Two accessions, Foster and 2562, both had the highest ratio of 0.87.

Comparison of the nine carbohydrate parameters [monosaccharide concentration (rhamnose, fucose, arabinose, xylose, galactose, glucose), rhamnose/xylose ratio, arabinose/xylose ratio, and carbohydrate yield] with protein and oil contents of the 109 accessions (data not shown) showed poor correlation. The Pearson correlation coefficient of the carbohydrate parameters ranged from −0.004 to 0.145 and from −0.041 to −0.289 for protein and oil, respectively. The weak association between the carbohydrate parameters and both protein and oil contents suggests that, in flaxseed, changes in carbohydrates should have little effect on protein and oil content.

Principal component analysis was performed on the carbohydrate parameters of the 109 accessions. When the nine carbohydrate parameters for each accession were entered in the PCA program, four factors were returned that met the criteria of their eigenvalue exceeding 1.0 (Table 2). It is customary to consider only those components that have an eigenvalue of 1.0 or greater for selecting principal component. The four components accounted for 90% of the total variance.

The first component had large positive loadings for galactose, rhamnose, and rhamnose/xylose ratio (Table 2) and negative loadings for arabinose and xylose. The correlation with galactose (0.931) is especially high. The second and third components were primarily concerned with arabinose/xylose ratio, 0.901 and 0.277, respectively. In addition, the third component was also related to the rhamnose/xylose ratio, while parameters contributing to the fourth component were carbohydrate yield and glucose content.

The scatterplot of the first two principal components (Figure 4) distinguishes the accessions when grouped by geographical areas of origin or type of flax. Accessions from North America (Canada and the United States), those originating from eastern Europe, and yellow-seeded types were not unambiguously discriminated. Since the first component is related to galactose and rhamnose and the second component to arabinose/xylose ratio, i.e. arabinoxylan, the PCA plot discriminates the accessions by the acid to neutral ratio of their flaxseed gum (Cui et al., 1994). As an example, the accession from China had a high arabinose xylose ratio (0.62), while that from India had a low ratio (0.27). In Figure 4, these accessions are located diagonally from each other. The plot of the first and fourth components (figure not shown) was the only one to clearly distinguish the accession by place of origin or type of flax. This suggests that flax accessions when grouped by place of origin or type can be distinguished from their carbohydrate composition and yield. Plots of the other components showed no patterns of interest.

From this study, it is evident that considerable and significant variations exist in monosaccharide composition, carbohydrate yield, and quality (i.e. arabinose/xylose and rhamnose/xylose ratios) among accessions from the world collection of flaxseed. By careful selection of seed whose carbohydrate composition is known, the breeder will have a greater probability of obtaining the desired carbohydrate qualities without sacrificing protein or oil content.

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