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Phytochemical and Fiber Components in Oat Varieties in the HEALTHGRAIN Diversity Screen

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The levels and compositions of a range of phytochemicals (sterols, tocols, avenanthramides, folates, phenolic acids) and dietary fiber components were determined in five oat cultivars (four husked and one naked) grown on a single site in 2005. The total levels of tocols, phenolic acids, and avenanthramides varied by over 2-fold between cultivars, but less variation occurred in total sterols and total folates. Limited variation was also observed in the dietary fiber content and composition of the four husked lines. These results indicate that it may be possible to selectively breed for lines with high contents of dietary fiber and specific groups of phytochemicals.

KEYWORDS: Dietary fiber; oats; phytochemicals

INTRODUCTION

Oats is only sixth in world cereal production (after wheat, maize, rice, barley, and sorghum) but differs from the more widely grown cereals in that it is consumed almost exclusively in wholegrain products. It contains significant quantities of dietary fiber and associated antioxidative phenolics and other components such as folates and sterols that contribute to health benefits.

Mixed linkage (1−3)(1−4)-β-D-glucans (β-glucans) together with arabinoxylans (AX) are the most important nonstarch polysaccharides (NSP) in oats, and both are important sources of soluble as well as insoluble dietary fiber (*1, 2*). Oats have long been considered as a healthy food ingredient, with an increasing body of evidence that the β-glucans have health-promoting properties, for example, by reducing risks of developing type 2 diabetes and heart disease (reviewed in ref *3*). These physiological effects are related to increased intestinal viscosity, which is dependent, among other things, on concentration and molecular weight of β-glucan (*4*), and similar properties have also been ascribed to AX (*5*). In addition to their nutritional importance, these NSP are known to have a significant impact on cereal processing and the quality of processed food products (*6*).

Oats also contain several groups of antioxidants including tocopherols and tocotrienols (tocols), phenolic acids, and avenanthramides. Although current recommendations consider that only 2*R*-α-tocopherol meets the vitamin E requirement, all tocols have high antioxidant activity in vivo and in vitro (*7*). Although they are only moderate sources of α-tocopherol, cereals are good sources of some other tocols, especially tocotrienols (*8*), which possess other potential health benefits (*9*).

Phenolic acids derived from hydroxybenzoic and hydroxycinnamic acids occur throughout the plant kingdom including cereals, and early studies showed that the phenolic acids in oats had antioxidant properties (reviewed in ref *10*).

The avenanthramides, a group of *N*-cinnamoylanthranilate alkaloids, are unique to oats among the cultivated cereals (*11, 12*). They are related to the flavor of oat products (*13*) and may act as antioxidants in vivo (*14*). They may also have anti-

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Table 1. Characteristics of the Oat Cultivars[a]

origin	cultivar	type	yield (t/ha)	flour yield (%)	bran yield (%)	protein content	
						wholemeal	flour
Poland	Cacko	naked	5.42	36.4	50.9	18.7	11.9
Hungary	MV-Pehely	husked	5.15	35.3	51.9	18.1	13.4
China	Fengli	husked	4.30	38.6	50.6	18.4	nd
Austria	Expander	husked	5.72	42.1	46.6	17.0	11.7
Poland	Bajka	husked	5.28	42.8	46.2	16.9	nd

[a] Yields were determined on the basis of four replicate 6 m^2 plots.

inflammatory, antiatherogenic, and antiproliferative properties (*15, 16*).

Cereals are major dietary sources of B vitamins including folate. For example, cereal products have been calculated to account for about 22% of the daily dietary intake of folate in the United Kingdom (*17*) and for 36% of the intake for women and 43% for men, respectively, in Finland (*18*). Current interest in folate relates to two major biological roles, in preventing defects in neural tube development during pregnancy and in reducing levels of homocysteine in the serum, which is a risk factor in atherosclerosis (*19, 20*).

Plant sterols (phytosterols) have cholesterol-lowering properties with natural intake levels of >150 mg/day inhibiting cholesterol uptake from the intestinal digestive system (*21*). Plant sterols also decrease both total cholesterol and LDL-cholesterol levels in serum.

Alkylresorcinols (ARs) are 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring. They are only present in the outer layers of the grain, notably in wheat and rye, and are therefore suggested to be biomarkers for intake of whole grain food products (*22*). They may have biological and physiological effects, which makes them interesting also from a nutritional point of view (*22, 23*). ARs have not been found previously in oats (*24*), so the present study included analyses to confirm this observation.

The HEALTHGRAIN cereal diversity screen forms part of an Integrated Project supported by the European Union as part of the sixth Framework program (see ref *25* and http://www.healthgrain.org/pub/). The main focus of the project is to develop new healthy food products based on whole grains of wheat and rye. However, other cereal species have also been studied including oats. Five oat varieties were grown in Martonvásár (near Budapest, Hungary) in 2005, and their contents of dietary fiber and phytochemical components were determined. This provides a unique comparison of the detailed composition of a selection of varieties grown under the same agronomic and environmental conditions.

MATERIALS AND METHODS

Oat Samples. Single 6 m^2 plots of five winter oat cultivars (including the naked type cv. Cacko) were grown in the field at the Agricultural Research Institute of the Hungarian Academy of Sciences (2005), Martonvásár, Hungary (latitude, 47° 21′ N; longitude, 18° 49′ E; altitude, 150 m). The soil was of the chernozem type with a loam texture and pH 6.8−7.2. The previous crop was peas. Plots were fertilized with 80 kg/ha of fertilizer containing 34% N in the autumn and a further 60 kg/ha in the spring. 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) and insecticide (0.2 L/ha Karate containing 2.5% λ-cihalotrin). No infections or fungal pathogens were observed.

After harvest, the grains from each plot were mixed and single 2 kg samples removed for milling. These samples (including hulls) were conditioned to 14% moisture content before milling using a Retch ZM100 Mill to produce wholemeal and a Chopin CD1 Laboratory Mill to produce bran and flour fractions. Milled samples were immediately cooled to −20 °C to protect bioactive components from degradation. The high bran yields (46−52%, see **Table 1**) reflect the presence of the hulls, which were present in the wholemeal samples.

Chemical Composition of Wholemeals. Moisture, crude protein, and ash contents were determined according to AACC approved methods 44-15A, 46-10, and 08-01, respectively. Total lipids were determined gravimetrically by extraction with acid solvent consisting of 60:40:1 (v/v/v) chloroform, methanol, and hydrochloric acid as described by Marchello et al. (*26*). Available starch was determined according to the procedure of Megazyme without dimethyl sulfoxide, which is consistent with AACC approved method 76-13, and free sugars by gas chromatography (GC) as a sum of all mono- and disaccharides (i.e., fructose, glucose, maltose, and sucrose) (*27*). The fiber content of the wholemeal samples was estimated as the difference from the analyses of moisture, protein, ash, lipids, available starch, and free sugars according to the following formula: fiber, % of dry weight = 100 − (crude protein + ash + total lipids + available starch + free sugars). Klason lignin was determined gravimetrically using AACC approved method 32-25 and viscosity of grain water extracts as described by Boros et al. (*28*) and Saulnier et al. (*29*) 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. All analyses were performed in triplicate, except for analysis of available starch and free sugars which were carried out in duplicate.

Arabinoxylan and β-Glucan Contents. The water-extractable arabinoxylan (WE-AX) and total arabinoxylan (TOT-AX) contents of the flour and bran samples were calculated after acid hydrolysis of all sugars, derivatization of the monosaccharides to alditol acetates, and quantification of these alditol acetates by gas−liquid chromatography (*30*). For the determination of the TOT-AX content, the complete sample was subjected to hydrolysis, whereas for determination of the WE-AX content the water-extractable fraction of the sample was analyzed. However, no correction was made of the arabinose content for the presence of arabinogalactan peptide. The mixed linkage β-glucan content was determined on flour, bran, and wholemeal samples using the Megazyme mixed linkage β-glucan assay kit (Megazyme, Bray, Ireland), as described by Gebruers et al. (*30*).

The molecular weight distribution of β-glucans was determined essentially according to the method of Rimsten et al. (*31, 32*), except that the extraction time in hot water was 6 h instead of 1.5 h. The 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 values at which 10, 50, and 90% of the distribution fall below were calculated using a calibration curve according to the method of Rimsten et al. (*31*).

Phytochemicals. Sterols were determined as described previously (*33, 34*) and tocopherols and tocotrienols (α-, β-, γ-, and δ) by normal-phase HPLC using fluorescent detection (*35, 36*). Total folate contents were determined microbiologically on 96-well microtiter plates using *Lactobacillus rhamnosus* ATCC 7469 as the test organism (*37*). Alkylresorcinols were extracted from intact kernels with ethyl acetate and analyzed by GC as described by Andersson et al. (*38*). Dry matter content of whole kernels was determined by oven-drying crushed grains (coffee-type mill, Janke and Kunkel, IKA-WERK, Germany) at 105 °C for 16 h. Concentrations of free, soluble conjugated, and bound phenolic acids were determined as detailed in Li et al. (*39*), using

Table 2. Levels of Major Components in Wholemeal Samples of Oat Grain

	cultivar				
	Cacko	MV-Pehely	Fengli	Expander	Bajka
ash (%)	2.1	2.4	2.3	2.4	2.6
lipids (%)	9.6	8.8	7.6	6.6	5.8
available starch (%)	57.3	50.7	47.0	49.4	51.0
free sugars (%)	1.7	1.5	1.4	1.5	1.4
estimated dietary fiber (%)	10.6	18.5	23.4	23.1	22.2
Klason lignin (%)	2.6	4.2	5.9	4.4	4.7
viscosity (mP·s)	7.0	5.7	2.7	3.6	4.6

reversed-phase HPLC-DAD chromatography using an acidic water/acetonitrile elution gradient.

Avenanthramides were extracted and determined with a modified version of the method described by Dimberg et al. (*40*). In brief, samples of the flours (2.0 g) were extracted with 80% (v/v) ethanol. The supernatants were evaporated and the residues suspended in 2 mL of methanol. After centrifugation, the compounds in the extracts were separated using a reversed-phase C-18 column and a linear gradient from 5 to 38% acetonitrile in 0.01 M fumaric acid (pH 2.9) (including 5% acetonitrile) over 35 min as mobile phase. Identification and quantification of the three main avenanthramides (2c, 2p, and 2f) were performed using synthetic compounds as external standards (*12*).

With the exception of the avenathramides, further details of analytical methods are given in the accompanying papers (*30, 33, 34, 36*−*39*). All analyses were carried out in duplicate or triplicate (as appropriate), and the standard errors of means were below 5% of the means in all cases. Unless otherwise stated, all results are expressed on a dry weight basis.

RESULTS AND DISCUSSION

Selection and Characteristics of Oat Samples. Five oat cultivars, all spring type, were selected for study. Cacko is a naked oat variety with high protein content. It has a high soluble dietary fiber (including β-glucan) content, but it is also sensitive to the environment and may give lower yields than other cultivars. Bajka is an intensive type with good protein content and seed yield. Expander is also an intensive type with good yield and is less sensitive to environmental effects. MV-Pehely has high protein content, whereas Fengli is a tall type.

The yields from the plots at MV ranged from 4.30 t/ha (Fengli) to 5.72 t/ha (Expander) (**Table 1**). All cultivars were milled to give wholemeal flours and separate bran and flour components, as described by Ward et al. (*41*). The yields of bran (including hulls) and flour varied from about 35 to 43% and from about 46 to 52%, respectively (**Table 1**).

Chemical Composition of Wholemeals. Wholemeal samples of the lines contained about 17−18% protein (**Table 1**) and 2−3% ash, the lowest ash level being in the naked cultivar Cacko (**Table 2**). Similarly, Cacko also contained higher proportions of protein and oil and a lower content of lignin than the other cultivars. The differences between Cacko and the other cultivars can be explained by the absence of the hull, which can account for up to 30% of the total kernel weight and is rich in ash and fiber but contains only 1−2% protein and no starch or oil (*42*). However, there were also differences in the oil contents of the four hulled cultivars (MV-Pehely, Fengli, Expander, Bajka), which were within the range reported for commercial oat cultivars (*43, 44*). Free sugars were low (<2%) in all lines.

Subtraction of the amounts of protein, lipid, ash, sugars, and enzyme digestible starch from the dry weight indicated that the estimated fiber content ranged from 10.6% dw in the naked variety Cacko to 18.5−23.4% in the hulled cultivars.

Table 3. Amounts and Properties of β-Glucan and Arabinoxylans (AX) in Wholemeal, Bran, and Flour Samples

	cultivar				
	Cacko	MV-Pehely	Fengli	Expander	Bajka
wholemeal β-glucan %	5.5	5.6	4.5	5.1	5.3
wholemeal β-glucan molecular weight ($\times$ 10^6g/mol)					
Mcf[a]	1.73	1.71	1.77	1.69	1.74
p10[b]	0.51	0.54	0.53	0.50	0.56
p50	1.65	1.62	1.69	1.62	1.66
p90	2.99	2.93	3.03	2.94	2.98
flour WE-AX (%)	0.16	0.18	0.18	0.15	0.18
flour TOT-AX (%)	0.97	1.26	1.14	1.05	1.15
flour β-glucan (%)	1.09	1.12	1.03	1.03	0.96
bran WE-AX (%)	0.19	0.20	0.21	0.19	0.21
bran TOT-AX (%)	3.83	13.20	11.52	8.75	8.02
bran β-glucan (%)	8.33	8.09	6.20	8.18	8.39

[a] Calcofluor average molecular weight. [b] Percentiles (p10, p50, and p90) describing the molecular weight values below which 10, 50, and 90% of the distribution falls.

Table 4. Levels of Major Groups of Phytochemicals in the Oat Cultivars

	cultivar				
	Cacko	MV-Pehely	Fengli	Expander	Bajka
sterols					
total sterols (μg/g)	618	646	657	682	662
stanols (%)	2.6	2.4	2.3	1.9	2.1
tocols					
total tocols (μg/g)	36.1	30.7	23.7	23.0	16.1
trienols (%)	71.2	65.2	74.4	68.7	68.1
folates					
total folates (ng/g)	495	571	604	571	588
phenolics					
total phenolics (μg/g)	612	423	351	395	874
total bound phenolics (μg/g)	248	175	131	169	640
total soluble phenolics (μg/g)	314	138	111	136	180
total free phenolics (μg/g)	50	110	109	90	53
avenanthramides					
total (μg/g)	42	44	85	48	91

Table 5. Compositions of Sterols and Tocols (Micrograms per Gram) in the Oat Cultivars

	cultivar				
	Cacko	MV-Pehely	Fengli	Expander	Bajka
individual sterols					
sitosterol	365	408	414	442	410
campesterol	52	60	50	62	53
stigmasterol	23	27	36	23	22
Δ^5-avenasterol	34	24	26	30	34
Δ^7-avenasterol	12	8	11	10	11
stanols	16	16	15	13	14
other sterols	115	103	103	103	117
individual tocols					
α-tocopherol	9.5	9.8	5.5	6.2	4.5
β-tocopherol	0.9	0.9	0.6	1.0	0.6
α-tocotrienol	23.0	18.2	16.3	14.2	9.4
β-tocotrienol	2.7	1.8	1.3	1.6	1.6

Dietary Fiber Components. The β-glucan contents of the wholemeal flours were similar in the five lines and ranged from 4.5 to 5.6%. This is consistent with previous studies, which have shown that oat hulls (which were present in four of the lines) are rich in hemicellulose, lignin, and cellulose but not in β-glucan (*1, 42*). Similarly, no significant differences in β-glucan contents were found between hull-less (naked) and hulled barley varieties, which were also analyzed in the HEALTHGRAIN study (*32*), although some workers have reported a tendency

Table 6. Compositions of Free, Bound, and Conjugated Phenolics Fractions (Micrograms per Gram) in the Oat Cultivars

	cultivar				
	Cacko	MV-Pehely	Fengli	Expander	Bajka
individual free phenolics					
4-hydrobenzoic acid	7.2	44.9	50.6	30.6	6.2
vanillic acid	nd	10.1	7.4	3.9	93.8
syringic acid	4.6	7.9	7.1	7.4	nd
syringaldehyde	nd	3.2	4.6	4.4	21.2
caffeic acid	13.0	15.7	10.8	14.8	5.2
2,4-dihydrobenzoic acid	5.0	nd	nd	nd	nd
sinapic acid	10.0	10.3	9.7	10.2	9.1
ferulic acid	6.6	7.8	6.6	8.4	nd
p-coumaric acid	2.4	9.3	11.9	8.6	nd
2-hydroxycinnamic acid	1.2	0.5	nd	2.0	6.7
individual conjugated phenolics					
4-hydrobenzoic acid	59.7	32.2	37.1	57.2	39.0
vanillic acid	27.7	22.0	21.9	18.1	19.4
syringic acid	49.6	34.3	30.8	27.1	25.4
syringaldehyde	4.0	1.7	2.0	3.1	2.5
2,4-dihydrobenzoic acid	35.1	21.7	13.6	9.8	12.1
sinapic acid	52.5	45.3	41.2	33.7	41.7
ferulic acid	119.7	68.7	44.9	53.9	68.0
p-coumaric acid	12.6	17.2	24.2	20.7	22.1
2-hydroxycinnamic acid	3.1	4.5	4.4	2.4	3.0
individual bound phenolics					
4-hydrobenzoic acid	10.8	6.1	7.8	8.6	43.1
vanillic acid	4.1	2.9	1.7	3.7	6.0
syringic acid	5.7	3.8	1.1	3.7	5.6
syringaldehyde	nd	nd	nd	nd	nd
2,4-dihydrobenzoic acid	21.5	nd	nd	nd	16.6
sinapic acid	17.5	12.9	10.3	9.6	18.8
ferulic acid	143.5	79.7	44.7	61.0	259.7
p-coumaric acid	41.7	67.2	62.7	79.7	287.3
2-hydroxycinnamic acid	2.9	2.7	2.4	2.6	3.3

Table 7. Composition of Avenanthramides (Micrograms per Gram) in the Oat Cultivars

	cultivar				
avenanthramide	Cacko	MV-Pehely	Fengli	Expander	Bajka
2c	16.3	15.8	44.9	18.7	37.2
2p	13.4	13.0	23.8	15.7	30.0
2f	12.7	15.6	16.1	13.6	24.0

toward lower contents in hulled samples (*45*). In contrast, the β-glucan contents of the 10 rye and 150 wheat cultivars included in the same diversity screen were much lower, varying between 1.7 and 2.0% (*46*) and between 0.5 and 1.0%, respectively (*30*).

The molecular weight distribution of the β-glucan was unimodal, and the $\overline{M}_{cf}$ ranged from 1.69×10^6 to 1.77×10^6 g/mol (**Table 2**). This is higher than reported for β-glucans in Swedish oats, which had an average molecular weight of about 1.49×10^6 g/mol (*47*). There was also less variation between the average molecular weights of the β-glucans in the oat cultivars in the present study than between samples grown in Sweden and Hungary, suggesting that the molecular weight is affected more by environmental factors than by genetics. This is in agreement with Ajithkumar et al. (*47*), who reported that β-glucans from samples grown in 2001 had higher molecular weights than those from samples grown in 2000. A high molecular weight is considered to be of importance for the physiological effects of β-glucan, because it affects the viscosity of the intestinal contents (*4*). However, several studies have also shown that low molecular weight β-glucans have a cholesterol-lowering effect (*48*). No relationship was found between β-glucan molecular weight and the viscosity of grain extracts, which ranged from 2.7 to 5.7 mP·s in the four hulled lines, but was higher in the naked line Cacko (7.0 mP·s).

The contents of β-glucan and WE-AX and TOT-AX were also determined on flour and bran samples of the lines. The contents of β-glucan showed little variation, being about 1% in all of the flour samples and 8.09−8.39% in all of the bran samples except for that from Fengli, for which it was 6.20%. This is consistent with the analyses of the wholemeal samples, which showed that Fengli had 4.5% β-glucan compared with >5% in the other cultivars. The WE-AX and TOT-AX contents of the flour samples and the WE-AX contents of the bran samples were also similar in the five cultivars, with low levels of WE-AX (about 0.2% in bran and <0.2% in flour). WE-AX accounted for about 15% of the TOT-AX in flour (~0.97−1.26%) of all cultivars. The TOT-AX of the bran fractions was 3.83% in Cacko but ranged from about 8.02 to 13.2% in the four hulled cultivars. By analogy with barley (*32*), this presumably reflects the presence of high levels of highly cross-linked AX in the hulls. The contents of WE-AX and TOT-AX in bran and of WE-AX in flour were comparable to those in barley (*32*) but lower than those in wheat (*30*) and rye (*46*).

Phytochemicals. *Folate.* The total folate contents of the five oat genotypes ranged from 495 to 604 ng/g with the mean content being 566 ng/g (**Table 4**). The lowest content was in the naked cultivar Cacko, whereas the contents of the other cultivars were quite similar. There are several published studies of folate content in oats, mostly on oatmeal or rolled oats. Cerna and Kas (*49*) reported that unspecified oat grains contained 670 ng/g of folate. In contrast, Gujska and Kuncewics (*50*) reported much less folate (140 ng/g) in oatmeal using an HPLC method, whereas rolled oats was reported to contain 640 ng/g (*49*) and 870 ng/g of fw (*51*) of folate. The measured mean content in this study was close to those of winter and spring wheats (561 and 551 ng/g) (*37*) but lower than in rye (693 ng/g) (*46*) and barley (675 ng/g) (*32*).

Tocols. The total tocol contents of the five oat genotypes ranged from 16.1 to 36.1 μg/g in wholemeals (**Table 4**). The average content was 25.9 μg/g. Oat grains contained only α- and β-vitamers. The major tocol was α-tocotrienol, contributing 57−69% of total tocols, followed by α-tocopherol with 23−32% of total tocols (**Table 4**). The average proportion of tocotrienols was 70% of total tocols (**Table 3**).

The total tocol content, the range in content (1.6-fold), and the proportions of vitamers in the five oat genotypes (**Table 5**) are comparable with those reported in earlier studies. In a German study, oats contained 38.0 μg of tocols/g of fw (*52*), whereas the average values for 12 oat cultivars grown in the United States were 26 μg/g (*53*), for seven grown in Sweden were 18.4 μg/g (*54*), and for 19 genotypes grown in the United States were 28.3 μg/g (*55*). The proportion of α-tocotrienol in these studies ranged between 57 and 77%, respectively, indicating that this vitamer is predominant in oats. Furthermore, the amount of tocotrienols has been shown to correlate positively with the oil content (*54, 56*), which is an important issue for plant breeding. Comparison of the values for lipid and α-tocotrienol in **Tables 2** and **5** shows a similar relationship in the present study. In contrast, no correlation was found in a recent study (*55*). This correlation also clearly does not exist across cereal species, with oats having the lowest levels of tocols but the highest oil contents of the species studied in the HEALTHGRAIN diversity screen.

Sterols. The total content of sterols in the five oat genotypes varied from 618 to 682 μg/g with an average of 653 μg/g (**Table 4**). Sitosterol was the most abundant type, contributing 59.1−64.9% of total sterols, followed by campesterol (7.6−9.1%)

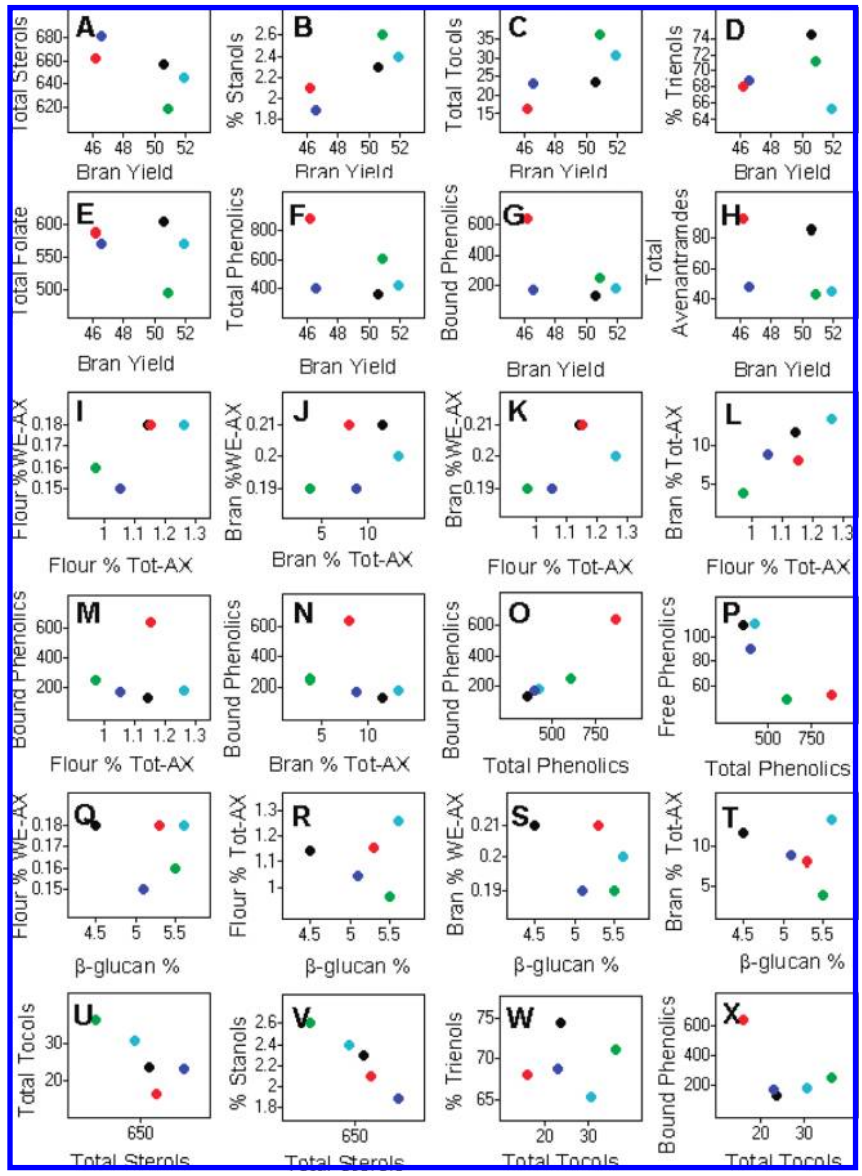

Figure 1. Associations between the concentrations of selected phytochemical and dietary fiber components in the five oat cultivars: red, Bajka; dark blue, Expander; black, Fengli; light blue, MV-Pehely; green, Cacko; (**A**) total sterols (μg/g of dw) vs bran yield (%); (**B**) % stanols vs bran yield (%); (**C**) total tocols (μg/g of dw) vs bran yield (%); (**D**) % trienols vs bran yield (%); (**E**) total folates (ng/g of dw) vs bran yield (%); (**F**) total phenolics (μg/g of dw) vs bran yield (%); (**G**) total bound phenolics (μg/g of dw) vs bran yield (%); (**H**) total avenanthramides (μg/g of dw) vs bran yield (%); (**I**) flour water-extractable arabinoxylan (%) vs flour total arabinoxylan (%); (**J**) bran water-extractable arabinoxylan (%) vs bran total arabinoxylan (%); (**K**) bran water extractable arabinoxylan (%) vs flour total arabinoxylan (%); (**L**) bran total arabinoxylan (%),vs flour total arabinoxylan (%); (**M**) total bound phenolics (μg/g of dw) vs flour total arabinoxylan (%); (**N**) total bound phenolics (μg/g of dw) vs bran total arabinoxylan (%); (**O**) total bound phenolics (μg/g dw) vs total phenolics (μg/g of dw); (**P**) total free phenolics (μg/g of dw) vs total phenolics (μg/g of dw); (**Q**) flour water-extractable arabinoxylan (%) vs β-glucan (%); (**R**) flour total arabinoxylan (%) vs β-glucan (%); (**S**) bran water-extractable arabinoxylan (%) vs β-glucan (%); (**T**) bran total arabinoxylan (%) vs β-glucan (%); (**U**) total tocols (μg/g of dw) vs total sterols (μg/g of dw); (**V**) % stanols vs total sterols (μg/g of dw); (**W**) % trienols vs total tocols (μg/g of dw); (**X**) total bound phenolics (μg/g of dw) vs total tocols (μg/g of dw).

(**Table 4**). The proportion of stanols (saturated sterols) was 2.6% or less of total sterols.

The total sterol content of the oat genotypes in this study was higher than in previous papers. Määttä et al. (*57*) reported that total sterols in seven oat cultivars ranged from 350 to 491 μg/g with significant differences in the contents of different cultivars but not between samples of the same cultivar grown in different locations. In another study, Piironen et al. (*33*) reported sterol contents for two oat genotypes of 527 and 483 μg/g, respectively.

The sterol composition of oat was similar to that of barley (*32*), but significantly different from those of wheat and rye (*34, 46*). As expected from the previous studies of oats and barley (*32, 57*), the proportion of Δ^5-avenasterol (which is named after oats, *Avena sativa*) was high, ranging from 3.2 to 7.5% of the total sterols (**Table 5**). This was higher than in wheat and rye (3.4% or less). Furthermore, the proportion of stanols (saturated sterols) was significantly lower in oats and barley (<3%), compared to wheat and rye (12−30%) (*32, 34, 46*).

Alkylresorcinols. Alkylresorcinols (ARs) were not found in any of the oat cultivars, which is in agreement with an earlier study (*24*). ARs are present only in the outer layers of wheat and rye and can therefore be used as biomarkers for the intake of wholegrain wheat and rye products (*22*). However, it is clearly necessary to develop alternative biomarkers for oats.

Phenolic Acids. Wholemeal flours were analyzed to determine the total amounts (**Table 4**) and compositions (**Table 6**) of free, conjugated, and bound phenolics acids.

Wide variation (351−873 μg/g) in total phenolic acid content was observed, with the mean value being 531 μg/g. Free phenolic acids comprised on average 19% of the total phenolic acids, with a range of 50−110 μg/g (**Table 4**). This was a higher proportion than in other cereals in the HEALTHGRAIN diversity screen (*32, 39, 46*).

Soluble conjugated phenolic acids comprised on average approximately 34% of the total phenolic acid content, with levels ranging from 111 to 314 μg/g. Despite having the lowest levels of free phenolic acids, the two Polish lines (Cacko and Bajka) contained the highest levels of soluble conjugated phenolics (180 and 314 μg/g, respectively).

Bound phenolic acids comprised approximately 47% of the total phenolic acid content of the wholemeal oat flours in this study with concentrations ranging from 131 to 640 μg/g.

The total phenolic acid content was in agreement with the previous studies (*58*) but significantly higher than that reported by Soulski et al. (*59*), although the latter study analyzed branless material.

The proportions of individual phenolic acids in the different classes (free, conjugated, and bound) varied greatly (**Table 6**).

In the free phenolic acid fraction the major component was 4-hydroxybenzoic acid, representing on average 32% of the free phenolic acid content across the oat lines. In contrast, ferulic acid, which is often a major component of the free phenolic acid fractions of wheat and other cereals (*32, 39, 46*), represented on average only 12% of the total free phenolic acid content of the oat samples.

The ferulic acid content was higher (26%) in the soluble conjugated fraction and in the bound phenolic acid fraction (43%). The other dominant phenolic acid in the bound fraction was *p*-coumaric acid, corresponding to 39% of the fraction.

The total ferulic acid concentrations across the five oat cultivars ranged between 96 and 334 μg/g, with a mean value of 196 μg/g. The results obtained for ferulate were in agreement with previously reported studies of Xing and White (*58*), who reported 147 μg/g of ferulate from ground groats.

Avenanthramides. The total content of the three main avenanthramides (2c, 2p, and 2f) ranged from 42 to 91 μg/g in the five different cultivars, with the highest amounts present in the cultivars Fengli and Bajka. In most cultivars, the avenanthramide 2c was present in the highest amount (**Table 7**). These results are in agreement with other studies, which have reported variation of 3−300 μg/g total avenanthramides in dried grain of different oat samples and that 2c usually comprises the largest fraction in mature grains. However, it is also known that the content and composition of avenanthramides vary greatly with, for example, seed development stage and various environmental and crop management practices such as fertilization, location, and year (*60, 61*).

Correlations between Components. Although only five oat cultivars were studied, it is nevertheless possible to identify both negative and positive correlations between components. Many phytochemicals are known to be enriched in the bran, and hence it is not surprising to find that the contents of tocols (**Figure 1C**) were greatest in the lines in which the bran yield was highest. These include the naked Cacko. Because the hull may account for up to 30% of the crop, an allowance for this should be made when analyses of naked and covered varieties are compared. However, an association with bran yield was not observed for sterols (**Figure 1A**), folates (**Figure 1E**), and avenanthramides (**Figure 1H**) or for total or bound phenolics (**Figure 1F**,**G**). In fact, a negative relationship with bran yield was observed for total sterols (**Figure 1A**). In contrast, the percentage of stanols (**Figure 1B**) was greatest in the lines with the highest bran yields. Clear inverse relationships were observed between total sterols and both total tocols (**Figure 1U**) and percent stanols (**Figure 1V**), but the percentage of trienols was not related to the bran yield (**Figure 1D**) or to total tocols (**Figure 1W**).

Total phenolics showed positive and negative relationships, respectively, with bound phenolics (**Figure 1O**) and free phenolics (**Figure 1P**). However, no relationships between total and bound phenolics and T-AX were observed (**Figure 1M**,**N**), despite the fact that bound phenolic compounds (**Figure 1M**) are primarily esterified to the arabinose residues of arabinoxylans.

Comparisons between fiber components (**Figure 1I−T**) showed a positive relationship between total AX in bran and flour (**Figure 1L**), but no apparent relationships between the amounts of β-glucan in the whole grain and AX in the flour (**Figure 1Q**,**R**) or bran (**Figure 1S**,**T**).

Conclusions. Substantial variation was demonstrated in the contents of phytochemicals and more limited variation in the contents of fiber components of five oat cultivars grown in adjacent plots on the same site. Although some of this variation may relate to environmental impacts, the fact that adjacent sites were used means that at least some of the differences were genetically determined. This indicates that there is sufficient genetic variation in the contents of bioactive components to be exploited by breeders to develop varieties with enhanced quality for human nutrition.

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