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Distribution of Bound Hydroxycinnamic Acids and Their Glycosyl Esters in Barley (*Hordeum vulgare* L.) Air-Classified Flour: Comparative Study between Reversed Phase-High Performance Chromatography−Mass Spectrometry (RP-HPLC/MS) and Spectrophotometric Analysis

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The level of bound hydroxycinnamic acid was determined by spectrophotometry (as total hydroxycinnamic compounds and free-radical-scavenging activity) and reversed-phase high-performance chromatography (RP-HPLC) coupled to mass spectrometry (MS) in barley flours (whole meals and air-classified fractions: coarse fraction and fine fraction). Hydroxycinnamic acids and their derivatives were the main bound phenols in barley flours. A total of 12 different hydroxycinnamic acids were identified and quantified by HPLC/diode array detector (DAD)−MS within 90 min. Ferulic acid (as a simple and glycosylated derivative) was the main phenolic acid in barley flours, representing 89−93% of total hydroxycinnamic acids. The amount of total hydroxycinnamic acid in air-classified coarse fraction was 2 and 3 times higher than those of whole meal and the air-classified fine fraction, respectively. Similarly, the coarse fraction showed higher antioxidant activity (650.03 μmol of TEAC/100 g of flour) compared to whole meal and the fine fraction (388.78 and 320.27 μmol of TEAC/100 g of flour, respectively).

KEYWORDS: High performance liquid chromatography (HPLC); mass spectrometry; barley; hydroxycinnamic acids; milling fractions; air classification

1. INTRODUCTION

Barley contains a number of phytochemicals (*1−3*) and finds extensive uses in functional food products development. Whole grain products are recommended for healthy diets, such as being recognized sources of dietary fibers and antioxidant substances (*4, 5*). Air classification has been successfully used for obtaining barley fractions (coarse fraction) enriched in β glucans (*2*). In the coarse fraction, cell walls of starchy endosperm and external layers of the kernel (aleurone and bran) are concentrated.

Different phenolics, such as benzoic acid, cinnamic acid, flavonoid, and tannin classes of compounds, are present in free, esterified, or insoluble bound forms (*3−7*).

Phenolic acids are the major phenylpropanoid components in cereals: the starchy endosperm contains low levels of them, whereas the outer layers of the grain (pericarp, aleurone layer, and germ) contain the highest portion. Hydroxycinnamic acids are a group of phenolic compounds that exhibit a wide range of *in vitro* chemoprotective and antioxidant properties. Epidemiological studies suggest a link between the consumption of whole grain products and the prevention of chronic diseases, such as coronary heart disease and certain forms of cancer (*8*). The most abundant cinnamic acid derivative, ferulic acid (FA), and the second most abundant phenolic acid, *p*-coumaric acid (*p*-CA), are mostly concentrated in the aleurone layer and 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 (*7, 9*).

Thus, regular consumption of cereal bran and bran-enriched products would result in the ingestion of significant amounts of these plant components, in particular, ester-linked ferulic and diferulic acids.

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Ferulic acid (FA) is reported as a good antioxidant (*10*), absorbed by human or rat, but only few data deal with the influence of the food matrix on its bioavailability and its potential protection against cardiovascular diseases and cancer (*11*).

The increasing interest in barley phenolic acids and their beneficial effects on the human body have led to many analytical developments for their determination. In the literature, bound phenols from cereals have been analyzed by spectrophotometric analysis (*12*), reversed-phase high-performance liquid chromatography (RP-HPLC) (*7*), and capillary gas chromatography (CGC) (*13*).

Several authors reported the presence of feruloyl oligosaccharides in wheat bran. Interest in these oligosaccharides is due to their biological activities and functional applications (*14*).

These hydroxycinnamic acids exhibit *in vitro* chemoprotective and antioxidant properties, and it is suspected that they may contribute toward the beneficial effects of a diet rich in cereal bran. Ferulic acid, the main phenolic acid in barley bran, is esterified to arabinose residues in the cell-wall arabinoxylan. Treatment of barley flours with hydrochloric acid released feruloylated oligosaccharides. They are a potential source of antioxidants, able to inhibit the peroxidation of low-density lipoproteins (*15*) and to protect normal rat erythrocytes against oxidative damage *in vitro* (*16*).

Therefore, the aim of this work is to evaluate the performance of RP-HPLC to characterize and quantify the amount of bound phenolic acids and their glycosyl esters in air-classified barley flour extracts. The best separation conditions were investigated to achieve the highest resolution and sensitivity using ultraviolet (UV) detection and mass spectrometry (MS) detection for the HPLC system. The amount of phenolic acids and the antioxidant activity of barley extracts by spectrophotometric determinations (hydroxycinnamic acids at 320 nm, and free-radical-scavenging activity by the DPPH discoloration assay) were also estimated, and statistical analysis was carried out.

2. MATERIALS AND METHODS

2.1. Samples and Sample Preparation. Hulled grain of barley (cv. Gotic) was dehulled and pin-milled (whole meal). Air classification was developed to separate flours into various particle sizes using air currents. Classification of flour is achieved under the influence of two opposing forces, air traction and centrifugal force. Selected particle-size ranges are obtained by adjusting baffles that rotate, forming a barrier that permits specific particle-size ranges to pass. Whole meal (WM) was air-classified into coarse fraction (CF) (43%, w/w) and fine fraction (FF) (57%, w/w), according to Marconi et al. (*2*). Particle-size ranges were 120−477 and 45−120 μm for coarse and fine fractions, respectively.

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

2.2. Reagents and Chemicals. All solvents were pro-analysis-grade from Merck (Darmstad, Germany). Ferulic acid, caffeic acid, and *p*-coumaric acid were from Sigma-Aldrich (St. Louis, MO).

2.3. Extraction of Bound Phenolic Compounds. Once extraction with aqueous ethanol was performed to discharge free phenolic compounds, bound phenols were collected by acid hydrolysis, as reported by Bonoli et al. (*17*). A total of 1 g of whole flour was shaken with 6 mL of 96% ethanol and 30 mL of 25% hydrochloric acid at 65 °C for 30 min. Then, 10 mL of 96% ethanol diethyl ether/petroleum benzine (1:1, v/v) at 40−60 °C was added to the digested samples. The organic fractions were pooled and evaporated to dryness. The phenolic compounds were reconstituted with 5 mL of 99.7:0.3 water/formic acid (v/v). Each fraction was extracted 4 times ($n = 4$). Extracts were stored at −18 °C until use.

2.4. Spectrophotometric Determinations. The spectrophotometric analyses were performed using a UV-1601 spectrophotometer from Shimadzu (Duisburg, Germany), and they were replicated 4 times for each extract ($n = 4$) and 3 times for each calibration point ($n = 3$). The hydroxycinnamic index (HI) was carried out at 320 nm, according to Maillard et al. (*18*), with some modifications, as described by Bonoli et al. (*6*). To assess HI, a ferulic acid calibration curve was set.

The free-radical-scavenging activity (FRSA) of extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following Bonoli et al. (*6*). To assess the FRSA, a Trolox calibration curve was arranged, and the results were expressed as micromoles of Trolox equivalent/100 g of flour.

2.5. RP-HPLC Analysis with UV-Diode Array Absorption and Mass Spectrometry Detections (UV-DAD/MSDs). RP-HPLC analysis was performed by a HP 1100 Series (Agilent Technologies, Palo Alto, CA), equipped with a binary pump delivery system, a degasser, an autosampler, a UV−vis DAD and a MSD. A C18 Luna column, 5 μm, 250 × 3.00 mm ID (Phenomenex, Torrance, CA), with a C18 precolumn filter was used. All solvents were HPLC-grade and filtered through 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 linear gradient elution system was reported by Verardo et al. (*19*). UV spectra were recorded from 210 to 600 nm. The injection volume was 10 μL. HPLC analysis was performed at room temperature. MS analysis was performed using an electrospray ionization (ESI) interface (using both positive and negative polarity) at the following conditions: drying gas flow (N_2), 9.0 L/min; nebulizer pressure, 50 psig; 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, 60 V and *m/z* 50−1000; 100 V and *m/z* 50−1000; negative mode, 80 V and *m/z* 50−1000; 140 V and *m/z* 50−1000.

The calibration curve for ferulic acid was evaluated from 1 to 500 μg/mL, at six concentration levels, plotting peak area versus the analyte concentration. The HPLC analysis was replicated 3 times for each extract and calibration points ($n = 3$).

2.8. Statistical Analysis. Unless otherwise stated, the results reported in this study are the averages of four repetitions ($n = 4$). Tukey's honest significant difference multiple comparison [one-way analysis of variation (ANOVA)] and Pearson's linear correlations, both at the $p < 0.05$ level, were evaluated using the Statistica 6.0 software (2001, StatSoft, Tulsa, OK).

3. RESULTS AND DISCUSSION

3.1. Analysis (Separation and Identification) of Bound Hydroxycinnamic Barley Phenols by HPLC. Bound phenolic acids in flour can be extracted under hydrolytic conditions using alkaline or acid reagents. In fact, several authors reported that the hot acid conditions degrade the cinnamic acid derivatives (*20*).

Preliminarily, the extraction yield of bound phenols by cold alkaline (*17*) and hot acid extraction was compared. Effectively, alkaline treatment led to a higher recovery of phenolic acids (161.9 ± 19.5, 59.4 ± 5.2, and 39.4 ± 1.6 mg/100 g of dry weight flour in coarse fraction, whole meal, and fine fraction, respectively) than acid hydrolysis. However, the chromatograms of basic extracts showed only four main peaks, with $(M - H)^-$ at *m/z* 163 and 193 corresponding to *p*-coumaric acid isomers (two peaks) and ferulic acid isomers (two peaks), respectively.

According to other authors (*21−23*), soft-acid treatment can extract hydroxycinnamic acids and some glycosyl esters of phenolic acids, such as feruloylated oligosaccharides.

Soft-acid hydrolysis may break most of the linkages between hydroxycinnamic phenolic compounds and cell-wall material of grains.

As **Figure 1** shows, ferulic acid was the most abundant hydroxycinnamic compound detected by HPLC in soft-acid hydrolysis extracts, as already reported in the literature (*12, 24−29*). HPLC−DAD spectral analysis confirmed this result, and

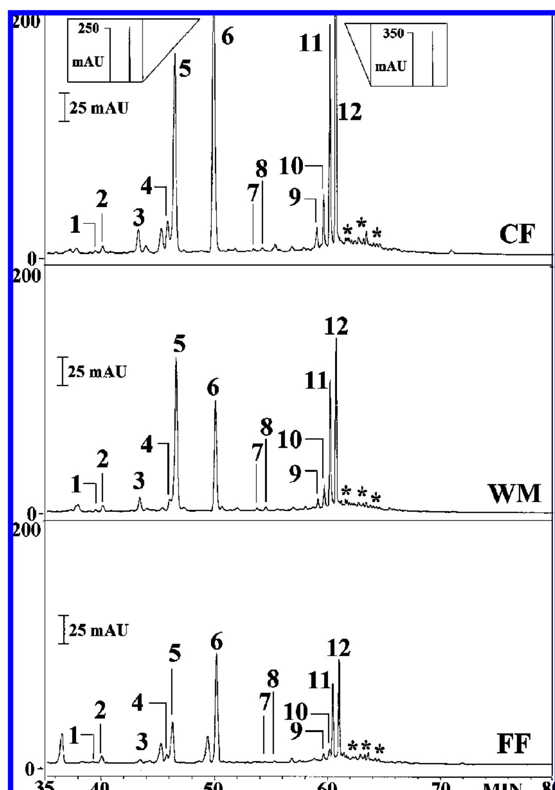

Figure 1. Chromatograms at 280 nm of hydroxycinnamic acids in soft-acid hydrolysis barley extracts. Peak identification: 1, caffeoyl-pentose; 2, caffeic acid; 3, *p*-coumaroyl-pentose; 4, *p*-coumaric acid; 5, feruloyl-pentose; 6, ferulic acid; 7 and 8, caffeoyl-hexose isomers; 9 and 10, *p*-coumaroyl-hexose isomers; 11 and 12, feruloyl-hexose isomers. Conditions are indicated in the Materials and Methods. Abbreviations: CF, coarse fraction; WM, whole meal; FF, fine fraction.

HPLC−ESI/MSD analysis in negative- and positive-ion modes showed typical ions of this compound (**Table 1**).

HPLC−MSD analysis confirmed the presence of ester-linked phenolic compounds (ferulic, *p*-coumaric, and caffeic acids) to pentose (arabinose and/or xylose) and hexose (glucose) saccharides (*24, 28*). Thus, as **Table 1** reports, a caffeoyl-pentose, a *p*-coumaroyl-pentose, a feruloyl-pentose, two caffeoyl-hexose isomers, two *p*-coumaroyl-hexose isomers, and two feruloyl-hexose isomers were identified by HPLC−ESI/MSD analysis.

Negative-mode ionization of compound **1** (see **Table 1** and **Figure 1**) showed a base peak with *m/z* 311 and a fragment at *m/z* 179 corresponding to [caffeic acid − H]$^-$, which matched a *m/z* 181 ion using the positive ionization mode. Therefore, this compound was identified as caffeoyl pentose.

Compound **2** showed two main fragments at *m/z* 179 and 135 corresponding to [M − H]$^-$ and [M − COOH]$^-$, respectively. Different authors, such as Troung et al. (*30*), Ma et al. (*31*), and Sanchez -Rebaneda et al. (*32*), assigned this typical ionization to the caffeic acid.

Compound **3** had a mass spectrum, using the negative ionization mode, with [M − H]$^-$ at *m/z* 295 and two main fragments at *m/z* 253 and 163 (correspond to deprotonated *p*-coumaric acid), which is compatible with a *p*-coumaroyl pentose. In the same way, using the positive ionization mode, the MS spectrum of peak 3 showed two main adducts with *m/z* 335 and 319 corresponding to [M + K]$^+$ and [M + Na]$^+$, respectively, which confirmed the identification of *p*-coumaroyl pentose.

The mass spectrum of compound **4** showed the deprotonated molecule at *m/z* 163 and a decarboxylated ion at *m/z* 119 corresponding to *p*-coumaric acid, as already reported by Ma et al. (*31*). The mass spectrum, under positive ionization conditions, had a [M + H]$^+$ ion with *m/z* 165 and the adduct [M + Na]$^+$ at *m/z* 187, which confirmed the identification of *p*-coumaric acid.

Peak 5 showed a MS profile with [M − H]$^-$ at *m/z* 325 and two fragments at *m/z* 193 and 175. The ion at *m/z* 193 [ferulic acid − H]$^-$ may derivate from the break of the ester bond. The positive-mode spectrum presented a series of ions, with *m/z* 365 [M + K]$^+$, 349 [M + Na]$^+$, and 177. Gallardo et al. (*33*) affirmed that the fragment *m/z* 177 corresponds to feruloyl derivate. Hence, compound **5** can be classified as feruloyl pentose.

Compound **6** with a deprotonated molecular ion at *m/z* 193 has been previously described by Bystrom et al. (*34*) as ferulic acid.

The [M − H]$^-$ ion (*m/z* 341) and two fragments at *m/z* 179 and 161 were detected for compounds **7** and **8**. The fragmented ion with *m/z* 179 corresponds to deprotonated caffeic acid. The positive mode showed a [M + Na]$^+$ ion with *m/z* 365. The same pattern was identified by Määttä and co-workers (*35*) as caffeoyl hexose.

Compounds **9** and **10** had negative ionization mass spectra, with [M − H]$^-$ at *m/z* 325 and fragments at 163, corresponding to deprotonated *p*-coumaric acid, and *m/z* 145. Seeram et al. (*36*) identified these compounds as *p*-coumaroyl hexose. Määttä and co-workers (*35*) confirmed this pattern and reported the [M + Na]$^+$ with *m/z* 349 in positive mode.

The MS fragmentation of compounds **11** and **12** showed a molecular ion [M − H]$^-$ at *m/z* 353 and fragments at 193 and 175. The fragment 193 corresponded to [ferulic acid − H]$^-$. The positive ionization gave an ion at *m/z* 379 and fragments at *m/z* 309 and 177. Gallardo et al. (*34*) identified the fragment 177 as feruloyl derivate. Määttä and co-workers (*35*) reported the fragment 349 as *p*-coumaroyl hexose.

Interestingly, several minor peaks (marked with asterisks in **Figure 1**) were observed in chromatograms. These peaks were identified as ferulic acid dehydrodimers by HPLC−MSD analysis because they showed *m/z* 385 ([M − H]$^-$), 341 ([M − COOH]$^-$), 193 ([ferulic acid fragment − H]$^-$, at higher fragmentor voltage) ions under negative polarity and *m/z* 409 ([M + Na]$^+$), 387 ([M + H]$^+$) ions using positive ionization. Moreover, their HPLC−UV spectra were confirmed by a comparison to published data (*37*), even if the single identification of ferulic acid dehydrodimers was behind the aim of this work. Those peaks marked with asterisks exhibited two main absorbance maxima around 290 and 325 nm and an absorbance minimum around 260 nm, similar to the UV spectrum of the ferulic acid. Thus, these peaks may correspond to ferulic acid dehydrodimers identified by HPLC−MSD analysis.

3.2. Repeatability and Sensitivity of the HPLC Method. The repeatability was assessed on a hydrolyzed extract obtained from organic whole barley flour, as reported by Bonoli et al. (*17*). The extract was injected 6 times on the same day (intraday precision, $n = 6$) and for 3 consecutive days (interday precision, $n = 18$). The percent relative standard deviations (% RSD) on the peak areas (UV detection) and the retention times were determined for each peak detected.

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

Table 1. HPLC−ESI/MSD (Negative and Positive) Data (*m*/*z* Ions) of Bound Hydroxycinnamic Barley Phenols[a]

phenolic compounds	peak number (RT)	ESI^- major fragment ions (*m*/*z*)	ESI^+ major fragment ions (*m*/*z*)
caffeoyl-pentose	1 (39)	311 $[M-H]^-$, 179	351 $[M+K]^+$, 335 $[M+Na]^+$, 181
caffeic acid	2 (40)	179 $[M-H]^-$, 135 $[M-COOH]^-$	
p-coumaroyl-pentose	3 (43)	295 $[M-H]^-$, 235, 163	335 $[M+K]^+$, 319 $[M+Na]^+$
p-coumaric acid	4 (45)	163 $[M-H]^-$	187 $[M+Na]^+$, 165 $[M+H]^+$
feruloyl-pentose	5 (46)	325 $[M-H]^-$, 193, 175	365 $[M+K]^+$, 349 $[M+Na]^+$, 177
ferulic acid	6 (50.5)	193 $[M-H]^-$	217 $[M+Na]^+$, 195 $[M+H]^+$
caffeoyl-hexose isomer A	7 (54)	341 $[M-H]^-$, 179, 161	365 $[M+Na]^+$
caffeoyl-hexose isomer B	8 (55)	341 $[M-H]^-$, 179, 161	365 $[M+Na]^+$
p-coumaroyl-hexose isomer A	9 (59)	325 $[M-H]^-$, 163, 145	365 $[M+K]^+$, 349 $[M+Na]^+$
p-coumaroyl-hexose isomer B	10 (60)	325 $[M-H]^-$, 163, 145	365 $[M+K]^+$, 349 $[M+Na]^+$
feruloyl-hexose isomer A	11 (60.5)	353 $[M-H]^-$, 193, 175	379 $[M+Na]^+$, 309, 177
feruloyl-hexose isomer B	12 (61.5)	353 $[M-H]^-$, 193, 175	379 $[M+Na]^+$, 309, 177

[a] Abbreviations: RT, retention time.

Table 2. Spectrophotometric Values (HI, Hydroxycinnamics Index; FRSA, Free-Radical-Scavenging Activity) and HPLC Quantification of Bound Hydroxycinnamic Acid Barley Phenols, Expressed as Average ± Standard Deviation ($n = 4$ for Spectrophotometry and HPLC, Respectively)[a]

samples	HI[b]	HPLC[c]	FRSA[d]
CF	128.8 ± 12.5	103.6 ± 4.6	650.0 ± 120.4
WM	76.4 ± 1.5	51.1 ± 2.6	388.8 ± 27.9
FF	45.0 ± 3.3	30.8 ± 4.8	320.3 ± 34.7

[a] CF, coarse fraction; WM, whole meal; FF, fine fraction. [b] In units of milligrams of ferulic acid/100 g of dw flour. [c] In units of milligrams of ferulic acid/100 g of dw flour. [d] In units of micromoles of Trolox equivalents/100 g of dw flour.

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

The sensitivity of methods was assessed for ferulic acids, which represent the phenolic acid class identified by UV-DAD spectral analysis. Solutions at 0.1 μg/mL gave a signal-to-noise ratio of approximately 3 (S/N ≈ 3) corresponding to the limit of detection (LOD) of the method.

Hydroxycinnamic acid content was calculated by

$$A = mc \pm q$$

where A is the peak area, c is the analyte concentration expressed as μg/mL, q is the y intercept, and r^2 is the correlation coefficient.

Linear regression results for ferulic acid were as follows:

$$A = 57.45c + 19.76\ (r^2 = 0.999)$$

3.3. Quantification of Bound Phenols in Barley by HPLC and Statistical Correlation with Spectrophotometric Determinations. Because most of compounds found in the hydrolytic samples were hydroxycinnamic acids or their derivatives, HPLC quantification of bound hydroxycinnamic phenols was carried out using a ferulic acid calibration curve. Moreover, the absorption at 320 nm (HI) was chosen as a spectrophotometric index to quantify the total hydroxycinnamic phenolic compounds.

As **Table 2** reports, the soft-acid hydrolysis coarse fraction extracts showed the highest HPLC, HI, and FRSA values (103.6 ± 4.6 and 128.8 ± 12.5 mg of ferulic acid/100 g of dw flour and 650.7 ± 120.4 μmol of Trolox equivalents/100 g of dw flour), which were between 2 and 3 times significantly higher than those of WM and FF ($p < 0.05$), respectively. The amount of bound phenols was in the following order: CF > WF > FF (**Figure 2**).

Similar to free phenolic extracts (*19*), it was confirmed that bound phenols are localized in external layers (aleurone and bran) of the kernel and, during milling, they are crushed into bigger particles, in consideration to their hardness, which give the coarse fraction (*19*). As it can be seen in **Table 2**, the HPLC values were close to those obtained with the hydroxycinnamics index; therefore, it could be pointed out that the selected compounds represent the profile of the barley total bound phenolic compounds in these extraction conditions (*38*). The positive correlations found between HPLC and HI ($r^2 = 0.961$, $p < 0.0001$) confirmed the correct selection of phenolic compounds quantified by the two analytical techniques. Moreover, the existing positive correlations between HPLC and FRSA ($r^2 = 0.822$, $p < 0.0001$) confirmed that the compounds detected and quantified by HPLC as bound phenols (mainly hydroxycinnamic acids and their derivatives) had significant antioxidant properties, expressed as free-radical-scavenging activity by the DPPH assay. A positive correlation between FRSA and HI ($r^2 = 0.872$, $p < 0.0001$) was obviously found, denoting that the hydroxycinnamic acids (which exhibit selective absorption at 320 nm) were the most representative class of bound phenols in barley flour, and they also gave the highest contribution to the antioxidant capacity (as free-radical-scavenging activity) of hydrolytic extracts.

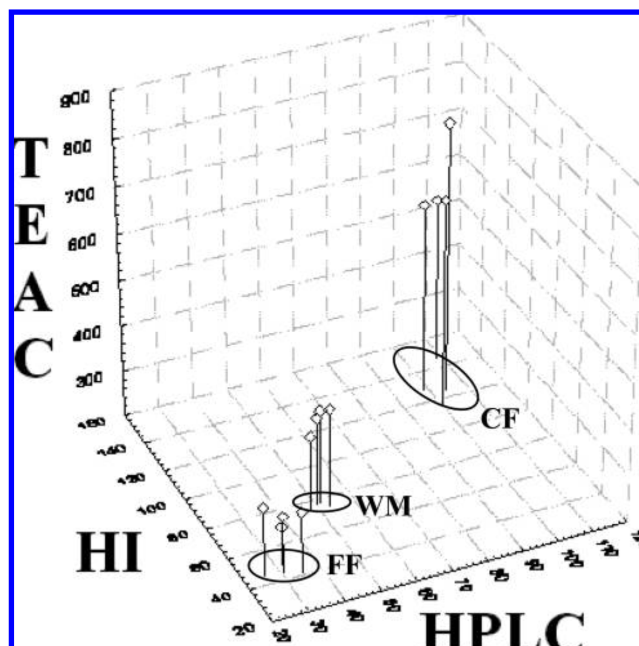

Figure 2. Scatterplot of the three samples applied to the bound hydroxycinnamic acids in barley samples. Abbreviations: WM, whole meal; CF, coarse fraction (43%); FF, fine fraction (57%).

Ferulic acid and its derivatives were the main hydroxycinnamic compounds in air-classified barley flours (**Table 3**).

In the present study, the optimum analytical conditions for the determination of bound barley phenolic compounds have been investigated by HPLC. Evidence shows that this method

Table 3. Hydroxycinnamic Acids (Milligrams of Ferulic Acid/100 g of Flour) in Soft-Acid Hydrolysate of Different Barley Flours[a]

compounds	coarse fraction	whole meal	fine fraction
p-coumaroyl-pentose	2.5 ± 0.2 (a)	1.4 ± 0.1 (b)	0.4 ± 0.1 (c)
p-coumaric acid	1.0 ± 0.2(a)	0.4 ± 0.2 (a)	0.8 ± 0.1 (a)
feruloyl-pentose	25.5 ± 2.6 (a)	17.1 ± 2.0 (b)	5.5 ± 0.8 (c)
ferulic acid	34.1 ± 1.5 (a)	11.0 ± 0.3 (b)	13.1 ± 3.1 (b)
caffeoyl-hexose isomer A	0.1 ± 0.0 (a)	0.0 ± 0.0 (b)	nd
caffeoyl-hexose isomer B	0.2 ± 0.1 (a)	0.1 ± 0.0 (b)	nd
p-coumaroyl-hexose isomer A	1.9 ± 0.5 (a)	1.0 ± 0.1 (a,b)	0.6 ± 0.4 (b)
p-coumaroyl-hexose isomer B	3.8 ± 0.6 (a)	1.6 ± 0.3 (b)	1.2 ± 0.3 (b)
feruloyl-hexose isomer A	13.3 ± 1.3 (a)	8.6 ± 0.6 (b)	4.0 ± 0.6 (c)
feruloyl-hexose isomer B	20.0 ± 2.5 (a)	9.2 ± 0.3 (b)	4.8 ± 1.0 (c)
others	3.8 ± 0.2 (a)	0.5 ± 0.3 (b)	0.3 ± 0.1 (b)

[a] Means in the same row with different letters are significantly different ($p \leq 0.05$).

was sensitive enough to detect and identify 12 bound phenolic compounds in barley flour.

From a technology point of view, the present work confirmed that air classification is a effective method to enrich barley flours in phenolic compounds. In fact, the air-classified coarse fraction showed the highest amount of ferulic acid and its derivatives, which were the main hydroxycinnamic acids found.

In conclusion, it has been demonstrated that the air-classified flours should be suitable for inclusion in bakery products, as functional ingredients. The use of the coarse fraction instead of only wheat flours should provide nutritionally better bakery products and pasta.

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