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Flavanol and Bound Phenolic Acid Contents in Different Barley Varieties

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Different barley varieties, consisting of hulled and hull-less types, of normal, waxy, and high amylose starch, as well as two-rowed and six-rowed types, were analyzed for their main proanthocyanidins and bound phenolic acids. Variations in proanthocyanidin and phenolic acid contents were studied in different barley types as well as inter-relationships between the phytochemicals and polysaccharides. The main flavanols found in the analyzed barley varieties were two dimeric as well as four trimeric forms in addition to catechin. The total amount of flavanols ranged from 325 to 527 μg/g of fresh weight of barley flour. No evident associations were found between variations in proanthocyanidin levels and different barley types. The total amount of phenolic acids ranged from 604 to 1346 μg/g of fresh weight of barley flour, with ferulic acid as the dominating acid. The amount of phenolic acids varied according to occurrence or lack of hull, with significantly higher levels in the hulled varieties.

KEYWORDS: Hulled and hull-less barley; phytochemicals; flavanols; proanthocyanidins; phenolic acids; HPLC-DAD-MS^n

INTRODUCTION

Interest in barley as a food grain is emerging. Over the past decade an increasing interest in barley for human consumption has been observed, mainly due to its content of health-related bioactive components (*1*−*3*). The health benefits normally associated with barley are attributed to high amounts of dietary fibers. However, antioxidants or phenolic structured antioxidant compounds are also detected in barley (*4*−*6*), and recent studies have shown that cereals contain more phytochemicals than previously considered (*7*, *8*). These constituents are considered to be the most important source of antioxidants in cereals and exist in free as well as bound form. The majority of the free phenolics are flavanols, whereas the bound phenolics are mainly phenolic acids. Both of these groups are known to have antioxidant activity and therefore, possibly, health benefits (*9*−*11*), and cereals are therefore claimed to be good sources of natural antioxidants (*1*).

The flavanols exist as monomers (mainly catechin and gallocatechin, abbreviated C and G) or as polymers (proanthocyanidins). Proanthocyanidins are known to have antioxidant activity and health benefits (*9*). In barley, except for catechin, proanthocyanidin oligomers have been detected. These include the two dimers (procyanidin B3 and prodelphinidin B3) as well as four trimers (procyanidin C2, prodelphinidin C2, and two other prodelphinidins) as the main proanthocyanidins (*12*, *13*). Phenolic acids are the major phenylpropanoid components in cereals, and different levels of these phenolics are found in different fractions of cereals (*14*, *15*). In different cereals, the starchy endosperm contains low levels, whereas the outer layers of the grain (pericarp, aleurone layer, and germ) contain the highest (*16*, *17*). The most abundant cinnamic acid derivative is ferulic acid (FA). FA and the second most abundant phenolic acid, *p*-coumaric acid (*p*-CA), are mostly concentrated in the aleurone layer and in the pericarp. Both FA and *p*-CA are associated with cell wall constituents because they are ester-linked to them, especially to arabinoxylans and lignin (*18*, *19*). The arabinose side chains of the arabinoxylans are substituted at O-5 by the phenolic acids.

The covalently bound FA can dimerize to form diferulic acid (DFA) bridges, cross-linking the arabinoxylan chains and thus fortifying the cell wall. This also influences the solubility of the fibers (*18*, *20*). The main dehydrodimers identified in plant material are 8-*O*-4′-DFA, 8−5′-DFA benzofuran, 8−5′-DFA, and 5−5′-DFA forms, as well as 8−8′-DFA (*21*). The two different 8−5′-DFAs are, however, artifacts of the alkali treatments as reported by Ralph et al. (*22*), and the native form in plants is 8−5′-DFA in benzofuran form. The amounts vary with type of cereal and between varieties of one cereal. In

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addition, the amounts of these compounds (dehydrodimers) can vary with different fractions of dietary fibers (soluble, insoluble) (*20*, *23*, *24*).

Preliminary results suggest that these phenolic acids are absorbed in humans (*25*−*27*) and that their antioxidant activity may reduce the risk of coronary heart diseases, cancers, and aging processes (*28*). The antioxidant activity is, however, dependent on the structure. The different phenolic acids have different numbers and positions of the hydroxyl groups on the aromatic ring. Furthermore, the presence of a CH═CH−COOH group in the cinnamic acid derivate gives higher antioxidant activities than the COOH group in benzoic acids. Also, phenols with *o*- or *p*-dihydroxylic groups as well as alkoxyl phenols containing one free and one alkylated hydroxyl group (usually methoxy) are effective antioxidants (*29*). Thus, for the cinnamic acid derivatives, caffeic acid has a higher antioxidant activity than FA, which has a higher antioxidant activity than *p*-CA. This may explain the different antioxidant activities found in different fractions of the grain (bran) because the different phenolic acids are unevenly distributed in the layers of the bran. Also, DFAs can be more efficient antioxidants than the monomeric FA (*30*). It should be noted that analytical procedures can affect the antioxidant activity because different methodologies can result in different amounts of antioxidants as well as extract different components with different antioxidant activities.

In addition to the possible health benefits associated with the phenolic acids, these compounds also have important functional properties. As mentioned, the dehydrodimers of phenolic acids reduce the digestibility of cell walls by the ability to cross-link. Furthermore, this can affect rumen physiology and provide a better plant resistance to insects (*24*). Phytochemicals in the grain are also contributors to product quality, in terms of color, flavor, and taste (*31*, *32*). The phenolic acids are perceived as sour, bitter, and astringent (*33*). In addition, they are reported to influence bread quality by interfering with dough and gluten formation (*34*−*37*). Applications in gels are also being explored because arabinoxylans can gel through the cross-linking of the dehydrodimers (*38*). Furthermore, the proanthocyanidins are important agronomically, due to their contribution to yield as well as increasing grain resistance (*39*). Proanthocyanidin-free barley mutants have been developed by breeding mainly due to an antinutrient effect of the proanthocyanidins observed in some animal trials (*40*). In contrast, others claim that proanthocyanidins are beneficial for animals (*39*). In any case, negative correlations are found between the protein digestibility and flavanol contents (*40*). For beer production, proanthocyanidin-free varieties can be beneficial because these polyphenols bind protein and contribute to haze formation. Still, these polyphenols are known to influence the flavor, flavor stability, physical stability, and color of beer (*32*). As polyphenols in beer, they are natural antioxidants, which can react with oxygen and thus protect other beer constituents against oxidation.

Proanthocyanidins and phenolic acids in barley as well as in other cereals have been studied. However, most of the literature lacks analyses of different genotypes within one cereal species, and knowledge on variations in different genotypes is therefore scarce. In this study variations in main flavanol and phenolic acid contents in different barley genotypes were analyzed, and differences in relation to quantity in different barley types were determined. In addition, possible relationships within the different phytochemicals as well as between the different phytochemical contents and different polysaccharide were studied.

Table 1. Different Barley Varieties and Their Characteristics

variety	**2**-rowed/**6**-rowed **h**ulled/**h**ull-less **w**axy/**n**ormal/**h**igh amylose	breeding company,[a] country
1. Thule	6 H n	NK, Norway
2. Olsok	6 H n	NK, Norway
3. NK96300	6 H n	NK, Norway
4. Åker	6 H n	NK,Norway
5. Tyra	2 H n	NK, Norway
6. Justina	2 H n	Nordsaat, Denmark
7. Olve	2 H n	NK, Norway
8. Otira	2 H n	Sejet, Denmark
9. CDC Dolly	2 H n	CDC, Canada
10. NK95003	2 H–L n	NK, Norway
11. CDC Dawn	2 H–L n	CDC, Canada
12. CDC Gainer	2 H–L n	CDC, Canada
13. CDC McGwire	2 H–L n	CDC, Canada
14. CDC Candle	2 H–L w	CDC, Canada
15. CDC Alamo	2 H–L w	CDC, Canada
16. SB94897	2 H–L h	CDC, Canada

[a] NK, Norsk Kornforedling; CDC, Crop Development Center.

MATERIALS AND METHODS

Samples. The barley samples analyzed consisted of 16 varieties of diverse origin, grown in the same field trial in Norway in 2002. These 16 varieties were chosen from a larger variety collection of well-characterized varieties with differences in polysaccharide contents (*41*) and included different barley types: hulled and hull-less, six-rowed and two-rowed, normal as well as waxy and high amylose varieties (**Table 1**).

Analysis. *General Methods.* The whole grains were ground on a Retsch centrifugal mill (model ZM1; Retsch GmbH, Haan, Germany) with a 0.5-mm sieve. Nitrogen was determined by total combustion at the Norwegian Centre for Soil and Environmental Research (Ås, Norway). Protein content was calculated as N × 6.25. To avoid unwanted samples of shriveled kernels that could affect the results differently, grain weights were determined by counting a sample of 250−300 kernels using a Numerical Seed Counter, EPL, weighing the samples and calculating kernel weight in milligrams per grain.

The determination of the mixed-linked β-glucan, total starch, and amylose/amylopectin contents in the barley samples was conducted by enzyme-based assay kits from Megazyme International Ireland Ltd., (Bray, Co. Wicklow, Ireland). The amounts of total nonstarch polysaccharides (T-NSP) and insoluble nonstarch polysacchardies (I-NSP) and their constituent sugars were determined according to the method described by Englyst et al. (*42*). Soluble nonstarch polysaccharides (S-NSP) was calculated by difference.

Extraction of Flavanols. The pressurized liquid extraction (PLE) was carried out using an accelerated solvent extractor (ASE) (ASE 200, Dionex, Idstein, Germany) as described in ref *43*, with minor adjustments. Four grams of sample was mixed with 1.8 g of diatomaceous earth and extracted with acetone/water (60:40, v/v) after a pre-extraction with pentane, at 60 °C and with a pressure of 200 bar. After the ASE extraction, the extracts were purified and concentrated by solid-phase extraction (SPE) on commercial polyamid cartridges.

Extraction of Bound Phenolic Acids. The extractions of phenolic acids were based on the method described by Adom and Liu (*7*) with small adjustments. Ground barley samples (0.5 g) were extracted twice with 80% ethanol (10 mL), and the residue was subjected to alkali treatment (2 M NaOH, 10 mL) for 1 h. The suspension was then neutralized and acidified with 6 M HCl and washed with hexane (10 mL). Finally, the phenolic acids were extracted with ethyl acetate (3 × 10 mL), concentrated to dryness, and resolved in 5 mL of DMSO.

HPLC-DAD-MS^n Analysis. The flavanols and the liberated bound phenolic acids were analyzed by subsequent HPLC-DAD-MS^n analysis with negative electrospray ionization as described by Papagiannopoulos et al. (*44*). The phenolics were identified by MS and quantified by

Table 2. Amounts of Flavanols in Different Barley Varieties as Micrograms per Gram of Fresh Weight of Barley Flour[a]

barley variety	C	GC prodelphinidin B3	CC procyanidin B3	GGC prodelphinidin C2	GCC	CGC	CCC procyanidin C2	T-F
1	26	102	105	89	104	48	53	527
2	14	59	63	58	67	33	31	325
3	16	70	76	83	90	45	39	419
4	34	106	81	105	90	45	32	493
5	22	91	88	80	83	46	36	446
6	15	61	69	59	67	38	36	345
7	24	73	71	78	76	42	31	395
8	23	72	103	64	87	56	59	464
9	22	70	99	70	88	52	52	453
10	22	70	75	55	65	35	34	356
11	27	70	104	58	81	49	54	443
12	20	48	88	40	66	41	59	362
13	24	57	92	47	69	42	50	381
14	24	68	94	53	73	44	48	404
15	41	62	126	53	96	54	81	513
16	28	80	107	63	92	62	68	500

[a] G, gallocatechin; C, catechin; T-F, total flavanols.

HPLC-DAD. The analytical column used was an Aqua 3 μm C18, 150 mm, 2 mm i.d. equipped with a guard column (Security Guard, C18, 4 mm, 2 mm i.d.) at 25 °C, both from Phenomenex (Aschaffenburg, Germany). Mobile phase A consisted of 1% acetic acid in high-purity water, whereas mobile phase B consisted of 1% acetic acid in acetonitrile. Flow rate was set to 0.2 mL/min. A linear gradient increased from 5 to 20% B after 45 min and up to 60% B after 77 min. Washing of the column was conducted by 100% B for 10 min. Ten microliters of sample solution was injected, and the quantification wavelengths were 280 nm for the flavanols and 310 nm for the phenolic acids. Due to low levels of cis-isomer of the acids, only the trans-isomers were quantified. For quantification, ferulic acid and *p*-coumaric acid were used as external standards for the phenolic acids, whereas catechin was the external standard for the flavanols. The molar-based detector responses of dimers were found to be twice that of monomers. Assuming a 3-fold molar response for trimers, this allowed quantification of both dimers and trimers by the use of monomeric external standards. All results are reported on a fresh weight basis and with standard deviations of <4%.

Statistics. Analysis of variance and significant differences among means were tested by one-way ANOVA, using Minitab (version 13.3; Minitab Inc., State College, PA). Significant differences were declared at $p < 0.05$. A simple correlation (Pearson correlation) was also conducted using Minitab.

RESULTS

Quantification of Proanthocyanidins. The main flavanols quantified were catechin, two dimers (procyanidin B3 and prodelphinidin B3), and four trimers (**Table 2**). The monomeric catechin was the minor flavanol constituent (**Table 2**) in all of the samples, ranging from 14 to 41 μg/g of fresh weight of barley flour. The lowest amounts were observed in a hulled six-rowed variety (sample 2) as well as in a hulled two-rowed sample (no. 6). The waxy, hull-less variety (sample 15) contained the highest catechin content, almost 3 times higher than that in varieties containing the lowest levels. Despite the results of the analysis of variance, which showed significantly lower amounts of catechin in barley varieties having a normal starch type compared to an atypical starch type ($p = 0.037$), no evident difference could be stated because this was mainly due to the high amount found in the waxy genotype 15. Occurrence or lack of hull was not related to the catechin contents. However, the catechin levels accounted for ~5% of the total flavanols in the different barley varieties, and this portion were related to occurrence or lack of hull ($p = 0.017$). These portions were lower than reported by Goupy et al. (*4*), who found 13−40% catechin of total flavanols.

Procyanidin B3 (CC) and prodelphinidin B3 (GC) (abbreviations are explained in **Table 2**) are the main dimers in barley (*4*, *45*, *46*). The former was usually in abundance in this study, especially among the hull-less varieties (samples 10−16). With the exception of a few varieties (which contained more GC than CC), the same was observed among the hulled varieties. The levels of prodelphinidin B3 and procyanidin B3 are in the ranges of 48−106 and 63−126 μg/g of fresh weight of barley flour, respectively. This is similar to results reported by Goupy et al. (*4*) and Friedrich (*32*), but somewhat lower than those reported in McMurrough et al. and Wollersen (*47*, *48*). The lowest amount of CC was detected in a hulled six-rowed variety (sample 2), whereas the waxy hull-less variety, sample 15, contained the highest level. The highest level of GC was observed in another hulled six-rowed variety (sample 4), whereas the lowest was in a normal starch hull-less variety (sample 12). A tendency toward lower levels of prodelphinidin B3 in the hull-less varieties compared to the hulled was observed, whereas the contrary was seen for procyanidin B3 contents. For both dimers, the six-rowed samples showed the largest variation among the hulled varieties and included high and low levels of both dimers. The total amount of dimers varied independent of barley type. The portion of dimers, in relation to total flavanol contents, was more or less constant (35−40%) and not related to differences in barley type.

The most abundant flavanols in the analyzed barley varieties were the trimers (**Table 2**). As much as 53−61% of the total flavanols were trimeric proanthocyanidins. This percentage share was, as also observed for catechin, related to the presence or lack of hull ($p = 0.027$). The dominating trimers were GGC and GCC, with the latter usually in abundance, except for the hulled samples 4 and 7. However, the trimer found in lowest amount depended on the barley type. For the hulled varieties, procyanidin C2 (CCC) showed the lowest values with few exceptions, whereas the opposite was observed for the hull-less samples except sample 10, which contained more CGC than CCC. Procyanidin C2 ranged from 31 to 81 μg/g of fresh weight of barley flour, prodelphinidin C2 varied between 40 and 105 μg/g of fresh weight of barley flour, and GCC and CGC were quantified between 65 and 104 μg/g of fresh weight of barley flour and between 33−62 μg/g of fresh weight of barley flour, respectively. This is higher than reported by Goupy et al. (*4*) but lower than reported by McMurrough et al. (*47*). The levels of the individual trimers were influenced by occurrence or lack of hull. Both GGC and CCC levels showed significant differences among hulled and hull-less barley varieties ($p = 0.002$ and 0.033, respectively). Hull-less varieties contained lower amounts of GGC but higher amounts of CCC compared to the hulled.

The total proanthocyanidin levels range from 325 to 527 μg/g of fresh weight of barley flour. This was lower than observed earlier (*45*, *47*), similar to the findings of Friedrich (*32*), and higher than the results of Goupy et al. (*4*). Both the lowest and the highest total amounts were detected in the six-rowed varieties. The amount of total flavanols was generally independent of type of barley. The hulled six-rowed variety, sample 2, contained low amounts of proanthocyanidins, whereas the waxy hull-less variety, sample 15, had high levels of proanthocyanidins. Finally, it was observed that the

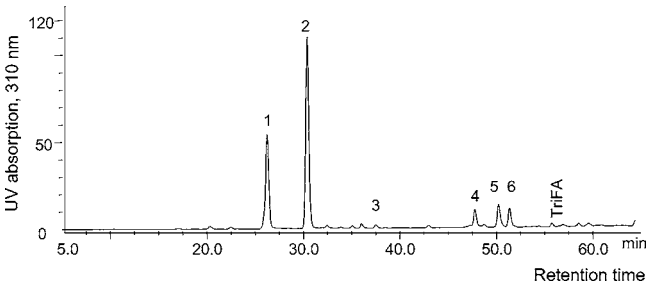

Figure 1. HPLC chromatogram (310 nm) of the alkali extracts of the barley flour, showing the different quantified phenolic acids: 1, *p*-CA; 2, FA; 3, 8–5′-DFA linear form; 4, 5–5′-DFA; 5, 8-*O*-4′-DFA; 6, 8–5′-DFA benzofuran form, TriFA.

individual variation in the hulled six-rowed representatives was higher than that of the hulled two-rowed varieties. It can be concluded on the basis of the large individual variations observed in flavanol contents within the different barley types that no evident or distinct relationships were detected between the variations in proanthocyanidin levels and the different barley types.

Quantification of Bound Phenolic Acids. In this study, two monomeric phenolic acids, four DFA (numbered 1−6), and one TriFA (identified by mass only) were detected and quantified (**Figure 1**). The MS spectra of the DFAs are shown in **Figure 2**.

The quantified amounts of bound phenolic acids of the barley samples are listed in **Table 3**. The monomeric FA was the most abundant phenolic acid, and the contents ranged between 403 and 723 μg/g of barley flour, which accounted for 52−69% of the total amount of phenolic acids (T-PA) (**Table** 4). As the content of FA was related to the occurrence or lack of hull, significantly higher levels of FA were observed in the hulled varieties compared to the hull-less samples ($p < 0.000$) (**Figure 3A**). Moreover, the highest percentage was observed in the hull-less varieties (sample 10−16) (**Figure 3B**). The content of FA

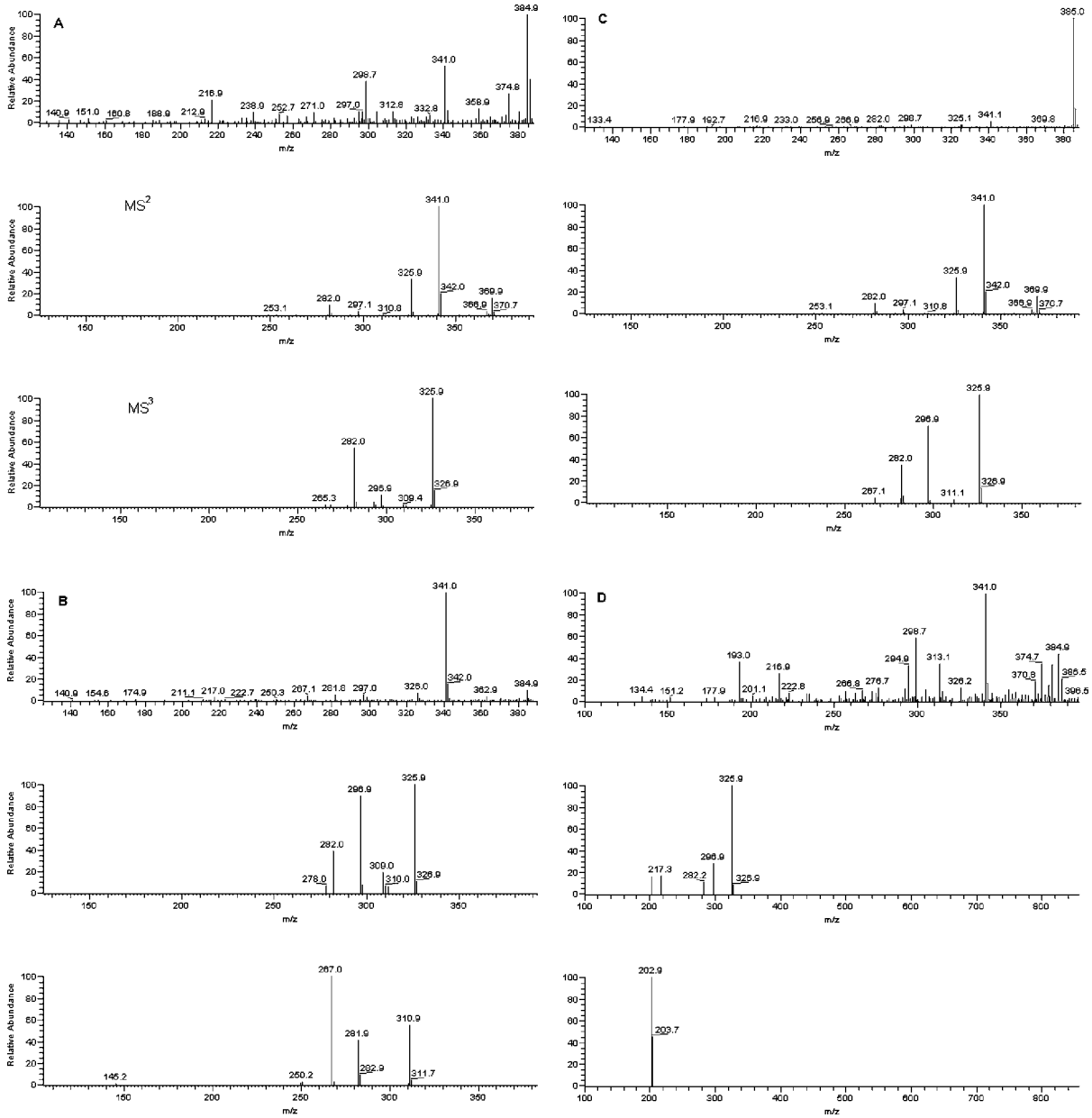

Figure 2. MS spectra obtained from peaks 3–6 in **Figure 1** after HPLC-DAD-MS^n analysis with negative electrospray ionization: (**A**) peak 3 at 37.6 min (8–5′-DFA linear form); (**B**) peak 6 at 51.5 min (8–5′-DFA benzofuran form); (**C**) peak 4 at 48 min (5–5′-DFA); (**D**) peak 5 at 50 min (8-*O*-4′-DFA). The *y*-axis is the relative abundance, whereas the *x*-axis shows the *m*/*z* (mass-to-charge ratio).

Table 3. Amount of Phenolic Acids (Micrograms per Gram of Barley Flour) in Different Barley Varieties[a]

barley variety	*p*-CA	FA	5–5′-DFA	8-*O*-4′-DFA	8–5′-DFA	TriFA	T-DFA	T-PA	T-P
1	374	696	64	79	104	28	247	1346	1873
2	199	594	45	67	87	24	199	1015	1340
3	198	512	42	64	82	18	188	915	1334
4	263	723	67	82	103	31	252	1270	1763
5	138	624	59	88	114	26	261	1049	1495
6	192	555	54	71	92	27	217	991	1336
7	114	723	62	85	104	34	251	1123	1518
8	244	512	50	65	91	25	206	988	1452
9	196	518	50	76	99	21	225	961	1414
10	27	447	42	53	79	17	174	665	1021
11	38	465	41	52	74	16	167	686	1129
12	21	403	37	53	75	15	165	604	966
13	19	444	39	50	72	17	161	641	1022
14	26	408	41	49	68	21	158	612	1016
15	31	436	39	49	76	16	164	646	1159
16	15	451	40	51	77	16	168	651	1151

[a] *p*-CA, *p*-coumaric acid; FA, ferulic acid; DFA, diferulic acid; TriFA, triferulic acid; T-DFA, total diferulic acids; T-PA, total phenolic acids; T-P, total phytochemicals.

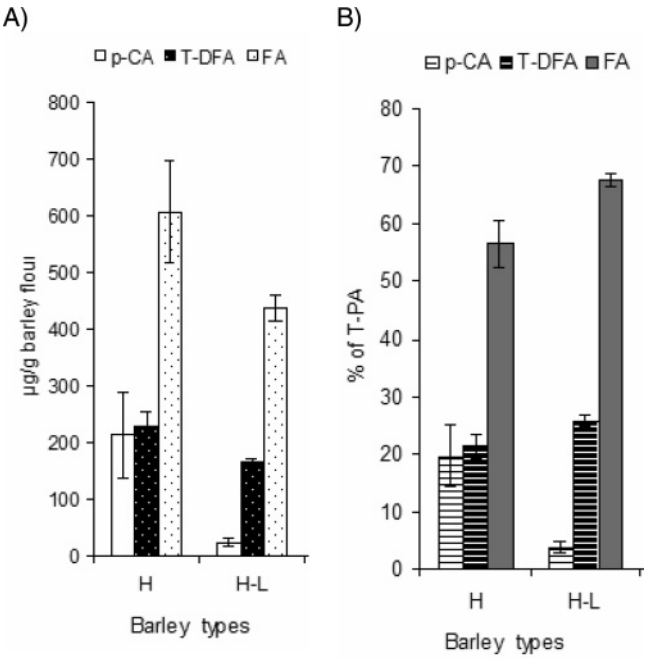

Figure 3. (**A**) Average amounts (μg/g of fresh weight of barley flour) of the main phenolic acids [*p*-coumaric acid (p-CA), ferulic acid as monomer (FA) and dimer (T-DFA) of the different barley types] and (**B**) phenolic acids in relation to total amount of bound phenolic acids (T-PA) for the different barley types (H, hulled; H-L, hull-less). Standard deviation for each group is denoted as bars.

in this study was lower than reported elsewhere (*20*), which was expected because Renger and Steinhart analyzed fractions (barley bran) and not whole grain. Our results were similar to those of Hernanz et al. (*49*). The other monomeric phenolic acid identified, *p*-CA, showed a total variation between 15 and 374 μg/g of barley flour (**Table 3**). A marked effect of occurrence or lack of hull was seen, and the content of *p*-CA was significantly higher in the hulled varieties than the hull-less samples ($p < 0.000$) (**Figure 3A**). These levels were similar to other literature data (*20*, *49*). Furthermore, the hull-less barley types had a percentage of *p*-CA in relation to T-PA of <10% (**Figure 3B**), whereas the hulled samples contained up to 10 times higher amounts of *p*-CA (**Table 4**). Because the results showed such a marked difference in *p*-CA contents between hulled and hull-less samples and because *p*-CA contents were coincident with the amounts found in barley bran mentioned above, we conclude that *p*-CA occurs more in the hulls compared to FA.

Table 4. Amount of Different Phenolic Acids as Shares of Total Phenolic Acids in the Different Barley Varieties[a]

barley variety	% *p*-CA of T-PA	% FA of T-PA	% T-DFA of T-PA
1	28	52	18
2	20	59	20
3	22	56	20
4	21	57	20
5	13	59	25
6	19	56	22
7	10	64	22
8	25	52	21
9	20	54	23
10	4	67	26
11	5	68	24
12	3	67	27
13	3	69	25
14	4	67	26
15	5	67	25
16	2	69	26

[a] *p*-CA, *p*-coumaric acid; FA, ferulic acid; T-DFA, total diferulic acids; T-PA, total phenolic acids.

Despite the identification of different trimers in the past (*50*−*52*), only one triferulic acid (TriFA)was found in quantitative amounts under the given conditions in this study. Also, the two different 8−5′-DFAs (peaks 3 and 6 in **Figure 1**) are, as noted in the Introduction, artifacts of the alkali treatment. The 8−5′-DFA benzofuran is the native form in the plant, whereas others like the open and carboxylated forms are induced by alkaline. Therefore, the sum of the two different 8−5′-DFAs (**Figure 1**) is given in **Table 3**. The TriFA was detected in the lowest amounts, and its contents were related to occurrence or lack of hull as observed for the other three DFAs. Significantly higher levels of TriFA were found in the hulled varieties compared to the hull-less samples ($p = 0.001$).

The dehydrodimers 5−5′-DFA and 8-*O*-4′-DFA were in general detected in similar levels in the barley flours and were approximately between 40 and 90 μg/g of barley flour (**Table 3**). Total amounts of 8−5′-DFAs varied between 68 and 114 μg/g of barley flour, with highest levels in the hulled samples. The total amount of ferulic acid dehydrodimers (T-DFA) varied between 158 and 261 μg/g of barley flour (**Table 3**). The lowest amounts of T-DFA were detected in the hull-less varieties, whereas sample 5 contained the highest. The amount of T-DFA was also related to hulls (**Figure 3A**) as there were significantly higher levels of T-DFA in the hulled varieties compared to the hull-less samples ($p < 0.000$). The percentage of the DFAs in relation to T-PA ranged between 18 and 27% (**Table 4**), and significantly higher levels were found in the hull-less samples ($p < 0.000$) (**Figure 3B**). Due to the low contents of *p*-CA in the hull-less samples, the largest portion of DFAs was observed in these hull-less genotypes.

The T-PA was highest in a six-rowed barley variety and lowest among the hull-less genotypes, especially varieties 12 and 14. The total range was between 604 and 1346 μg/g of barley flour, which is similar to the sum of the individual levels reported by Hernanz et al. (*49*). The T-PA content also showed

Table 5. Results of the Correlation Analysis between (**A**) Flavanol Contents and Polysaccharide Contents and (**B**) Phenolic Acid Contents and Polysaccharide Contents[a]

(A) Flavanol Contents and Polysaccharide Contents

flavanol	T-β-glucan	I-β-glucan	T-NSP	I-NSP	T-AX	I-AX	A/X (T-AX)	A/X (I-AX)
C	0.56*	0.61**	–	–	–	–	–	–
GC	–	–	0.52*	0.53*	0.54*	0.51*	–	–
CC	0.55*	0.55*	–	–	–	–	–	–
GGC	–	–	0.63**	0.77***	0.74***	0.74***	–0.68**	–0.69**
GCC	–	–	–	–	–	–	–	–
CGC	0.51*	0.57*	–	–	–	–	–	–
CCC	0.57*	0.60**	–	–0.53*	–0.54*	–0.57*	0.51*	0.52*
T-F	–	–	–	–	–	–	–	–

(B) Phenolic Acid Contents and Polysaccharide Contents

phenolic acid	starch	T-β-glucan	I-β-glucan	protein	T-NSP	I-NSP	S-NSP	T-AX	I-AX	A/X (T-AX)	A/X (I-AX)	cellulose
p-CA	–	–0.50*	–	–0.59*	0.53*	0.89***	–0.65**	0.77***	0.86***	–0.87***	–0.86***	–
FA	–0.58*	–	–	–	0.74***	0.91***	–	0.92***	0.91***	–0.77***	–0.79***	0.51*
5–5′**-DFA**	–	–	–	–	0.69**	0.84***	–	0.85***	0.84***	–0.75***	–0.74***	0.50*
8-*O*-4′**-DFA**	–	–	–0.50*	–0.50*	0.72**	0.89***	–	0.91***	0.90***	–0.86***	–0.86***	0.54*
8–5′**-DFA**	–	–	–	–	0.74**	0.84***	–	0.87***	0.85***	–0.82***	–0.82***	0.60*
TriFA	–0.52*	–	–0.49*	–	0.73***	0.87***	–	0.87***	0.87***	–0.77***	–0.78***	0.51*
T-PA	–0.52*	–	–0.50*	–0.52*	0.70**	0.97***	–0.55*	0.96***	0.96***	–0.89***	–0.89***	0.49*
T-P	–0.55*	–	–	–	0.71**	0.91***	–	0.87***	0.89***	–0.83***	–0.83***	0.51*

[a] T-, I-, S-NSP, total, insoluble, and soluble nonstarch polysaccharide content; T-, I-AX, total and insoluble arabinoxylan contents; T-, I-β-glucan, total and insoluble β-glucan contents; A/X (T-, I-AX), degree of branching in both total and insoluble arabinoxylans; C, catechin; G, gallocatechin; T-F, total flavanols; T-PA, total phenolic acids; T-P, total phytochemicals (T-PA + T-F); *, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; ***, $p \leq 0.001$.

relation to occurrence or lack of hull. Higher levels of total phenolic acids were observed in the hulled samples compared to the hull-less varieties ($p < 0.000$).

The total amount of phytochemicals (T-P) (**Table 3**) is the combined results of total flavanol (T-F) contents and T-PA. Total phenolics ranged between 966 and 1873 μg/g of barley flour. The hull-less varieties, especially sample 12, contained the lowest levels of T-P, whereas hulled genotypes, especially some of the six-rowed genotypes, had the highest amounts. Significant differences were observed between different barley types. Furthermore, the soluble flavanols accounted for 24−44% of T-P, whereas the bound phenolic acids varied between 56 and 76% of T-P. These ratios were again related to the occurrence and lack of hull, with higher levels of T-PA and lowest T-F in the hulled varieties ($p < 0.000$). These results showed that barley contains high amounts of bound PA and confirms recent studies showing that cereals contain more phytochemicals than previously considered (*7*, *8*).

Significant Correlations between Flavanols and Polysaccharides. Correlation analysis was conducted to compare the proanthocyanidin levels and the phenolic acid amounts to the quantity of other grain components, such as polysaccharide contents. This provided additional and beneficial information on how the variations found in the different compounds in the barley were related. **Table 5A** shows that both T-NSP and I-NSP, including total and insoluble β-glucan and arabinoxylan (AX), were correlated to the proanthocyanidin levels.

Furthermore, the correlations within the proanthocyanidins, that is, correlations to other proanthocyanidins, showed that GGC and GC only correlated positively to each other, whereas CGC, CCC, CC, and C all showed interaction with each other. Finally, GCC was related to all of the proanthocyanidins except procyanidin C2.

Significant Correlations between Phenolic Acids and Polysaccharides. In **Table 5B** the correlations between polysaccharides and the bound phenolic acids are listed. Especially T-NSP, I-NSP, T-AX, and I-AX, as well as the degree of branching (A/X) in AX, were related to the amount of phenolic acids. All phenolic acid levels increased with increased levels of the fibers, whereas the contents decreased upon increased substitution in AX. This indicated that the higher the amount of AX in the barley flour, the higher was the content of acids. However, the negative correlation to the A/X shows that lower amounts of phenolic acids occur when there is a high degree of arabinose substitution. A possible explanation for the higher levels of ferulic acids in the hulled varieties is related to the location of the AX. As seen above, the ferulic acids are associated with the AX in the grain, and because part of the AX is located in the hulls (*40*), a higher content of T-FA can occur in these genotypes.

Furthermore, the phenolic acids were all positively correlated to each other ($p < 0.02$, $r > 0.6$). If one increases, all others increase as well. By measuring and comparing one of these components, it is therefore possible to assume whether the levels of the other phenolic acids increase or decrease.

The variation in phenolic acid content as well as flavanol contents differed with different barley types. Furthermore, variations were also observed within the same types. Consequently, occurrence or lack of hull was the dominating factor for the contents of phenolic acids; however, individual differences occurred. The different barley types seemed to have no evident effect on the flavanol contents. This study showed that different barley varieties contain varying amounts of phytochemicals, and the choice of variety for human consumption will be of great importance with regard to the intake of phytochemicals. Even if the amount of phytochemicals is higher in the hulled compared to the hull-less varieties, the industry should consider the hull-less varieties. The necessarity of pearling of the hulled varieties will decrease the amounts of antioxidants in these genotypes considerably compared to the hull-less samples. Furthermore, the variations observed in the amount of phytochemicals within the different types of barley should provide an advantage for breeders to produce barley varieties of high antioxidant levels for food uses.

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