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Endosperm Structure Affects the Malting Quality of Barley (*Hordeum vulgare* L.)

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Twenty-seven barley (*Hordeum vulgare* L.) samples collected from growing sites in Scandinavia in 2001 and 2002 were examined to study the effect of endosperm structure on malting behavior. Samples were micromalted, and several malt characteristics were measured. Samples were classified as having a mealier or steelier endosperm on the basis of light transflectance (LTm). Because endosperm structure is greatly dependent on protein content, three barley sample pairs with similar protein contents were chosen for further analysis. During malting, the steelier barley samples produced less root mass, but showed higher respiration losses and higher activities of starch-hydrolyzing enzymes. Malts made from steelier barley had a less friable structure, with more urea-soluble D hordein and more free amino nitrogen and soluble protein. The reason for these differences may lie in the structure or localization of the hordeins as well as the possible effects of endosperm packing on water uptake and movement of enzymes.

KEYWORDS: Barley (*Hordeum vulgare* L.); endosperm structure; malting; protein; hordein; grain hardness

INTRODUCTION

To produce malt of good quality, the cell walls of the endosperm and a part of the small starch granules and surrounding protein matrix should be broken down during the malting process (*1*). This degradation of endosperm reserves involves coincident action of the enzymes hydrolyzing protein, starch, and cell wall structures. These enzymes are synthesized, or activated in the aleurone and scutellar cells, and secreted to starchy endosperm. In addition to the embryonic gibberellin-activated signal transduction pathways, breakdown of endosperm reserves is controlled by the structural pattern of tissues and stored macromolecules (*2*).

Good malting barley varieties should have an endosperm structure that is easy to modify and a good enzyme-synthesizing capacity to ensure fast modification. These quality parameters are, however, seldom analyzed when barley is purchased for malting purposes. Barley lots for malting purposes are currently purchased on the basis of total protein content, although mealiness has also traditionally been tested for in, for example, Great Britain. A suitable protein content of barley is known to be favorable for malting, whereas grains with a high protein content are often steely and are therefore a malting quality risk (*3*). However, barley samples of the same variety with similar protein contents may show very different modification patterns depending on crop year and growth environment. Good and poor malting quality barley cultivars, with similar protein contents, have been noted to differ in starch−protein adhesion and the patterns of fracture through the endosperm (*4*). These differences appear to relate to the properties of the endosperm storage proteins, hordeins in the case of barley, rather than total protein amount.

Grain hardness is related to the packing of the endosperm and affects endosperm modification because the dense structure of a steely endosperm limits water uptake and passage of hydrolyzing enzymes (*5*). Hardness of barley grains may also be linked to the protein−starch association as in wheat (*6*, *7*). Recently, grain hardness has been considered to be the most important variable for describing malting performance, despite the facts that a low correlation with chemical and physical grain characteristics has been previously noted and that this cannot be used as a single factor in the prediction of malting quality (*8*−*10*). For malting and brewing purposes, a method for quantifying the structure of the endosperm is available. This LTm method is based on the use of a light transflectance meter, which assesses the density of endosperm structure by its ability to transmit and reflect light (*11*).

This study was undertaken to investigate and explain the effect of endosperm structure on modification and malting

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behavior between barley lots of the same variety. For this purpose, different compositional, structural, and enzymatic properties of 27 barley samples and their corresponding malted samples were compared. Three sample pairs with similar protein contents were studied in more detail.

MATERIALS AND METHODS

Plant Material. Twenty-seven barley (*Hordeum vulgare* L.) samples of the varieties Barke, Scarlett, Wikingett, and Luberon were collected from different growing sites in Scandinavia in 2001 and 2002.

Barley Analyses. Protein and starch contents of the samples were determined by using near-infrared analysis (Foss Tecator, Infratec 1241 grain analyzer). Germinative capacity, kernel size distribution, and moisture of the samples were determined according to recommended methods of the European Brewing Convention (*12*). The relative proportions of steeliness and mealiness and the homogeneity of the endosperm were estimated with a light transflectance meter and are indicated as mean LTm values of the samples (*11*). The endosperm structure of 97 grains of each sample batch was analyzed. Chandra et al. (*11*) defined grains having average LTm values of <200 mV as mealy and those having LTm values of ≥300 mV as steely.

Micromalting Process and Malt Analyses. Grain samples of 1 kg (>2.5 mm screen) were micromalted (Joe White Malting System) at LP Research Centre Ltd., Lahti, Finland. The malting procedure consisted of steeping at 13−15 °C for 2 days, germination for 5 days at 14 °C, and kilning for 22 h to a final temperature of 82 °C to produce malts with ∼4% moisture. The moisture contents of the samples after steeping were determined by weighing. After kilning, the rootlets were removed. Weight losses (dry basis) caused by respiration and removal of rootlets were determined by weighing. Respiration losses were calculated as differences of total weight loss and weight loss in removing rootlets.

Malt samples were analyzed using the following EBC-recommended methods: moisture, fine/coarse extract, friability, modification, homogeneity, total protein, soluble nitrogen, protein solubility as Kolbach index, free amino nitrogen, wort β-glucan, wort color, and wort viscosity (*12*). Megazyme assay kits (Megazyme, Co. Wicklow, Ireland) were used to determine the activities of the following enzymes in malts: α-amylase (*13*; with modification of the extraction time to 30 min), free β-amylase (*14*, *15*), total and free limit dextrinase [*16*; with modification of the concentration of dithiothreitol (DTT) in extraction buffer to 62 mM], and β-glucanase (*17*, *18*). The endo-β-xylanase activity of malt samples was measured viscometrically according to the method described by Autio et al. (*19*).

Assay of Endopeptidase Activity. The crude endopeptidase extraction procedure was modified from the method of Zhang and Jones (*20*). Extracts were prepared by extracting 2.00 g of ground barley or malt with 9 mL of 50 mM sodium acetate buffer containing 2 mM cysteine with constant magnetic stirring at 4 °C for 1 h. After extraction, extracts were centrifuged (1590*g*, 15 min, 4 °C).

To determine the proportion of cysteine proteinases of the total endopeptidase activity, cysteine proteinases were inhibited by the specific inhibitor *trans*-epoxysuccinyl-L-leucylamido(4-guanidino)butane, E-64 (E3132, Sigma Chemical Co., St. Louis, MO). The inhibitor was added to crude enzyme extracts to give a final concentration of 24.9 μM (*21*). Extracts were incubated for 30 min at 4 °C before the assay. E-64-inhibited endopeptidase activity was defined as cysteine proteinase activity.

The endopeptidase activity was determined with the Megazyme assay kit using azo-casein substrate. The activity of endopeptidases was detected spectrophotometrically against reaction blank at 440 nm. Each assay was done in triplicate. For statistical analysis, initial hydrolysis rates between time points of 0 and 10 min were calculated.

Hordein Extraction Procedure. Barley and malt samples (>2.5 mm) were ground in a sample mill (Pulverisette 14, Fritsch) equipped with a 0.5 mm sieve. The moisture contents of the barley and malt samples were determined as described in *Analytica EBC* (*12*).

The extraction procedure used was modified from that of Marchylo et al. (*22*) and was done in triplicate for each sample. Ground grain and malt (0.2 g) were extracted sequentially at 60 °C for 30 min with vortexing at 10 min intervals, followed by centrifugation (10000*g*, 15 min, 4 °C) using 1.2 mL of the following extracting solutions: (1) 0.5 M sodium chloride (twice), (2) water, (3) 50% 1-propanol (HPLC grade, Rathburn) (twice), and (4) 50% 1-propanol containing 1% DTT. The residual samples were extracted overnight at room temperature with 1.2 mL of 8 M urea containing 1% DTT and centrifuged at room temperature (20200*g*, 15 min). The supernatants were stored at −20 °C and passed through 0.45 μm GHP Minispike filters (Pall Gelman Laboratory, Ann Arbor, MI) before RP-HPLC analysis.

RP-HPLC Separation of Hordeins. The protein extracts were analyzed in triplicate with an HT-Alliance 2795 HPLC chromatograph (Waters Associates, Inc., Milford, MA) including a Waters 996 photodiode array detector. A 250 mm × 4.6 mm i.d. SynChropak RP-P column [C18, 300 Å, 6.5 μm particle size (Waters)] preceded by a 4.0 μm Novapak C18 Guard-Pak precolumn (Waters) was used for separation. The column and the sample compartment temperatures were 25 and 20 °C, respectively. The injection volume was 25 μL. Running solvents consisted of water and acetonitrile (HPLC grade, Rathburn), each containing 0.1% trifluoroacetic acid (HPLC grade, Fluka). Elution was carried out by using a gradient extending from 31.2 to 54.0% acetonitrile in 105 min followed by a 20 min washing step. The initial conditions were restored, and the column was equilibrated for 10 min. Flow rate was 1 mL/min, and detection was at 210 nm. Data were collected and analyzed with Millenium[32] software (Waters). Hordein amounts are presented as chromatogram areas in arbitrary units (AU).

Analysis of Grain Microstructure. Twenty grains of each sample were prepared for microstructure analysis. A 2−3 mm thick section was cut from the middle of each grain, fixed in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated in a graded ethanol series, and embedded in hydroxyethyl methylacrylate as recommended by the manufacturer (Leica Historesin embedding kit, Heidelberg, Germany). Polymerized samples were sectioned (2 μm sections) in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife. The sections were transferred onto glass slides and stained with Acid Fuchsin and Calcofluor White or Light Green and Lugol's iodine solution (*23*−*25*).

Acid Fuchsin and Calcofluor White. Sections were pretreated in 2,4-dinitrophenylhydrazin and in 0.5% periodic acid for staining. Protein was stained with aqueous 0.1% (w/v) Acid Fuchsin for 1 min (Gurr, BDH Ltd., Poole, U.K.), and β-glucan was stained with aqueous 0.01% (w/v) Calcofluor White for 1 min (fluorescent brightener 28, Aldrich, Germany). In exciting light (excitation, 330−385 nm; fluorescence, >420 nm) intact cell walls stained with Calcofluor appear blue and proteins stained with Acid Fuchsin appear red. Starch is unstained and appears black.

Light Green and Iodine Staining. Protein was stained with aqueous 0.1% (w/v) Light Green for 1 min (Gurr, BDH Ltd.) and starch with 1:10 diluted Lugol's iodine solution (I_2, 0.33%, w/v; and KI, 0.67%, w/v). Light Green stains protein green. Iodine stains the amylose component of starch blue and amylopectin brown.

The samples were examined with an Olympus BX-50 microscope (Tokyo, Japan). Micrographs were obtained using a SensiCam PCO CCD camera (Kelheim, Germany) and the AnalySIS 3.0 image analysis program (Soft Imaging System, Münster, Germany). The micrographs shown were chosen to represent the average of the 20 grains analyzed of each sample.

Statistical Analysis. The statistical significance of the differences between the barley samples representing different endosperm structure as well as the differences in endopeptidase activities, hordein composition, and LTm values between the sample pairs chosen were calculated. Mean values were compared by two-tailed Student *t* test, and the differences were considered to be significant when $p < 0.05$.

RESULTS

The 27 barley samples of four different cultivars (Barke, Luberon, Scarlett, and Wikingett) from different growing sites in Scandinavia were arranged into two groups according endosperm structure as measured with a light transflectance meter. In 2001, samples with LTm mean values >210 mV were classified as "steelier" and those with values <160 mV as

Table 1. Barley and Malt Characteristics of Samples Classified as Steelier or Mealy in 2001 and 2002[a]

	2001			2002		
	steelier[b] ($n = 4$)	mealier ($n = 6$)	c	steelier ($n = 11$)	mealier ($n = 6$)	c
barley						
protein (% db[d])	12.0 ± 1.0	9.9 ± 1.3	*	11.4 ± 0.6	10.3 ± 0.6	*
starch (% db)	63.4 ± 1.2	64.7 ± 1.2	ns	63.9 ± 0.7	63.2 ± 1.1	ns
germination capacity (%)	99.0 ± 1.4	99.2 ± 0.8	ns	98.7 ± 0.5	98.7 ± 1.0	ns
grains over 2.8 mm (%)	69.1 ± 11.4	81.6 ± 5.6	*	41.2 ± 15.3	92.0 ± 5.3	*
LTm, mean (mV)	327 ± 66	106 ± 49	*	213 ± 34	52 ± 16	*
malt						
friability (%)	71 ± 10	93 ± 6	*	78 ± 4	91 ± 4	*
soluble N (mg/100 g)	890 ± 52	742 ± 72	*	880 ± 66	746 ± 52	*
free amino N (mg/L)	205 ± 16	176 ± 16	*	221 ± 20	185 ± 16	*
extract (%/average)	83.0 ± 0.7	84.3 ± 1.1	ns	82.7 ± 0.5	84.1 ± 1.1	*
wort color (°EBC)	4.5 ± 0.4	2.8 ± 0.3	*	4.5 ± 0.4	2.8 ± 0.3	*

[a] Results are expressed as means ± SD. [b] Refers to the endosperm structure as measured by LTm. In 2001, samples with LTm mean values >230 mV were classified as "steelier" and those with LTm values <160 mV as "mealy". In 2002, "steelier" samples had LTm values >150 mV and "mealy" samples <100 mV. [c] Statistical significance: ns, not significant; *, significant for $p < 0.05$. [d] Dry basis.

Table 2. Barley Characteristics

	Barke 2001 1A[a]	Barke 2001 1B	Barke 2002 2A	Barke 2002 2B	Scarlett 2002 3A	Scarlett 2002 3B	
moisture (%)	11.7	12.8	9.9	9.7	10.1	9.6	± 0.12[b]
protein (% db[c])	10.8	11.3	11.2	11.0	11.2	10.6	± 0.09
starch (% db)	64.8	64.0	64.2	62.7	64.2	62.5	± 1.62
grains over 2.8 mm (%)	80.9	84.7	47.2	95.4	70.8	97.5	nd[d]

[a] The A samples had a steelier endosperm than the B samples. [b] Standard deviation of the standard malt sample. [c] Dry basis. [d] Not determined.

Table 3. Endosperm Structure of Barley Samples as LTm Values[a]

	Barke 2001 1A ($n = 97$)	Barke 2001 1B ($n = 97$)	Barke 2002 2A ($n = 96$)	Barke 2002 2B ($n = 97$)	Scarlett 2002 3A ($n = 97$)	Scarlett 2002 3B ($n = 97$)
LTm (mV)	233 ± 109	160 ± 110	226 ± 118	64 ± 88	239 ± 102	73 ± 74
mealy grains[b] (%)	46	71	43	91	42	94
steely grains[c] (%)	25	10	31	5	24	3

[a] Results are expressed as means ± SD. For differentiation of A and B samples, see **Table 2**. [b] LTm value < 200 mV. [c] LTm value ≥ 300 mV.

"mealy". In 2002, barley samples were in general mealier than in 2001, and therefore samples with an LTm value >150 mV were included in the "steelier" group and those with an LTm mean value <100 mV were classified as mealy. As a result, four samples were classified as steelier and six samples as mealy in 2001. In 2002, 11 samples were classified as steelier and six samples as mealy.

Endosperm structure was related to several barley and malt characteristics (**Table 1**). The steelier barley samples had higher protein content, smaller grain size, lower friability, darker wort color, higher soluble nitrogen content, and higher free amino nitrogen content than the mealy barley samples. All of these differences were statistically significant at the 95.0% confidence level ($p < 0.05$). Some of the malting quality parameters, such as grain size, were also affected by the cultivar (data not shown). However, in general, the effect of growth site was much stronger than that of the cultivar. The majority of the analysis parameters, especially endosperm structure, are greatly affected by protein content. To eliminate the effect of protein content only three barley sample pairs with similar protein contents of 11% were studied in detail. Three steelier barley samples (A) and three mealy barley samples (B) were selected as follows: Barke 2001 (samples 1A and 1B), Barke 2002 (2A and 2B), and Scarlett 2002 (3A and 3B).

Table 4. Moisture after Steeping and Weight Losses in Malting Process[a]

	Barke 2001 1A	Barke 2001 1B	Barke 2002 2A	Barke 2002 2B	Scarlett 2002 3A	Scarlett 2002 3B
moisture (%)	41.4	41.7	44.1	44.8	45.8	45.6
respiration loss (%)	5.4	4.0	9.1	3.2	5.2	4.2
loss in rootlet removal (%)	3.3	3.7	4.5	4.7	3.8	4.2
total weight loss	8.6	7.7	13.6	7.9	9.0	8.4

[a] A and B samples as in **Table 2**.

Barley Analyses. Despite the similar protein contents (**Table 2**), the differences in LTm values were significant ($p < 0.001$) between barley sample pairs chosen. Samples 1B, 2B, and 3B consisted mostly of grains with a mealy endosperm, whereas samples 1A, 2A, and 3A were less homogeneous and had a steelier endosperm structure (**Table 3**). On average, the mealy B samples had slightly lower starch contents than the steelier A samples.

Table 5. Malt Characteristics[a]

	Barke 2001 1A	Barke 2001 1B	Barke 2002 2A	Barke 2002 2B	Scarlett 2002 3A	Scarlett 2002 3B	
friability (%)	85	92	86	92	78	90	± 1.02[b]
modification (%)	96	99	97	97	92	95	± 3.25
homogeneity (%)	81	93	84	85	76	83	± 4.86
protein (% db[c])	10.4	10.8	11.3	10.4	11.2	10.1	± 0.17
soluble N (mg/100 g)	828	741	910	719	866	815	± 0.76
Kolbach index (% soluble protein/total protein)	50	43	50	43	48	50	± 0.91
free amino N (mg/L)	193	160	237	167	220	199	± 3.62
α-amylase (units/g db)	240	216	340	255	306	314	± 13.2
β-amylase (units/g db)	942	984	941	943	745	678	± 45.1
total limit dextrinase (APU/kg db)	491	477	689	553	600	530	nd[d]
free limit dextrinase (APU/kg db)	61	45	69	44	71	68	nd
xylanase (VU/g db)	28 ± 0.4	11 ± 0.5	41 ± 2.2	11 ± 0.4	46 ± 3.6	16 ± 1.5	nd
β-glucanase 30°C (units/g db)	518	505	573	524	590	590	± 19.1
extract (%/average)	83.6	82.9	82.5	82.1	83.5	84.8	± 0.3
wort β-glucan (mg/L)	142	72	88	219	255	186	± 20.81
wort color (°EBC)	4.4	2.5	4.7	2.5	4.4	2.8	± 0.12
wort viscosity (mPa.s)	1.45	1.46	1.42	1.47	1.46	1.49	± 0.01

[a] A and B samples as in **Table 2**. [b] Standard deviation of the standard malt sample. [c] Dry basis. [d] Not determined.

Malting Process, Malt Characteristics, and Enzyme Activities. The steelier barley samples showed higher respiration losses but lower losses due to rootlet removal than the mealy samples (**Table 4**). The total weight loss was higher in steelier samples compared with mealy samples. The moisture contents determined after steeping did not show any differences between mealy and steelier samples.

Results of malt analyses are presented in **Table 5**. The friabilities of the mealy samples were higher than those of the steelier ones. The differences in homogeneity and Calcofluor modification were less evident. During malting, the protein content of the mealy barley samples decreased slightly more than that of the steelier samples. However, malts produced from steelier barley contained more soluble and free amino nitrogen. The degree of proteolysis was also reflected in the wort color, which was higher for the steelier samples. In Barke, α-amylase, β-glucanase, and xylanase activities were slightly higher in the steelier than in the mealy sample pairs. In all steelier samples examined, the total and free limit dextrinase activities were higher than in the mealy samples, whereas differences in β-amylase activities were ambiguous. Wort viscosity was lower for all steelier samples, but the extract and the β-glucan content of the wort did not correlate with steeliness.

Total endopeptidase activities of unmalted barley samples were very low, and the differences between samples were not statistically significant (data not presented). Even though activities of different malts showed similar patterns, there were noticeable and statistically significant ($p < 0.01$) differences between mealy and steelier Barke samples (**Figure 1**). The specific cysteine proteinase inhibitor E-64 reduced greatly the total endopeptidase activities (**Figure 2**). Differences in E-64-inhibited activities between sample pairs 2A and 2B and 3A and 3B were statistically significant ($p < 0.001$).

Hordein Patterns. Total hordein amounts of the samples varied slightly, showing no clear correlation with steeliness (**Figure 3A,B**). The steelier barley samples 2A and 3A contained significantly more hordein than their mealy sample pairs ($p < 0.01$ for both sample pairs). B hordein was present in all barley

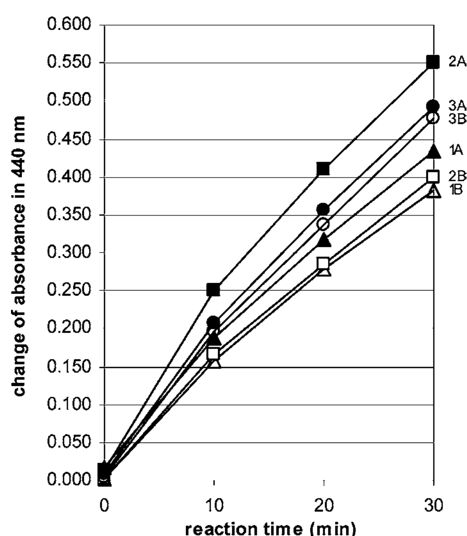

Figure 1. Total endopeptidase activities in malt samples (mean, $n = 3$). Standard deviations of absorbance values varied between 0.001 and 0.010. Samples: (▲) 1A Barke 2001; (△) 1B Barke 2001; (■) 2A Barke 2002; (□) 2B Barke 2002; (●) 3A Scarlett 2002; (○) 3B Scarlett 2002. The steelier A samples are indicated by solid symbols and the mealy B samples by open symbols.

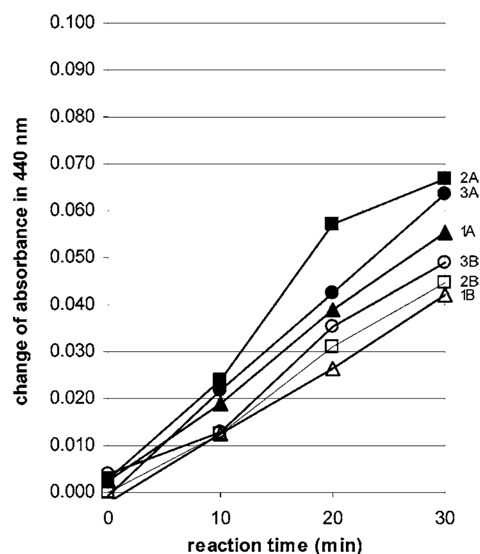

Figure 2. E-64 inhibited endopeptidase activities in malt samples (mean, $n = 3$). Standard deviations of absorbance values varied between 0.000 and 0.003. Samples are as in **Figure 1**.

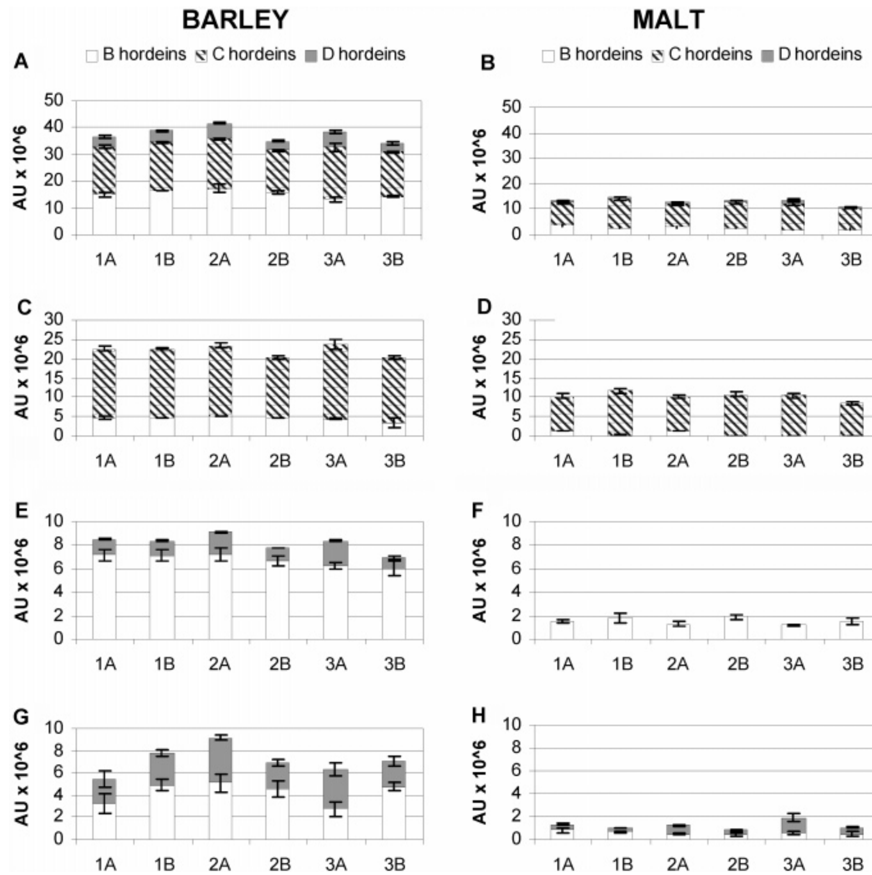

Figure 3. Total hordein composition in barley and malt samples and hordein composition in the extraction fractions in arbitrary units (AU) (means, $n = 9$): (**A**, **B**) total hordein composition; (**C**, **D**) propanol-soluble hordeins; (**E**, **F**) propanol–DTT-soluble hordeins; (**G**, **H**) urea–DTT-soluble hordeins. Samples: 1A Barke 2001, 1B Barke 2001, 2A Barke 2002, 2B Barke 2002, 3A Scarlett 2002, and 3B Scarlett 2002. The A samples had a steelier endosperm structure and the B samples a mealy structure.

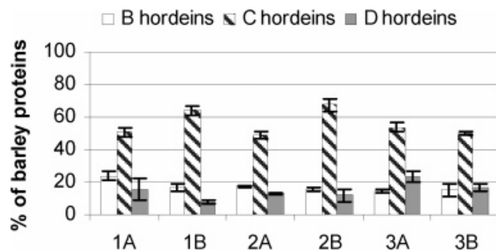

Figure 4. Hordeins in malt samples as proportions of respective barley hordein amount (means, $n = 9$). Samples are as in **Figure 3**.

and malt extracts, and C hordein was present only in propanol extracts. D hordein was present in propanol–DTT and urea–DTT extracts of barley samples but only in urea–DTT extracts of malt samples (**Figure 3C–H**). Some differences in the solubility of hordeins were observed between steelier and mealy samples. The steelier barley samples 2A and 3A contained more C hordein ($p < 0.01$ and $p < 0.05$, respectively), and both propanol–DTT-soluble ($p < 0.001$ for both sample pairs) and urea–DTT-soluble ($p < 0.01$ and $p < 0.05$, respectively) D hordein than their mealy sample pairs. Differences in B hordein solubilities were not significant.

During malting, C hordein was proportionally the least degraded hordein in all samples, whereas D hordein was the most degraded during malting in all Barke samples and B hordein was the most degraded in Scarlett samples (**Figure 4**). Steelier Barke malt samples contained significantly more D hordein ($p < 0.05$ and $p < 0.01$, respectively) and B hordein ($p < 0.05$ for both) than their mealy sample pairs. The difference in D hordein amounts in Scarlett malt samples was not statistically significant. In the steelier Barke malt samples equal amounts of B hordein were present in the propanol extracts, whereas in the other malt samples propanol-soluble B hordein was hardly present (**Figure 3D**). C hordein amounts were lower in steelier Barke malt samples ($p < 0.01$ and $p < 0.05$, respectively), but higher in steelier Scarlett samples ($p < 0.01$) than in their corresponding mealy sample pairs.

Grain Microstructure. On the basis of the visual examination of 20 grains of each sample, there was a clear difference in the structure of cell walls of starchy endosperm between the two cultivars Barke and Scarlett. In Barke barley (**Figure 5A**) the cell walls in the starchy endosperm appeared to be thinner than in Scarlett and usually already partially degraded in the area surrounding the crease. In Scarlett (**Figure 5B**) the cell walls appeared to be thicker and the grains had an even cell wall thickness throughout the endosperm. No systematic differences in cell wall structure of aleurone layer or starchy endosperm were observed between the mealy and steelier pairs of barley grains (**Figure 5C,D**). The packing of endosperm cells with starch granules and protein matrix was also similar in all samples, and no systematic differences between the two cultivars or the mealy and steelier sample pairs could be seen (**Figure 5E,F**). The subaleurone cells contained mostly small, B-type starch granules, whereas the cells of the middle part of the endosperm were evenly packed with small and large starch granules surrounded by protein matrix.

DISCUSSION

A large barley sample collection grown in 2001 and 2002 was analyzed for its barley and malt characteristics, and six of

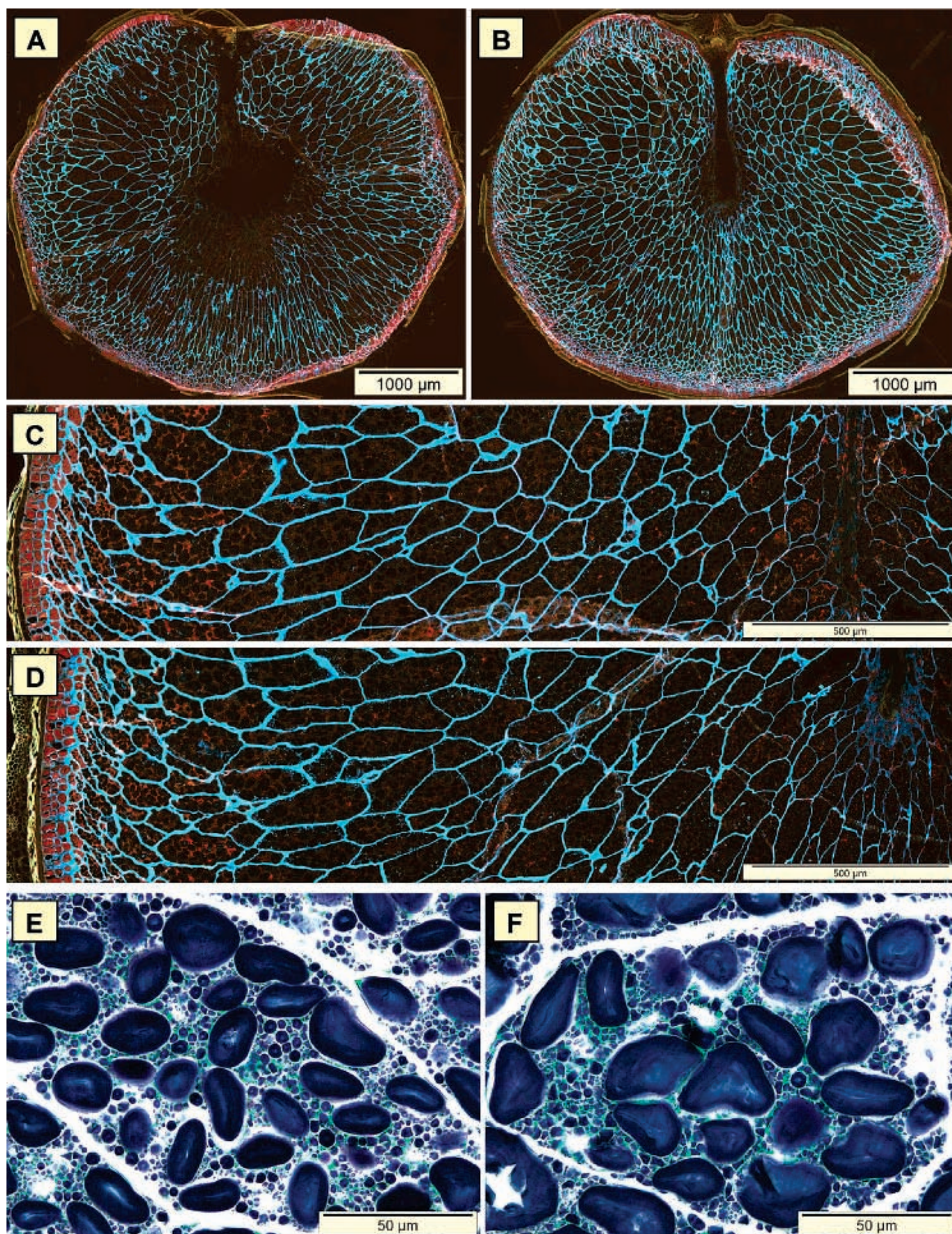

Figure 5. Micrographs of cross sections of the barley samples: (**A**–**D**) stained with Acid Fuchsin and Calcofluor to show protein (red) and cell walls (light blue); (**E**, **F**) stained with Light Green and iodine to show protein (green) and starch (blue-violet); (**A**) 1B Barke 2001; (**B**) 3B Scarlett 2002; (**C**) 3A Scarlett; (**D**) 3B Scarlett 2002; (**E**) 2A Barke 2002; (**F**) 2B Barke 2002.

the samples were chosen for further analysis. According to our results the endosperm structure measured by LTm analysis is an essential factor of malting performance as mealy and steelier samples consistently had different characteristics in several analyses. Steeliness was related to higher starch content, even though this correlation was not observed in results concerning the whole sample collection. This difference in starch content could not be detected in the microstructure, but it may be a consequence of the denser packing of a steelier than a mealy endosperm.

All samples were micromalted using the same malting schedule. The weight loss during the malting process showed that even though the steelier samples had a higher respiration rate and a higher total malting loss, the growth of their rootlets was lower than in the mealy samples. Recently, low respiratory losses have been linked to a low grain protein content (*26*), but our results suggest that endosperm structure may also affect respiratory losses. Apparently the high respiration rate of steelier barley was not related to the growth of the embryo but possibly to an increased requirement of energy for endosperm degradation caused by the tight structure of endosperm. Wallwork et al. (*27*) reported higher total malting loss for heat-treated barley than controls.

The malts made of steelier barley were less modified than the mealy samples, on the basis of friability parameters. The Barke samples could, however, all be defined as well modified, whereas the steelier Scarlett sample was slightly undermodified according to the friability values. The corresponding difference in Calcofluor modification was slightly smaller, and there was no difference at all between mealy and steelier Barke 2002 samples. Whereas the Calcofluor method is based on the detection of unhydrolyzed β-glucan, the friability test measures the hardness of the malted grain. It may be concluded that malts made of steelier barley had a harder, less friable, structure, although they were not necessarily undermodified in terms of β-glucan degradation. The worts from the steelier samples also had a darker color, which according to Palmer (*28*) is a sign of uneven pattern of modification.

The average decrease in protein content during malting was larger for the mealy samples. This observation is consistent with earlier studies showing that the hydrolysis of storage proteins proceeds more rapidly in a soft-textured endosperm than in a hard one (*7*). In germinating barley, the hydrolyzed protein is directed to the growing roots and shoot. Because the rootlets were removed after malting, the decrease in protein content of the mealy samples can be attributed to the more extensive rootlet growth compared with the steelier samples. However, the steelier malt samples contained a higher amount of free amino acids and soluble nitrogen than the mealy samples. The free amino nitrogen and soluble protein were extracted from the malt using a temperature-programmed mash procedure by which the temperature was maintained at 45 °C for 30 min, allowing the action of proteolytic enzymes. The difference in proteolysis between mealy and steelier samples could therefore be a consequence of the mashing procedure as well as the malting procedure. It is possible that the steelier samples contained more slowly hydrolyzable storage proteins that were not hydrolyzed until during mashing. This difference can be only partially explained by the small differences in endopeptidase activities, most of which consisted, expectedly, of cysteine proteinase activities (*29*). It has been stated that differences in rates of hordein degradation refer more to differences in hordein structures than to the amount of endopeptidases (*30*). It is also possible that the spatial patterns of endoproteolytic activity development or substrate localization differed between mealy and steelier sample pairs. Additionally, the differences in protein degradation could be caused by differential activity of carboxypeptidases needed for further degradation of peptides (*31*, *32*).

Visually recognized steely grains have been found to contain more nitrogen, especially hordein proteins, than mealy grains (*33*). However, in this study the mealy and steelier grain samples had equal protein contents, and only minor differences were observed in total protein or hordein contents. Steelier barley and malt samples did not differ systematically in their total hordein composition from mealy samples, but certain differences in hordein classes and solubilities were detected.

Both steelier Barke malt samples contained more D hordein than the corresponding mealy samples. In Scarlett samples this difference was not statistically significant. This result could be associated with the incompelete degradation of D hordein in steely kernels proposed in earlier studies (*5*). Malted samples contained only urea−DTT-soluble D hordein, although barley samples contained both propanol−DTT- and urea−DTT-soluble D hordein. This may be an indication that propanol−DTT-soluble D hordein is more easily degraded than urea−DTT-soluble D hordein. In addition, there were fewer propanol−DTT- and more urea−DTT-soluble D hordeins left in steelier Barke malt samples, possibly indicating that the D hordeins remaining after malting in steelier samples were those most difficult to degrade. It remains to be explained whether the detected differences in hordein solubilities were a reason for, or a consequence of, the differences in the endosperm structure and malting performance. The properties of storage proteins have indeed been considered to be a more important factor influencing endosperm structure than the total protein content of the grain (*4*). Both C hordein and gel protein composed of B and D hordeins are suggested to restrict hydration and access of hydrolases (*34*−*37*).

Overall, the degradation of hordeins during malting proceeded in the order observed in earlier studies, with D hordeins being hydrolyzed more readily than B hordeins and C hordeins being the most resistant to degradation (*22*, *38*, *39*). Barke 2002 and Scarlett 2002 barley samples had many similarities in hordein composition, whereas, after malting, Barke 2001 and 2002 samples had most in common with each other. This could be an indication of varietal characteristics, which appear in malting performance. For example, differences in hordein degradation patterns can be explained by varietal preferences in hordein degradation during malting. In this study, the D hordein in Barke was more susceptible to degradation than the D hordein in Scarlett. These differences in substrate preferences may be linked to differences in the proteolytic enzyme spectrum. Scarlett was found to lack one proteolytic enzyme activity that was clearly observed in Barke using edestin gel electrophoresis at pH 5.0 (Holopainen and Wilhelmson, unpublished results).

In earlier studies, β-amylase activity has been linked to steeliness and total protein and hordein contents (*33*). In this study, a relationship between free β-amylase activity and steeliness was not observed. However, the α-amylase and limit dextrinase activities were in general higher in the steelier than in the mealy samples of the large sample collection analyzed (data not shown). The activities of these starch-hydrolyzing enzymes are controlled by gibberellic acid in the germinating grain, and their increased activities may be linked to the higher respiration losses observed in the steelier grains.

The relationship between β-glucanase activity and steeliness was not clear. Recently, malt β-glucanase activity was found to correlate positively with Kolbach index and negatively with viscosity (*40*). These relationships between malt quality parameters were also observed in this study, but their possible association with grain hardness remains to be determined. Xylanases, hydrolyzing cell wall arabinoxylans, are released from the aleurone cells during the final stage of endosperm mobilization and linked to the gibberellin-controlled programmed cell death of aleurone cells (*41*−*43*). Higher activities of xylanases may be an indication of a further proceeded programmed cell death in steely grains. Xylanase activities may also be partly of microbiological origin.

The differences in steeliness were not clearly seen in the microscopic examination of grain microstructure; that is, mealy and steelier barley grains did not differ in cell wall thickness or in packing of endosperm cells. This is consistent with an earlier observation that the modification rate of barley grain does not correlate with cell wall thickness, although thicker cell walls may be more resistant to enzymic degradation than thinner ones (*1*). However, the differences observed in biochemical analyses between malts of cultivars Barke and Scarlett may be partially explained by the difference in the cell wall thickness. Cell wall degradation before maturation in the area surrounding the crease seems to be a varietal characteristic of Barke. However, its significance for endosperm modification cannot be estimated on the basis of these results.

In conclusion, a clear relationship between the structure of endosperm and the malting quality was observed in this study. Endosperm structure determined by the LTm method proved to be linked to many differences in malting performance. The steelier samples showed higher respiration loss during malting, higher activities of starch-hydrolyzing enzymes, and possibly a further proceeded programmed cell death. Malts made from steelier barley had a less friable structure, with more slowly degradable, urea-soluble D hordein depending on cultivar. Steelier barley produced less root mass during malting, and more free amino nitrogen and soluble protein were released during mashing. Hordein degradation was also at least partially a cultivar-dependent feature. On the basis of these results, it seems that grains with steelier structured endosperms have an equal

or better capacity to produce enzymes hydrolyzing starch, protein, and cell wall than mealy grains. Despite this, endosperm modification is slower in steelier grains than in mealy samples. The fundamental reason for this difference may lie in the structure or localization of the hordeins as well as the possible effects of endosperm packing on water uptake and movement of enzymes. Further experiments are needed to clarify this aspect.

ABBREVIATIONS USED

AU, arbitrary units; EBC, European Brewing Convention; DTT, dithiothreitol; LTm, light transflectance meter.

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