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Phytochemical and Dietary Fiber Components in Barley Varieties in the HEALTHGRAIN Diversity Screen

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Ten different barley varieties grown in one location were studied for their content of tocols, folate, plant sterols, alkylresorcinols, and phenolic acids, as well as dietary fiber components (arabinoxylan and β-glucan). The samples included hulled and hull-less barley types and types with normal, high-amylose, and waxy starch. The aim was to study the composition of raw materials, and therefore the hulls were not removed from the hulled barleys. A large variation was observed in the contents of all phytochemicals and dietary fibers. Two varieties from the INRA Clermont Ferrand barley program in France (CFL93-149 and CFL98-398) had high content of tocopherols and alkylresorcinols, whereas the variety Dicktoo was highest in dietary fiber content and phenolics. Positive correlations were found between 1000 kernel weight, alkylresorcinols, and tocols, as well as between dietary fiber content and phenolic compounds. The results demonstrate that the levels of phytochemicals in barley can likely be affected by breeding and that the contents of single phytochemicals may easily be adjusted by a right selection of a genotype.

KEYWORDS: Phytochemical; barley; *Hordeum vulgare* L.; genetic variation; dietary fiber; β-glucan; arabinoxylan; folate; tocopherol; tocotrienol; alkylresorcinol; phenolic acid; plant sterol

INTRODUCTION

Barley is the fourth most produced cereal in the world (after wheat, maize, and rice) and is generally classified as six-rowed or two-rowed and as hulled or hull-less. A further distinction can be made between types containing normal, waxy, and high-amylose starch (*1*). In the Western world today barley is mostly used for feed, but a limited amount is also used for malt and food. In other parts of the world barley is the staple food, for example, in Tibet and Morocco, where the per capita consumptions are 155 and 39 kg/year, respectively (*2*). However, there is also considerable interest in increasing the consumption of barley for food in Western countries, mainly due to its high content of dietary fiber, especially partly soluble β-glucan, which can lower serum cholesterol, glucose, and insulin levels in humans (*3, 4*), and arabinoxylan, which has been shown to decrease postprandial serum glucose and insulin response in subjects with impaired glucose tolerance (*5*). The endosperm cell walls in barley are also more resistant to degradation than in wheat and maize, and thereby the rate of release of nutrients in the upper gastrointestinal tract may be reduced. Two novel specific barley varieties (Sustagrain and Himalaya 292), which are lacking activity of a key enzyme of starch synthesis giving a grain containing less total starch, more amylose, and higher

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total dietary fiber, have been shown to have enhanced health potential (*6, 7*). As for oats, whole grain barley and barley-containing products are today allowed to claim that they may reduce the risk of coronary heart disease (*8, 9*). Typical food applications of barley are the production of beer, porridges, breakfast cereals, bread, and other bakery products (*10*). Low-value byproducts (e.g., spent brewery grains) are also generated and utilized by the feed industry.

Barley varies widely in chemical composition, due to genotype and environment and interactions between the two (*11*−*14*). In normal hulled barley, starch is the major constituent, accounting for about 60% of dry matter (dm), followed by total dietary fiber and protein, which comprise about 20 and 11% of dm, respectively. The most important dietary fiber components in barley are mixed-linkage (1→3),(1→4)-β-D-glucan (hereafter referred to as β-glucan) and arabinoxylan (AX), two partly soluble nonstarch polysaccharides (NSP) occurring in the cell walls. β-Glucan is a linear homopolysaccharide of glucose with approximately 70% β-(1→4)-linkages and 30% β-(1→3)-linkages. On average, three or four (1→4)-linked units are separated by a single (1→3)-linkage. AX is composed of a backbone of (1→4)-linked β-D-xylopyranosyl residues, of which some are substituted at positions O-2 and/or O-3 with α-L-arabinofuranosyl residues. The latter residues can be substituted at O-5 by ferulic (or more rarely coumaric) acid residues (*15*). Both NSP have profound impacts on cereal processing (e.g., impairing wort filtration during beer production and affecting dough properties during breadmaking) and product properties (e.g., beer hazes, bread loaf volume) (*16*−*18*).

A range of different phytochemicals, including tocols, folate, sterols, phenolic acids, and alkylresorcinols (AR), are also found in barley in smaller amounts. Tocopherols and tocotrienols (i.e., tocols) are vitamin E active compounds that each occur in four forms called α-, β-, γ-, and δ-vitamers. Although current recommendations consider 2*R*-α-tocopherol as the only vitamer that meets the vitamin E requirement; the antioxidant activity of all tocols is acknowledged (*19*). In addition to acting as effective antioxidants, tocotrienols possess other potential health benefits shown in model systems, including inhibition of cholesterol synthesis, neuroprotection and anticarcinogenetic properties (*20, 21*). The tocol composition of barley is interesting due to the presence of all eight vitamers.

Folate is a B vitamin, which acts as a coenzyme in several single carbon transfer reactions in the metabolism of nucleic acids and amino acids. It occurs as several vitamers, reduced derivatives of folic acid (pteroyl-L-glutamic acid, PGA), which mainly exist as polyglutamates in cereals. Folate is the focus of active research because of its role in prevention of neural tube defects in babies. Several countries, for example, the United States, Canada, and Chile, have launched mandatory folate fortification programs. Suboptimal folate intake is also associated with an increased risk of cardiovascular diseases, stroke, and colorectal cancer and may be associated with dementia and Alzheimer's disease (*22*−*24*). Although cereals are generally regarded as important folate sources, little is published on folate in barley.

Plant sterols (phytosterols) are secondary plant metabolites that are of interest because of their health-promoting properties. They are added to functional foods to aid in lowering serum cholesterol levels in humans. Recent studies have also shown that natural intakes of dietary plant sterols can have a positive effect on serum cholesterol levels (*25*). Cereals are the main sources of plant sterols, together with vegetable oils, contributing up to 40% of daily intake of plant sterols (*26*).

Phenolic compounds are believed to be responsible for some of the beneficial effects derived from the consumption of whole grains, fruits, and vegetables and may also contribute to the flavor of for examples cereal foods. They have strong antioxidant activities associated with their ability to scavenge free radicals. Consumption of a high level of phenolic compounds has been correlated with a reduced risk of certain cancers and cardiovascular diseases (*27, 28*). Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. Several of these phenolic acids are also commonly found in grain products other than barley, with ferulic acid being the most abundant (*29, 30*). The phenolic acids present in cereals are found in both the free and bound forms, with the majority existing in the insoluble bound form. Ferulic acid and *p*-coumaric acid are reported to be the major low molecular weight phenolic acids in barley grain, being concentrated mainly in the outer layers, but they are also present in the endosperm (*31*).

Finally, alkylresorcinols (AR) are major phenolic compounds in certain cereals. They are 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring. They are present only in the outer cuticula of testa/inner cuticula of pericarp (*32*) and are therefore suggested to be biomarkers for intake of wholegrain rye and wheat food products (*33*). AR are present in high levels in rye, wheat, and durum wheat, but have also been found in smaller amounts in barley (*34, 35*).

The HEALTHGRAIN cereal diversity screen forms part of an Integrated Project supported by the European Union as part of the sixth Framework Program (*36*−*38*). The main focus of the project is to develop new healthy food products based on wholegrains of wheat and rye. However, other cereal species have also been studied in the project, including barley. Whole grains contain many types of dietary fibers and bioactive components, which may be of importance for the human health. However, how these individual components work together is not yet known. In this study, 10 barley genotypes were grown in Martonvásár (Hungary) in 2005 as part of a wider diversity screen of cereals, and their contents of dietary fiber and phytochemical components were determined. This provides a unique comparison of the detailed composition of a selection of genotypes grown under the same agronomic and environmental conditions.

MATERIALS AND METHODS

Barley Samples. The barley lines were grown in two replications in the breeding nursery at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary (latitude, 47° 21′ N; longitude, 18° 49′ E; altitude, 150 m). The plots were 2.5 m long, with six rows spaced at a distance of 20 cm. The soil was of the chernozem type with a loam texture and pH 6.8−7.2. The previous crop was pea. Normal agronomic practices were applied. The experiments were treated with herbicide (4 L/ha U-46-M Fluid containing 500 g/L MCPA; 15 g/ha Granstar containing 75% tribenuron methyl), insecticide (0.2 L/ha Karate containing 2.5% λ-cyhalothrin), and fungicide (1 L/ha Eminent containing 125 g/L tetraconazole, 1 L/ha Tango Star containing 84 g/L epoxyconazole and 250 g/L fenpropimorph). The winter barley genotypes were sown in late September 2004 and the spring lines in the beginning of March 2005. The grains were conditioned to 14% moisture content and milled with a Chopin CD1 Laboratory Mill to produce flour and bran. Wholemeal samples were prepared with the Perten 3100 Laboratory Mill using a 0.5 mm sieve. To avoid loss of parts of the outer layers of the grains, they were not dehulled before milling. Therefore, bran and wholemeal samples from hulled barleys contain hull, whereas the flour samples do not contain any hulls. Hull-less barleys contained only some grains with the hulls

remaining after threshing (about 9 and 1%, respectively, for Rastik and CFL98-450). Milled samples were immediately cooled and stored at −20 °C to protect the bioactive components.

Basic Chemical Composition. To simplify the analysis of the HEALTHGRAIN samples an indirect approach was taken to measure the whole complex of fiber constituents. The dietary fiber content (estimated dietary fiber) was therefore measured in wholemeal samples 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 (*39*). Total lipids were analyzed gravimetrically by extraction with acid solvent consisting of 60:40:1 (v/v/v) chloroform/methanol/hydrochloric acid as described by Marchello et al. (*40*). Available starch was determined with the procedure of Megazyme (Bray, Ireland), consistent with AACC approved method 76-13 (*39*), whereas free sugar was analyzed by a GLC procedure as a sum of all mono- and disaccharides, that is, fructose, glucose, maltose, and sucrose (*41*). Amylose content (*42, 43*) was measured with the concanavalin method using the Megazyme kit. Klason lignin was analyzed gravimetrically according to AACC method 32-25 (*44*), whereas viscosity was measured in the grain water extract as described earlier (*45, 46*) using the Brookfield Cone/Plate Digital Viscometer, model LVDV-II+ (Stoughton, MA), with an 0.8° cone spindle and a shear rate of 225 s^{-1} at 25 °C.

***β*-Glucan and Arabinoxylan.** Total arabinoxylan (TOT-AX) and water-extractable arabinoxylan (WE-AX) contents were determined in the barley flour and bran samples after acid hydrolysis of the complete sample and the water-extractable fraction of the sample, respectively, derivatization of the produced monosaccharides to alditol acetates, and quantification of these alditol acetates by gas chromatography. The method was identical to that used for quantification of AX in wheat (*47*). The mixed-linkage β-glucan content was determined in the barley wholemeal samples using the Megazyme mixed-linkage β-glucan assay kit as described for wheat (*47*). The assay is based on the enzymic degradation of glucans with lichenase and β-glucosidase and the quantification of the released glucose using an oxidase/peroxidase reagent. The molecular weight distribution of β-glucan was measured in wholemeal samples essentially according to the method of Rimsten et al. (*48*) with extraction of β-glucan in hot water and thermostable α-amylase for 1.5 h. Calcofluor average molecular weight ($\overline{M}$cf) including only molecules large enough to be detected with Calcofluor (>10000 g/mol), and percentiles (p10, p50, and p90) describing the molecular weight at which 10, 50, and 90% of the distribution fall below were calculated by using a calibration curve with narrow molecular weight β-glucan standards (*48*).

Phytochemicals. α-, β-, γ-, and δ-tocopherols and tocotrienols were determined by normal-phase HPLC using fluorescent detection. Wholemeal samples were subjected to hot saponification, and the tocols were solvent extracted prior to HPLC analysis (*49, 50*). Details of the analytical method and its performance during the study were reported by Lampi et al. (*50*).

Total folate contents were determined microbiologically on 96-well microtiter plates using *Lactobacillus rhamnosus* ATCC 7469 as the test organism (*51, 52*). The sample preparation procedure included heat extraction followed by trienzyme treatment using first α-amylase and chicken pancreas conjugase and after that protease. A certified reference material and an in-house reference sample were used to confirm the quality of the analysis as reported by Piironen et al. (*52*).

Plant sterols were determined by GC as described earlier (*53, 54*). In brief, wholemeal samples 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 identities of the reported 15 sterols were confirmed by GC-MS. Details of the analytical method and its performance during the study were reported by Nurmi et al. (*54*).

Alkylresorcinols (AR) were extracted from intact kernels and analyzed by GC essentially according to the method of Ross et al. (*55*) with only minor modifications (*56*). Briefly, whole grains were extracted with ethyl acetate, and an internal standard was added. Extracts were thereafter analyzed on GC. Dry matter content of whole grains was determined by oven-drying of crushed grains (coffee-type mill, Janke and Kunkel, IKA-WERK, Germany) at 105 °C for 16 h.

Free and conjugated phenolic acids were extracted from wholemeal using aqueous ethanol and sonication (*57*). Acidification of the supernatant and extraction into 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, into ethyl acetate. Bound phenolic acids were obtained by hydrolyzing the residue generated after initial aqueous ethanol extraction. Internal standards were employed to quantify the individual phenolic acid components of each fraction. Phenolic acid fractions were analyzed using reverse phase HPLC-DAD chromatography using an acetonitrile/acidic water elution gradient.

Statistical Analysis. To study the variation in chemical composition, including dietary fiber components and phytochemicals, and to find correlations between different variables, principal component analysis (PCA, The Unscrambler v9.5, CAMO Process AS, Oslo, Norway) was performed. All samples and all variables in **Tables 1−3**, except AX content and arabinose to xylose (A/X) ratio in flour and bran, were included in the analysis.

RESULTS AND DISCUSSION

Selection and Characteristics of Varieties. Ten barley varieties with different countries of origin, both spring and winter types, as well as two-rowed and six-rowed types, were selected for the study (**Table 1**). For the sake of clarity the term variety is used for all samples, even though some of the samples are not commercial varieties. Rastik and CFL98-450 were hull-less barley types, whereas all the others were hulled. CFL93-149 had a high amylose content (38% of the starch), and CFL98-398 and CFL98-450 were waxy type lines (6 and 9% amylose of the starch, respectively). All other barleys had amylose contents of 24−25% of the starch (**Table 1**). The three CFL samples were from the INRA Clermont Ferrand barley program in France. Erhard Frederichen is a Polish variety, and Borzymowicki comes from the United States (a selection from the Polish cultivar Hanna). Dicktoo, also from the United States, has excellent winter hardiness with no vernalization requirement. It is an important genotype in barley genetic studies as a doubled-haploid parent, like Plaisant, Igri, and Morex. Plaisant is a winter type barley from France with a good quality for brewing. Rastik is a spring type barley from Poland and is also used for brewing. Igri is a winter type German model barley plant. There was large variation in yield in the field trial, ranging from 1.1 (CFL93-149) to 5.1 (Borzymowicki) kg/plot (**Table 1**). Thousand kernel weight and test weight varied greatly, with 31−53 g/1000 kernels and 60−86 g/L, respectively. All lines were milled to give wholemeal as well as separate bran and flour fractions, as described by Ward et al. (*36*). The yields of bran and flour varied from 27 to 63% and from 18 to 44%, respectively (**Table 1**). The flour yield was very low for some of the genotypes compared to earlier reports on milling of hull-less barley (37−48%) (*58*). This was probably due to the fact that the milling procedure was not optimized for barley.

Basic Chemical Composition. The gross chemical composition of wholemeal samples of the 10 barley varieties is shown in **Table 1**. Available starch was the major constituent (51−57%), followed by estimated dietary fiber (15−24%) and protein (15−19%). Lipids, ash, and free sugars were only minor constituents with contents of 3−4.5, 1.8−2.5, and 2.2−2.8%, respectively. Estimated dietary fiber contents were determined using an indirect approach and may contain some components that are generally not considered to be dietary fiber. The estimated dietary fiber content was significantly higher in the eight hulled barley genotypes (20−24% dm) than in the hull-

Table 1. Characteristics and Chemical Composition of the Different Barley Varieties[a]

	Dicktoo	Plaisant	Igri	Rastik	CFL93-149	CFL98-398	CFL98-450	Erhard-Frederichen	Borzymowicki	Morex
barley type	winter 6-rowed hulled	winter 6-rowed hulled	winter 2-rowed hulled	winter 6-rowed naked	spring 2-rowed hulled	spring 2-rowed hulled	spring 2-rowed naked	spring 2-rowed hulled	spring 2-rowed hulled	spring 6-rowed hulled
starch type	normal	normal	normal	normal	high-amylose	waxy	waxy	normal	normal	normal
country of origin	USA	France	Germany	Poland	France	France	France	Poland	USA	USA
yield (kg/plot)	1.77	1.62	1.96	2.69	1.13	4.66	4.29	2.97	5.09	3.01
1000 kernel wt (g/1000 kernels)	34.6	31.4	43.1	35.8	53.0	47.3	41.4	38.2	43.7	41.6
test weight (kg/hL)	61.6	60.2	62.8	77.3	67.6	66.5	76.9	85.7	67.6	66.4
Chopin flour yield (%)	35.6	44.1	31.3	36.4	18.2	29.4	22.7	34.3	23.3	31.6
Chopin bran yield (%)	41.2	37.7	42.6	39.0	50.5	45.8	49.2	63.1	39.7	47.2
protein (%)	15.2	15.0	17.9	18.3	16.9	16.3	18.4	18.2	18.7	16.3
ash (%)	2.37	2.50	2.25	2.01	2.27	2.19	1.84	2.02	2.37	2.06
lipids (%)	3.63	3.58	3.55	3.59	4.13	4.15	4.54	3.32	3.64	3.12
starch (%)	52.3 (26)	53.4 (24)	51.9 (25)	58.5 (24)	51.1 (38)	52.3 (6.4)	56.8 (9.0)	54.3 (25)	51.3 (25)	53.5 (24)
free sugars (%)	2.83	2.77	2.35	2.59	2.23	2.63	2.99	2.21	2.22	2.27
estimated dietary fiber (%)	23.8	22.8	22.1	15.0	23.4	22.4	15.4	20.0	21.8	22.8
viscosity (mP · s)	2.96	2.23	2.45	2.72	2.92	3.20	4.66	2.47	3.78	2.26
Klason lignin (%)	4.68	4.23	4.02	3.27	3.86	4.10	3.83	4.45	4.14	3.90
β-glucan (%)	4.0	3.7	4.6	4.6	6.4	5.4	6.5	4.7	5.4	5.1
av MW of β-glucan, g/mol $\times 10^6$	1.84	1.77	1.70	1.58	1.71	1.52	1.52	1.83	1.69	1.66
flour water-extractable arabinoxylan (%)	0.32	0.38	0.24	0.15	0.24	0.27	0.15	0.26	0.2	0.24
flour total arabinoxylan (%) (A/X)	2.16 (0.63)	2.13 (0.64)	1.83 (0.64)	1.4 (0.68)	2.24 (0.63)	2.01 (0.62)	2.01 (0.74)	1.69 (0.67)	2.12 (0.58)	1.53 (0.66)
bran water-extractable arabinoxylan (%)	0.29	0.35	0.25	0.19	0.22	0.28	0.15	0.27	0.24	0.24
bran total arabinoxylan (%) (A/X)	9.03 (0.58)	9.84 (0.56)	8.06 (0.62)	6.05 (0.67)	5.81 (0.58)	8.38 (0.54)	4.84 (0.74)	7.59 (0.55)	7.90 (0.54)	7.80 (0.62)

[a] The amylose content of total starch (%) is given in parentheses. All data are expressed on dry weight.

Table 2. Content and Composition of Tocols, Folates, Sterols, and Alkylresorcinols (AR) in the Different Barley Varieties[a]

	Dicktoo	Plaisant	Igri	Rastik	CFL93-149	CFL98-398	CFL98-450	Erhard-Frederichen	Borzymowicki	Morex
total tocol content (μg/g)	48.0	47.5	52.4	48.9	68.8	65.9	61.0	46.2	54.3	57.1
trienols (μg/g)	38.2	38.1	40.0	35.9	53.5	50.6	48.0	35.6	38.3	44.0
individual tocopherols (μg/g)										
α-tocopherol	7.9	7.4	10.2	10.0	10.1	10.6	9.1	7.7	10.4	10.2
β-tocopherol	0.2	0.3	0.3	0.5	0.6	0.5	0.3	0.4	0.7	0.4
γ-tocopherol	1.7	1.7	1.7	2.3	3.9	3.7	3.3	2.3	4.4	2.2
δ-tocopherol	0.0	0.0	0.2	0.2	0.6	0.5	0.3	0.3	0.5	0.3
α-tocotrienol	31.6	27.1	26.3	24.9	33.4	31.9	35.2	27.4	25.9	29.5
β-tocotrienol	1.9	4.4	4.7	3.6	10.6	5.3	3.3	3.0	4.1	5.6
γ-tocotrienol	4.4	6.1	7.6	6.9	8.3	12.0	9.0	4.8	7.5	7.9
δ-tocotrienol	0.2	0.5	1.4	0.5	1.3	1.4	0.5	0.4	0.8	1.1
total folate content (ng/g)	785	690	738	518	789	535	724	533	645	609
total sterol content (μg/g)	1120	1043	1019	899	1153	1153	1034	1024	996	1038
individual sterols (μg/g)										
sitosterol	595	597	618	510	676	691	618	622	573	604
campesterol	217	207	181	167	199	176	173	141	203	169
Δ^5-avenasterol	84	50	33	65	53	56	54	47	39	54
stigmasterol	45	41	41	25	44	42	33	45	47	38
stanols	25	25	24	10	24	25	17	30	15	21
other sterols	153	121	123	121	158	162	139	139	119	152
total AR content (μg/g)	55.9	48.3	59.2	56.9	68.6	103.1	40.9	41.1	33.5	32.2
AR homologues (μg/g)										
C17:0	3.8	1.2	4.9	0.3	2.2	0.6	3.8	3.7	4.6	3.9
C19:0	3.5	4.0	5.7	3.9	7.8	15.5	1.5	2.7	3.9	2.4
C21:0	14.1	10.6	15.7	12.8	18.5	34.1	9.5	9.7	8.5	8.1
C23:0	7.7	8.3	8.4	10.5	10.9	17.3	7.0	5.5	4.2	3.8
C25:0	26.8	24.3	24.4	29.5	29.2	35.6	19.0	19.5	12.4	14.0

[a] All data are expressed on dry weight.

less genotypes Rastik and CFL98-450 (15% dm) (**Table 1**). The trend of higher dietary fiber content in hulled barleys has been reported previously (*9, 59, 60*) and is due to the fact that Klason lignin, AX, and cellulose are concentrated in the hull (*59, 61*). This was also observed for hulled and hull-less oat varieties in the HEALTHGRAIN diversity screen (*61*).

***β*-Glucan and Arabinoxylan.** β-Glucan was determined on wholemeal samples, whereas WE-AX and TOT-AX contents were determined in flour as well as bran samples. The β-glucan contents of the 10 barley wholemeal samples ranged from 3.7 to 6.5% dm (**Table 1**). Earlier studies described slightly higher β-glucan levels in hull-less barley genotypes compared to their hulled counterparts, which was attributed to the fact that β-glucans are found principally in the starchy endosperm cell walls. The waxy (CFL98-398 and CFL98-450) and high-amylose (CFL93-149) barley varieties contained the highest β-glucan levels, which is consistent with results described earlier (*62*). The β-glucan contents in the barley samples were much higher than those in the 150 wheat and 10 rye samples that varied between 0.5 and 1.0% dm (*47*) and between 1.7 and 2.0%

Table 3. Content and Composition of Total, Free, Conjugated, and Bound Phenolic Acids in the Different Barley Varieties[a]

	Dicktoo	Plaisant	Igri	Rastik	CFL93-149	CFL98-398	CFL98-450	Erhard-Frederichen	Borzymowicki	Morex
phenolic acids (μg/g)										
total phenolics	675.3	512.0	395.2	304.2	549.0	377.9	253.5	422.8	520.1	621.2
total bound phenolics	463.8	375.9	272.1	212.0	442.5	276.2	132.9	313.7	423.2	522.8
total conjugated phenolics	197.7	113.1	115.0	87.6	101.1	94.6	113.0	96.1	86.4	92.8
total free phenolics	13.8	23.0	8.1	4.6	5.4	7.0	7.6	13.0	10.6	5.6
individual free phenolic acids (μg/g)										
4-hydrobenzoic acid	nd[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd
vanillic acid	3.24	4.71	2.60	1.45	1.72	1.47	2.80	3.15	3.22	2.84
syringic acid	2.67	3.74	0.46	1.19	nd	2.43	1.96	2.70	1.78	nd
syringaldehyde	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
caffeic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2,4-dihydrobenzoic acid	2.62	1.69	0.04	nd	nd	nd	nd	nd	nd	nd
sinapic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
ferulic acid	4.73	5.87	2.87	1.32	2.19	1.36	1.86	2.61	2.47	1.57
p-coumaric acid	0.57	7.01	2.14	0.65	1.52	1.49	1.03	3.23	2.82	1.18
2-hydroxycinnamic acid	nd	nd	nd	nd	nd	0.27	nd	1.31	0.26	nd
individual conjugated phenolic acids (μg/g)										
4-hydrobenzoic acid	26.7	12.1	14.1	6.4	9.6	8.5	10.3	7.7	10.1	5.8
vanillic acid	30.2	12.6	12.8	11.5	11.9	9.8	11.2	10.8	8.9	10.4
syringic acid	2.2	10.0	7.8	3.6	3.1	6.5	3.6	3.8	6.3	6.7
syringaldehyde	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2,4-dihydrobenzoic acid	61.6	31.8	10.9	7.0	6.8	8.0	13.4	28.2	8.1	26.2
sinapic acid	18.7	16.1	20.6	18.2	18.9	17.4	24.4	13.8	20.1	12.4
ferulic acid	42.5	21.7	38.6	37.7	41.0	34.0	42.5	23.5	25.2	25.4
p-coumaric acid	13.1	5.6	8.1	1.7	8.3	8.2	6.1	5.9	5.2	4.6
2-hydroxycinnamic acid	2.7	3.2	2.1	1.5	1.5	2.3	1.4	2.3	2.4	1.2
individual bound phenolic acids (μg/g)										
4-hydrobenzoic acid	2.3	5.4	4.8	0.8	3.9	2.9	0.5	4.7	3.0	2.0
vanillic acid	7.5	5.3	5.4	3.9	4.5	4.8	0.5	6.6	4.4	4.5
syringic acid	3.0	1.1	0.8	0.0	1.2	0.8	0.4	0.6	0.6	1.0
syringaldehyde	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2,4-dihydrobenzoic acid	19.2	21.5	13.5	16.4	31.9	18.4	11.1	26.0	74.4	61.6
sinapic acid	8.9	15.1	13.6	11.7	14.4	13.0	10.7	12.3	17.8	14.1
ferulic acid	365.4	234.3	191.5	172.7	327.5	192.9	104.3	197.9	227.7	326.8
p-coumaric acid	52.8	90.4	39.7	2.9	55.3	39.0	2.7	62.2	91.6	109.7
2-hydroxycinnamic acid	4.7	2.8	2.7	3.5	3.8	4.3	2.6	3.4	3.9	3.1

[a] All data are expressed on dry weight. [b] nd, not detected.

dm (*63*), respectively. The molecular weight distribution for β-glucan was unimodal for all barley samples and ranged from about 1×10^5 to 5×10^6 g/mol. The average molecular weight ($\bar{M}$cf) varied between 1.52 and 1.84×10^6 g/mol (**Table 1**). This was in the same range as reported earlier for barley (*48*) and also similar to the oat samples in the HEALTHGRAIN diversity screen [$(1.69-1.77) \times 10^6$ g/mol] (*61*). In another study, Swedish oats were shown to have somewhat lower molecular weights (*64*). The two hull-less varieties Rastik and CFL98-450, together with one hulled variety (CFL98-398), had the lowest average molecular weights [$(1.52-1.58) \times 10^6$ g/mol] in this study. The molecular weight percentiles at 10, 50, and 90% were also lower for these three varieties [$(0.48-0.51) \times 10^6$, $(1.38-1.47) \times 10^6$, and $(2.70-2.78) \times 10^6$ g/mol, respectively], than for the other samples [$(0.58-0.70) \times 10^6$, $(1.56-1.76) \times 10^6$, and $(2.86-3.11) \times 10^6$ g/mol] (individual results not shown).

The TOT-AX content in the barley flours varied between 1.4 and 2.2% dm (**Table 1**), which is in the range determined for wheat, whereas the rye and oat flours included in the HEALTHGRAIN diversity screen had higher and lower levels, respectively. The A/X values of TOT-AX of the different barley flour samples were similar (0.58−0.74). Analysis of the bran fractions (which also contained the hulls of the hulled lines) gave lower TOT-AX contents for the hull-less varieties (Rastik and CFL98-450) than for the hulled ones, which is due to he fact that AX is concentrated in the hull. The high-amylose variety CFL93-149 was also characterized by low TOT-AX. This is in contrast with the results from Oscarsson et al. (*9*), who analyzed a set of barley samples and reported the highest xylose and arabinose contents for a high-amylose barley. Bran of the waxy hulled barley genotype CFL98-398 had a TOT-AX content comparable to the regular barley genotypes. This is consistent with the results of Xue et al. (*59*), who did not observe any effect of the waxy gene on TOT-AX content. The A/X values of barley bran TOT-AX varied between 0.54 and 0.74 (**Table 1**). The two hull-less barleys were at the upper limit of this range, which agrees with data from Holtekjølen et al. (*1*). However, they also reported higher degrees of branching for waxy and high-amylose barleys, which is not the case for the barley samples analyzed in this study. In comparison to wheat and rye (*47, 63*), the barley flour and bran fractions contained significantly lower levels of WE-AX, that is, varying between 0.15 and 0.38% dm and between 0.15 and 0.35% dm, respectively. These levels compare well with those reported for oats (*61*). The hull-less variety Rastik and the waxy variety CFL98-450 have the lowest WE-AX levels, whereas the hulled cultivar Plaisant has the highest level, both in flour and in bran.

Phytochemicals. *Tocols.* The total tocol contents of the 10 barley varieties ranged from 46.2 to 68.8 μg/g of dm in wholemeals with an average value of 55.0 μg/g of dm (**Table 2**). The barley grains generally contained all eight vitamers, with α-tocotrienol being the major one contributing to ≥47.7% of total tocols, and followed by γ- and β-tocotrienols and α-to-

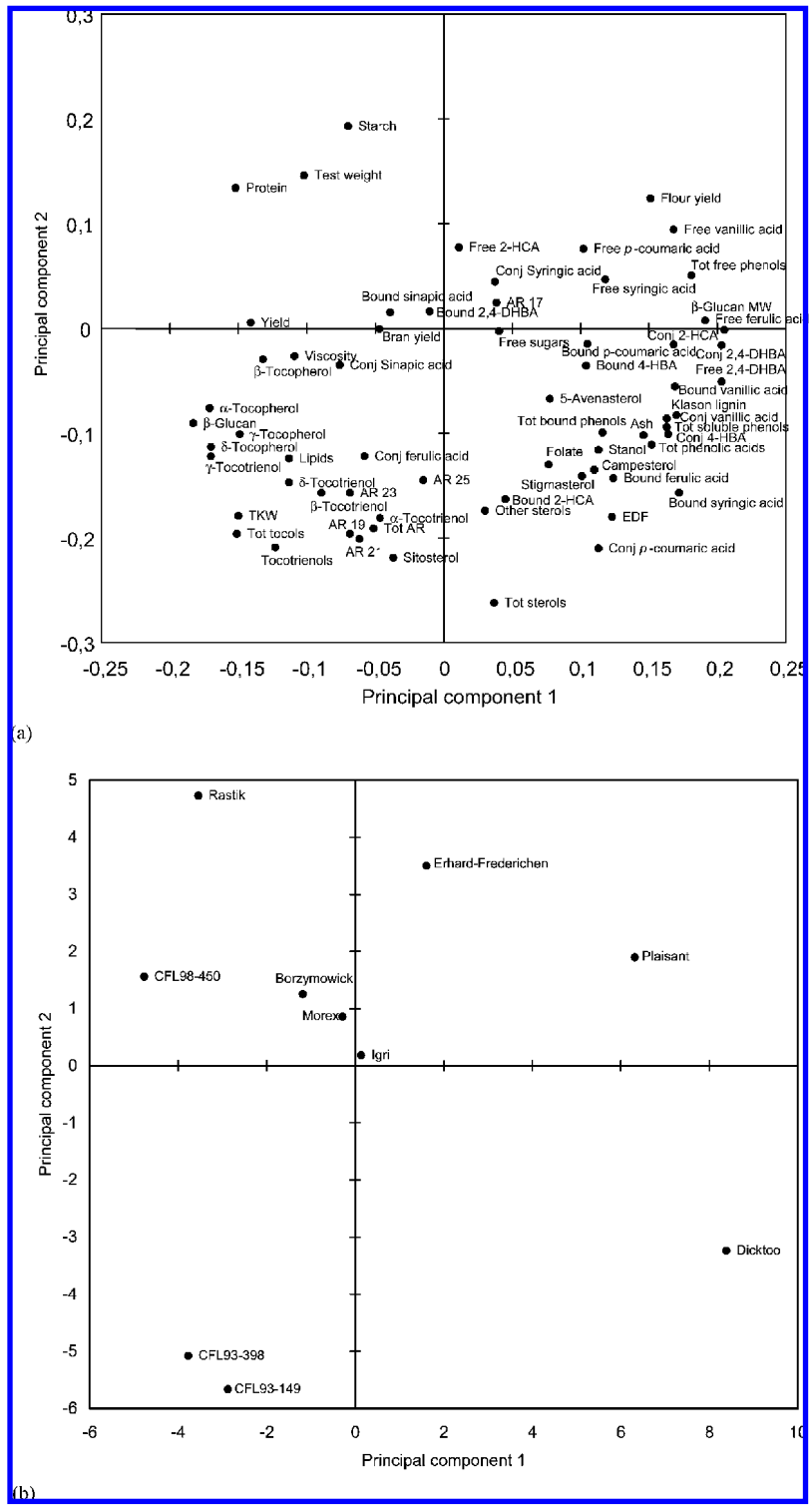

Figure 1. (**a**) Loading plot and (**b**) score plot of the first two principal components from analysis of 10 different barley samples. EDF, estimated dietary fiber; MW, molecular weight; AR, alkylresorcinol; AR17−25, alkylresorcinol homologues; Conj, conjugated; 2,4-DHAB, 2,4-dihydrobenzoic acid; 2-2-HCA, 2-hydroxycinnamic acid; 4-HBA, 4-hydrobenzoic acid.

copherol (**Table 2**). The average proportion of tocotrienols was 76.8% of total tocols, indicating that barley is one of the richest sources of tocotrienols among cereal grains (see also ref *36*).

The tocol compositions of the 10 barley varieties were in line with those obtained in earlier studies. The total tocol contents were greater than those reported for two commercial barley meal samples, being 32 and 36.9 μg/g of fw (*65, 66*), but they showed a similar range as samples derived from six spring barley cultivars grown in Italy containing 51.0−61.4 μg/g of dm of tocols (*67*) and 12−13 lines grown in Czech Republic containing 45.8−68.1 μg/g of dm of tocols (*68, 69*). Similarly, α-tocotrienol was always the major vitamer, and the proportion of tocotrienols ranged between 72 and 81% as in the other published studies of barley grain (*67*−*70*). In general, total tocol contents of barley grains were among the highest in HEALTHGRAIN diversity screen samples. In the Italian study referred to above, hulled barley genotypes contained more total tocols than the hull-less genotypes (*67*), whereas in the Czech study the waxy

(both hulled and hull-less) lines had greater levels than hulled malting lines (*68, 69*). In this study the total tocol contents of the two hull-less varieties (Rastik and CFL98-450) did not differ from those of the other genotypes, whereas both the high-amylose and waxy types contained more tocols than barley varieties with normal starch.

Folates. The total folate contents of the 10 barley genotypes ranged from 518 − to 789 ng/g of dm, with the mean folate content being 657 ng/g of dm (**Table 2**). The highest contents were measured in CLF93-149 and Dicktoo and the lowest contents in Erhard-Frederichen and Igri. The mean content was higher than those in the wheat and oat genotypes (mean contents of 551 and 566 ng/g of dm, respectively) (*57, 61*) and close to that of the rye genotypes (*63*) in the HEALTHGRAIN diversity screen. Previously, only a few values have been published on total folate in barley grains. Cerna and Kas (*71*) reported a value 360 ng/g and Hegedüs et al. (*72*) a value 730 ng/g of fw, which are within the range of our results. Barley groats (i.e., grains) were reported to contain 250−370 ng/g of fw (*73*) and 210 ng/g of dm (*74*) of folate. Food composition tables report folate contents of barley groats ranging from 190 to 340 ng/g of fw (http://www.ktl.fi/fineli/, http://www.foodcomp.dk/, http://www.ars.usda.gov/nutrientdata). There are no data on variation in different varieties and climate conditions.

Sterols. The average content of plant sterols in the barley varieties was 1048 μg/g of dm, ranging from 899 to 1153 μg/g of dm (**Table 2**). As in most cereals, sitosterol was the most abundant sterol form, contributing 53−61% of total sterols, followed by campesterol (14−20%). Interestingly, in all of the varieties, except Borzymowicki, the proportion of Δ^5-avenasterol (range of 3.2−7.5%) was higher than that of stigmasterol (2.8−4.7%), which is generally thought to be one of the abundant sterols in plants. Furthermore, the percentage of stanols (saturated sterols, commonly rich in cereals) in barley varieties was only 1−3%.

The sterol composition of barley varieties with a low percentage of stanols and high percentage of Δ^5-avenasterol was similar to the sterol composition of oat genotypes, although the overall content of plant sterols in oats was significantly lower (average = 653 μg/g of dm) (*61*). The content of sterols in the barley varieties was on average higher than in a previous study that reported total sterol contents for the barley cultivars Kustaa and Pokko of 820 and 910 μg/g of dm, respectively (*53*). In those cultivars the proportion of Δ^5-avenasterol was also higher than stigmasterol, and stanols contributed 2% of total sterols.

Alkylresorcinols. The content of alkylresorcinols in the 10 barley varieties ranged from 32 to 103 μg/g of dm, with an average of 55 μg/g of dm (**Table 2**). This is in accordance with earlier studies that have shown an alkylresorcinol content of 41−210 μg/g of dm in different barley varieties and with different methods used (*34, 35, 75*). This content is much lower than in wheat and rye, which have been reported to contain about 300−1000 and 360−3200 μg/g of dm, respectively (*35*). The highest content was found in CFL98-398, which is a hulled barley type with waxy starch. The relative composition of AR was quite similar for the different barley varieties (**Table 2**). The dominant AR was C25:0, with 35−48%, followed by C21:0, with 23−33% and C23:0, with 12−19%. The relative content of C17:0 and C19:0 was generally lower, but varied greatly between genotypes (1−14 and 6−15%, respectively). These results are in accordance with an earlier study on Swedish barleys (*35*). However, in another study of Polish barleys the dominant AR was C21:0 (34−43%), followed by C19:0 (27−37%) and C25:0 (15−25%), whereas C23:0 was lower (8−12%) and C17:0 was present only in very small amounts (0−2%) (*75*).

Phenolic Acids. Wholemeal samples of the 10 different barley varieties were analyzed for free, conjugated, and bound phenolic acids. In addition to total concentrations of each class of phenolic acids, individual measurements were also made for each phenolic acid. The phenolic acids quantified in this study included 4-hydroxybenzoic acid, vanillic acid, syringic acid, syringaldehyde, caffeic acid, 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.

A wide range (254−675 μg/g of dm) in total phenolic acid content was observed across the different barley samples in this study, with the mean value being 463 μg/g of dm (**Table 3**). These results are consistent with those previously reported for commercial barley flour from Finland (450 mg/kg of fw) (*76*). The cultivar with the highest total concentration of phenolic acids was Dicktoo (675 μg/g of dm), and that with the lowest concentration was CFL98-450 (254 μg/g of dm). Considering all samples, the varieties originating from the Unites States (Dicktoo, Morex, and Borzymowicki) contained a higher total concentration of phenolic acids (mean = 605 μg/g of dm) than the European cultivars.

Free phenolic acids comprised a very small (<3%) proportion of the total phenolic acids. Of the 10 varieties Plaisant had the highest concentration of free phenolic acids (23 μg/g of dm), whereas Rastik had the lowest (4.6 μg/g of dm). The range of concentrations of free phenolic acids did not vary according to the different countries of origin of the barley varieties. Soluble conjugated phenolic acid concentrations comprised approximately 25% of the total phenolic acid content and with levels ranging from 86 to 198 μg/g of dm. Again no correlation with the country of origin was observed. Nine of the varieties contained similar concentrations of soluble conjugated phenolic acids (86−115 μg/g of dm). The exception was Dicktoo, which originated from the United States and had a much higher concentration of soluble conjugated phenolic acids (198 μg/g of dm). Bound phenolic acids make up approximately 73% of the total phenolic acid content with concentrations ranging from 133 to 523 μg/g of dm. The genotype with greatest total concentration of phenolic acids was Morex (523 μg/g of dm), and that with the lowest was CFL98-450 (133 μg/g of dm). Across all of the samples the varieties originating from the United States (Dicktoo, Morex, and Borzymowicki) contained a higher total concentration of bound phenolic acids (mean = 470 μg/g of dm) than the European cultivars.

Phenolic Acid Composition. The breakdown of individual phenolic acids in the different classes (free, conjugated, and bound) varied greatly. The predominant phenolic acids are ferulic, vanillic, syringic, and *p*-coumaric acid, and the percentages of these vary in the different free or bound forms (**Table 3**). The free phenolic acids fraction contained four major phenolic acids (averages: ferulic, 27%; vanillic, 28%; syringic, 17%; and *p*-coumaric acid, 22%). Whereas the ferulic acid content increases slightly (to 31%) in the soluble conjugated fraction, the percentage levels of other dominant phenolic acids decrease slightly to accommodate the elevations of sinapic acid and 2,4-dihydroxybenzoic acid levels in the conjugated forms. Ferulic acid is the dominant form of bound phenolic acids, comprising 68% of the total fraction. These results are in agreement with previous studies of the *p*-coumaric acid and ferulic acid levels in barley (*77, 78*). The total ferulic acid

concentrations ranged between 149 and 413 μg/g of dm, with a mean value of 270 μg/g of dm. In general, the highest ferulic acid levels were observed for barley varieties of U.S. origin. The other major phenolic acid in barley, *p*-coumaric acid, ranged from 5.25 to 115.5 μg/g of dm with a mean concentration of 63.5 μg/g of dm. The results obtained for both of these components were in agreement with previously reported studies (*76*).

Principal Component Analysis. PCA was used to illustrate the variation in the material and to identify correlations between different variables. The first two principal components (PC1 and PC2) explained 29 and 19% of the variation, respectively (**Figure 1**). The loading plot shows the relationship between different variables (**Figure 1a**) and the score plot the relationship between different samples (**Figure 1b**). The loading plot shows that varieties with high levels of components concentrated in the hull and bran were present in the lower right square. These included estimated dietary fiber, Klason lignin, and ash, as well as several of the phenolic acids and sterol fractions, which were positively correlated. Total phenolics, total bound phenolics, and total conjugated phenolics were located close to each other in this square, whereas total free phenolics and most of the individual free phenolics were located in the upper right square and not correlated with the bound and conjugated phenolic acid fractions. Bound and conjugated phenolics are known to be concentrated in the outer layers of the grain, and they are therefore correlated to ash and estimated dietary fiber content. Most of the individual bound and conjugated phenolics were also positively correlated with these variables. Estimated dietary fiber, ash, and phenolics were located opposite the starch and protein content, which were found in the upper left square. These groups of components were thus negatively correlated to each other.

Total AR content was in the lower left square, together with all AR homologues except C17:0, which was present in very low amounts in all barley varieties. All other AR homologues correlated positively to each other and to the total content of AR. Total tocopherols, tocotrienols, and most of the individual tocols were also found in the lower left square together with 1000 kernel weight (TKW) and lipids, which means that they were positively correlated to each other and also to AR content. Larger grains thus contained more tocols and AR than smaller grains. All of these variables, including TKW, were in opposite part of the plot to flour yield. This is perhaps surprising as TKW is positively correlated to flour yield (as discussed in ref *36*). However, the flour yields from milling the barley lines in the current study were low and varied greatly between varieties; this probably reflects the fact that the milling procedure was not optimized for barley, rather than any intrinsic differences between the milling properties of the varieties.

The content of β-glucan as well as viscosity was also found in the lower left square, opposite the molecular weight of β-glucan, which was located in the lower right square. The viscosity is known to be affected by β-glucan content, which explains the positive correlation between these two variables, but the negative correlation of β-glucan content and molecular weight is more unexpected. Yao et al. (*79*) showed a relationship between pasting properties and both content and molecular weight of β-glucan.

The score plot shows that the hull-less barley varieties (Rastik and CFL98-450) are located together in the upper left square, whereas the French varieties CFL193-149 and CFL98-398 with high-amylose and waxy starch, respectively, are located in the lower left square (**Figure 1b**). Objects with high scores for one PC have large values for variables with large loadings for the same PC. Thus, CFL193-149 and CFL98-398 had high TKW and high levels of the phytochemicals AR and tocols. They were, however, lower in phenolic compounds. Dicktoo (from the United States) was the only cultivar in the lower right square, which means that it was higher in phenolic compounds and estimated dietary fiber than the other genotypes. The hull-less varieties Rastik and CFL98-450 were high in starch and protein and low in estimated dietary fiber and phenolics, due to the absence of a hull.

In summary, there was large variation between the barley samples and some interesting correlations between dietary fiber and phytochemical components. However, caution must be taken in drawing conclusions about the full range of variation in the composition of barley as only a small number of varieties was analyzed. The aim of the HEALTHGRAIN diversity screen was to study the composition of raw materials, and therefore the hulls were not removed from the hulled barleys. For human consumption of barley, further studies on flour with standardized ash content and without the inedible hull fraction have to be performed. Further studies are also required to determine the extent to which differences in the composition that were identified are affected by growth location, year, and other agroclimatic factors.

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