Hydroxycinnamic Acids and Ferulic Acid Dehydrodimers in Barley and Processed Barley

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Hydroxycinnamic acid content and ferulic acid dehydrodimer content were determined in 11 barley varieties after alkaline hydrolysis. Ferulic acid (FA) was the most abundant hydroxycinnamate with concentrations ranging from 359 to 624 μg/g dry weight. *p*-Coumaric acid (PCA) levels ranged from 79 to 260 μg/g dry weight, and caffeic acid was present at concentrations of <19 μg/g dry weight. Among the ferulic acid dehydrodimers that were identified, 8-*O*-4′-diFA was the most abundant (73−118 μg/g dry weight), followed by 5,5′-diFA (26−47 μg/g dry weight), the 8,5′-diFA benzofuran form (22−45 μg/g dry weight), and the 8,5′-diFA open form (10−23 μg/g dry weight). Significant variations ($p < 0.05$) among the different barley varieties were observed for all the compounds that were quantified. Barley grains were mechanically fractionated into three fractions: F1, fraction consisting mainly of the husk and outer layers; F2, intermediate fraction; and F3, fraction consisting mainly of the endosperm. Fraction F1 contained the highest concentration for ferulic acid (from 77.7 to 82.3% of the total amount in barley grain), *p*-coumaric acid (from 78.0 to 86.3%), and ferulic acid dehydrodimers (from 79.2 to 86.8%). Lower contents were found in fraction F2, whereas fraction F3 exhibited the lowest percentages (from 1.2 to 1.9% for ferulic acid, from 0.9 to 1.7% for *p*-coumaric acid, and <0.02% for ferulic acid dehydrodimers). The solid barley residue from the brewing process (brewer's spent grain) was ~5-fold richer in ferulic acid, *p*-coumaric acid, and ferulic acid dehydrodimers than barley grains.

Keywords: *Barley; brewer's spent grain; ferulic acid; p-coumaric acid; ferulic acid dehydrodimers*

INTRODUCTION

Ferulic acid (4-hydroxy-3-methoxycinnamic acid; FA) and *p*-coumaric acid (4-hydroxycinnamic acid; PCA) are the major low-molecular weight phenolic acids in barley grain, being concentrated mainly in the outer layers (husk, pericarp, testa, and aleurone), but they are also detected in endosperm (*1*). Other bound phenolic acids found in barley are vanillic, sinapic, and *p*-hydroxybenzoic acids (*2*). Barley grains also contain a range of flavan-3-ols from monomers [(+)-catechin and (−)-epicatechin], dimers (prodelphinidin B3 and procyanidin B3), and trimers (procyanidin C2), up to higher-molecular weight flavonoid-derived tannins (*2*, *3*). Although some forms of ferulic acid dehydrodimers have been detected in barley (*4*), we have found no data about the content and distribution of ferulic acid dehydrodimers in barley grain.

The main dehydrodimers identified in plant material are 8-*O*-4′-diFA, 8,5′-diFA, 8,5′-diFA dehydrobenzofuran form, 5,5′-diFA, 8,8′-diFA noncyclic form, and 4-*O*-5′-diFA (*5*−*9*). Like the monomer, ferulic acid dehydrodimers are mainly linked to sugars providing cross-linking between cell wall polymers (*10*), although they also form ether bonds and C−C linkages with the lignin (*11*, *12*). These cross-links seem to influence cell wall extensibility (*13*) and cell−cell adhesion, having significant effects on the texture of plant-derived foods (*14*). In addition, the esterified diferulates limit the enzymatic degradation of the cell wall polysaccharides (*15*). Antioxidant properties of ferulic acid dehydrodimers in different in vitro assays are greater in scale than those of the monomer and depend on dimer chemical structure (*7*, *16*).

The ester-linked hydroxycinnamates are thought to be formed by feruloylation of polysaccharides in the Golgi apparatus, feruloyl-CoA perhaps being the substrate (*17*). Once in the cell wall, ferulates can be oxidized by peroxidases, and the radical coupling produces a range of dehydrodiferulates (*18*). On the other hand, ferulate and diferulate esters can be hydrolyzed by specific cinnamoyl esterases (feruloyl esterases). These enzymes have been intensely studied in microorganisms (*19*). Recent studies indicate that endogenous cinnamoyl esterase activity occurs in barley and malt, although their role in cell wall degradation during brewing is still unknown (*20*−*22*). Solubilized ferulic acid and conjugates may preserve oxidant reactions in the wort and in the final beer (*23*), although ferulic acid can also undergo decarboxylation leading to the formation of off-flavor compounds (*24*).

The aim of this paper is to determine the content of ferulic acid dehydrodimers as well as that of hydroxycinnamic acids in whole barley grain and processed

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barley. We have evaluated the genetic variability of barley with respect to the content of hydroxycinnamic acids and ferulic acid dehydrodimers and determined the distribution of these compounds in the barley grain using three fractions obtained mechanically. Finally, the solid residue after the brewing process, brewer's spent grain, has also been analyzed for its hydroxycinnamic acid and ferulic acid dehydrodimer content.

MATERIALS AND METHODS

Materials. Eleven different barley varieties of high-quality malting (*Natasha*, *Volga*, *Chariot*, *Alexis*, *Halcyon*, and *Nevada*), low-quality malting (*Sunrise*, *Iranis*, and *Boira*), and feed (*Epic* and *Target*) grade were evaluated. Barley grain var. *Halcyon* was obtained from Plant Breeding International (Cambridge, U.K.); vars. *Chariot*, *Sunrise*, *Epic*, and *Target* were obtained from Brewing Research International (Surrey, U.K.), and vars. *Boira*, *Iranis*, *Volga*, *Natasha*, *Nevada*, and *Alexis* were obtained from Cargill España S. A. (Madrid, Spain). Three samples of brewer's spent grain from different grists of malt/corn (80%/20%) and malt (100%) were provided by Mahou SA (Madrid, Spain). This material was oven-dried at 60 °C for 18 h. Both barley and spent grain samples were milled to a fine powder with a particle size of <50 μm. Barley grains from the varieties *Boira*, *Iranis*, and *Volga* were mechanically fractionated using a Chopin CD1 mill (Tripette & Renaud, Villeneuve La Garenne, France) specially designed for wheat milling. Three fractions were obtained: F1, fraction consisting mainly of the husk and outer layers; F2, intermediate fraction; and F3, fraction consisting mainly of the endosperm.

Sample Preparation. Before alkali hydrolysis, samples were treated as described by Nordkvist et al. (*1*). Samples (50 mg) were extracted three times with 95% ethanol (550 μL, 30 min) and three times with hexane (550 μL, 30 min) in an ultrasonic bath. The materials were centrifuged (20000*g* for 15 min at 10 °C) after each extraction. The total alkali-extractable hydroxycinnamic acid content was determined by adding 2 M NaOH (550 μL) to the pellets followed by incubation at 20 °C for 16 h under N_2. After centrifugation (20000*g* for 15 min at 10 °C), the supernatant was collected, acidified with 6 M HCl (200 μL), and extracted five times with ethyl acetate (650 μL). The organic solutions were combined and evaporated to dryness in a rotary evaporator. The residue was dissolved in 0.5 mL of a methanol/water mixture (50/50, v/v) and filtered through a 0.45 μm filter, and 60 μL was injected into the HPLC column. Samples were prepared and analyzed in triplicate.

HPLC Conditions. A Waters (Milford, MA) HPLC chomatograph equipped with a 600-MS controller, a 717 plus autosampler, and a 996 photodiode-array detector was used. A gradient of solvent A (water/acetic acid, 98/2, v/v) and solvent B (water/acetonitrile/acetic acid, 78/20/2, v/v/v) was applied to a reversed-phase Nova-pack C_{18} column [30 cm × 3.9 mm (inside diameter)] as follows: 80% B linear (1.1 mL/min) from 0 to 55 min, 90% B linear (1.2 mL/min) from 55 to 57 min, 90% B isocratic (1.2 mL/min) from 57 to 70 min, 95% B linear (1.2 mL/min) from 70 to 80 min, 100% B linear (1.2 mL/min) from 80 to 90 min, and washing and re-equilibration of the column from 90 to 120 min. Detection was performed by scanning from 210 to 400 nm. Identification of chromatographic peaks was carried out by comparing the retention times and spectra to those of standards, except for ferulic acid dehydrodimers that were identified according to their UV spectra (*6*) and molecular mass (see below). Quantification of total ferulic and *p*-coumaric acids was carried out by area measurements at 280 nm of both *trans* and *cis* forms. Calibration curves for the cis forms were calculated using different fresh trans hydroxycinnamic acid solutions that were placed under the UV lamp overnight to ensure different trans/cis transformation ratios. Ferulic acid dehydrodimers were quantified according to the method of Waldron et al. (*6*) using the following response factors (RFs) against trans cinnamic acid at 280 nm: RF = 0.21 for 5,5′-diFA, RF = 0.14 for 8-*O*-4′-diFA, RF = 0.18 for 8,5′-diFA open form, and RF = 0.12 for 8,5′-diFA benzofuran form.

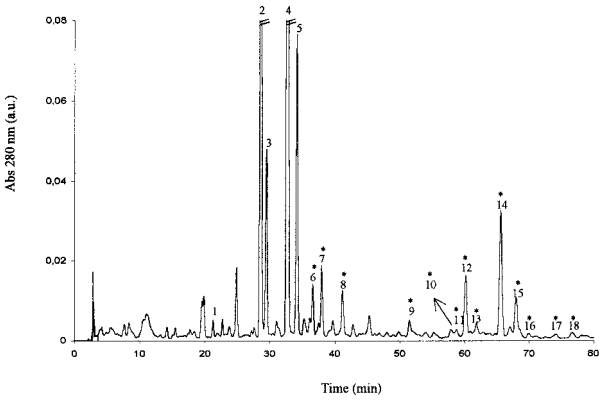

Figure 1. HPLC chromatogram at 280 nm of barley grain extract after alkaline hydrolysis: (1) caffeic acid, (2) *trans p*-coumaric acid, (3) *cis p*-coumaric acid, (4) *trans* ferulic acid, (5) *cis* ferulic acid, (8) 8,5′-diFA open form, (12) 5,5′-diFA, (14) 8-*O*-4′-diFA, (15) 8,5′-diFA benzofuran form, and (6, 7, 9−11, 13, and 16−18) unknown ferulic acid dehydrodimers.

HPLC−MS Conditions. A Hewlet-Packard series 1100 (Palo Alto, CA) chromatograph equipped with DAD and MS detectors was used. A gradient of solvent A (water/acetic acid, 99/1, v/v) and solvent B (acetonitrile/acetic acid, 99/1, v/v) was applied to a reversed-phase Nova-pack C_{18} column [15 cm × 3.9 mm (inside diamter)] as follows: 80% B linear (0.5 mL/min) from 0 to 60 min. Nitrogen was used as the nebulizing and drying gas. ES conditions were as follows: nitrogen pressure, 40 psi; drying gas, 10 L/min at 320 °C; ion spray voltage, 4000 V; and variable fragmentator voltage, 50 V (*m*/*z* <200), 80 V (*m*/*z* 200−350), and 200 V (*m*/*z* 350−1000).

Statistical Analysis. ANOVA and discriminate analyses of the data, corresponding to hydroxycinnamic acid and ferulic acid dehydrodimer content in the different barley varieties, were performed using the PC software package Statgraphics Plus 2.1 (Graphics Software Systems, Rockville, MD).

RESULTS AND DISCUSSION

Identification of Hydroxycinnamic Acids and Ferulic Acid Dehydrodimers in Barley. An HPLC chromatogram at 280 nm of barley grain extract after alkaline hydrolysis is shown in Figure 1. According to their retention times and UV spectra, the hydroxycinnamic acids (ferulic, *p*-coumaric, and caffeic) were identified. No sinapic acid was detected. The trans and cis isomers of ferulic (**4** and **5**, respectively) and *p*-coumaric acid (**2** and **3**, respectively) were clearly separated. For identification of ferulic acid dehydrodimers, samples were also subjected to HPLC−MS analysis. Peaks marked with an asterisk (*) exhibited an *m*/*z* signal of 385.1 (Figure 1), which corresponds to the molecular mass of ferulic acid dehydrodimers (386.1). Considering both UV spectra and mass molecular data, we identified the following ferulic acid dehydrodimers: (*E*,*E*)-4,4′-dihydroxy-3,5′-dimethoxy-β,3′-bicinnamic acid (8,5′-diFA open form) (**8**), (*E*,*E*)-4,4′-dihydroxy-5,5′-dimethoxy-3,3′-bicinnamic acid (5,5′-diFA) (**12**), (*Z*)-β-{4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy}-4-hydroxy-3-methoxycinnamic acid (8-*O*-4′-diFA) (**14**), and *trans*-5-[(*E*)-2-carboxyvinyl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid (8,5′-diFA dehydrobenzofuran form) (**15**) (Figure 1). Nine other minor peaks were also attributed to ferulic acid dehydrodimer-type compounds, although final structures were not confirmed (Figure 1).

Table 1. Content (μg/g) of Hydroxycinnamic Acids and Ferulic Acid Dehydrodimers in Different Barley Grain Varieties[a]

variety	origin	quality	caffeic acid	*p*-coumaric acid	ferulic acid	8,5′-diFA open form	5,5′-diFA	8-*O*-4′-diFA	8,5′-diFA benzofuran form
Natasha	Spain	malting (high)	6.9 ± 3.2	143 ± 18	410 ± 33	10.6 ± 1.1	26.4 ± 2.2	73.5 ± 8.3	24.2 ± 5.4
Epic	U.K.	feed	18.3 ± 5.5	140 ± 29	603 ± 50	22.9 ± 5.3	34.3 ± 3.1	90.0 ± 6.5	33.1 ± 3.1
Sunrise	U.K.	malting (low)	10.9 ± 3.9	79.1 ± 11.1	454 ± 36	19.8 ± 5.9	39.4 ± 5.7	89.5 ± 8.4	22.6 ± 4.9
Iranis	Spain	malting (low)	16.1 ± 1.3	260 ± 33	624 ± 47	23.3 ± 3.8	47.4 ± 4.8	118 ± 7	45.4 ± 6.1
Volga	Spain	malting (high)	12.7 ± 3.1	155 ± 19	571 ± 39	21.4 ± 1.6	42.1 ± 4.8	108 ± 4	39.3 ± 3.8
Target	U.K.	feed	15.5 ± 4.6	152 ± 31	593 ± 72	19.1 ± 5.9	36.1 ± 4.4	111 ± 12	42.0 ± 5.1
Chariot	U.K.	malting (high)	14.9 ± 3.0	166 ± 28	498 ± 70	21.8 ± 6.3	35.1 ± 6.3	93.4 ± 8.9	26.5 ± 3.6
Boira	Spain	malting (low)	7.0 ± 5.0	89.2 ± 9.7	359 ± 8	19.0 ± 2.2	28.4 ± 2.1	84.4 ± 8.3	33.5 ± 4.3
Alexis	Spain	malting (high)	7.0 ± 3.2	246 ± 22	562 ± 52	20.9 ± 0.9	31.8 ± 4.5	92.1 ± 9.5	33.4 ± 4.7
Halcyon	U.K.	malting (high)	14.6 ± 3.4	190 ± 21	537 ± 66	19.5 ± 7.8	35.0 ± 5.5	90.1 ± 11.7	31.7 ± 4.3
Nevada	Spain	malting (high)	7.1 ± 1.7	196 ± 24	523 ± 33	16.2 ± 5.2	31.8 ± 5.3	89.1 ± 8.0	28.6 ± 0.9
p value (ANOVA analysis)			0.0000	0.0000	0.0000	0.0246	0.0007	0.0001	0.0007

[a] Means ± standard deviation ($n = 3$).

Genetic Variation. Barley is used mainly for malting (brewing and distilling) and animal feed. One of the most import criteria for malting barley is a rapid and uniform enzymatic degradation of the cell wall (β-glucans and arabinoxylans) and of the protein matrix (*25*). In the case of animal nutrition, there is some evidence which suggests that phenolic acids may limit the digestibility of the plant cell wall in the ruminants (*26*).

Ferulic acid was the most abundant phenolic acid in all 11 malting and feed barley varieties that were investigated, followed by *p*-coumaric acid (Table 1). Under the extraction conditions described in this paper, ~81% of the total quantifiable ferulic acid and 85% of the *p*-coumaric acid was in the *trans* form. The levels of ferulic acid ranged from 359 to 624 μg/g dry weight and those of *p*-coumaric acid from 79.1 to 260 μg/g dry weight. The content of caffeic acid ranged from 7.0 to 18.3 μg/g dry weight (Table 1). The *Iranis* variety (low-quality malting grade) exhibited the highest ferulic acid content, followed by the feed varieties (*Epic* and *Target*). The lowest levels of ferulic acid were found in the *Boira*, *Natasha*, and *Sunrise* varieties. Similar patterns among varieties were found for *p*-coumaric and caffeic acids. Other authors have reported comparable data for ferulic and *p*-coumaric acid content in barley grain. Using acid hydrolysis, Zupfer et al. (*27*) found concentrations of ferulic acid ranging from 343 to 580 μg/g dry weight in 18 different malting and feed barleys. Using 2 N NaOH hydrolysis, Maillard and Berset (*23*) determined the ferulic acid (225 μg/g dry weight) and *p*-coumaric acid (78.5 μg/g dry weight) contents in germinated barley var. *Triumph*. In other cereals, Lempereur et al. (*28*) observed a range of ferulic acid content from 784 to 1980 μg/g dry weight in durum wheat and Andreasen et al. (*29*) from 895 to 1174 μg/g dry weight in rye, both using 2 N NaOH hydrolysis.

Among the ferulic acid dehydrodimers, 8-*O*-4′-diFA was the most abundant in all the barley varieties that were studied, with concentrations ranging from 73.5 to 118 μg/g dry weight (Table 1). 5-5′diFA was found at concentrations ranging from 26.4 to 47.4 μg/g dry weight, 8,5′-diFA benzofuran form at concentrations ranging from 22.6 to 45.4 μg/g dry weight, and 8,5′-diFA open form in concentrations ranging from 10.6 to 23.3 μg/g dry weight. The *Iranis* variety exhibited the highest ferulic acid dimer content, followed by the *Volga*, *Epic*, and *Target* varieties. The lowest contents were found for the *Boira* and *Natasha* varieties. In general, barley varieties with high ferulic acid content also had high levels of ferulic acid dehydrodimers. We calculated that the ratio of dehydrodimers to ferulic acid (which represents an index of dimerization) was between 0.30 and 0.37 for the barley varieties that were studied, except for *Boira* (0.46). These values are in the same range as the ratio of *p*-coumaric acid to ferulic acid (0.17−0.43). The same four ferulic acid dehydrodimers identified in this paper in barley (8,5′-diFA open form, 5,5′-diFA, 8-*O*-4′-diFA, and 8,5′-diFA dehydrobenzofuran form) have also been detected as main dimers in rye (*29*) and durum wheat (*30*). From these studies, we calculated that the ratio of dehydrodimers to ferulic acid found in barley is similar to the one in rye (0.27−0.37) but lower than the one in durum wheat (0.44−0.61). However, rye exhibited a lower *p*-coumaric acid content and a higher sinapic acid content with regard to ferulic acid than reported here for barley.

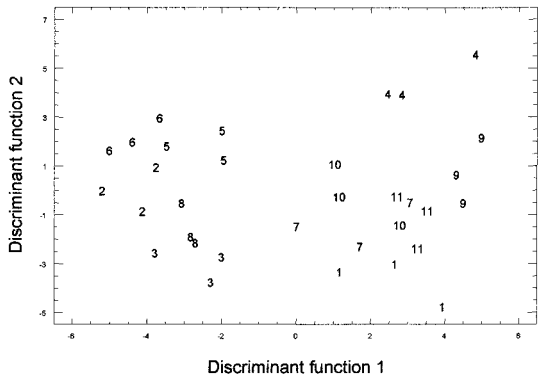

Figure 2. Plot of discriminant function scores for samples of barley varieties: (1) *Natasha*, (2) *Epic*, (3) *Sunrise*, (4) *Iranis*, (5) *Volga*, (6) *Target*, (7) *Chariot*, (8) *Boira*, (9) *Alexis*, (10) *Halcyon*, and (11) *Nevada*.

ANOVA analysis showed significant differences ($p < 0.05$) among the different barley varieties for all the compounds that were studied (Table 1). This indicated that the concentration of ferulic acid dehydrodimers in barley, which may reflect the extent of cross-linking in the cell wall, was influenced by the genotype. Other authors have observed genetic variation in the content of ferulic acid in barley (*27*) and durum wheat (*28*), and in the content of ferulic acid dehydrodimers in rye (*29*). It has also been confirmed that these genetic variations cannot be attributed to differences in the grain caryopsis structure since no correlation between kernel weight and hydroxycinnamate concentration was found (*27*, *29*). In our study, in an attempt to investigate the existence of groups according to variety, a discriminate analysis was applied to the variables for content of ferulic and

Table 2. Distribution of Ferulic and *p*-Coumaric Acids and Ferulic Acid Dehydrodimers in the Different Fractions of Barley Grain Obtained Mechanically[a]

variety		% fraction[b]	% *p*-coumaric acid[c]	% ferulic acid[c]	% 8,5′-diFA open form[c]	% 5,5′-diFA[c]	% 8-*O*-4′-diFA[c]	% 8,5′-diFA benzofuran form[c]
Boira	F1	47.5	78.0	77.7	84.4	85.9	83.7	81.3
	F2	27.0	20.5	20.4	15.6	14.1	16.3	18.7
	F3	25.5	1.49	1.89	0.01	0.01	0.02	0.01
Iranis	F1	54.4	81.4	78.7	79.9	82.5	82.6	79.2
	F2	27.2	16.9	20.1	20.1	17.5	17.4	20.8
	F3	18.4	1.74	1.21	0.01	0.01	0.01	0.01
Volga	F1	47.6	86.3	82.3	85.9	85.9	85.4	86.8
	F2	29.7	12.9	16.4	14.1	14.1	14.6	13.2
	F3	22.7	0.87	1.35	<0.01	0.01	0.01	0.01

[a] F1 consists mainly of the husk and outer layers, F2 of an intermediate fraction, and F3 of the endosperm. [b] % fraction = (g of fraction/g of barley) × 100. [c] % = (g in a fraction/g in the three fractions) × 100.

Table 3. Content (μg/g) of Ferulic and *p*-Coumaric Acids and Ferulic Acid Dehydrodimers in Brewer's Spent Grain

sample	grain composition	*p*-coumaric acid	ferulic acid	8,5′-diFA open form	5,5′-diFA	8-*O*-4′-diFA	8,5′-diFA benzofuran form
1	80% malt, 20% corn	565 ± 39	1867 ± 163	119 ± 7	169 ± 7	443 ± 27	181 ± 11
2	80% malt, 20% corn	794 ± 58	1948 ± 143	171 ± 7	154 ± 8	526 ± 35	220 ± 19
3	100% malt	721 ± 144	1860 ± 190	159 ± 19	150 ± 10	501 ± 43	232 ±17

p-coumaric acids and ferulic acid dehydrodimers. Figure 2 displays the distribution of the samples in a two-coordinate system defined by the two functions with the greatest discriminating power (78%). Barley varieties of high-quality malting grade except *Volga* (*Natasha*, *Chariot*, *Alexis*, *Halcyon*, and *Nevada*) were located close together. Low-quality malting varieties (*Sunrise* and *Boira*) were very close together, except *Iranis*, which was fully separated. Feed varieties (*Epic* and *Target*) were located together, but were not fully distinguishable from *Volga*, *Sunrise*, and *Boira*. These findings indicate that the levels of hydroxycinnnamic and ferulic acid dehydrodimers may be related to the use of a barley variety for malting or feed, but there are indeed some other factors involved.

In a previous paper (*31*), endogenous feruloyl esterase activity was determined in the same 11 barley varieties used in this study. Significant differences were found among varieties, but they were not associated with malting quality or barley origin. We have now investigated the possible correlation between these activity data and the content of hydroxycinnamic acid and ferulic acid dehydrodimers reported in this paper. No significant correlation ($p > 0.05$) was found for any of these individual compounds or for the total content. This indicated that cinnamoyl esterase activity is not dependent on the total level of extractable hydroxycinnamates present in the grain, notwithstanding a possible relationship between enzyme and phenolic acid levels in specific tissues (i.e., endosperm). The existence of several endogenous esterases specific for different cinnamic structures may also explain these findings.

Distribution of Phenolic Acids in Fractionated Barley Grain. The distribution of ferulic and *p*-coumaric acids and ferulic acid dehydrodimers in the three different mechanical fractions that were obtained was expressed as a percentage of the content of the whole grain (Table 2). Fraction F1, consisting mainly of the husk and outer layers, contained the highest concentration for ferulic acid (from 77.7 to 82.3% of total), *p*-coumaric acid (from 78.0 to 86.3%), and ferulic acid dehydrodimers (from 79.2 to 86.8%). Lower contents were found in the intermediate fraction F2, whereas fraction F3, consisting mainly of the endosperm, exhibited the lowest percentages for ferulic acid (from 1.2 to 1.9%) and *p*-coumaric acid (from 0.9 to 1.7%) and traces for ferulic acid dehydrodimers (<0.02%). The results indicate that ferulic acid dehydrodimers were slightly more concentrated in the husk and outer layers (fraction F1) than the monomer for the three barley varieties that were studied, while the opposite behavior was observed for the rest of the grain (fractions F2 and F3). The distribution of ferulic acid reported here for barley is similar to the one found in durum wheat (*28*) and rye (*32*).

Content in Brewer's Spent Grain. During the mashing process in brewing, starch and reserve proteins are extensively degraded and solubilized into the wort. The solid residue after mashing and filtration, brewer's spent grain, consists mainly of cell walls (*33*). Our results indicate that this material exhibits ~5-fold higher levels of ferulic and *p*-coumaric acids and ferulic acid dehydrodimers than the unprocessed barley grains described above (Table 3). Differences among the three samples were attributed to the use of different barley varieties (or mixes of barley varieties) and to the addition of crude grain such as corn. Values for ferulic and *p*-coumaric acids were similar to those reported previously (*34*). The *p*-coumaric acid/ferulic acid ratio in brewer's spent grain (0.30−0.40) was similar to the one found for barley grains. However, the dehydrodimer/ferulic acid ratio in spent grain (>0.48) is markedly higher than the one found for barley grains, which indicates that solubilization of ferulic acid structures during mashing is more extensive for the monomer than for the dimers.

In summary, this paper reports novel data concerning hydroxycinnamic acids and ferulic acid dehydrodimers in barley. Although FA dehydrodimers are present at a much lower concentration than the monomer, their contribution to the total phenolic content is at the same level as that of the flavan-3-ols, the major class of flavonoids in barley. Their presence might limit the enzymatic degradation of barley cell walls during malting, hence limiting the amount of sugars that is solubilized. Released ferulic acid dehydrodimers, although at a low concentration, may act as a naturally occurring antioxidant in beer.

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