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# Phytochemicals and Dietary Fiber Components in Rye Varieties in the HEALTHGRAIN Diversity Screen

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Ten rye varieties grown in one location were analyzed for their contents of dietary fiber (arabinoxylan and $\beta$-glucan) and phytochemicals (folate, tocols, phenolic acids, alkylresorcinols, and sterols). The varieties included old and modern varieties from five European countries. Significant differences were observed in the contents of all phytochemicals in whole grains and in the fiber contents in the flour and bran. The old French varieties Haute Loire and Queyras had high contents of most phytochemicals, whereas the Polish varieties Dankowskie-Zlote and Warko were relatively poor in phytochemicals. The varieties with a high content of folate tended to have low alkylresorcinol contents and vice versa. Furthermore, high contents of arabinoxylans were associated with high contents in tocols and sterols. The 10 selected rye samples comprising old populations and old and modern varieties from different ecological regions of Europe demonstrate high natural variation in their composition and show that landraces and old populations are useful genetic resources for plant breeding. The contents of single phytochemicals can likely be affected by breeding, and they may be adjusted by the right selection of genotype.

**KEYWORDS: Phytochemical; rye; *Secale cereale* L.; genetic variation; old genetic resources; dietary fiber; arabinoxylan; folate; tocopherol; tocotrienol; alkylresorcinol; phenolic acid; plant sterol**

## INTRODUCTION

On a worldwide basis, rye is the eighth most important cereal crop, after millet, oat, sorghum, barley, rice, wheat, and maize (in increasing order of production) (*1*). It is an especially important cereal crop in the eastern and northern European countries. From an agronomic point of view, rye is the most widely adapted of the cereals because of its extreme winter hardiness and ability to grow on very marginal soils (*2*). Typical food applications of rye are the production of bakery products, breakfast cereals, and alcoholic beverages (*3*). Rye is also a recognized source of dietary fiber, vitamins, and minor phytochemicals with positive health attributes.

The most important dietary fiber components in rye are arabinoxylans (AX), representing the largest part of the building blocks of cell walls (*3*). In general, rye has the highest level of AX compared to other cereals (*4*). AX have received an increasing amount of attention as dietary fiber, as they may promote health by reducing the risk of different cancers, coronary heart disease, and diabetes (*5, 6*). In addition, they are a major determinant of the functionality of cereals in biotechnological processes and applications. It is generally acknowledged that the behavior of rye in breadmaking can be attributed mainly to the high water-binding capacity of the AX,

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which affects water partitioning in dough, and to the high viscosity of the water extractable AX (WE-AX) fraction, which strongly influences gas retention and loaf volume (*3*). Rye AX comprise a backbone of β-1,4-linked D-xylopyranosyl residues substituted at O-2 and/or O-3 with α-L-arabinofuranosyl residues. The structure is similar to that of wheat AX (*7*), but differences in the occurrence of the differently bound arabinofuranosyl residues are observed (*3*). Some arabinofuranosyl residues are substituted at the O-5 position with ferulic acid moieties.

The second fraction of rye dietary fiber is mixed-linkage β-glucans, which have also been ascribed health-promoting properties. As in barley and oat, the rye β-glucans are linear homopolysaccharides of glucose residues linked by β-(1→4)- and β-(1→3)-linkages, although the ratio of cellotriosyl to cellotetrasyl units may be somewhat lower in rye β-glucans (*8*).

Tocopherols and tocotrienols (i.e., tocols) are vitamin E active compounds that each occur as four vitamers. Current recommendations consider 2*R*-α-tocopherol as the only compound that meets the vitamin E requirement (*9*). Nevertheless, all tocols are considered as good lipid-soluble antioxidants. Although cereals are only moderate sources of α-tocopherol, they are good sources of other tocols and especially of tocotrienols (*10*). This is important because tocotrienols are known to possess other potential health benefits including inhibition of cholesterol synthesis, neuroprotection, and anticancer properties (*11, 12*).

Interest in folates has been stimulated by recent research on the association of insufficient or suboptimal folate intake with the risk of several important diseases. Sufficient folate intake prevents neural tube defects in babies while suboptimal folate intake is known to be associated with an increased risk of cardiovascular diseases, stroke, and colorectal cancer and may also be associated with dementia and Alzheimer's disease (*13*−*15*). Cereals are major sources of folate. For example, in a Finnish study cereal products accounted for 36% of the daily dietary folate intake for women and 43% of that for men, respectively (*16*). The main single source in Finland is rye because rye is mainly consumed as wholegrain bread (*17*).

Plant sterols (phytosterols) are secondary plant metabolites with a structure closely similar to that of cholesterol. They are currently of interest primarily in relation to their health-promoting properties. Plant sterols are added to functional foods to aid in decreasing serum cholesterol levels, and a daily dose of 1−3 g has been shown to significantly reduce both the total and LDL-cholesterol levels in humans. However, recent studies have shown that natural intakes of dietary plant sterols can also have a positive effect on serum cholesterol levels, and hence, an increasing amount of research is being carried out on plant sterols from nonenriched sources (*18*). Cereals are the main sources of plant sterols together with vegetable oils, contributing up to 40% of daily intake of plant sterols (*19*).

Alkylresorcinols (ARs), 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring, represent one of the major groups of phenolic compounds in wheat and rye. The alkyl chain is 15−25 carbon atoms long and mainly saturated, but some alkylresorcinols have a modified alkyl chain that can be mono-, di-, and triunsaturated or may have a hydroxyl or keto group substituted on the alkyl chain. Alkylresorcinols are present in high amounts in the outer layers of wheat and rye and low levels in barley, but not in other foods. They have therefore been used as biomarkers for the human intake of wholegrain wheat and rye food products (*20*). They may also have other biological and physiological effects, for example, antimicrobial properties and effects on biological membranes, which make them interesting compounds from a nutritional point of view (*20*).

Phenolic compounds are recognized to possess beneficial effects as potent antioxidants due to their ability to scavenge free radicals (*21, 22*). In addition, consumption of a high level of phenolic compounds has been associated with a reduced risk of certain cancers and cardiovascular diseases (*23, 24*). These benefits are derived from the consumption of whole grains, fruits, and vegetables. Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. Hydroxycinnamic acids are more common than hydroxybenzoic acids and consist chiefly of *p*-coumaric, caffeic, ferulic, and sinapic acids. The phenolic acids in cereals are found in both the free and bound (ester bonded) forms, with the majority existing in the insoluble bound form. In the majority of cases ferulic acid is the most abundant (*25, 26*). In rye grain the major phenolic acids are ferulic acid, sinapic acid, and *p*-coumaric acid (*27*).

Systematic studies of the diversity of phytochemicals and dietary fiber composition in cereals are scarce. The HEALTHGRAIN project (http://www.healthgrain.org) focuses on improving health benefits of cereal foods (*28*) and has therefore carried out an extensive diversity screen to determine the extent of variation in these components in a wide range of cereal varieties. The data from this study can be used to select varieties with high levels of specific fiber and phytochemical components, either for cultivation or as sources of variation to develop novel varieties with enhanced health benefits. In this paper we compare the contents of phytochemical and fiber components of 10 rye varieties grown at the same location.

## MATERIALS AND METHODS

**Samples.** Ten different rye varieties and populations were chosen for the study. The older populations, Portugaise-3 and Portugaise-6 from Poland and Haute Loire, Grandrieu, and Queyras from France, are genetically more heterogeneous than modern varieties or hybrids. Nikita and Rekrut are modern high-yielding German varieties, and Warko is a modern Polish cultivar. Dankowskie-Zlote is an old Polish cultivar with wide adaptability and Lovaszpatonai-1 an old, stable Hungarian cultivar. For the sake of clarity the term variety is used for all samples, although the old populations (Haute Loire, Grandrieu, Queyras, Portugaise-3, and Portugaise-6) are not commercial varieties. All rye varieties were grown in the field at Martonvasar, Hungary, in 2005. The plots were 2 m long, with six rows spaced at a distance of 20 cm. The soil was of chernozem type with a loam texture and pH 6.8−7.2. The previous crop was peas. The weather was of continental type with a rainy harvest period. The samples were milled as described in detail by Ward et al. (*29*). Briefly, samples were conditioned to 14% moisture content before milling. Milling was carried out using a Perten Laboratory Mill 3100 (with 0.5 mm sieve). Flour and bran were produced with a Chopin CD1 Laboratory Mill. After milling, the samples were immediately cooled to −20 °C to protect bioactive components from heat damage. Samples were stored in sealed plastic bags until further analysis. A winter wheat sample (MV-Emese) was used as a reference sample in phytochemical analyses in addition to the in-house reference samples. Furthermore, a 10% difference was accepted between sample duplicates.

**Analytical Methods.** *Basic Composition.* Estimated dietary fiber in wholemeal samples was measured with an indirect method by difference from the analyses of moisture, protein, ash, lipids, available starch, and free sugars. Moisture, crude protein, and ash contents of wholemeal were determined according to AACC approved standard methods 44-15A, 46-10, and 08-01, respectively (*30*). Total lipids were analyzed gravimetrically by extraction with acidic solvent consisting of 60:40:1 (v/v/v) chloroform/methanol/hydrochloric acid as described by Marchello et al. (*31*). Available starch was determined with the procedure of Megazyme (Bray, Ireland), consistent with the AACC approved method 76-13, whereas free sugar was analyzed by a GC

procedure as a sum of all mono- and disaccharides, that is, fructose, glucose, maltose, and sucrose (*32*). Klason lignin was analyzed gravimetrically with the AACC method 32-25, whereas viscosity was measured in the grain water extract as described by Boros et al. (*33*) and Saulnier et al. (*34*) using a Brookfield Cone/Plate Digital Viscometer, model LVDV-II+ (Stoughton, MA), with an 0.8° cone spindle and shear rate of 225 s at 25 °C.

*Arabinoxylan.* The total arabinoxylan (TOT-AX) and water-extractable arabinoxylan (WE-AX) contents of the rye flour and bran samples were determined by acid hydrolysis of the whole sample and the water-extractable fraction of the sample, respectively, followed by derivatization of the released monosaccharides to alditol acetates and quantification by GC. The content of TOT-AX in MV-Emese reference sample was 2.06 ± 0.05% for flour and 18.46 ± 0.31% for bran ($n = 3$). The method is described in detail by Gebruers et al. (*7*).

*Mixed-Linkage β-Glucans.* The mixed-linkage β-glucan content in the wholemeal samples was determined using the Megazyme mixed linkage β-glucan assay kit, as described by Gebruers et al. (*7*) for wheat. The assay is based on the enzymatic degradation of glucans with lichenase and β-glucosidase, and the quantification of the released glucose using an oxidase/peroxidase reagent. The mixed-linkage β-glucan content of MV-Emese was 0.68% ($n = 2$).

*Tocols.* α-, β-, γ-, and δ-tocopherols and tocotrienols were determined by normal-phase HPLC using fluorescent detection. Wholemeal samples of 0.5 g were subjected to hot saponification, and the tocols were solvent extracted prior to HPLC analysis (*35, 36*). A rapeseed oil sample was analyzed in each analytical batch in addition to the reference sample MV-Emese to verify the performance of the method. The content of total tocols in MV-Emese was 33.8 ± 2 μg/g of fw ($n = 63$). Details of the analytical method and its performance during the study are reported by Lampi et al. (*36*).

*Folate.* Total folate contents were determined microbiologically on 96-well microtiter plates using *Lactobacillus rhamnosus* ATCC 7469 as the test organism (*37, 38*). The sample preparation procedure included heat extraction of 1 g wholemeal samples followed by trienzyme treatment using first α-amylase and chicken pancreas conjugase and then protease. A certified reference material and the reference sample (MV-Emese) were used to confirm the quality of the analysis as reported by Piironen et al. (*37*). The total folate content of MV-Emese reference sample was 475 ± 40 ng/g of dm ($n = 40$).

*Plant Sterols.* Plant sterols were determined by GC as described in detail by Piironen et al. (*39*) and Nurmi et al. (*40*). In brief, wholemeal samples of 0.5 g were subjected to acid and alkaline hydrolyses, and after purification of the nonsaponifiable lipids, the sterols were converted to trimethylsilyl ethers prior to GC analysis. The reference sample (MV-Emese) was analyzed in each analytical batch, and the identities of the 15 reported sterols were confirmed by GC-MS. The total sterol content of MV-Emese was 651 ± 14 μg/g of fw ($n = 46$). Details of the analytical method and its performance during the study are reported by Nurmi et al. (*40*).

*Alkylresorcinols.* Alkylresorcinols were extracted from intact kernels using ethyl acetate and analyzed by gas chromatography essentially according to the method of Ross et al. (*20*) with some modifications (*41*). Dry matter content of whole kernels was determined by oven-drying of crushed grains (coffee-type mill, Janke and Kunkel, IKA-WERK, Germany) at 105 °C for 16 h. The total content of alkylresorcinols in the reference sample MV-Emese was 389 ± 33 μg/g of dm ($n = 12$).

*Phenolic Acids.* Concentrations of free, soluble conjugated, and bound phenolic compounds were determined as detailed in Li et al. (*42*). In brief, free and conjugated phenolic acids were extracted from wholemeal flour using aqueous ethanol and sonication. Acidification of the supernatant and extraction with ethyl acetate allowed the isolation of the free phenolic acids. To isolate the conjugated acids, the aqueous ethanol fraction was hydrolyzed with sodium hydroxide. Conjugated phenolic acids were returned after acidification and extraction with ethyl acetate. Bound phenolic acids were obtained by hydrolyzing the residue generated after the initial aqueous ethanol extraction. Phenolic acid fractions were analyzed using reversed phase HPLC-DAD chromatography using an acetonitrile/acidic water elution gradient. Internal standards were employed to quantify the individual phenolic acid components of each fraction. Conditions for the HPLC analysis are detailed in Li et al. (*42*). The contents of phenolic acids in reference sample MV-Emese were as follows ($n = 25$): total phenolic acids 651 ± 23 μg/g of dm, of which total bound phenolic acids 524 ± 47 μg/g of dm, total conjugated phenolic acids 133 ± 8 μg/g of dm, and total free phenolic acids 6 ± 0.4 μg/g of dm.

*Data Analysis.* Correlations (Pearson's correlation coefficients) between components were studied using Statgraphics 4.0 software (Manugistics, Inc., Rockville, MD).

## RESULTS AND DISCUSSION

**General Characteristics.** There were differences between the basic composition of the rye varieties as well as their grain characteristics, such as 1000 kernel weight and test weight (**Table 1**). The basic composition of the whole grains was 55−60% starch, 11−16% protein, 2.5−2.8% lipids, and 1.6−2.2% ash. Thousand kernel weights (TKW) ranged from 30.0 to 39.6 g and were highest in the Portuguese varieties and lowest in the modern German varieties Nikita and Rekrut. The Portuguese varieties also had a high test weight and a high protein content, whereas the high-yielding German varieties had low test weight and low protein contents. The test weights ranged from 64.9 to 74.7 kg/hL. The three French varieties had the highest bran yields and, correspondingly, the lowest flour yields of all varieties. The basic composition and kernel characteristics of the Hungarian cultivar Lovaszpatonai-1 were close to the mean values in all variables, and it did not appear in either the high or low end of the range in any aspect.

**Dietary Fiber.** Dietary fiber and β-glucan quantification was performed on wholemeal samples, whereas WE-AX and TOT-AX contents were measured in flour as well as bran samples. The main groups of dietary fiber components are the nonstarch polysaccharides (including AX and β-glucans) and the oligosaccharides derived from them, resistant starch, lignins, and substances associated with them (*43, 44*). Dietary fiber content was estimated as the difference between the sample dry weight and the amounts of crude protein, ash, total lipids, enzyme digestible starch, and free sugars. Dietary fiber contents in wholemeal samples ranged from 20.4 to 25.2%, being lowest in the Hungarian cultivar Lovaszpatonai-1 and highest in the German cultivar Rekrut (**Table 1**).

The WE-AX and TOT-AX contents of the flours varied between 1.05 and 1.49% dm and between 3.11 and 4.31% dm, respectively (**Table 1**). These values are significantly higher than those determined for wheat (*7*), barley (*45*), and oat flours (*46*). Of the 10 rye samples analyzed, the Haute Loire population and the varieties Nikita and Rekrut, selected as varieties with high bioactive compounds, tended to have higher TOT-AX content in the flour.Although the latter two varieties also contain high WE-AX levels, the Haute Loire population had a moderate WE-AX concentration in flour. These three rye samples also had high WE-AX and TOT-AX concentrations in the bran. Of the 10 bran samples, the WE-AX and TOT-AX levels varied between 1.04 and 1.47% dm and between 12.06 and 14.76% dm, respectively (**Table 1**). The Portugaise-6 population was characterized by low AX contents both in flour and in bran. The rye bran WE-AX levels were greater than those determined for wheat, barley, and oat brans. Furthermore, the TOT-AX contents of the rye bran samples were higher than those for barley and oat bran, but on average below those for wheat bran (*7, 45, 46*). The low TOT-AX content in rye bran compared to wheat bran may be caused by the higher yield of bran and hence higher dilution with nonbran components (a yield of about 40% for rye compared with 25% yield for wheat). The A/X

**Table 1.** Characteristics and Chemical Composition [Contents of β-Glucan, Water-Extractable Arabinoxylan (WE-AX), Total Arabinoxylan (TOT-AX), and Arabinose/Xylose Ratio of Arabinoxylans (A/X)] of Rye Varieties on a Dry Matter Basis

| | rye cultivar | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dankowskie-Zlote | Warko | Rekrut | Nikita | Lovaszpatonai-1 | Grandrieu | Queyras | Haute Loire | Portugaise-3 | Portugaise-6 | mean | range |
| country of origin | Poland | Poland | Germany | Germany | Hungary | France | France | France | Portugal | Portugal | | |
| 1000 kernel wt | 37.5 | 33.6 | 30 | 31 | 34 | 34.3 | 33.1 | 37.6 | 38.8 | 39.6 | 35 | 30.0−39.6 |
| test wt (kg/hL) | 73.8 | 69.4 | 64.9 | 65.2 | 70.8 | 73.4 | 68.7 | 67.1 | 73.8 | 74.4 | 70.2 | 64.9−74.4 |
| Chopin bran yield (%) | 36.4 | 37.9 | 40.4 | 41.5 | 39.7 | 42.9 | 41.7 | 42.8 | 39.8 | 38 | 40.1 | 36.4−42.9 |
| Chopin flour yield (%) | 48.1 | 46.1 | 46.9 | 45.9 | 45.7 | 41.6 | 45.4 | 43.1 | 47 | 45.5 | 45.5 | 41.6−48.1 |
| protein (%) | 12.2 | 11.5 | 11.4 | 11.9 | 14.1 | 15.8 | 12.5 | 14.4 | 15 | 15.2 | 13.4 | 11.4−15.8 |
| ash (%) | 1.6 | 1.7 | 1.7 | 1.8 | 1.8 | 1.9 | 1.9 | 2.2 | 2.1 | 2.1 | 1.9 | 1.6−2.2 |
| lipids (%) | 2.5 | 2.5 | 2.7 | 2.8 | 2.6 | 2.7 | 2.5 | 2.8 | 2.6 | 2.6 | 2.6 | 2.5−2.8 |
| digestible starch (%) | 60.3 | 59.6 | 57.2 | 58.2 | 58.8 | 55.4 | 56.3 | 54.9 | 56.8 | 57.0 | 57.5 | 54.9−60.3 |
| free sugars (%) | 1.9 | 1.7 | 1.8 | 2.0 | 2.2 | 2.4 | 1.7 | 2.3 | 2.3 | 2.5 | 2.1 | 1.7−2.5 |
| Klason lignin (%) | 2.0 | 2.6 | 2.9 | 2.2 | 2.4 | 2.7 | 2.9 | 2.3 | 2.3 | 2.1 | 2.4 | 2.0−2.9 |
| estimated dietary fiber (%) | 21.6 | 23 | 25.2 | 23.3 | 20.4 | 21.9 | 25.1 | 23.6 | 21.2 | 20.5 | 22.6 | 20.4−25.2 |
| water extract viscosity (mP·s) | 34.2 | 63.1 | 45.7 | 49.4 | 41.2 | 45.1 | 50.1 | 64.8 | 37.2 | 36 | 46.7 | 34.2−64.8 |
| wholemeal moisture content (%) | 8.2 | 8.8 | 8.6 | 8.3 | 8.4 | 8.8 | 8.8 | 10.1 | 9.8 | 9.3 | 8.9 | 8.2−10.1 |
| wholemeal | | | | | | | | | | | | |
| β-glucan (%) | 2.0 | 2.0 | 1.9 | 1.7 | 1.9 | 1.7 | 2.0 | 1.7 | 1.7 | 1.7 | 1.8 | 1.7−2.0 |
| flour | | | | | | | | | | | | |
| WE-AX (%) | 1.18 | 1.15 | 1.49 | 1.43 | 1.05 | 1.22 | 1.1 | 1.17 | 1.24 | 1.05 | 1.21 | 1.05−1.49 |
| A/X ratio | 0.57 | 0.68 | 0.62 | 0.64 | 0.58 | 0.62 | 0.62 | 0.67 | 0.63 | 0.67 | 0.63 | 0.57−0.68 |
| TOT-AX (%) | 3.19 | 3.11 | 3.98 | 4.31 | 3.68 | 3.62 | 3.6 | 3.98 | 3.74 | 3.19 | 3.64 | 3.11−4.31 |
| A/X ratio | 0.66 | 0.76 | 0.71 | 0.73 | 0.7 | 0.7 | 0.71 | 0.74 | 0.72 | 0.74 | 0.72 | 0.66−0.76 |
| bran | | | | | | | | | | | | |
| WE-AX (%) | 1.21 | 1.14 | 1.44 | 1.4 | 1.1 | 1.04 | 1.42 | 1.46 | 1.47 | 1.06 | 1.27 | 1.04−1.47 |
| A/X ratio | 0.71 | 0.82 | 0.76 | 0.78 | 0.75 | 0.78 | 0.77 | 0.83 | 0.75 | 0.84 | 0.78 | 0.71−0.84 |
| TOT-AX (%) | 12.4 | 12.08 | 13.44 | 14.76 | 12.89 | 13.97 | 14.41 | 14.06 | 13.41 | 12.06 | 13.35 | 12.06−14.76 |
| A/X ratio | 0.54 | 0.58 | 0.48 | 0.5 | 0.52 | 0.58 | 0.52 | 0.56 | 0.51 | 0.55 | 0.53 | 0.48−0.58 |

values for flour and bran WE-AX and TOT-AX showed limited variation between the different samples (**Table 1**).

The β-glucan contents of the wholemeal flours of the different rye varieties varied between 1.7 and 2.0% dm, which is considerably lower than in oats (4.5−5.6% dm) and barley (3.7−6.5 dm), but higher than in wheat (0.5−1.0% dm) (*7, 45, 46*). The Portugaise-3, Portugaise-6, and Grandrieu populations and the cultivar Nikita were at the lower end of this narrow concentration range, whereas the Warko, Dankowskie-Zlote, and Queyras-72 populations were at the upper end. Similar β-glucan levels have been reported for rye also previously (*47*).

**Tocols.** The total tocol contents of the 10 rye varieties ranged from 43.6 to 67.2 μg/g dm in wholemeals, with an average value of 52.0 μg/g of dm (**Table 2**). Only α- and β-vitamers were present above the determination limit of 0.7 μg/g in rye grains. α-Tocotrienol and α-tocopherol were the major forms in all varieties contributing on average 38 and 32%, respectively, of the total tocols. The average proportion of tocotrienols was 59.6% of the total tocols.

The tocol profiles of the 10 rye varieties were consistent with those reported in earlier studies (*10*), but there was more variation in the contents of total tocols. The total tocol contents were also somewhat higher than those of commercial rye meal and flour samples, which ranged from 28.7 to 32.4 μg/g of fw (*35, 48, 49*). For example, the total tocol contents of wholegrain flours of 10 rye varieties grown in Finland ranged from 39.9 to 54.3 μg/g of fw with an average of 48.8 μg/g of fw (*35*). The α-vitamer dominated also in these samples, and the average percentage of tocotrienols was 62%. However, whole grains of rye varieties grown in Poland contained much less total tocols, that is, from 17.4 to 27.7 μg/g of dm, with both α-tocols and β-tocotrienol as the major vitamers (*50*). Moreover, two of the varieties studied, namely, Warko and Dańkowskie-Złote, contained only 43 and 49%, respectively, of the amounts of total tocols compared to the same varieties analyzed in the present study. The difference between tocol amounts may be partly due to real differences in the composition of the grains, but may also be due to the differences in the analytical methods used. Saponification combined with solvent extraction, which was used in this study, has been shown to liberate more tocols from oats than the methanol extraction method used in the Polish study (*51*), and this may also apply to rye grains. The total tocol contents of the rye samples were almost as high as those of barley varieties grown at the same time and at about the same level as in most of the HEALTHGRAIN wheat varieties (*36, 45*).

**Folate.** The total folate content of the 10 rye varieties ranged from 574 to 775 ng/g of dm, with the mean content being 693 ng/g of dm (**Table 2**). This was higher than in winter and spring wheat or oats (*37, 46*) and close to that in barley (*45*). The magnitude of the range is in good agreement with the previously published data of Kariluoto et al. (*38*). They reported that folate contents of 10 rye varieties ranged from 630 to 780 ng/g of fw, with the mean being 700 ng/g. On a dry matter basis the contents were therefore somewhat higher than in the present study. Similarly, Hegedüs et al. (*52*) reported a value 650 ng/g of fw for 100% rye flour. The folate contents in this study were only slightly higher than the values in some food composition tables, for example, the USDA National Database (600 ng/g) and the Danish Food Composition Databank (560 ng/g) (*53, 54*). On the other hand, much higher contents have also been reported for rye grains by other workers: 920 ng/g of fw (*55*), 1430 ng/g of fw (*56*), and 1226 and 1349 ng/g of dm (*57*). These

**Table 2.** Content and Composition of Tocols, Folate, Sterols, and Alkylresorcinols (AR) in Wholemeal Rye Flours on a Dry Matter Basis

| | rye cultivar | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dankowskie-Zlote | Warko | Rekrut | Nikita | Lovaszpatonai-1 | Grandrieu | Queyras | Haute Loire | Portugaise-3 | Portugaise-6 | mean | range |
| tocols ($\mu$g/g) | 48.4 | 49.6 | 55 | 67.2 | 53.7 | 43.6 | 56.1 | 56.5 | 44.5 | 45 | 52 | 44−67 |
| $\alpha$-tocopherol | 15.0 | 15.6 | 14.8 | 19.1 | 18.7 | 15.9 | 18.4 | 20.1 | 13.3 | 14.3 | 16.5 | 13.3−20.1 |
| $\beta$-tocopherol | 3.5 | 3.4 | 3.8 | 4.7 | 4.9 | 4.9 | 4.5 | 5.8 | 4.0 | 3.8 | 4.3 | 3.4−5.8 |
| $\alpha$-tocotrienol | 18.4 | 19.7 | 23.0 | 27.4 | 19.2 | 14.2 | 22.4 | 20.4 | 16.8 | 17.2 | 19.9 | 14.2−27.4 |
| $\beta$-tocotrienol | 11.6 | 10.9 | 13.3 | 16.0 | 10.8 | 8.5 | 10.7 | 10.2 | 10.4 | 9.6 | 11.2 | 8.5−16.0 |
| folate (ng/g) | 666 | 755 | 614 | 626 | 723 | 775 | 574 | 760 | 721 | 717 | 693 | 574−775 |
| total sterols $\mu$g/g | 1098 | 1212 | 1211 | 1295 | 1153 | 1186 | 1222 | 1420 | 1275 | 1214 | 1228 | 1098−1420 |
| individual sterols[a] | | | | | | | | | | | | |
| sitosterol | 508 | 563 | 576 | 601 | 550 | 604 | 588 | 712 | 619 | 592 | 591 | 508−712 |
| campesterol | 188 | 206 | 238 | 246 | 220 | 223 | 216 | 259 | 241 | 213 | 225 | 188−259 |
| stigmasterol | 36 | 43 | 53 | 55 | 40 | 30 | 43 | 41 | 36 | 35 | 41 | 30−55 |
| stanols | 218 | 247 | 224 | 249 | 192 | 163 | 229 | 227 | 199 | 215 | 216 | 163−249 |
| other sterols | 149 | 154 | 121 | 144 | 152 | 165 | 145 | 181 | 180 | 158 | 155 | 121−181 |
| total AR ($\mu$g/g) | 1065 | 1123 | 1207 | 1444 | 864 | 796 | 1231 | 881 | 821 | 868 | 1030 | 797−1231 |
| relative composition (%) | | | | | | | | | | | | |
| C17:0 | 26.8 | 27.9 | 24.6 | 23.5 | 24.1 | 25.7 | 22.6 | 23.9 | 22.1 | 23.8 | 24.5 | 22.1−27.9 |
| C19:0 | 33.2 | 33.6 | 31.8 | 31.9 | 32.2 | 32.8 | 32.2 | 31.5 | 32.4 | 32 | 32.4 | 31.5−33.6 |
| C21:0 | 22 | 20.7 | 23.7 | 24.6 | 23.2 | 21.7 | 24.7 | 23.5 | 26.7 | 25 | 23.6 | 20.7−26.7 |
| C23:0 | 9.6 | 9.4 | 10.8 | 11.2 | 10.7 | 10.2 | 10.7 | 11.2 | 10.9 | 11 | 10.6 | 9.4−11.2 |
| C25:0 | 8.4 | 8.3 | 9.1 | 8.9 | 9.8 | 9.6 | 9.7 | 9.8 | 8 | 8.2 | 9 | 8.0−9.8 |
| ratio C17:0/ C21:0 | 1.2 | 1.3 | 1 | 1 | 1 | 1.2 | 0.9 | 1 | 0.8 | 0.9 | 1 | 0.8−1.3 |

[a] A total of 15 individual sterols were determined.

differences may in part be explained by differences in the analytical methods. Furthermore, only a few samples were analyzed in the previous studies, and these varied in origin.

**Plant Sterols.** The content of total plant sterols determined in wholemeal rye flours of the different varieties ranged from 1098 to 1420 $\mu$g/g of dm (**Table 2**). The highest content of plant sterols was found in Haute Loire, a French genotype, and the lowest content in Dankowskie-Zlote, an old Polish variety.

The contents of total sterols in the rye varieties of this study were somewhat higher than was determined in previous studies. Piironen et al. (*39*) reported the plant sterol content of 10 rye varieties to range from 774 to 937 $\mu$g/g of dm. A similar range, 761−967 $\mu$g/g of fw, was reported for seven varieties by Zangenberg et al. (*58*). The latter study concluded that the range of plant sterol contents in rye varieties is 700−1000 $\mu$g/g of fw. However, all of the rye varieties analyzed in this study contained higher amounts of sterols (>1000 $\mu$g/g of fw), suggesting that there are significant differences between the sterol contents of different rye varieties and between years of cultivation. Significant differences between sterol contents were also observed in the study of Zangenberg et al. (*58*), who compared different years of cultivation. However, the contents still fell within the 700−1000 $\mu$g/g range and were therefore lower than in the current study. The content of total sterols was also on average the highest in rye (1228 $\mu$g/g of dm), when different cereals in the HEALTHGRAIN diversity screen were compared (*40, 45, 46*).

Little variation in sterol composition was observed between the rye varieties (**Table 2**). Sitosterol contributed about half of the total sterols in all rye varieties (range of 508−712 $\mu$g/g of dm, corresponding to 46−51% of total sterols determined in the rye varieties). The other sterols in order of decreasing proportion were campesterol (17−20%), sitostanol (8−12%), campestanol (6−9%), and stigmasterol (3−4%) and other minor sterols (10−14%). The percentage of stanols (sitostanol and campestanol) was on average 18% of total sterols (range of 14−20%). These sterol compositions are similar to those reported in earlier studies by Piironen et al. (*39*) and Nyström et al. (*59*) and close to the composition of rye sterols reported by Zangenberg et al. (*58*).

**Alkylresorcinols.** The total content of alkylresorcinols (**Table 2**) varied widely between 796 and 1231 $\mu$g/g of dm (mean = 1030 $\mu$g/g of dm), with Nikita, Rekrut, and Queyras having the highest and Grandrieu having the lowest contents. However, the relative proportions of the alkylresorcinol homologues varied less than the total alkylresorcinol content (**Table 2**). The dominant alkylresorcinol homologues in all samples were C17:0, C19:0, and C21:0, accounting for 22−28% (mean = 25%), 32−34% (mean = 32%), and 21−27% (mean = 24%) of total alkylresorcinols, respectively. The homologues C23:0 and C25:0 were present in smaller amounts, with 9−11% (mean = 11%) and 8−10% (mean = 9%), respectively. Dankowskie-Zlote, Warko, and Grandrieu had slightly higher relative contents of C17:0 and lower contents of C21:0 than the other varieties, and therefore had higher ratios of C17:0/C21:0 (**Table 2**).

The total contents of alkylresorcinols in this set of samples were higher than previously reported for rye varieties grown in Sweden, which had contents of 568−1022 $\mu$g/g of dm (mean = 726 $\mu$g/g of dm) (*60*). Much higher contents were also reported earlier by Evans et al. (*61*) (3220 $\mu$g/g of dm) and Gohil et al. (*62*) (2000 $\mu$g/g of dm). These differences may relate to the use of different varieties and growth under different agroclimatic conditions. The varieties Nikita and Rekrut, which had the high contents of alkylresorcinols, are modern high-yielding German varieties with high content of bioactive components. Another cultivar that was high in alkylresorcinols, Queyras, and a cultivar low in alkylresorcinols, Grandrieu, are French old varieties. These old varieties are genetically more heterogeneous than the modern varieties or hybrids. A negative correlation existed between alkylresorcinol content and 1000 kernel weight (TKW) ($R^2 = 0.5$), which has also been reported earlier in wheat, where winter wheat had a lower content of

**Table 3.** Content and Composition of Total, Free, Conjugated, and Bound Phenolic Acids in Rye Varieties (Micrograms per Gram of Wholemeal Flour, Dry Matter Basis)

| | rye cultivar | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dankowskie-Zlote | Warko | Rekrut | Nikita | Lovaszpatonai-1 | Grandrieu | Queyras | Haute Loire | Portugaise-3 | Portugaise-6 |
| **total phenolic acids** | | | | | | | | | | |
| total phenolic acids | 542 | 760 | 601 | 491 | 593 | 1082 | 884 | 497 | 542 | 853 |
| total free phenolic acids | 11 | 21 | 23 | 25 | 21 | 23 | 19 | 22 | 29 | 26 |
| total conjugated phenolic acids | 153 | 198 | 208 | 193 | 203 | 349 | 271 | 259 | 231 | 218 |
| total bound phenolic acids | 378 | 541 | 370 | 273 | 369 | 711 | 593 | 216 | 282 | 610 |
| **individual free phenolic acids** | | | | | | | | | | |
| vanillic acid | 1.3 | 1.3 | 1.4 | 1.2 | 1.3 | 1.7 | 1.1 | 1.5 | 1.6 | 1.5 |
| syringic acid | 1.4 | 1.8 | 1.8 | 1.9 | 2.3 | 2.2 | 1.1 | 1.8 | 2.0 | 1.2 |
| syringaldehyde | | | | 1.1 | | 1.5 | | 1.5 | 2.5 | 2.9 |
| caffeic acid | | 3.1 | 3.6 | 3.7 | | | 3.1 | | | |
| 2,4-dihydroxybenzoic acid | 1.9 | 0.5 | 1.1 | 1.8 | 1.4 | | 1.1 | 1.7 | 5.0 | 3.4 |
| sinapic acid | | 9.5 | 9.3 | 9.2 | 8.9 | 9.4 | 8.8 | 9.4 | 9.6 | 9.2 |
| ferulic acid | 4.9 | 5.1 | 4.8 | 4.8 | 5.6 | 6.4 | 3.0 | 5.1 | 6.4 | 5.4 |
| *p*-coumaric acid | 0.9 | | 0.9 | 0.8 | 1.1 | 1.4 | 0.7 | 1.1 | 1.7 | 1.5 |
| 2-hydroxycinnamic acid | 0.2 | 0.1 | 0.2 | 0.3 | 0.2 | 0.0 | 0.2 | 0.2 | 0.2 | 0.5 |
| **individual conjugated phenolic acids** | | | | | | | | | | |
| 4-hydroxybenzoic acid | 5.5 | 6.1 | 8.2 | 7.8 | 7.9 | 13.0 | 8.5 | 11.1 | 9.9 | 12.5 |
| vanillic acid | 4.6 | 4.6 | 5.8 | 6.4 | 6.2 | 6.1 | 7.8 | 6.8 | 5.5 | 5.0 |
| syringic acid | 0.6 | 1.4 | 3.0 | 2.0 | 0.2 | 0.2 | 2.5 | 2.1 | 0.8 | 1.1 |
| syringaldehyde | 0.7 | | 1.0 | 0.3 | 0.9 | | 1.2 | | | |
| 2,4-dihydroxybenzoic acid | 44.6 | 66.8 | 64.0 | 57.5 | 63.4 | 87.6 | 106.7 | 78.4 | 64.4 | 58.3 |
| sinapic acid | 51.6 | 66.5 | 73.1 | 67.3 | 59.8 | 91.4 | 69.4 | 71.2 | 61.3 | 58.1 |
| ferulic acid | 34.8 | 44.8 | 46.9 | 46.4 | 53.5 | 122.7 | 61.0 | 73.5 | 75.8 | 71.1 |
| *p*-coumaric acid | 9.0 | 9.1 | 7.4 | 7.6 | 9.1 | 25.8 | 15.4 | 13.4 | 12.0 | 10.8 |
| 2-hydroxycinnamic acid | 1.6 | 2.0 | 2.0 | 1.7 | 2.2 | 2.4 | 2.2 | 2.3 | 2.0 | 1.9 |
| **individual bound phenolic acids** | | | | | | | | | | |
| 4-hydroxybenzoic acid | 2.4 | 9.1 | 5.5 | 1.2 | 7.6 | 14.0 | 5.6 | 0.0 | 3.6 | 6.3 |
| vanillic acid | 1.4 | 2.2 | 2.1 | 1.8 | 1.5 | 10.4 | 3.4 | 9.3 | 2.6 | 2.8 |
| syringic acid | 0.4 | 2.7 | 0.8 | | 2.0 | 12.4 | 1.4 | 8.3 | 0.3 | 1.1 |
| syringaldehyde | | | | | 0.4 | | | | | |
| 2,4-dihydroxybenzoic acid | 28.7 | 109.3 | 19.5 | 18.2 | 77.3 | 53.0 | 38.8 | 0.0 | 15.4 | 37.8 |
| sinapic acid | 27.0 | 36.3 | 37.1 | 27.3 | 30.0 | 60.1 | 57.8 | 26.1 | 31.1 | 33.3 |
| ferulic acid | 312.1 | 372.1 | 276.1 | 200.6 | 240.7 | 476.1 | 443.0 | 145.8 | 204.3 | 521.4 |
| *p*-coumaric acid | 2.7 | 4.4 | 24.9 | 18.6 | 5.2 | 59.5 | 35.8 | 18.3 | 20.0 | 3.1 |
| 2-hydroxycinnamic acid | 3.3 | 4.8 | 4.4 | 5.2 | 4.6 | 25.7 | 7.7 | 7.9 | 4.7 | 4.4 |

alkylresorcinol and a higher TKW than spring wheat (*63*). This may result from dilution by the higher levels of starch and protein deposited during kernel development, whereas the content of alkylresorcinols remains constant (*63*). The proportions of the different homologues were in the same range as reported previously (*60*), although some varieties had slightly higher relative contents of C17:0 and lower contents of C21:0 than were reported for Swedish rye samples (*60*). The ratio between C17:0 and C21:0 was 0.9 ± 0.1 (mean ± SD) in the Swedish material, whereas in this study the ratio was 1.0 ± 0.2 (mean ± SD). The pattern of alkylresorcinol homologues of rye is known to be very different from that in wheat and durum wheat (*60, 63, 64*), which was confirmed in this study. In wheat the dominant alkylresorcinol homologues are C19:0 and C21:0, with relative proportions of about 35 and 50% (*41, 63*). The dominant alkylresorcinol homologue in durum wheat is C21:0, with a relative content of about 60%, whereas the proportions of C19:0 and C23:0 are about 14 and 21%, respectively. The ratio between the C17:0 and C21:0 homologues is characteristic for each cereal, being about 1.0 for rye, 0.1 for wheat, and 0.01 for durum wheat (*60, 63, 64*). This ratio may be used in combination with the total alkylresorcinol content of a cereal product to estimate whether the product contains wholegrain wheat, rye, or a mixture of the two cereals (*63*).

**Phenolic Acids.** The wholemeal rye samples were analyzed for free, conjugated, and bound phenolic acids (**Table 3**). In addition to total concentrations of each class of phenolic acids, individual measurements were made for each phenolic acid within the classes of free, conjugated, or bound phenolic acid. The phenolic acids quantified in this study included 4-hydroxybenzoic acid, syringic acid, syringaldehyde, 2,4-dihydroxybenzoic acid, sinapic acid, ferulic acid, *p*-coumaric acid, and 2-hydroxycinnamic acid. The concentrations of each individual phenolic acid were summed to give the total phenolic acid content for each class and total phenolic acids calculated by summing the total concentrations of the free, conjugated, and bound phenolic acid fractions.

A wide range (491−1082 μg/g of dm) in total phenolic acid contents was observed across the different wholemeal rye flours, with the mean value being 685 μg/g of dm. These results were generally slightly lower than those previously reported for commercial rye flour from Finland (1000 μg/g of dm) (*27*). The cultivar with the highest total concentration of phenolic acids was Grandrieu (1082 μg/g of dm), and that with the lowest was Nikita (491 μg/g of dm).

Free phenolic acids comprised a very small (<4%) proportion of the total phenolic compounds. Among the 10 varieties in this study, Portugaise-3 and Portugaise-6, originating from Portugal, contained the highest concentrations of free phenolic acids (29 and 26 μg/g of dm), whereas a Polish line, Dankowskie-Zlote, contained the lowest (10.6 μg/g of dm).

The soluble conjugated phenolic acids comprised approximately 35% of the total phenolic acids, ranging from 153.0 to 348.5 μg/g of dm. It is of interest that the varieties with the highest concentrations (>250 μg/g of dm) of soluble conjugated phenolic acids all originated from France.

Bound phenolic acids comprised approximately 62% of the total phenolic acids with concentrations ranging from 216 to

711 μg/g of dm. The cultivar with the highest total concentration of phenolic acids was Grandrieu (711 μg/g of dm) and that with the lowest was Haute Loire (216 μg/g of dm). However, there was no relationship between the amounts of bound phenolic compounds and the country of origin.

Analyses of individual phenolic acids in the different classes (free, conjugated, and bound) showed wide variation in the compositions of the rye varieties (**Table 3**). The predominant phenolic acids were ferulic, sinapic, and 2,4-dihydroxybenzoic acid, and the percentages of these in free and bound forms varied.

Three major components (sinapic, 39%; ferulic, 23%; and 2,4-dihydroxybenzoic acid, 8%) were present in the free phenolic acid fraction. However, whereas the ferulic acid content was slightly higher (27%) in the soluble conjugated fraction, the proportion of sinapic acid decreased slightly due to an increase in 2,4-dihydroxybenzoic acid (to 28%). The bound phenolic acid fraction was dominated by ferulic acid, comprising 74% of the fraction.

Total ferulic acid concentrations in the 10 rye varieties ranged between 229 and 612 μg/g of dm with a mean value of 393 μg/g of dm. The highest concentration was in Grandrieu, and the lowest was in Haute Loire. The second major phenolic acid in rye, sinapic acid, ranged from 79 to 170 μg/g of dm with a mean concentration of 120 μg/g of dm. The results obtained for both of these components were in agreement with previously reported studies (*27*).

**Trends in the Contents of Phytochemicals and Fiber.** Considerable variation was observed in the contents of phytochemicals and dietary fiber components in rye varieties. The greatest variation was seen in phenolic acids, where the highest content (Grandrieu) was 120% greater than the lowest content (Nikita) (**Table 3**). The contents of alkylresorcinols and tocols (which are both also phenolic compounds) varied widely, with 81 and 52% differences between the highest and lowest values, respectively. In contrast, less variation was observed in the contents of folates (35%) and sterols (30%).

A statistically significant correlation between the TKW and phytochemical content was observed with alkylresorcinols ($r = -0.71$, $p = 0.02$). This indicates that large kernels with a high TKW have also, for example, a high protein content (correlation $r = 0.65$, $p = 0.04$), and thus the proportion of bran layer components such as alkylresorcinols is smaller. This is indicated also by the negative correlation between alkylresorcinols and protein content ($r = -0.86$, $p = 0.001$). On the other hand, low TKWs were associated with a low content of folate, but high flour and bran yields. Generally the varieties with low folate contents, such as the German varieties Rekrut and Nikita, were rich in alkylresorcinols, whereas varieties rich in folate, such as the Portuguese populations, had low contents of alkylresorcinols ($r = -0.77$, $p = 0.01$).

The French varieties had the highest flour and bran yields, but varied in their contents and compositions of phytochemicals. The Haute Loire population was rich in all phytochemicals, except phenolic acids, being among the top three varieties for sterol, folate, and tocol contents. Queyras was among the top three varieties for contents of tocols, alkylresorcinols, and total phenolic compounds, but had the lowest folate content of all studied varieties. The third French variety, Grandrieu, was in the top three for contents of folate and total phenolic compounds, but in the lowest three for contents of sterols, tocols, and alkylresorcinols. These observations suggest further studies of old rye varieties such as old landraces or old variety populations to carry out selection for high or low content in phytochemicals. Dissection of old heterogeneous populations may contribute to the selection of wider genetic variations for these traits.

There were some relationships between the contents of phytochemicals in wholemeal and total AX contents in the bran and flour fractions of the corresponding varieties. The positive correlations between tocols (in wholemeal) and total AX (in bran and flour) were the most significant with coefficients of correlation $r = 0.71$ ($p = 0.02$) and $r = 0.61$ ($p = 0.05$) in flour and bran, respectively. Positive, but less significant, correlation was also observed between wholemeal sterol content and total AX in flour ($r = 0.59$, $p = 0.07$) and bran ($r = 0.52$, $p = 0.12$). Furthermore, a negative correlation was observed between wholemeal total phenolic acids and the flour AX content ($r = 0.44$, $p = 0.17$), but no correlation was observed between the phenolic acids and bran AX ($r = 0.06$, $p = 0.87$). The French cultivar Haute Loire, which had a low content of phenolic acids but a high content of all other phytochemicals, was also rich in AX in both flour and bran. Furthermore, the Polish varieties Dankowskie-Zlote and Warko that were relatively poor in phytochemicals also had low AX contents in flour and bran.

The results of this study show that there are marked differences in phytochemical and dietary fiber contents between rye varieties, with the highly heterogeneous older types being particularly variable. The data from this study can be used to select varieties with high levels of fiber and single phytochemical components or a compromise containing a maximal content of each. Further studies are, however, needed to see how much variation may be caused by growth location, year, and other agroclimatic factors.

## ABBREVIATIONS USED

AR, alkylresorcinol; TKW, 1000 kernel weight; TOT-AX, total arabinoxylan; WE-AX, water-extractable arabinoxylan; A/X, arabinose/xylose ratio; dm, dry matter; fw, fresh weight; GC, gas chromatography.

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