JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Free Flavan-3-ol Content in Barley (*Hordeum vulgare* L.) Air-Classified Flours: Comparative Study of HPLC-DAD/MS and Spectrophotometric Determinations

VITO VERARDO,[†] MATTEO BONOLI,[†] EMANUELE MARCONI,[§] AND MARIA FIORENZA CABONI*,[†]

Dipartimento di Scienze degli Alimenti, Alma Mater Studiorum Università di Bologna, Piazza Goidanich 60, 47023 Cesena (FC) Italy, and Dipartimento di Scienze e Tecnologie Agro-Alimentari Ambientali e Microbiologiche (DISTAAM), Università del Molise, via De Sanctis, 89100 Campobasso (CB) Italy

The determination of free flavan-3-ol compounds in barley flours (cv. Gotic) and two resulting milling fractions (fine fraction 57% and coarse fraction 43%, w/w) obtained by air classification of dehulled grain is herein described. The determinations were carried out using reversed phase high-performance liquid chromatography coupled with both diode array detection and ESI-MS compared with conventional spectrophotometric determinations (total phenolic compounds by the Folin−Ciocalteu method and free radical scavenging activity with the DPPH assay). Significant correlations among the HPLC quantification, the spectrophotometric data, and the antioxidant capacity of extracts were revealed by Pearson's analysis. Nine flavan-3-ols were identified by HPLC-MS. Catechins and their derivatives were found to make a substantial contribution to the antioxidant power of extracts. The coarse fraction showed larger concentrations of flavan-3-ols (221%) with respect to the fine fraction. This was confirmed by the antioxidant activity of the analyzed flours. The coarse fraction showed the greatest antioxidant activity (1200.1 ± 66.2 μmol of Trolox equiv/100 g of flour) with respect to whole meal and fine fraction (1025.9 ± 18.3 and 761.7 ± 55.3 μmol of Trolox equiv/100 g of flour, respectively).

KEYWORDS: HPLC-DAD/MSD; spectrophotometric determinations; barley; flavan-3-ols; air classification

INTRODUCTION

Current interest in barley for food purposes stems mainly from the many health benefits associated with certain components of this cereal. Barley is widely consumed both as a whole grain and as a food ingredient for the production of processed functional foods (pasta, baked products) (*1, 2*).

It has a high content of bioactive compounds (β-glucans and tocols) (*3*) and a large range of antioxidant compounds having a phenolic structure (*4, 5*). Barley phenols exist in the free or bound form. Generally, free phenolic compounds are flavonoids, whereas bound phenolics are ester-linked to cell wall polysaccharides (*6–8*). Zimmermann and Galensa (*9*) described the presence of different proanthocyanidins in barley and malt. An increased consumption of these phenolic compounds has been correlated with a reduced risk of cardiovascular diseases and certain cancers (*10, 11*) due to antioxidant activities that have been demonstrated both in vitro and in vivo.

Air classification is a physical separation technique that uses air currents in combination with centrifugal force to achieve the separation of flour particles according to size and density. Air classification has been successfully employed for legumes to produce low- or high-protein and -starch flour fractions (*12–15*) and for cereals (barley and oat) to produce fractions enriched in β-glucans (*2, 3*).

Several investigations have analyzed the phenols in cereals by spectrophotometric analysis, reverse phase-high performance liquid chromatography (RP-HPLC) (*4, 9, 16–19*).

Whole barley meal can be air classified into two fractions (fine and coarse fractions) to produce flour fractions enriched in β-glucans (*20*). The fine fraction was rich in starch (from starchy endosperm), whereas concentrated cell walls of starchy endosperm and external layers (aleurone, bran) of kernel (rich in dietary fiber and β-glucans) are concentrated in the coarse fraction. In this work, it was determined if the coarse fraction was also enriched with phenolic compounds. Accordingly, spectrophotometric indices and liquid chromatography quantifications were carried out to evaluate the level of flavan-3-ols in whole meal and air-classified fractions. The performances

* Corresponding author (telephone +39-0547-338117; fax +39-0547-382348; e-mail maria.caboni@unibo.it).
[†] Alma Mater Studiorum Università di Bologna.
[§] Università del Molise.

10.1021/jf8006344 CCC: $40.75
Published on Web 07/16/2008

of HPLC were evaluated to characterize and quantify free flavan-3-ols in barley extracts, and the best separation conditions were investigated to achieve the highest resolution and sensitivity using UV detection for both the instrumental techniques and MS detection for the HPLC system. The amount of flavan-3-ols and the antioxidant activity of barley extracts by spectrophotometric determinations (total phenolic compounds by the Folin−Ciocalteu method and free radical scavenging activity by the DPPH discoloration assay) were also estimated.

MATERIALS AND METHODS

Samples and Sample Preparation. Hulled grain of barley (cv. Gotic) was dehulled and pin-milled (whole meal). Whole meal (WM) was air-classified into coarse fraction (CF) (43%) and fine fraction (FF) (57%) according to the method of Marconi et al. (*2*).

The most representative particle sizes of the coarse and fine fractions were in the ranges of 120−477 and 45−120 μm, respectively.

The barley samples (WM, CF, and FF) were stored at −18 °C until use.

Reagents and Chemicals. Unless otherwise stated, all solvents were of analytical grade from Merck (Darmstadt, Germany). Procyanidins B1 and B2 were from Extrasynthese (Genay, France); prodelphinidin B3, (+)-catechin, (−)-epicatechin, and gallic acid were from Sigma-Aldrich (St. Louis, MO).

Extraction of Free Phenolic Compounds. To collect free phenolic compounds the optimized extraction protocol by Bonoli et al. (*21*) was used.

Briefly, 5 g of barley flour was extracted twice in an ultrasonic bath using 40 mL of acetone/water (4:1, v/v) (Ac). The supernatants of the extracts were pooled, evaporated, and reconstituted with 5 mL of water/formic acid (99.7:0.3, v/v). Each extraction was replicated four times ($n = 4$), and the extracts were stored at −18 °C until use.

Spectrophotometric Determinations. Spectrophotometric analyses were performed using a UV-1601 spectrophotometer from Shimadzu (Duisburg, Germany) and were repeated four times for each extract ($n = 4$) and three times for each calibration point ($n = 3$). Total phenolic compounds (TPC) were determined at 750 nm using the Folin−Ciocalteu spectrophotometric method according to the guidelines of Bonoli et al. (*22*). To assess TPC, a gallic acid calibration curve was made.

The free radical scavenging activity of extracts (FRSA) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with some modifications as reported by Bonoli et al. (*22*). To assess the FRSA a Trolox calibration curve was made, and the results were expressed as micromoles of Trolox equivalents per 100 g of flour.

Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) Analysis with UV−Diode Array Absorption and Mass Spectrometry Detections (UV-DAD/MSD). RP-HPLC analysis was performed by a HP 1100 series instrument (Hewlett-Packard, Wilmington, DE) equipped with a binary pump delivery system, a degasser (model G1322A), an autosampler (Automatic Liquid Sampler, ALS, model G1312A), a HP diode array UV−vis detector (DAD, model G1315A), and a HP mass spectrometer detector (MSD, model G1946A); integration and data elaboration were performed using Chemstation software (Hewlett-Packard).

A C18 Luna column, 5 μm, 250 × 3.00 mm i.d. (Phenomenex, Torrance, CA), with a C18 precolumn filter was used. All solvents were of HPLC grade and filtered with a 0.45 μm filter disk. A gradient elution was carried out using the following solvent system: mobile phase A, water/acetic acid (99:1, v/v); mobile phase B, mobile phase A/acetonitrile (60:40, v/v). The gradient program (flow = 0.5 mL/min) was as follows: from 0 to 14 min, 2% B; from 14 to 16 min, 6% B; from 16 to 20 min, 10% B; from 20 to 24 min, 17% B; from 24 to 38 min, 36% B; from 38 to 40 min, 38.5% B; from 40 to 53 min, 60% B; from 53 to 58 min, 100% B; from 58 to 78 min, 5% B; from 78 to 85 min, 2% B; post run for 5 min. UV spectra were recorded from 210 to 600 nm, whereas the chromatograms were registered at 280 nm. The injection volume was 10 μL. All analyses were carried out at room temperature. MS analysis was carried out using an electrospray ionization (ESI) interface (using both positive and negative ionization) at the following conditions: drying gas flow (N_2), 9.0 L/min; nebulizer pressure, 50 psi; gas drying temperature, 350 °C; capillary voltage, 4000 V; fragmentor voltage and scan range variables. The fragmentor and *m/z* ranges used for HPLC-ESI/MSD analyses were as follows: positive mode, 80 V and *m/z* 50−1000, 120 V and *m/z* 50−1000, 120 V and *m/z* 1000−2000, 120 V and *m/z* 2000−3000; negative mode, 80 V and *m/z* 50−1000, 140 V and *m/z* 50−1000, 140 V and *m/z* 1000−2000, 140 V and *m/z* 2000−3000.

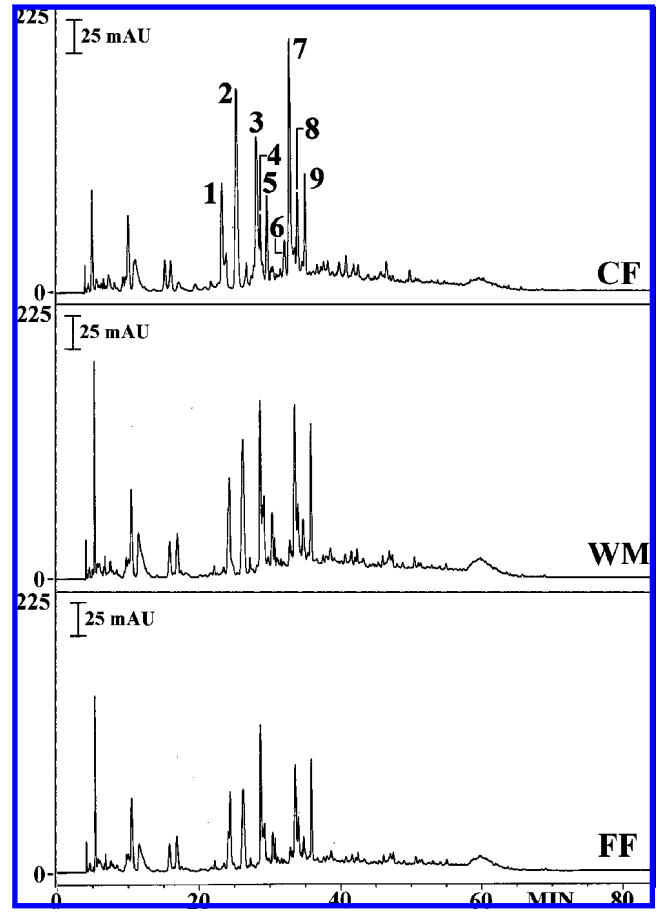

Figure 1. Chromatograms at 280 nm of flour barley samples. Peak identification: 1, 2 GC/1 C prodelphinidin trimer; 2, 1 GC/1 C prodelphinidin dimer B; 3, 1 GC/1 C prodelphinidin dimer B (prodelphinidin B3); 4, 1 GC/2 C prodelphinidin trimer B; 5, 1 GC/2 C prodelphinidin trimer B; 6, procyanidin dimer monoglycoside; 7, procyanidin dimer B2; 8, procyanidin trimer B; 9, (+)-catechin. Conditions are those reported under Materials and Methods. Abbreviations: CF, coarse fraction; WM, whole meal; FF, fine fraction.

The calibration curve of (+)-catechin was arranged from 5 to 500 mg/L, at six concentration levels, plotting peak area versus analyte concentration. The HPLC analysis was replicated three times for extracts and calibration points ($n = 3$).

Statistical Analysis. The results reported are the averages of four repetitions ($n = 4$), unless otherwise stated. Tukey's honest significant difference multiple comparison (one-way ANOVA) and Pearson's linear correlations were performed using Statistica 6.0 software (2001, StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Analysis (Separation and Identification) of Free Barley Flavan-3-ols by HPLC. Figure 1 shows the chromatograms (at 280 nm) of the Ac extracts of the three milling barley fractions analyzed (whole meal, fine fraction, and coarse fraction). As previously reported, aqueous acetone can selectively extract flavan-3-ol derivatives from barley flour (*22*).

Table 1. HPLC-ESI/MSD (Negative and Positive) Data (*m/z* Ions) of Free Barley Flavan-3-ols[a]

flavan-3-ol	peak (RT)	ESI^- major fragment ions, *m/z*	ESI^+ major fragment ions, *m/z*
GC−GC−C	1 (24.5)	897 $[M-H]^-$, 729, 711, 593 [M − GC (1 GC/1 C prodelphinidin dimer fragment) − $H]^-$, 289 $[C-H]^-$	731, 595 [M − GC (1 GC/1 C prodelphinidin dimer fragment) + $H]^+$, 443, 425, 305 [1 GC/1 C prodelphinidin dimer fragment − C + $H]^+$, 291 $[C+H]^+$
GC−C	2 (26)	593 $[M-H]^-$, 467, 289 $[C-H]^-$	633 $[M+K]^+$, 617 $[M+Na]^+$, 595 $[M+H]^+$, 443, 427, 291 $[C+H]^+$
prodelphinidin B3	3 (28)	593 $[M-H]^-$, 467, 303 $[GC-H]^-$, 289 $[C-H]^-$	617 $[M+Na]^+$, 595 $[M+H]^+$, 443, 427, 289 $[M-GC+H]^+$
GC−C−C	4 (28.5)	881 $[M-H]^-$, 751, 729, 713, 577 [M − GC (procyanidin dimer aglycone fragment) − $H]^-$, 289 $[C-H]^-$	595 [M − C (1GC/1 C prodelphinidin dimer fragment) + $H]^+$, 579 [M − GC (procyanidin dimer fragment) + $H]^+$, 307
C−GC−C	5 (29)	881 $[M-H]^-$, 751, 729, 713, 593 [M − C (1 GC/1 C prodelphinidin dimer fragment) − $H]^-$, 467, 289 $[C-H]^-$	731, 595 [M − C (1 GC/1 C prodelphinidin dimer fragment) + $H]^+$, 443, 425, 289
procyanidin dimer monoglycoside	6 (32)	739 $[M-H]^-$, 451, 425, 407, 289 $[C-H]^-$	453, 291 $[C+H]^+$
procyanidin dimer B2	7 (32.5)	577 $[M-H]^-$, 425 (RDA fragment), 407, 289 $[C-H]^-$	617 $[M+K]^+$, 601 $[M+Na]^+$, 579 $[M+H]^+$, 427, 291 $[M-C+H]^+$
C−C−C	8 (33.5)	865 $[M-H]^-$, 713, 739, 577 [M − C (procyanidin dimer fragment) − $H]^-$, 289 $[C-H]^-$	579, 291 $[C+H]^+$
(+)-catechin	9 (35)	289 $[M-H]^-$, 245, 221, 203, 137	329 $[M+K]^+$, 313 $[M+Na]^+$, 291 $[M+H]^+$, 255, 237, 139

[a] Abbreviations: GC, (epi)gallocatechin subunit; C, (epi)catechin subunit; RT, retention time.

The former, from 20 and 50 min for HPLC, were flavan-3-ols (usually called catechins) and their oligomers (usually called proanthocyanidins).

HPLC UV-DAD spectral analysis indicated that most compounds had spectra typical of flavan-3-ols according to de Pascual-Teresa and co-workers (*23*).

Because most phenolic compounds found in cereals have complex structures (proanthocyanidins) and are not available as commercial standards, simple phenols and three proanthocyanidin dimers were employed to determine the optimal MS ionization conditions. The fragmentor was varied from 60 to 200 V. The fragmentation degree increased with the fragmentor voltage, even if at high values the sensitivity was drastically affected and the ionization was too severe. However, those compounds with a high degree of polymerization (high molecular weight proanthocyanidins) needed higher fragmentor voltages to obtain satisfactory ionization. Thus, 80 and 120 V were set for low and high molecular weight compounds, respectively, and the *m/z* range was properly varied.

In this investigation, HPLC-ESI/MSD analysis was performed to collect structural information about the molecular weight and typical fragmentation of peaks eluted. HPLC-ESI/MSD analysis in negative and positive ion mode established that barley flavan-3-ols and proanthocyanidins were four dimers and four trimers having (epi)catechin and/or (epi)gallocatechin (C and/or GC) subunits.

Identified flavan-3-ols compounds in negative and positive mode are shown in **Table 1**.

The main fragmentation pathways for proanthocyanidins are (a) retro-Diels−Alder (RDA) cleavage and subsequent elimination of a water molecule, (b) interflavanic bond cleavage through the quinone-methine (QM) mechanism, and (c) heterocyclic ring fission (HRF) $[M - C_6H_6O_3 - H]$.

As **Table 1** reports, a 2GC/1 C proanthocyanidin trimer (molecular weight = 898), two isomeric forms of 1 GC/2 C proanthocyanidin trimer (molecular weight = 882), and two isomeric forms of procyanidin trimer (molecular weight = 866) pattern fragmentations in negative and positive mode were shown.

Peak 1 (*m/z* 897 $[M-H]^-$) mass spectra in negative mode reported the ion *m/z* 729 corresponding to RDA fission followed by water elimination (*m/z* 711). Other fragment ions were detected; resulting dimeric fragment at *m/z* 593 corresponding to the $[(GC-G)-H]^-$ fragment and the $[C-H]^-$ fragment with *m/z* 289 were obtained. This fragmentation pattern was studied by Friedrich et al. (*24*), and the GC−GC−C trimer was identified. The same compound was detected in positive mode. Fragment ions with *m/z* 595, 443, 305, and 291 were observed. The identical pattern was reported by Li and Deinzer (*25*).

Two isomeric forms of GC−C prodelphinidin dimer (peak 2, molecular weight = 594, and the second isomer eluted identified as prodelphinidin B3 by spiking with the commercial standard) were determined. These dimers were identified (in negative mode) on the basis of the *m/z* 289 derived from the QM cleavage. RDA and HRF cleavage on the extension unit of the dimer gave rise to *m/z* 425 and 467 according to the Gu et al. (*26*) and Friedrich et al. (*24*) patterns. The fragmentation pattern of prodelphinidin B3 obtained in positive mode was in agreement with that of de Pascual-Teresa and co-workers (*23*).

Peaks 4 and 5 gave spectra showing $[M-H]^-$ at *m/z* 881. They showed a different fragmentation pattern. Peak 4 presented, in negative mode, fragmentation ions with *m/z* 577 and 303; these fragmentation data establish that peak 4 is a GC−C−C trimer in accordance with Gu et al. (*26*). Fragmentation data in positive mode were in agreement with the de Pascual-Teresa pattern (*23*).

Peak 5 showed the fragmentation ions (in negative mode) of C−GC−C reported by Gu and collaborators (*26*). The positive fragmentation data reported the ions with *m/z* 595 and 443 reported by Li and Deinzer (*25*).

Peak 6 (t_R = 32 min) showed the presence of the *m/z* 451 fragment anion and the molecular ion at *m/z* 453 ($[M+H]^+$) in positive ion mode. Friedrich and Galensa (*27*) identified, for the first time, the (2*R*,3*S*)-catechin-7-*O*-*β*-D-glucopyranoside in barley. For this, the procyanidin dimer monoglycoside was detected The negative mode fragmentation pattern obtained confirmed the results of Salminen et al. (*28*).

A procyanidin dimer (molecular weight, 578) has been identified by observing, in negative mode, the molecular anion at *m/z* 577. Fragmentation of this anion yielded a monomer catechin (*m/z* 289) due to the cleavage of the interflavanoid C−C linkages with losses of 288 amu. The fragment of *m/z* 425 resulted from a RDA fission of the flavonoid nucleus giving rise to a fragment of *m/z* 425. From the *m/z* 425 fragment, after water elimination, the *m/z* 407 fragment was obtained. Tomás-Barberán and co-workers (*29*) linked this fragmentation pattern to procyanidin B2. The fragmentation pattern was confirmed in positive mode; the *m/z* value selected was 579. This compound showed a major ion fragment at *m/z* 427 corresponding to RDA reaction. Thus, the fragment at *m/z* 409 corresponds to the loss of a water molecule. Finally, the ion at 289 (breaking of the C−C linkage between two catechin units) confirmed the nature of

Table 2. Spectrophotometric Values (TPC, Total Phenolic Compounds; FRSA, Free Radical Scavenging Activity), and HPLC Quantification of Free Flavan-3-ol Barley Phenols, Expressed As Average ± Standard Deviation ($n = 4$)

	HPLC[a]												
sample	GC−GC−C	GC−C	PD B3	GC−C−C	C−GC−C	PC glycoside	PC B2	C−C−C	(+)-CAT	others	sum	TPC[b]	FRSA[c]
CF	3.9 ± 0.7	3.7 ± 0.4	19.3 ± 3.9	32.8 ± 7.4	18.6 ± 5.4	16.6 ± 6.0	11.3 ± 2.5	8.2 ± 2.0	32.5 ± 6.0	37.9 ± 6.5	185.0 ± 37.5	168.7 ± 29.2	1200.1 ± 66.2
WM	3.7 ± 0.5	4.7 ± 0.5	11.7 ± 1.3	17.6 ± 1.9	13.9 ± 2.6	8.9 ± 2.2	5.8 ± 0.6	4.6 ± 0.4	17.9 ± 2.4	30.3 ± 3.6	119.0 ± 12.8	117.1 ± 28.6	1025.9 ± 18.3
FF	2.7 ± 0.3	3.8 ± 0.2	8.0 ± 1.3	10.2 ± 1.7	9.8 ± 1.2	5.9 ± 1.4	3.0 ± 0.5	3.5 ± 0.5	11.2 ± 2.9	20.4 ± 2.0	78.6 ± 10.9	76.0 ± 8.0	761.7 ± 55.3

[a] Milligrams of (+)-catechin/100 g of flour. [b] Milligrams of gallic acid/100 g of flour. [c] Micromoles of Trolox equivalents/100 g of flour.

the dimeric catechin of this compound. The fragmentation data obtained by positive electrospray ionization confirmed the data reported by de Pascual-Teresa et al. (*23*).

Peak 8 was identified to be a C−C−C procyanidin trimer. Its mass spectrum showed $[M - H]^-$ m/z 865 and a fragment ion m/z 577 corresponding to $[(C - C) - H]^-$.

The (+)-catechin (molecular weight = 290) was identified. Several reports on the catechin ESI-MS fragmentation in the negative mode can be found in the literature. Deprotonated catechin anion was dissociated into major fragment ions. The fragment peak at m/z 245 is proposed to be the possible result of the loss of a $-CH_2-CHOH-$ group in the benzofuran skeleton. ESI-MS analysis in the positive mode revealed a fragment ion at m/z 139 obtained from RDA reaction of catechin.

Quantification of Flavan-3-ols Phenols by HPLC and Statistical Correlation with Spectrophotometric Determinations. To compare the quantification performance, **Table 2** shows the sum of flavan-3-ols [peaks were quantified using the (+)-catechin calibration curve] detected by HPLC and the spectrophotometric determinations.

HPLC quantification considered the peaks identified by mass spectrometry analysis and other peaks that had a typical UV spectrum of flavan-3-ols but unidentified by MS analysis.

HPLC detected the highest amount of catechins and proanthocyanidins, extracted by aqueous acetone, in the coarse fraction. In particular, the amount of flavan-3-ols of the coarse fraction found by HPLC [185.0 ± 37.5 mg of (+)-catechin/100 g of flour] was about 2 times greater than the whole and fine fractions (significant at $p < 0.05$), respectively. Moreover, the content of flavan-3-ols found by HPLC in the whole meal [119.0 ± 12.8 mg of (+)-catechin /100 g of flour] was significantly different ($p < 0.05$) from that of the fine fraction [78.6 ± 10.9 mg of (+)-catechin/100 g of flour]. Results obtained for whole meal were in agreement with Zimmermann and Galensa. Similarly, the TPC and the FRSA contents of the three fractions were all significantly different ($p < 0.05$) from each other; the CF sample had the highest values ($p < 0.05$; TPC, 168.7 ± 29.2 mg of gallic acid/100 g of flour; FRSA, 1200.1 ± 66.2 μmol of Trolox equiv/100 g of flour). Notably, the quantification indices selected (spectrophotometry and HPLC) were in strong agreement, and the order of the amount of flavan-3-ols for the three fractions was CF > WM > FF.

However, TPC and HPLC values of the three fractions were not significantly different from each other, whereas the CF-FRSA value was significantly higher (1200.1 ± 66.2 μmol of Trolox equiv/100 g of flour, $p < 0.05$) than the WM and FF-FRSA values.

Because HPLC and spectrophotometric determinations showed that the coarse fraction generally had higher amounts of free phenolic compounds than the other two fractions (WM and FF), this confirmed that free flavan-3-ols are localized in the external layers (aleurone, bran) and in the cell wall of the starchy endosperm.

As can be seen in **Table 2**, the HPLC values were close to those obtained with the Folin−Ciocalteu method. The positive

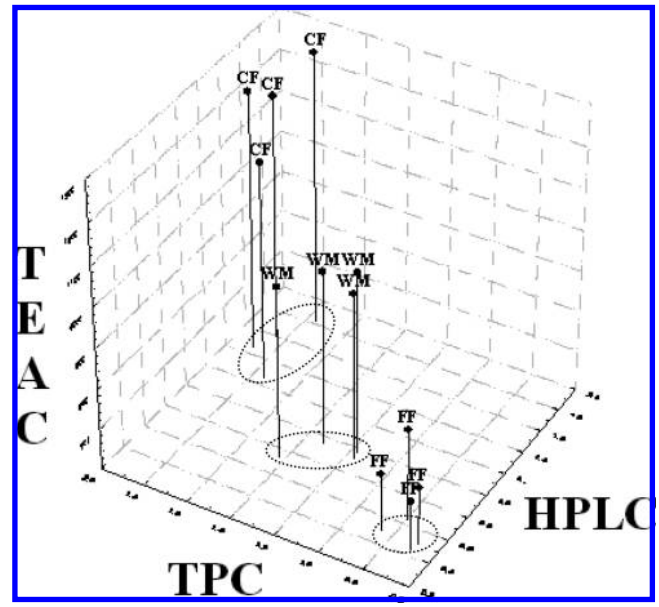

Figure 2. Scatterplot of the three samples applied to the free flavan-3-ol barley sample determinations.

correlations found between HPLC and TPC ($r^2 = 0.836$, $p < 0.0001$) confirmed the good selection of these phenolic compounds quantified by the two techniques. Moreover, positive correlations between HPLC and FRSA ($r^2 = 0.922$, $p < 0.0001$) were found, confirming that the compounds detected and quantified by HPLC as catechins and proanthocyanidins had significant antioxidant properties, expressed as free radical scavenging activity by the DPPH assay. A positive correlation between FRSA and TPC ($r^2 = 0.898$, $p < 0.0001$) was found, denoting that the whole pattern of simple free phenolic compounds (detected by the Folin−Ciocalteu reagent) made a significant contribution to the antioxidant activity (as free radical scavenging capacity).

Figure 2 shows a graphical interpretation of the results previously discussed.

In particular, the extracts formed three well-defined clusters in consideration of the different levels of simple flavan-3-ols, which determined the differences of the other spectrophotometric indices. Moreover, it is possible to determine the localization of these phenolic compounds in kernels.

Therefore, the air classification conditions used in this work can be effectively used to obtain flour fractions selectively enriched in free flavan-3-ols, which might be used as ingredients for the production of functional foods. Previous assessments using the air classification system to obtain antioxidant ingredients are limited (*30, 31*), and to our knowledge this is the first work investigating the morphological distribution of free flavan-3-ols (catechins and their oligomers) in air-classified barley flour.

On the basis of these results, free flavan-3-ols are localized in the external layers (aleurone, bran) and in the cell walls of the endosperm of the kernel. Furthermore, the air classification

conditions used in this work [previously standardized for production of a flour fraction enriched in β-glucans (*2*)] can be effectively used to obtain flour fractions selectively enriched in this phenol class (and β-glucans), which might be used as an ingredient for the production of functional foods, with positive implications on the nutraceutical characteristics and shelf life.

Repeatability and Sensitivity of HPLC. The repeatability was assessed for an extract obtained from organic whole barley flour. The extract was injected on the same day (intraday precision) and for three consecutive days (interday precision, $n = 12$). The percent relative standard deviations (% RSD) of the peak areas (UV detection) and retention times were determined for each peak detected.

The intraday repeatability (expressed as % RSDs) of the retention times was from 0.22 to 2.74%, whereas the interday repeatability was from 1.14 to 2.96%.

The intraday repeatability (expressed as % RSDs) of the total peak area was 0.52%, whereas the interday repeatability was 1.29%.

As expected, the intraday precision was higher than the interday precision.

LITERATURE CITED

(1) Marconi, E.; Graziano, M.; Cubadda, R. Composition and utilization of barley pearling by-products for making functional pastas rich in dietary fiber. *Cereal Chem.* **2000**, *77*, 133–139.

(2) Marconi, E.; Panfili, G.; Ferrante, M.; Raponi, F.; Falasca, L.; Fratianni, A.; Cubadda, R. Functional pasta and bakery products from barley flour enriched in bioactive compounds *Proceedings of the Second International Workshop "Durum Wheat and Pasta Quality: Recent Achievements and New Trends"*; 2003; pp 229−232.

(3) Panfili, G.; Fratianni, A.; Di Criscio, T.; Marconi, E. Tocol and β-glucan levels in barley varieties and in pearling by-products. *Food Chem.* **2008**, *107*, 84–91.

(4) McMurrough, I.; Madigan, D.; Smyth, M. R. Semipreparative chromatographic procedure for the isolation of dimeric and trimeric proanthocyanidins from barley. *J. Agric. Food Chem.* **1996**, *44*, 1731–1735.

(5) Goupy, P.; Hugues, M.; Boivin, P.; Amiot, M. J. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *J. Sci. Food Agric.* **1999**, *79*, 1625–1634.

(6) McMurrough, I.; Madigan, D.; Kelly, R. J. The role of flavonoid polyphenols in beer stability. *J. Am. Soc. Brew Chem.* **1996**, *54*, 141–148.

(7) Waldron, K. W.; Parr, A. J.; Ng, A.; Ralph, J. Cell wall esterified phenolic dimers: identification and quantification by reverse phase high performance liquid chromatography and diode array detection. *Phytochem. Anal.* **1996**, *7*, 305–312.

(8) Jadhav, S. J.; Lutz, S. E.; Ghoparde, V. M.; Salunkhe, D. K. Barley: chemistry and value-added processing. *Crit. Rev. Food Sci.* **1998**, *38*, 123–171.

(9) Zimmermann, B. F.; Galensa, R. One for all−all for one: proof of authenticity and tracing of foods with flavonoids. Analysis of proanthocyanidins in barley and malt. *Eur. Food Res. Technol.* **2007**, *224*, 385–393.

(10) Tapiero, H.; Tew, K. D.; Nguyen Ba, G.; Mathé, G. Polyphenols: do they play a role in the prevention of human pathologies? *Biomed. Pharmacother.* **2002**, *56*, 200–207.

(11) Duthie, G. G.; Duthie, S. J.; Kyle, J. A. M. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutr. Res. Rev.* **2000**, *13*, 79–106.

(12) Sessa, D. J. Processing of soybean hulls to enhance the distribution and extraction of value-added proteins. *J. Sci. Food Agric.* **2004**, *84*, 75–82.

(13) Wolf, W. J.; Sessa, D. J.; Wu, Y. V.; Thompson, A. R. Air classification of pin-milled soybean hulls. *Cereal Chem.* **2002**, *79*, 439–444.

(14) Vasanthan, W. H.; Bhatty, R. S. Starch purification after pin milling and air classification of waxy, normal, and high amylose barleys. *Cereal Chem.* **1995**, *72*, 379–384.

(15) Létang, C.; Samson, M. F.; Lassere, T. M.; Charand, M.; Abécassis, J. Production of starch with very low protein content from soft and hard wheat flours by jet milling and air classification. *Cereal Chem.* **2002**, *79*, 535–543.

(16) McMurrough, I. High-performance liquid chromatography of flavonoids in barley and hops. *J. Chromatogr., A* **1981**, *218*, 683–693.

(17) Jerumanis, J. Quantitative analysis of flavonoids in barley, hops and beer by high-performance liquid chromatography (HPLC). *J. Inst. Brew.* **1985**, *91*, 250–252.

(18) Mulkay, P.; Touillaux, R.; Jerumanis, J. Proanthocyanidins of barley: separation and identification. *J. Chromatogr., A* **1981**, *208*, 419–423.

(19) Holtekjølen, A. K.; Ninitz, C.; Knutsen, S. H. Flavanol and bound phenolic acid contents in different barley varieties. *J. Agric. Food Chem.* **2006**, *54*, 2253–2260.

(20) Marconi, E.; Carcea, M. Pasta from non traditional raw materials. *Cereal Foods World* **2001**, *46*, 522–529.

(21) Bonoli, M.; Verardo, V.; Marconi, E.; Caboni, M. F. Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. *J. Agric. Food Chem.* **2004**, *52*, 5195–5200.

(22) Bonoli, M.; Marconi, E.; Caboni, M. F. Free and bound phenolic compounds in barley (*Hordeum vulgare* L.) flours. Evaluation of the extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry. *J. Chromatogr., A* **2004**, *1057*, 1–12.

(23) de Pascual-Teresa, S.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Prodelphinidins and related flavanols in wine. *Int. J. Food Sci. Technol.* **2000**, *35*, 33–40.

(24) Friedrich, W.; Eberhardt, A.; Galensa, R. Investigation of proanthocyanidins by HPLC with electrospray ionization mass spectrometry. *Eur. Food Res. Technol.* **2000**, *211*, 56–64.

(25) Li, H. J.; Deinzer, M. L. Tandem mass spectrometry for sequencing proanthocyanidins. *Anal. Chem.* **2007**, *79*, 1739–1748.

(26) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51*, 7513–7521.

(27) Friedrich, W.; Galensa, R. Identification of a new flavonol glucoside from barley (*Hordeum vulgare* L.) and malt. *Eur. Food Res. Technol.* **2002**, *214*, 388–393.

(28) Salminen, J. P.; Karonen, M.; Lempa, K.; Liimatainen, J.; Sinkkonen, J.; Lukkarinen, M.; Pihlaja, K. Characterisation of proanthocyanidin aglycones and glycosides from rose hips by high-performance liquid chromatography−mass spectrometry, and their rapid quantification together with vitamin C. *J. Chromatogr., A* **2005**, *1077*, 170–180.

(29) Tomás-Barberán, F. A.; Gil, M. I.; Cremin, P.; Waterhouse, A. L.; Hess-Pierce, B.; Kader, A. A. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *J. Agric. Food Chem.* **2001**, *49*, 4748–4760.

(30) Gamel, T. H.; Linssen, J. P.; Mesallam, A. S.; Damir, A. A.; Shekib, L. A. Seed treatments affect functional and antinutritional properties of amaranth flours. *J. Sci. Food Agric.* **2006**, *86*, 1095–1102.

(31) Shahidi, F.; Chavan, U. D.; Naczk, M.; Amarowicz, R. Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). *J. Agric. Food Chem.* **2001**, *49*, 926–933.

Received for review February 29, 2008. Revised manuscript received June 10, 2008. Accepted June 13, 2008.

JF8006344