JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Analysis of Volatile Compounds from Various Types of Barley Cultivars

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We identified volatile compounds of barley flour and determined the variation in volatile compound profiles among different types and varieties of barley. Volatile compounds of 12 barley and two wheat cultivars were analyzed using solid phase microextraction (SPME) and gas chromatography. Twenty-six volatiles comprising aldehydes, ketones, alcohols, and a furan were identified in barley. 1-Octen-3-ol, 3-methylbutanal, 2-methylbutanal, hexanal, 2-hexenal, 2-heptenal, 2-nonenal, and decanal were identified as key odorants in barley as their concentration exceeded their odor detection threshold in water. Hexanal (46−1269 μg/L) and 1-pentanol (798−1811 μg/L) were the major volatile compounds in barley cultivars. In wheat, 1-pentanol (723−748 μg/L) was a major volatile. Hulled barley had higher total volatile, aldehyde, ketone, alcohol, and furan contents than hulless barley, highlighting the importance of the husk in barley grain aroma. The proanthocyanidin-free varieties generally showed higher total volatile and aldehyde contents than wild-type varieties, potentially due to decreased antioxidant activity by the absence of proanthocyanidins.

KEYWORDS: Barley; volatile compounds; solid phase microextraction; hexanal; 1-pentanol

INTRODUCTION

Barley grains are readily available, fairly inexpensive, and present numerous health benefits when consumed in the human diet. Nonetheless, with only 2% of the total production used for human consumption, barley remains underutilized as a food ingredient. When incorporated in food products, barley gives a relatively distinct flavor as compared to other cereals, thus lowering consumer acceptance of barley-based products. Food flavor is commonly defined as a complex combination of different taste and aroma perceptions. According to Hornstein (*1*), aroma is the most important parameter, as a food with no odor has a very bland flavor and is simply described with the basic tastes such as sweet, sour, salty, or bitter.

Aroma characteristics of various cereals such as corn, rye, triticale, wheat, roasted barley, malted barley, or rice have been investigated on a volatile compounds composition standpoint, mainly using laborious and expensive solvent extraction techniques (*2*). A solvent-free, fast, and inexpensive method called solid phase microextraction (SPME) is based on the adsorption of volatile compounds onto a coated fused silica fiber. SPME offers the possibility to detect compounds at the ultratrace level. As most cereal grains are characterized by a very low concentration of aroma volatiles (*3*), SPME has opened up new avenues to offer to researchers interested in studying cereal aroma. The SPME method for headspace analysis of volatile compounds was successfully applied for the identification of volatiles in processed oats (*4*), distillers grains (*5*), and bread crumbs (*6*).

The volatile compound composition of roasted and malted barley has been widely studied (*7*−*10*). However, investigations on barley flavor among unprocessed grains and barley-based food products have been neglected.

The objectives of this study were (i) to optimize SPME experimental conditions to determine aroma volatile compounds of barley and wheat flours, (ii) to identify and quantify volatile compounds present in barley flours, and (iii) to investigate potential differences in volatile compounds content and composition among various barley cultivars.

MATERIALS AND METHODS

Materials. Twelve barley cultivars and breeding lines including one six-row hulled proanthocyanidin-containing (Steptoe), three two-row hulled proanthocyanidin-containing (Harrington, Baronesse, and Farmington), four two-row hulled proanthocyanidin-free (Radiant, CA803803, Caminant, and 98NZ015), two two-row hulless regular (Bear and CDC McGwire), and two two-row hulless waxy (SH97110 and CDC Candle) were grown in 2001 in the Royal Slope area of Washington and used for this study. A hard red wheat cv. Tara and a hard white wheat cv. Macon grown in 2001 were also included. Whole barley and wheat grains were ground to flour using a cyclone sample mill (Udy Co., Fort Collins, CO) equipped with a 0.5 mm screen for composition analysis.

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10.1021/jf0506939 CCC: $30.25
Published on Web 08/19/2005

Chemical Analyses. The moisture content was determined by oven drying for 1 h at 130 °C according to the approved method 44-15A (*11*), and the ash content was determined by dry combustion for 16 h at 580 °C according to the approved method 08-01. The free lipid content was determined by petroleum ether extraction in a Soxhlet apparatus followed by evaporation under nitrogen to a constant weight according to the approved method 30-25. The protein content ($N \times 6.25$) of flours was determined using a nitrogen analyzer (Leco Corporation, St. Joseph, MI). The β-glucan content was measured enzymatically using a β-glucan assay kit from Megazyme (Megazyme International Ireland Ltd. Co., Wicklow, Ireland).

Polyphenols. The total polyphenol content was determined according to Bendelow (*12*) with modifications. Barley flour (1 g, db) was extracted with 30% dimethylformamide (10 mL). The mixture was agitated for 45 min at 22 °C using a gyrotary shaker at speed 8 (model S-3, New Brunswick Scientific Co., New Brunswick, NJ) and centrifuged at 5000*g* for 10 min. The supernatant (0.5 mL) was collected and added with 15% ammonia (1.0 mL), 2% 4-aminoantipyrine (0.5 mL, w/v), 2% $K_3Fe(CN)_6$ (0.5 mL, w/v), and water (7.5 mL). After thorough agitation, the mixture was left to stand for 30 min at 22 °C and the absorbance was measured at 505 nm using a UV−visible spectrophotometer (model UV-1601, Shimadzu Corporation, Kyoto, Japan). A calibration curve was constructed using gallic acid (Sigma-Aldrich Inc., Milwaukee, WI) as a reference.

Fatty Acid Analysis. The fatty acid composition of lipids extracted from barley and wheat flours with petroleum ether was analyzed using gas chromatography (GC). Lipid extracts (~20 mg) were methylated in test tubes by heating with 0.5 N sodium methoxide in methanol (2.0 mL) for 3 h at 55−60 °C. After cooling to 22 °C, fatty acid methyl esters (FAMEs) were extracted using water (5.0 mL) and 1:1 (v/v) diethyl ether/hexane (3.0 mL). After the tubes were inverted 10 times, the organic phase was passed through a Pasteur pipet containing 2.5 cm of silica gel and 1.5 cm of charcoal/$MgSO_4$ (1:10 v/v), retained by glass wool. The FAMEs were eluted with diethyl ether/hexane 1:1 (v/v) (2.0 mL), and the solvent was collected.

The FAMEs were analyzed using a Hewlett-Packard HP 6890 GC (Agilent, Willmington, DE) coupled with a flame ionization detector (FID) and equipped with a Quadrex cyanopropyl bonded capillary column (60 m × 0.25 mm i.d.). Helium was used as the carrier gas at a flow rate of 1.1 mL/min. The injector and detector temperature was 260 °C. The initial column temperature of 50 °C increased to 150 °C at a rate of 10 °C/min and then to 190 °C at a rate of 1 °C/min. The fatty acids were identified using the retention time matching to a Matreya FAMEs standard mix 4210 (Matreya Inc., Pleasant Gap, PA). The proportion of each fatty acid was calculated by dividing their peak area by the total peak area of all fatty acids detected.

Analysis of Volatile Compounds. To identify and quantify barley volatile compounds, the headspace (HS) SPME technique was adopted. For this study, the temperature was maintained at 18 ± 1 °C to avoid formation of flavor compound artifacts. The barley slurry was added with salt and agitated to improve the release of volatile compounds and the adsorption of the released compounds to the SPME fiber. Because of the viscous nature of the slurry, HS-SPME was used instead of the immersion-SPME method.

A slurry was prepared by blending freshly ground flour (3.60 g), sodium chloride (3.60 g), and distilled water (14.8 mL) in a 25 mL flask with cap and Teflon-faced silicone rubber septa (Supelco, Co., Bellefonte, PA). The flask containing sodium chloride, flour, and water was placed on a magnetic stir plate (model PC-220, Corning, NY) and stirred at 1100 rpm for 20 min. An internal standard *n*-tridecane (50 mg/L) was added to the slurry after stirring for 18 min to preequilibrate volatiles. A SPME fiber was then exposed to the headspace of the barley slurry. Three fibers from a flavor assortment kit (Supelco Co.) were tested: 100 μm poly(dimethylsiloxane) (PDMS), 65 μm poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB), and 75 μm carboxen/ poly(dimethylsiloxane) (CAR/PDMS). The selection of the fiber to be used for further analysis was based upon its affinity for the barley volatiles and the symmetry of chromatographic peaks. To compare the three fibers, a flour slurry of barley cv. Farmington was prepared as described above. The fiber exposure time was arbitrarily fixed to 1 h. Once the fiber was selected, different exposure times (30, 45, 60, and 90 min) were investigated in order to optimize the extraction conditions for the volatile compounds.

The volatiles adsorbed to the SPME fiber were thermally desorbed into the injection port of a Varian 3400 (Varian, Inc., Walnut Creek, CA) gas chromatograph connected to a FID detector and equipped with a DB-1 column (30 m × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA). Helium was used as the carrier gas. The injector and detector temperatures were 200 and 250 °C, respectively, according to the method of Yang and Peppard (*13*). The column temperature was initially maintained at 33 °C for 5 min before increasing to 50 °C at a rate of 2 °C/min and to then 225 °C at a rate of 5 °C/min. Volatile compounds were first identified with a preliminary study using GC/MS under the same condition as described above with the exception that the column length was 60 m. Barley volatile compounds were identified by comparing their spectra with those present in the Wiley-NBS library. Also, the retention index was calculated based on Kovats retention indicies using a series of straight chain alkanes (C4−C14) under the chromatographic conditions described above.

When FID was used, identification and quantification of volatiles were accomplished using detector response factors established from standard compounds and their retention times. The compounds were identified using GC/MS. The compounds 2-methylbutanal, hexanal, *trans*-2-hexenal, heptanal, *trans*-2-heptenal, *trans*-2-octenal, nonanal, *trans*-2-nonenal, 3-methyl-2-pentanone, 3-hexanone, 6-methyl-2-heptanone, 6-methyl-5-hepten-2-one, 2-octanone, *trans*-3-octen-2-one, 2-pentylfuran, and 1-octen-3-ol were purchased from Sigma-Aldrich Inc. The compound pentanal was purchased from Alltech (Deerfield, IL). 2-Hexanone was purchased from Ultra Scientific (North Kingston, RI). The compounds 2-pentanone, 1-pentanol, 1-hexanol, 3-heptanone, 2-heptanone, decanal, 4-methyl-2-pentanone, benzaldehyde, and 3-methylbutanal were purchased from Chem Service (West Chester, PA). The internal standard *n*-tridecane was purchased from Alfa Aesar (Ward Hill, MA). The key odorants in whole grain flours of barley and wheat were identified as volatiles appearing in higher concentration than their odor detection thresholds reported by Leffingwell and Associates (*14*) and Buttery et al. (*15*).

Statistical Analysis. Data were analyzed using the SAS package for Windows (version 8.01) by performing analysis of variance and the least significant differences test. Statistically significant differences were determined at ($P < 0.05$).

RESULTS AND DISCUSSION

Composition of Whole Grain Flours. The composition of whole barley and whole wheat flours is summarized in **Table 1**. The ash content was significantly higher in barley flours than in wheat flours. The ash content ranged from 2.28 to 2.55% in hulled barley and from 1.79 to 1.87% in hulless barley cultivars. The protein content of barley flours ranged from 12.8% in Steptoe to 16.5% in SH97110. The hulled barley cultivars contained significantly less protein than the hulless varieties. The free lipid content was significantly lower in wheat than in barley. The lipid content ranged from 1.99 to 2.87% in barley and was 1.70% in wheat varieties. Among hulless cultivars, the waxy barleys contained significantly more free lipids than regular barley. The β-glucan content was significantly greater in barley flours than in wheat, ranging from 4.59% in Baronesse to 7.37% in SH97110. The β-glucan content was significantly greater in the hulless waxy cultivars than in the hulled or hulless regular barley. Analogous results have previously been reported by Czuchajowska et al. (*16*), Baik et al. (*17*), and Klamczynski (*18*). With more than 0.42% polyphenols as gallic acid, hulless barley exhibited a higher total polyphenol content than hulled barley (0.10−0.39%). Among hulled barley, the proanthocyanidin-free cultivars contained significantly less polyphenols (<0.14%) than proanthocyanidin-containing barley cultivars (<0.39%) since proanthocyanidins are major phenolic com-

Table 1. Composition of Barley and Wheat Varieties[a,b]

cultivars	class[c]	ash (%)	protein (%)	free lipids (%)	β-glucan (%)	total polyphenol (% gallic acid)
			hulled barley			
Steptoe[d]	Pro+	2.55 a	12.8 j	1.96 hi	5.0 ef	0.37 bc
Harrington	Pro+	2.41 e	14.2 f	2.27 e	5.3 d	0.36 cd
Baronesse	Pro+	2.50 b	13.3 h	1.99 h	4.6 h	0.39 b
Farmington	Pro+	2.50 b	13.5 g	2.34 d	5.7 c	0.34 d
Radiant	Pro–	2.44 d	12.4 k	1.95 i	4.8 g	0.12 ef
CA803803	Pro–	2.46 cd	13.2 i	2.25 f	5.1 de	0.10 f
Caminant	Pro–	2.48 bc	13.5 g	2.16 g	4.1 j	0.13 ef
98NZ015	Pro–	2.28 f	13.5 g	1.97 hi	4.3 i	0.14 e
			hulless barley			
CDC Candle	waxy	1.87 g	16.1 c	2.66 b	7.1 b	0.45 a
SH97110	waxy	1.85 gh	16.5 b	2.83 a	7.4 a	0.43 a
Bear	regular	1.83 h	15.4 e	2.13 g	4.5 h	0.42 a
CDC McGwire	regular	1.79 i	15.9 d	2.48 c	4.9 fg	0.43 a
			hard wheat			
Tara	red	1.72 j	16.8 a	1.70 j	0.5 k	
Macon	white	1.47 k	15.5 e	1.70 j	0.4 k	

[a] Values with different letters within the same column are significantly different ($p < 0.05$). [b] Ash, protein, lipids, and β-glucan contents are expressed in dry weight basis. [c] Pro+, proanthocyanidin containing; Pro–, proanthocyanidin-free. [d] Steptoe is a six-row barley. All other varieties are two-row barleys.

Table 2. Fatty Acid Composition of Lipids of Barley and Wheat Varieties[a]

		fatty acid composition (% of total fatty acids)						
cultivars	class[b]	C16:0	C18:0	C18:1tr	C18:1c	C18:2	C18:3	C20:1
				hulled barley				
Steptoe[c]	Pro+	17.37 i	1.26 d	16.95 c	0.71 h	57.44 c	5.42 c	0.86 e
Harrington	Pro+	18.96 g	1.10 h	17.86 b	0.77 d	55.75 e	4.67 i	0.88 cd
Baronesse	Pro+	20.28 b	1.18 f	16.46 f	0.75 d	55.16 j	5.51 b	0.87 c
Farmington	Pro+	21.25 b	1.17 f	16.42 e	0.72 h	54.42 i	5.20 e	0.83 fg
Radiant	Pro–	21.15 b	1.14 g	14.68 i	0.77 de	55.69 ef	5.71 a	0.87 de
CA803803	Pro–	20.09 e	1.32 ab	16.40 e	0.73 g	55.36 g	5.29 d	0.81 gh
Caminant	Pro–	20.48 d	1.31 b	15.61 h	0.72 h	55.57 f	5.47 b	0.84 f
98NZ015	Pro–	21.97 a	1.22 e	16.01 g	0.81 c	53.59 k	5.49 b	0.92 b
				hulless barley				
CDC Candle	waxy	20.13 e	1.33 a	14.94 i	0.77 de	57.05 d	4.98 g	0.80 h
SH97110	waxy	20.72 c	1.34 a	16.86 cd	0.75 efg	54.67 h	4.82 h	0.84 f
Bear	regular	19.07 g	1.28 c	16.82 d	0.76 def	55.82 e	5.28 d	0.97 a
CDC McGwire	regular	19.47 f	1.10 h	18.43 a	0.75 fg	54.16 j	5.13 f	0.97 a
				hard wheat				
Tara	red	17.80 h	0.96 i	14.80 j	0.94 a	60.50 b	4.21 j	0.79 h
Macon	white	16.53 j	1.16 g	16.41 e	0.87 b	60.87 a	3.48 k	0.67 i

[a] Values with different letters within the same column are significantly different ($p < 0.05$). [b] Pro+, proanthocyanidin containing; Pro–, proanthocyanidin-free. [c] Steptoe is a six-row barley. All other varieties are two-row barleys.

pounds in barley. No difference in polyphenol content was noticed between waxy and regular hulless barley varieties.

Fatty Acid Composition. Table 2 summarizes the fatty acid composition of lipids in whole barley and wheat flours. Palmitic (C16:0), stearic (C18:0), elaidic (C18:1tr), oleic (C18:1c), linoleic (C18:2), linolenic (C18:3), and eicosenoic (C20:1) acids were detected in lipids of barley and wheat flours. The fatty acid compositions of the barley cultivars were similar to those presented by De Man et al. (*19*). As previously noticed by Palmer (*20*), wheat and barley contained mostly unsaturated fatty acids, which constitute 76.8−82.3% of the total fatty acids. Linoleic acid (C18:2) was the most abundant fatty acid, ranging from 53.9% in 98NZ015 to 57.4% in Steptoe and from 60.5 to 60.9% in wheat cultivars. Wheat cultivars exhibited generally higher oleic (C18:1c) and linoleic (C18:2) acids content and a lower linolenic acid (C18:3) content than barley. These results agree with Morrison et al. (*21*) who reported that barley and wheat show very comparable fatty acid compositions although wheat contains less linolenic acid.

Optimization of SPME Experimental Conditions. Different fiber coatings and exposure times were investigated prior to analyzing barley and wheat slurries for their volatile compositions. The chromatogram obtained with the PDMS fiber showed the least number (*11*) of peaks. Furthermore, the PDMS-coated fiber resulted in lower area counts of individual volatile compounds than with the PDMS/DVB or the CAR/PDMS coatings. The PDMS/DVB and the CAR/PDMS fibers exhibited 30 and 25 peaks on their respective chromatograms.

When comparing peak retention times, the PDMS/DVB fiber adsorbed different compounds than the CAR/PDMS fiber. According to the fiber manufacturer, the CAR/PDMS coating is more appropriate for the detection of low molecular weight compounds such as acetone or 2-butanone, whereas the PDMS/DVB absorbs compounds of higher molecular weight such as nonanal or decanal. Furthermore, the lowest molecular weight compound detected in this study was 3-methyl butanal, and the highest molecular weight compound detected was decanal. On the basis of the three different fibers tested, the PDMS/DVB-

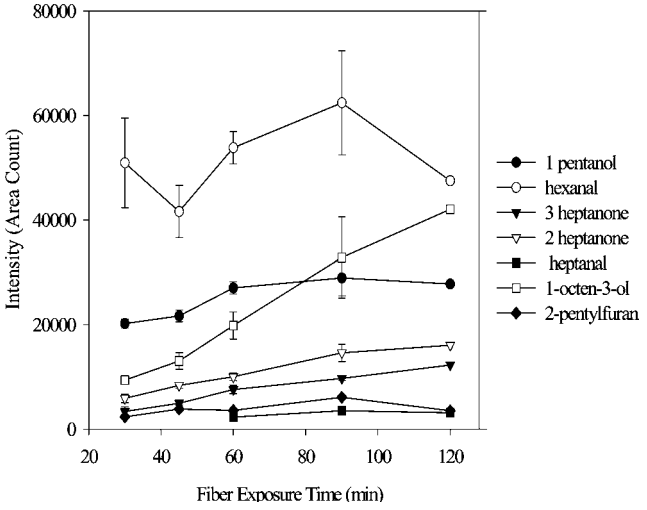

Figure 1. Effect of exposure time on the amount of selected compounds absorbed by a PDMS/DVB fiber. The intensities of volatile compounds at 30, 45, 60, and 90 min were the average values of three replicated runs.

coated fiber was far superior for the analysis of barley and wheat slurry volatiles.

The effect of the SPME fiber exposure time to the headspace of barley slurries was investigated with GC-FID. **Figure 1** shows the effect of various exposure times on the detection intensity of selected compounds in barley slurries. After exposing a PDMS/DVB fiber to the headspace of a barley slurry for 30 and 45 min, most volatile compounds were detected. However, compounds such as heptanal were detectable only at 60 min of exposure. An exposure time of 90 min appeared to be appropriate for the SPME analysis of a barley slurry as the area count of most of the compounds such as heptanal, 3-heptanone, 2-heptanone, and 2-pentylfuran exhibited little changes after 90 min of exposure. As the area count of hexanal or 1-octen-3-ol was still affected by the exposure time after 90 min, the exposure time was carefully controlled throughout the experiment to limit variability among replications.

Volatile Compounds of Whole Barley and Whole Wheat Flours. Twenty-six and 12 volatiles comprising aldehydes, ketones, alcohols, and a furan were respectively identified in barley and wheat. The concentrations of identified aldehydes, ketones, alcohols, and furan in barley and wheat slurries are presented in **Table 3**. Hougen et al. (*22*) noticed that different cereals or different grain varieties commonly have similar volatile compounds but in different concentrations. Similar observations were made in this study when comparing wheat and barley or different barley varieties.

Barley generally showed higher total volatile compound contents (953−3339 μg/L) than wheat (913−1017 μg/L). The total volatile content of barley ranged from 1178 to 3339 μg/L in hulled cultivars and from 953 to 1211 μg/L in hulless varieties, suggesting that some volatile compounds may originate from the husk of barley grains. Among hulled barley, with the exception of Radiant (1718 μg/L), the proanthocyanidin-free cultivars contained more volatile compounds than proanthocyanidin-containing barley cultivars, indicating that proanthocyanidins present in barley may have an impact on barley flour aroma. No significant differences were observed between the total volatile compounds content of waxy and regular hulless barley. Overall, the lowest amounts of volatile compounds were detected in hulless regular barley.

Alcohols were the major quantitative constituents of barley volatiles, followed by aldehydes, ketones, and furans. In wheat, alcohols were also the major constituents followed by ketones. Neither aldehydes nor furan were detected in wheat flour. Barley flour exhibits a higher content of aldehydes, alcohols, and furans and a lower content of ketones as compared to wheat flour. In general, hulled barley cultivars contained more aldehydes, ketones, alcohols, and furan, possibly due to the presence of volatile compounds in the husk of barley grains. Proanthocyanidin-free cultivars showed higher aldehydes, ketones, and alcohols contents than proanthocyanidin-containing barley cultivars with the exception of Radiant. Because aldehydes, alcohols, and ketones are common lipid oxidation products, the absence of proanthocyanidins, which could work as antioxidants to prevent lipid oxidation in barley flour, may be responsible for the higher volatile content in proanthocyanidin-free barley cultivars.

Table 3. Aldehydes, Ketones, Alcohols, Furan, and Total Volatile Content (in μg/L) of Barley and Wheat Flours[a]

cultivars	class[b]	aldehydes	ketones	alcohols	furans	total
			hulled barley			
Steptoe[c]	Pro+	564 c	26 f	1365 d	0.63 abc	1955 c
Harrington	Pro+	380 d	58 ef	1248 de	0.51 cd	1686 d
Baronesse	Pro+	51f g	40 ef	1087 ef	0.48 d	1178 e
Farmington	Pro+	199 e	91 cdef	1393 cd	0.55 abcd	1684 d
Radiant	Pro−	313 d	34 ef	1371 d	0.53 bcd	1718 cd
CA803803	Pro−	965 b	190 ab	1548 bc	0.50 d	2703 b
Caminant	Pro−	1361 a	152 bcd	1825 a	0.65 ab	3339 a
98NZ015	Pro−	928 b	116 bcde	1630 b	0.67 a	2675 b
			hulless barley			
CDC Candle	waxy	200 e	20 f	991 fg	ND[d]	1211 e
SH97110	waxy	115 ef	23 f	890 gh	ND	1027 ef
Bear	regular	74 fg	44 ef	852 gh	0.27 e	970 ef
CDC McGwire	regular	g	71 def	874 gh	0.28 e	953 ef
			hard wheat			
Tara	red	ND	170 abc	742 h	ND	913 f
Macon	white	ND	239 a	777 h	ND	1017 ef

[a] Values with different letters within the same column are significantly different ($p < 0.05$). [b] Pro+, proanthocyanidin containing; Pro−, proanthocyanidin-free. [c] Steptoe is a six-row barley. All other varieties are two-row barleys. [d] Not detected.

Hulled barley showed a higher alcohol content (1087−1825 μg/L) than hulless barley (852−991 μg/L) and wheat (742−777 μg/L). The aldehyde content of hulled barley exhibited a large variation among varieties, ranging from 51.0 μg/L in Baronesse to 1361 μg/L in Caminant. Baronesse exhibited the lowest aldehyde content among hulled barley cultivars. Ranging from 115 to 200 μg/L, the aldehyde content of hulless waxy barley was significantly greater than the aldehyde content of regular hulless barley (8−74 μg/L). In cereal grains, the presence of aldehydes is often related to lipid oxidation. In barley, polyunsaturated fatty acids, including linoleic, linolenic, and eicosenoic acids, represent about 60% of the fatty acids. As emphasized by Baxter (*23*) and Yang et al. (*24*), barley contains lipoxygenases and hydroperoxide isomerases, which are responsible for the oxidation of unsaturated fatty acids leading to the production of volatile compounds such as hexanal. Consequently, it is highly probable that most odorants of barley result from lipid oxidation. Wheat flour contained significantly more ketones than hulless barley as well as hulled proanthocyanidin-containing barley flours. With the exception of Radiant, proanthocyanidin-free barley exhibited a higher ketones content than proanthocyanidin-containing cultivars. 2-Pentylfuran was the only furan identified in barley flour. Hulled barley contained significantly more 2-pentylfuran than hulless barley. 2-Pentylfuran was not detected either in waxy hulless barley or in wheat flours.

Table 4. Volatile Compounds of Barley Flour Identified Using SPME GC FID

peak no.	KI[a]	compound
1	622	3-methyl-butanal
2	631	2-methyl-butanal
3	649	2-pentanone
4	661	pentanal
5	713	4-methyl-2-pentanone
6	742	1-pentanol
7	754	3-hexanone
8	756	2-hexanone
9	766	hexanal
10	820	2-hexenal
11	852	1-hexanol
12	860	3-heptanone
13	864	2-heptanone
14	873	heptanal
15	917	benzaldehyde
16	921	2-heptenal
17	929	6-methyl-5-hepten-2-one
18	957	6-methyl-2-heptanone
19	959	1-octen-3-ol
20	963	2-octanone
21	973	2-pentylfuran
22	994	3-octen-2-one
23	1023	2-octenal
24	1074	nonanal
25	1123	2-nonenal
26	1185	decanal

[a] Kovats retention indexes relative to a *n*-alkane series calculated on a 60 m DB-1 column.

The compounds identified in barley flour are listed in **Table 4**. The compounds 3-methylbutanal, 2-methylbutanal, pentanal, hexanal, 2-hexenal, benzaldehyde, nonanal, 2-pentylfuran, 1-pentanol, 1-hexanol, and 2-nonenal were previously identified in roasted or malted barley (*7*−*10*, *25*−*27*) and were also identified in this study in the flour of barley grains.

The compounds 3-methylbutanal, 2-methylbutanal, pentanal, 3-hexanone, hexanal, 2-hexenal, heptanal, benzaldehyde, 2-heptenal, 2-pentylfuran, 2-octenal, nonanal, 2-nonenal, and decanal were detected in barley flours only. The compounds 2-pentanone, 4-methyl-2-pentanone, 1-pentanol, 2-hexanone, 1-hexanol, 3-heptanone, 2-heptanone, 6 methyl-2-heptanone, 6-methyl-5-hepten-2-one, 1-octen-3-ol, 2-octanone, and *trans*-3-octen-2-one were found in both wheat and barley. All volatile compounds detected in wheat flours were also present in barley flours.

Key Odorants of Whole Barley and Whole Wheat Slurries. Hexanal and 1-pentanol were the major compounds detected in barley cultivars, and their content ranged from 46 to 1269 μg/L and from 798 to 1811 μg/L, respectively. 1-Pentanol detected in the range of 723−748 μg/L was also a major volatile compound in wheat. Aroma active compounds of barley and wheat flours were identified by comparing the concentration of detected volatile compounds to their odor detection threshold in water (*14*, *15*). When the concentration of a compound was greater than its odor detection threshold, the compound was considered to be a potential key odorant of the barley or wheat flour.

3-Methylbutanal, 2-methylbutanal, hexanal, 2-hexenal, 2-heptenal, 2-nonenal, and decanal were identified as key odorants of barley flour. 1-Octen-3-ol was a potential aroma active compound of both barley and wheat flours. Using aroma extract dilution analysis, however, Czerny and Schieberle (*28*) reported a number of odor active compounds including (*E*)-2-nonenal, (*E*,*Z*)- and (*E*,*E*)-2,4-decadienal, (*E*)-2,4-decadienal, (*E*)-4,5-epoxy-(*E*)-2-decenal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, and vanillin in whole wheat meal. Even though 1-pentanol was the major compound detected in both wheat and barley, its concentration was still much lower than the odor detection threshold of 3256 μg/L (*15*). No conclusion could be drawn for 4-methyl-2-pentanone, 3-hexanone, 2-hexanone, 3-heptanone, 6-methyl-2-heptanone, and trans 3-octen-2-one as their odor detection threshold was not available in the literature.

Table 5 illustrates varietal differences in the concentration of potential key odorants of barley and wheat flours. Varietal differences among barley were observed when comparing the concentration of each aroma active compounds. 3-Methylbutanal was detected in two proanthocyanidin-free hulled barley cultivars, Caminant and 98NZ015. Only Radiant and 98NZ015

Table 5. Key Odorants in Various Barley and Wheat Varieties[a]

		concentration in barley slurry (in μg/L)							
cultivars	class[b]	3-methyl butanal	2-methyl butanal	hexanal	2-hexenal	2-heptenal	1-octen-3-ol	2-nonenal	decanal
DT[c]		0.2–2	1–3	4.5–5	17	13	1	0.08–1	0.1–2
					hulled barley				
Steptoe[d]	Pro+	ND[a]	ND	508.53 c	16.67 de	30.08 bc	3.84 ab	0.35 a	0.14 a
Harrington	Pro+	ND	ND	342.47 d	19.55 cd	11.69 d	3.94 ab	0.33 a	0.13 a
Baronesse	Pro+	ND	ND	46.13 gh	ND	3.44 ef	3.59 ab	0.17 bc	0.09 a
Farmington	Pro+	ND	ND	191.43 ef	2.84 f	4.17 e	3.71 ab	0.19 b	0.12 a
Radiant	Pro−	ND	16.98 b	304.39 d	3.51 f	5.83 e	3.80 ab	0.11 cd	0.12 a
CA803803	Pro−	ND	ND	898.75 b	34.94 b	28.87 c	4.36 a	0.15 bcd	0.13 a
Caminant	Pro−	44.33 b	27.61 a	1269.44 a	50.14 a	37.86 a	3.52 ab	0.12 bcd	0.13 a
98NZ015	Pro−	64.54 a	ND	859.80 b	30.35 bc	33.71 ab	4.29 ab	0.14 bcd	0.11 a
					hulless barley				
CDC Candle	waxy	ND	ND	194.49 e	7.66 ef	0.56 f	1.53 c	0.16 bc	0.13 a
SH97110	waxy	ND	ND	113.33 efg	0.94 f	0.45 f	1.27 c	0.08 d	0.11 a
Bear	regular	ND	ND	95.33 fgh	1.67 f	0.64 f	2.01 c	0.14 bcd	0.10 a
CDC McGwire	regular	ND	ND	9.34 h	ND	0.56 f	1.82 c	0.16 bc	ND
					hard wheat				
Tara	red	ND	ND	ND	ND	ND	1.87 c	ND	ND
Macon	white	ND	ND	ND	ND	ND	1.80 c	ND	ND

[a] Values with different letters within the same column are significantly different ($p < 0.05$); ND, nondetected. [b] Pro+, proanthocyanidin containing; Pro−, proanthocyanidin-free. [c] Odor detection threshold in water appeared in the *Odor & Flavor Detection Thresholds in Water* by Leffingwell and Associates. [d] Steptoe is a six-row barley. All other varieties are two-row barleys.

exhibited 2-methylbutanal. With the exception of Radiant, hulled proanthocyanidin-free barley cultivars contained significantly greater amount of hexanal, 2-hexenal, and 2-heptenal than proanthocyanidin-containing barley. Hulled barley exhibited a significantly higher 1-octen-3-ol content than hulless barley or wheat cultivars. Decanal was the only key odorant detected in barley flour without significant varietal differences.

3- and 2-Methylbutanals were determined as the most odor active compounds in malted or roasted barley (*8*, *9*, *25*, *26*). In this study, the methylbutanals were only detected in the proanthocyanidin-free barley cultivars. This could be explained by the potential role of proanthocyanidins as antioxidant but also by the fact that 2- and 3-methylbutanals have low molecular weights and the PDMS/DVB fiber used for the SPME analysis is more adequate for higher molecular weight compounds. The fiber selected for this study has possibly been a limiting factor in the detection and quantification of methylbutanals.

LITERATURE CITED

(1) Hornstein, I. In *Flavor Chemistry*; Hornstein, I., Ed.; American Chemical Society: Washington, DC, 1966.

(2) Maga, J. A. Cereal volatiles, a review. *J. Agric. Food Chem.* **1978**, *26*, 175−178.

(3) Zhou, M.; Robards, K.; Glennie-Holmes, M.; Helliwell, S. Analysis of volatile compounds and their contribution to flavor in cereals. *J. Agric. Food Chem.* **1999**, *47*, 3941−3953.

(4) Sides, A.; Robards, K.; Helliwell, S.; An, M. Changes in the volatile profile of oats induced by processing. *J. Agric. Food Chem.* **2001**, *49*, 2125−2130.

(5) Biswas, S.; Staff, C. Analysis of headspace compounds of distillers grains using SPME in conjunction with GC/MS and TGA. *J. Cereal Sci.* **2001**, *33*, 223−229.

(6) Ruiz, J. A.; Quilez, J.; Mestres, M.; Guasch, J. Solid-phase microextraction method for headspace analysis of volatile compounds in bread crumb. *Cereal Chem.* **2003**, *80*, 255−259.

(7) Fickert, B.; Schieberle, P. Identification of the key odorants in barley malt (caramalt) using GC/MS techniques and odour dilution analyses. *Nahrung* **1998**, *42* (6S), 371−375.

(8) Beal, A. D.; Mottram, D. S. Compounds contributing to the characteristic aroma of malted barley. *J. Agric. Food Chem.* **1994**, *42*, 2880−2884.

(9) Wang, P.-S.; Kato, H.; Fujimaki, M. Studies on flavor components of roasted barley Part II. The major volatile carbonyl compounds. *Agric. Biol. Chem.* **1968**, *32* (4), 501−506.

(10) Wang, P.-S.; Kato, H.; Fujimaki, M. Studies on flavor components of roasted barley Part IV. The volatile sulfur compounds, fatty acids and neutral non-carbonyl oxygenated compounds. *Agric. Biol. Chem.* **1970**, *34* (4), 561−567.

(11) American Association of Cereal Chemists. *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2002; Methods 44-15A, 08-01, and 30-25.

(12) Bendelow, V. M. Automated procedure for the estimation of total polyphenol content in beer, wort, malt and barley. *J. Am. Soc. Brew. Chem.* **1977**, *35*, 150−152.

(13) Yang, X.; Peppard, T. Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem.* **1994**, *42*, 1925−1930.

(14) Leffingwell and Associates. Odor and Flavor Detection Thresholds in Water (In Parts per Billion). http://www.leffingwell.com/odorthre.htm.

(15) Buttery, R. G.; Louisa, C.; Stern, D. J. Studies on popcorn aroma and flavor volatiles. *J. Agric. Food Chem.* **1997**, *45*, 837−843.

(16) Czuchajowska, Z.; Szczodrak, J.; Pomeranz, Y. Characterization and estimation of barley polysaccharides by near-infrared spectroscopy. I. Barleys, starches, and β-D-glucans. *Cereal Chem.* **1992**, *69*, 413−418.

(17) Baik, B.-K.; Czuchajowska, Z. Barley in udon noodles. *Food Sci. Technol. Int.* **1997**, *3*, 423−435.

(18) Klamczynski, A. P. Composition, thermal properties, and processing of nonwaxy, high-amylose and waxy barley for food products. Ph.D. dissertation, Washington State University, Pullman, WA, 1998.

(19) De Man, W.; Bruyneel, P. Fatty acid content and composition in relation to grain size of barley. *Phytochemistry* **1987**, *26*, 1307−1310.

(20) Palmer, G. H. In *Cereal Science and Technology*; Palmer, G. H., Ed.; Aberdeen University Press: Aberdeen, Scotland, 1989; Cereals in malting and brewing, pp 61−242.

(21) Morrison, W. R. In *Barley: Chemistry and Technology*; McGregor, A. W., Bhatty, R. S., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1993; Barley lipids, pp 199−236.

(22) Hougen, F. W.; Quilliam, M. A.; Curran, W. A. Headspace vapors from cereal grains. *J. Agric. Food Chem.* **1971**, *19* (1), 182−183.

(23) Baxter, E. D. Lipoxidases in malting and mashing. *J. Inst. Brew.* **1982**, *88*, 390−396.

(24) Yang, G.; Schwarz, P. B.; Vick, B. A. Purification and characterization of lipoxygenase isoenzymes in germinated barley. *Cereal Chem.* **1993**, *70*, 589−595.

(25) Kim, Y.; Lee, Y. C.; Kim, K. O. Optimum roasting and extraction conditions and flavor characteristics of roasted malt extract. *Cereal Chem.* **1998**, *75* (3), 282−288.

(26) Collins, E. Steam volatile components of roasted barley. *J. Agric. Food Chem.* **1971**, *19* (3), 533−535.

(27) Olsson, J.; Börjesson, T.; Lundstedt, T.; Schnürer, J. Volatiles for mycological quality grading of barley grains: Determinations using gas chromatography−mass spectrometry and electronic nose. *Int. J. Food Microbiol.* **2000**, *59*, 167−178.

(28) Czerny, M.; Schieberle, P. Important aroma compounds in freshly ground wholemeal and white wheat flour—Identification and quantitative changes during sourdough fermentation. *J. Agric. Food Chem.* **2002**, *50*, 6835−6840.

Received for review March 28, 2005. Revised manuscript received June 15, 2005. Accepted June 19, 2005.

JF0506939