J. Fac. Agr., Kyushu Univ., **60** (1), 57–63 (2015)

Comparison of nutritional composition of premature, mature and de–hulled barley in Korea

Lu–Jing GAN[1], Xiang–Yu WANG[1], Dan YANG[1], Jung–Ah SHIN[1], Ki–Teak LEE[1], Jeung– Hee LEE[2], Takeo YAMAKAWA* and Jin–Woong CHO[1]*

Laboratory of Plant Nutrition, Division of Molecular Biosciences, Department of Biosciences & Biotechnology, Faculty of Agriculture, Kyushu University,
6–10–1 Hakozaki, Fukuoka 812–8581, Japan
(*Received October 27, 2014 and accepted November 14, 2014*)

So far, there are lots of paper reported the nutritional composition of mature barley and de–hulled barley, but little research on premature barley. For the purpose of exploring nutritional potential of premature barley, we analyzed the nutritional status of premature green, mature yellow, and de–hulled barley grown in the Jeollabuk–Do Agricultural Research & Extension Services (Iksan, Korea). Compared to de–hulled and mature barley, premature barley contained higher amounts of lysine (41.46 mg g^{-1} protein), total essential amino acids (351.75 mg g^{-1} protein), vitamin C (0.93 mg 100 g^{-1} DW), vitamin B_2 (0.10 mg 100 g^{-1} DW), and vitamin B3 (2.16 mg 100 g^{-1} DW), and β–carotene (69.79 μg 100 g^{-1} DW) was only detected in premature barley. Statistically higher quantities of Na and K were present in premature barley compared to the other two grain–types ($P \leq 0.05$), whereas mature barley contained the highest amount of P and Fe ($P \leq 0.05$). All three grain–types contained similar levels of Ca and Cu. Mature barley contained the highest betaine and choline (49.44 mg 100 g^{-1} DW and 34.61 mg 100 g^{-1} DW, respectively). The results indicate that premature barley is nutritionally superior to de–hulled barley.

Key words: betaine, choline, essential amino acids, premature barley

Abbreviations: ^{1}H NMR; ^{1}H nuclear magnetic resonance, GC; gas chromatography, HPLC; high performance liquid layer chromatography, IPC–AES; inductively coupled plasma atomic emission spectroscopy.

INTRODUCTION

Since ancient times, barley (*Hordeum vulgare* L.) has been an important cereal grain, primarily used as animal feed and for malting processes. However, the nutritional value of barley has recently been shown to be an important human food resource. Barley contains considerable levels of dietary fiber that contributes toward reducing coronary heart disease and diabetes (Huth *et al*., 2000; Knutsen and Holtekjølen, 2007). In particular, the beneficial health effect of β–glucans in lowering blood cholesterol levels has widely been recognized (Behall *et al*., 2004). Furthermore, barley provides several types of antioxidants, primarily tocopherols and other polyphenol compounds (Petti and Scully, 2009).

Because barley grains have hard hulls, these are usually removed, along with small amounts of bran, germ, and endosperm. Furthermore, after de–hulling, the remaining hull, bran, germ, and part of the endosperm are often removed during the subsequent pearling process (Baik and Ullrich, 2008). Such de–hulled or pearled barley is usually used in breakfast cereals or added to various food products, such as stews, soups, and breads. In Korea, de–hulled barley grains are usually used in food products. However, the de–hulling process causes other nutritive compounds, such as fiber and phenol, lost from the grains. For instance, whole barley grain contains 11% to 34% total dietary fiber, of which 3% to 20% is soluble dietary fiber. In comparison, de–hulled barley grain generally contains 11% to 20% total dietary fiber and 3% to 10% soluble dietary fiber (Baik and Ullrich, 2008). Furthermore, Panfili *et al*. (2008) reported that α– and β–tocopherol contents of barley pearling by–products were about seven and five–folds higher than the hulled grain, and the pearled kernel contained 4.82% β–glucans. These results indicate that the pearling process dramatically reduced the nutritive values of barley.

Because premature barley grain has a soft pericarp, and hence softer texture than mature grain, the pearling process may not be required. Yang *et al*. (2012) reported that premature green wheat had significantly higher content of dietary fiber, Vitamin C, β–carotene than mature yellow wheat, indicated that premature green wheat has potential for the human diet because of its desirable nutritional value. To date, research has primarily focused on the biochemical changes of barley during grain development, few comparison has been studied on the nutritional compounds between premature barley and matured barley. In this study, in order to identify the grain type with the highest nutritional value, we compared the relative content of various compounds such as sugars, protein, dietary fiber, amino acids, vitamins, minerals, phytosterols, and fatty acids—found in premature–green barley (premature barley), mature–yellow barley (mature barley), and de–hulled barley grown in Korea.

[1] College of Agricultural and Life Sciences, Chungnam National University, Daejeon, Korea
[2] Department of Food Nutrition, Daegu University, Daegu, Korea
* Joint Corresponding author (E–mail: yamakawa@kyushu–u.ac.jp)
[1]* Joint Corresponding author (E–mail: jwcho@cnu.ac.kr)

MATERIALS AND METHODS

Plant materials and collection

The barley grains samples were produced by the variety of barley (*Hordeum distichum* L. cv. Pungsanchalssalbori) grown in the Jeollabuk–Do Agricultural Research & Extension Services (Iksan, Korea). Premature barleys were harvested on May 13th, 2010, which is 20 days after heading date (April 23th, 2010) and mature barleys were harvested on June 6th, 2010, which is 40 days after heading date. Steam blanching, partial drying and de–hulling process were carried out using a newly developed continuous system. The steam blanching system (1,200 W×10,000 L×1,900 H mm) with a capacity of 3,500 kg hr^{-1} consists of input, blanching and cooling chamber, blanching process was carried out at 95±1.5°C for 2 min. Drying process was performed below 70°C with a system consists of drying chamber (2.0 W×2.0 L×2.5 H m), equipped with a fan (1.3 H×1.5 L m), an exhaust fan (1.2 H×1.3 L m) and a heater (1.0 W×0.8 L m). After blanching and drying, mature barleys were de–hulled to remove the whole hull and approximately 20% of bran, and the product was called de–hulled barley. For the following analysis of nutrients, 3 kinds of barley grains were ground by an electric miller (FM–909T, Hanil Co., Ltd., Korea), and screened using a sifter (bore diameter: 0.8 mm). The subsamples were stored at –20°C.

Color and water, protein, ash, and dietary fibre analysis

The content of water, protein, ash, and dietary fibre were analyzed according to Helrich (1990). The color of barley was measured as Hunter L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) values with a JC801 colorimeter (Color Techno System Corp., Tokyo, Japan).

Amino acids analysis

High–speed amino acid analyzer (L–8900 A, Hitachi) was used to obtain amino acid profiles of grain protein. Sample was prepared by hydrolysis of HCl at 105°C for 24 h according to the method slightly modified by Jiang *et al*. (2013). Hydrolysate was dissolved in distilled water. After centrifuged and filtered, supernatant was used for analysis.

Fatty acid composition analysis

Analysis was conducted following the method from Zhu *et al*. (2009). Hewlett–Packard 6890 GC (Agilent Technologies, Little Falls, DE, USA) with a fused–silica capillary column (SP–2560, 100 m × 0.25 mm i.d., 0.2–mm film thickness, Supelco, Bellefonte, PA, USA) and a flame–ionization detector (at 285°C) was used. The oven was set at 100°C and held for 4 min, then increased to 240°C at the rate of 3°C/min and held again for 15 min. Helium was used for carrier gas with flow rate of 0.75 mL/min in constant mode.

Selected minerals analysis

The ash from each sample was used for analysis. The contents of Na, Ca, Fe, K, P and Cu were determined using an ICP–AES (Spectro Analytical Instruments, USA) according to methods of Afshar–Mohammadian & Rahimi–Koldeh (2010).

Vitamin C, B1, B2, B3 and B6 analysis

Vitamin C (ascorbic acid) was determined by the indophenols titration method (Hewitt and Dickes, 1961). For vitamin B_1 (thiamine) and vitamin B_3 (niacin) determination, each sample (1 g) was settled to 50 mL by sodium 1–hexanesulfonate. After 30 min incubation the upper–layer was filtered. Samples were chromatographically analyzed using a YMC–Pack ODS–AM column (250 mm×4.6 mm, Waters Corporation, Milford, MA, USA) with mobile phase consisted of 5 mM sodium 1–hexanesulfonate and 5 mM sodium 1–hexanesulfonate in 50% methanol. UV detector was set at 270 nm. For vitamin B_2 (riboflavin), samples were chromatographically analyzed using a Capcellapk C18 column (250 mm× 4.6 mm, 5 μm) with mobile phase consisted of 10 mM NaH_2PO_4 (pH 5.5) and methanol (75:25, v:v). Vitamin B_2 was detected using a fluorescence detector set to $\lambda_{excitation}$ = 445 nm and $\lambda_{emission}$ = 530 nm. For vitamin B6 (pyridoxine), each sample was analyzed by HPLC with 50 mM NaH_2PO_4 (pH 2.5) as elutant. The fluorescence detector was operated at $\lambda_{excitation}$ = 290 nm and $\lambda_{emission}$ = 396 nm.

Vitamin E (α–, γ–tocopherol), provitamin A (β–carotene) and phytosterols analysis

Each barley sample (2 g) was weighted and 6% pyrogallol in ethanol (10 mL) was added as an antioxidant. 5α–cholestane (1 mg mL^{-1}) was used as internal standard for phytosterol quantification. After sonication, the mixture was saponified with 60% KOH solution at 80°C for 1 h. Then, 5–7 mL of solvent mixture (hexane: ethyl acetate, 85:15 v:v) was used to extract three times. Beta–carotene was analyzed using a HPLC system with Nova–Pak C18 column (4 μm, 3.9 mm × 150 mm, Waters, Milford, Massachusetts, Ireland) and Yonglin UV830 detector set at 450 nm. The elution solvents were A (acetonitrile: dichloromethane: methanol, 70:30:10, v:v:v) and B (acetonitrile: methanol: dichloromethane, 75:25:10) with the following gradient: 0% A hold 3.5 min, 0% to 100% A from 3.5 to 18.5 min. Keep for 10 min and decline to 0% from 28.5 to 30.0 min. α– and γ–tocopherol were analyzed using HPLC system with a Chromsep Cartridge, LiChrosorb Diol column (5 μm, 3 mm × 100 mm, Chromapack, Rartian, NJ, USA) and Yonglin UV830 detector set at 295 nm. The mobile phase was a mixture of hexane fortified with 0.1% acetic acid (1000:1 v/v). Phytosterols analysis were carried out by M600D (Yonglin, Anyang, Korea) GC equipped with a flame–ionization detector and an Ultra–2 column (5% diphenyl, 95% dimethylsiloxane, 30 m × 0.25 mm i.d., 0.2–mm film thickness, Agilent Technologies, Santa Clara, CA, USA). Nitrogen was used as a carrier gas. Oven temperature was 285°C, and the detector and injector temperature were 250 and 300°C, respectively.

Choline and Betaine

The analysis was conducted according (Graham *et al*., 2009) by ^{1}H NMR. The spectra were recorded in D_2O on a JEOL JNM–AL400 spectrometer, operating at a ^{1}H frequency of 399.65 MHz with a relaxation delay of 3 s, a spectral width of 7992.00 Hz and a mixing time of 50 ms. The sample temperature was 293 K. The chemical shifts (δ) were referred to the TSP at δ=0 ppm.

Statistical analysis

Each experimental set was compared with one–way analysis of variance (ANOVA) and Least–significant difference (LSD) ($P \leq 0.05$) using SPSS 16.0 (SPSS inc. 2007). All data were expressed as means ± standard deviations of duplicate measurements.

RESULTS AND DISCUSSION

Proximate composition and color

Table 1 shows the proximate composition and color of premature barley, mature barley, and de–hulled barley. Premature barley contained less water than the other two grain–types. Furthermore, the ash content was higher in premature and mature barley compared to hulled barley. The crude protein content (8.81–10.72 g 100 g^{-1} fresh weight (FW), which is equivalent to 10.0–12.95 g 100 g^{-1} dry weight (DW)) of all three barley grains was similar to that previously reported by Baik and Ullrich (2008), in which 10–17 g 100 g^{-1} DW was recorded. Mature barley (10.72 g 100 g^{-1} DW) had the highest crude protein content. The total dietary fiber content ranged from 13 to 19.82 g 100 g^{-1} DW in all three grain–types. The dietary fiber content of premature barley was not significantly different to that of de–hulled barley, which may partly be explained by the soft texture of premature barley. There was a significant difference in color among the three barley types. De–hulled barley was much brighter (L*: 68.46) than mature and premature barley ($P \leq 0.05$). Mature barley was more red (a*: 3.41) and yellow (b*: 32.22) in color compared to the other two types ($P \leq 0.05$).

Amino acid profile

The amino acid profiles of the three types of grain are shown in Table 2. Even though premature barley contained the lowest amount of crude protein (Table 1), it had a significantly greater total essential amino acid (EAA) content (351.75 mg g^{-1} protein) compared to the other two grain–types ($P \leq 0.05$). There was variation in the amounts of EAAs in the three grain–types, with the EAA relative contents (%) [Level of total EAAs (mg g^{-1} of protein)/sum of all measured amino acids (mg g^{-1} protein) × 100] accounting for 34.73–35.98% of total amino acids. Glutamic acid was the most abundant amino acid among the three barley grains, with the highest content being recorded in premature barley (274.36 mg g^{-1} protein). Similar values have been obtained in previous studies (Van Wijk *et al*., 1998; Mariscal–Landín *et al*., 2005). Table 2 shows that the contents of most amino acids were significantly higher in premature barley compared to mature and de–hulled barley ($P \leq 0.05$). In contrast, mature barley had the highest contents of tyrosine, cysteine, and arginine. Lysine is the limiting amino acid in most cereal grains, and in this study, premature barley was found to have the highest lysine content (41.46 mg g^{-1} protein, equivalent to 35.53 μg g^{-1} DW grain). This content is comparable to other whole grains, with 20 μg g^{-1} DW being reported for rye grain, 20 μg g^{-1} DW for wheat grain, and 18 μg g^{-1} DW for oat grain (Mustafa *et al*., 2007). Furthermore, the levels of EAAs in premature, mature, and de–hulled barley were comparable to FAO/WHO (1991) specifications. The level of total EAAs in premature barley (351.75 mg g^{-1} protein) exceeded FAO/WHO requirements (328 mg g^{-1} protein, excludes tryptophan). Thus, premature barley seemed to be superior to mature and de–hulled barley grains regarding total EAA content.

Fatty acid composition

Table 3 shows the fatty acid contents of the three grain–types. Total fatty acids ranged from 1.50 to 2.94 mg 100 g^{-1} DW. The major fatty acids of all three analyzed barley grains were palmitic (16:0, 0.41–0.73 mg 100 g^{-1} DW), oleic (18:1, 0.17–0.39 mg 100 g^{-1} DW), and linoleic

Table 1. Proximate composition (g 100 g^{-1} DW) and color of premature, mature and de–hulled barley

	Premature barley	Mature barley	De–hulled barley
Water content	10.44 ± 0.06^c	12.05 ± 0.01^a	11.36 ± 0.03^b
Ash	1.70 ± 0.09^a	1.80 ± 0.02^a	1.00 ± 0.06^b
Crude protein (g 100 g^{-1} FW)	8.81 ± 0.11^c	10.72 ± 0.16^a	9.17 ± 0.04^b
Total Dietary Fibre	13.00 ± 0.06^b	19.82 ± 0.33^a	14.52 ± 0.93^b
Color			
L*	49.73 ± 0.14^c	55.05 ± 0.67^b	68.46 ± 0.56^a
a*	–3.25 ± 0.14^c	3.41 ± 0.18^a	–0.87 ± 0.63^b
b*	25.93 ± 0.17^b	32.22 ± 0.84^a	24.59 ± 4.37^b

Values are mean ± SD of duplicate. Means in rows with different letters (a–c) are significantly different ($P \leq 0.05$).

L*: lightness/darkness; a*: redness/greenness; and b*: yellowness/blueness.

Table 2. Amino acid profile (mg $^{g-1}$ protein) of premature, mature and de–hulled barley

Amino acid	Premature barley	Mature barley	De–hulled barley	FAO/WHO (1991) requirement pattern*
Essential amino acid				
Isoleucine	36.23 ± 0.07^{a}	32.23 ± 0.32^{c}	33.85 ± 0.54^{b}	28
Leucine	79.59 ± 0.19^{a}	74.68 ± 0.57^{b}	72.62 ± 1.03^{c}	66
Lysine	41.46 ± 0.07^{a}	38.10 ± 0.29^{b}	32.73 ± 0.36^{c}	58
Methionine	15.39 ± 0.05^{a}	14.60 ± 0.07^{b}	14.05 ± 0.43^{c}	25**
Phenylalanine	49.28 ± 0.18^{b}	46.84 ± 0.3^{c}	52.12 ± 0.75^{a}	
Threonine	38.53 ± 0.06^{a}	34.61 ± 0.25^{b}	34.00 ± 0.47^{b}	34
Valine	52.78 ± 0.12^{a}	49.33 ± 0.53^{b}	47.46 ± 0.75^{c}	35
Histidine	22.39 ±0.15^{a}	21.17 ± 0.12^{b}	20.91 ± 0.37^{b}	19
Tyrosine	15.85 ± 0.05^{b}	17.20 ± 0.34^{a}	17.02 ± 0.13^{a}	63***
Total EAAs	351.75 ± 0.53^{a}	329.56 ± 2.72^{b}	323.52 ± 4.58^{b}	328****
Nonessential amino acid				
Glutamic acid	274.36 ± 1.65^{a}	241.50 ± 1.18^{c}	269.50 ± 3.36^{b}	
Proline	110.41 ± 0.92^{b}	102.84 ± 0.16^{c}	117.11 ± 1.75^{a}	
Glycine	44.56 ± 0.13^{a}	41.65 ± 0.38^{b}	38.27 ± 0.48^{c}	
Alanine	51.74 ± 0.07^{a}	40.99 ± 0.28^{b}	36.95 ± 0.2^{c}	
Cystine	10.08 ± 0.4^{b}	11.05 ± 0.32^{a}	9.81 ± 0.29^{b}	
Aspartic acid	61.64 ± 0.16^{a}	59.38 ± 0.46^{b}	55.24 ± 0.72^{c}	
Arginine	41.37 ± 0.21^{b}	45.44 ± 0.47^{a}	40.84 ± 1.17^{b}	
Serine	46.13 ± 0.21^{a}	42.72 ± 0.24^{b}	42.19 ± 0.53^{b}	
Total amino acid	993.43 ± 3.65^{a}	915.91 ± 5.45^{c}	931.61 ± 12.77^{b}	

Values are mean ± SD of duplicate. Means in rows with different letters (a–c) are significantly different ($P \leq 0.05$).
* The suggest pattern for pre–school child (2–5 years).
** Cystine + methionine.
***Tyrosine + phenylalanine.
****Total essential amino acids (mg/g protein) exclude tryptophan.

acid (18:2, 0.82–1.62 mg 100 g^{-1} DW). These results are similar to those reported by Parsons and Price (1974). Interestingly, premature barley contained the lowest amount of total fatty acids (1.5 g 100 g^{-1} DW), whereas mature barley contained the highest amount (2.94 g 100 g^{-1} DW). Even de–hulled barley contained statistically higher amounts of total fatty acids than premature barley ($P \leq 0.05$). The percentages of saturated fatty acid (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were also obtained (Table 3). Premature barley contained the highest amount of PUFA (60.1%), whereas de–hulled barley contained the highest amount of SFA (28.98%).

Phytosterols and minerals

Phytosterols are a group of bioactive plant compounds that cannot be synthesized by humans. These compounds may potentially have beneficial health effects in terms of lowering LDL cholesterol levels (Lampi *et al*., 2004). In this study, levels of the phytosterols (β–sitosterol, campesterol, and stigmasterol) were measured, with total phytosterols representing the sum of these three compounds (Table 4). β–sitosterol was the most common phytosterol in barleys, as previously found by Ryan *et al*. (2007). Mature barley had the highest total phytosterol content (61.76 mg 100 g^{-1}) among the analyzed grains. The phytosterol content of all three barley types (50.24–61.76 mg 100 g^{-1} DW) was similar to that of maize (43.6 mg 100 g^{-1} DW), millet (57.8 mg 100 g^{-1} DW), and rye (75.9 mg 100 g^{-1} DW) (Ryan *et al*., 2007).

The calcium (Ca), iron (Fe), sodium (Na), potassium (K), phosphorus (P), and copper (Cu) contents of all three grain–types were analyzed (Table 4). K and P were the major minerals present in all three barley grain–types, whereas the contents of Fe and Cu were low. For example, K accounted for nearly 62% of the total mineral content in premature barley, 47.93% in mature barley, and 48.13% in de–hulled barley. Overall, premature barley contained significantly more Na and K than mature and de–hulled barley ($P \leq 0.05$), while mature barley contained significantly more P and Fe ($P \leq 0.05$). Similar Ca and Cu contents were recorded in all three barley grains.

Vitamins

The vitamin and β–carotene contents of the three grains are presented in Fig 1. Vitamin C (0.93 mg 100 g^{-1} DW), vitamin B_2 (0.10 mg 100 g^{-1} DW), and vitamin B_3 (2.16 mg 100 g^{-1} DW) levels were higher in premature bar-

Table 3. Fatty acid composition (mg 100 g^{-1} DW) and percentage of total fatty acids ofpremature, mature and de–hulled barley

Fatty acid	Premature barley	Mature barley	De–hulled barley
Myristic acid (14:0)	ND	0.01 ± 0.01[a]	0.01 ± 0.00[a]
Palmitic acid (16:0)	0.41 ± 0.01[c]	0.73 ± 0.01[a]	0.62 ± 0.01[b]
Stearic acid (18:0)	0.02 ± 0.01[b]	0.03 ± 0.01[a]	0.03 ± 0.01[a]
Oleic acid [18:1(n–9)c]	0.15 ± 0.01[c]	0.36 ± 0.01[a]	0.24 ± 0.01[b]
Vaccenic acid [18:1(n–7)c]	0.02 ± 0.00[b]	0.03 ± 0.00[a]	0.02 ± 0.00[b]
Linoleic acid [18:2(n–6)c]	0.82 ± 0.01[c]	1.62 ± 0.03[a]	1.20 ± 0.02[b]
Arachidic acid (20:0)	ND	0.01 ± 0.00[a]	ND
Gadoleic acid (20:1)	0.01 ± 0.00[b]	0.02 ± 0.01[a]	0.02 ± 0.00[a]
α–Linolenic acid [18:3(n–3)]	0.09 ± 0.01[b]	0.14 ± 0.01[a]	0.14 ± 0.01[a]
Total FAs	1.50 ± 0.01[c]	2.94 ± 0.04[a]	2.27 ± 0.03[b]
Percentage of total FAs (%)			
Σ SFA	28.53 ± 0.14[b]	26.29 ± 0.1[c]	28.98 ± 0.04[a]
Σ MUFA	11.36 ± 0.2[c]	13.9 ± 0.12[a]	12.21 ± 0.07[b]
Σ PUFA	60.1 ± 0.06[a]	59.82 ± 0.22[a]	58.81 ± 0.03[b]

Values are mean ± SD of duplicate. Means in rows with different letters (a–c) are significantly different ($P \leq 0.05$).
ND: not detected.
Σ SFA: total saturated fatty acid; Σ MUFA: total monounsaturated fatty acids; Σ PUFA: total polyunsaturated fatty acids.

Table 4. Contents (mg 100 g^{-1} DW) of phytosterols and selected minerals of premature, mature and de–hulled barley

	Premature barley	Mature barley	De–hulled barley
Phytosterol			
β–sitosterol	40.41 ± 0.51[b]	45.65 ± 4.84[a]	45.18 ± 0.84[a]
stigmasterol	4.60 ± 1.37[c]	7.41 ± 1.80[a]	6.91 ± 1.60[b]
campesterol	5.24 ± 0.49[b]	8.70 ± 2.52[a]	7.43 ± 0.33[a]
Total phytosterols	50.24 ± 2.37[b]	61.76 ± 8.48[a]	59.51 ± 0.43[a]
Mineral			
Ca	41.50 ± 4.01[a]	41.79 ± 3.58[a]	45.93 ± 0.79[a]
Fe	3.14 ± 0.13[b]	7.11 ± 0.12[a]	3.42 ± 0.03[b]
Na	19.43 ± 0.02[a]	11.52 ± 0.06[b]	8.54 ± 0.55[c]
K	547.29 ± 5.30[a]	430.52 ± 0.13[b]	247.27 ± 0.60[c]
P	279.12 ± 7.47[b]	406.88 ± 6.57[a]	208.35 ± 2.55[c]
Cu	0.33 ± 0.01[a]	0.33 ± 0.01[a]	0.24 ± 0.00[b]

Values are mean ± SD of duplicate. Means in rows with different letters (a–c) are significantly different ($P \leq 0.05$).

ley compared to mature and de–hulled barley. Vitamin E is a well–known antioxidant, generating important lipophilic quenchers of reactive oxygen species and lipid radicals (Kamal–Eldin and Appelqvist, 1996). It has been reported that α– and γ–tocopherol are the major tocopherols in most cereal grains (Falk *et al*., 2004). In this study, γ–tocopherol was only found in mature barley (0.21 mg 100 g^{-1} DW), whereas α–tocopherol was found in all three barley grains. Significantly higher levels of α–tocopherol were recorded in mature barley (0.85 ± 0.04 mg 100 g^{-1} DW) compared to the other two grain types (premature barley: 0.34 ± 0.02 mg 100 g^{-1} DW; de–hulled barley: 0.38 ± 0.15 mg 100 g^{-1} DW; $P \leq 0.05$). There was no significant difference in α–tocopherol content between premature and de–hulled barley ($P > 0.05$). These results are consistent with those of a previous study by Falk *et al*. (2004), indicating that tocopherols accumulate in barley grain during development. Of note, β–carotene (69.79 ± 1.09 μg 100 g^{-1} DW) was only found in premature barley. Therefore, premature barley is a rich source of vitamin C and vitamin B_3 compared to mature and de–hulled barley.

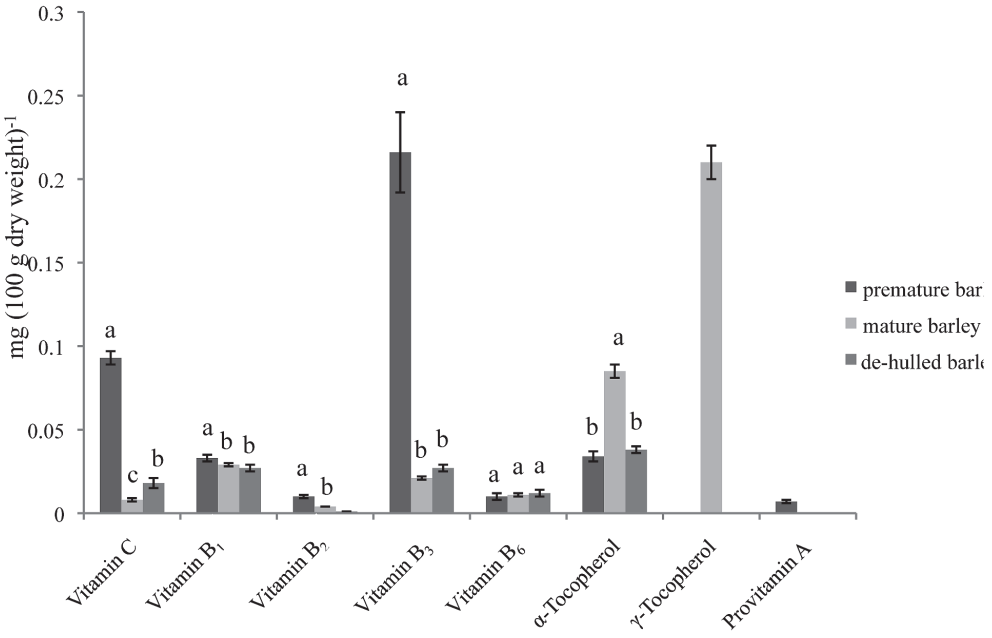

Fig. 1. The vitamins and provitamin contents of premature, mature and de–hulled barley. Different letters (a–c) on top of each column indicates significantly differences ($P \leq 0.05$).

Betaine and choline

Betaine (also known as trimethyl glycine or glycine betaine) is useful in the treatment of coronary artery disease (Vos, 2000) and fatty liver disease (Abdelmalek *et al*., 2001; Cave *et al*., 2007). Choline is structurally similar to betaine, and may be converted into betaine in vivo or metabolized into acetylcholine and phosphatidylcholine (Zeisel, 2006; Likes *et al*., 2007). The betaine and choline content of premature, mature, and de–hulled barley was quantified using ^{1}H NMR (Table 5). The highest betaine and choline contents were recorded in mature barley (49.44 and 34.61 mg 100 g^{-1} DW). Premature barley (35.04 mg 100 g^{-1} DW) contained significantly higher amounts of betaine than de–hulled barley (30.22 mg 100 g^{-1} DW) ($P \leq 0.05$). However, de–hulled barley (20.61 mg 100 g^{-1} DW) contained significantly higher levels of choline than premature barley (11.73 mg 100 g^{-1} DW) ($P \leq 0.05$). Bruce *et al*. (2010) measured total betaine and free choline in whole–grain barley using LC–MS, we obtained similar results for betaine but 60% less choline (10.97 mg 100 g^{-1} DW and 6.87 mg 100 g^{-1} DW, respectively) using ^{1}H NMR. This discrepancy may be due to the difficulty in determining total choline levels, as this compound has several forms, including choline, glycerophosphocholine, phosphocholine, and phosphatidylcholine, and ^{1}H NMR detects free and conjugated choline in a single peak (Graham *et al*., 2009). Therefore, the results indicate that a large component of choline in barley grains is present in the conjugated form.

Table 5. Contents (mg 100 g^{-1} DW) of betaine and choline of premature, mature and de–hulled barley

	Premature barley	Mature barley	De–hulled barley
Choline	11.73 ± 0.47^c	34.61 ± 1.72^a	20.61 ± 0.4^b
Betaine	35.04 ± 0.47^b	49.44 ± 0.25^a	30.22 ± 0.08^c

Values are mean ± SD of duplicate. Means in rows with different letters (a–c) are significantly different ($P \leq 0.05$).

CONCLUSIONS

The amino acid profile of premature barley is nutritionally better (particularly with respect to lysine and total EAAs) than that of mature and de–hulled barley. Premature barley also has higher contents of vitamins C, B_2, and B_3 compared to mature and de–hulled barley. Interestingly, β–carotene was found only in premature barley. In addition, higher Na and K levels were recorded in premature barley. These results indicate that premature barley could be a potentially important healthy food grain for human consumption, due to its soft texture, palatability, and beneficial nutritional composition.

ACKNOWLEDGMENTS

This research was supported by Technology Commercialization Support Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea. The authors thank Korea Food Research Institute and Joongang Institute of Life Science for analytical support.

REFERENCES

Abdelmalek M. F., P. Angulo, R. A. Jorgensen, P. B. Sylvestre and K. D. Lindor 2001 Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. *Am. J. Gastroenterol*. **96**: 2711–2717

Afshar–Mohammadian M, J. Rahimi–Koldeh 2010 The comparison of carbohydrate and mineral changes in three cultivars of kiwifruit of Northern Iran during fruit development. *Aust. J. Crop Sci*. **4**: 49–54

Baik B. K. and S. E. Ullrich 2008 Barley for food: Characteristics, improvement, and renewed interest. *J. Cereal Sci*. **48**: 233–242

Behall K. M., D. J. Scholfield and J. Hallfrisch 2004 Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. *Am. J. Clin. Nutr*. **80**: 1185–1193

Bruce S. J., P. A. Guy, S. Rezzi and A. B. Ross 2010 Quantitative measurement of betaine and free choline in plasma, cereals and cereal products by isotope dilution LC–MS/MS. *J Agric Food Chem*. **58**: 2055–2061

Cave M., I. Deaciuc, C. Mendez, Z. Song, S. Joshi–Barve, S. Barve and C. McClain 2007 Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition. *J. Nutr. Biochem*. **18**: 184

Falk J., A. Krahnstöver, T. A. van der Kooij, M. Schlensog and K. Krupinska 2004 Tocopherol and tocotrienol accumulation during development of caryopses from barley (*Hordeum vulgare* L.). *Phytochemistry*. **65**: 2977–2985

FAO/WHO 1991 Protein Quality Evaluation: Report of the Joint FAO/WHO Expert Consultation, Bethesda, Md., USA 4–8 December 1989, Food and Agriculture Organization of the United Nations

Graham S. F., J. H. Hollis, M. Migaud and R. A. Browne RA 2009 Analysis of betaine and choline contents of aleurone, bran, and flour fractions of wheat (*Triticum aestivum* L.) using 1H nuclear magnetic resonance (NMR) spectroscopy. *J. Agric. Food Chem*. **57**: 1948–1951

Helrich, K. 1990 Official Methods of Analysis, 15th edn., Association of Official Analytical Chemists Inc., Arlington

Hewitt E. and G. Dickes 1961 Spectrophotometric measurements on ascorbic acid and their use for the estimation of ascorbic acid and dehydroascorbic acid in plant tissues. *Biochem. J*. **78**: 384

Huth M., G. Dongowski, E. Gebhardt and W. Flamme 2000 Functional properties of dietary fibre enriched extrudates from barley. *J. Cereal. Sci*. **32**: 115–128

Jiang X. L., Z. Y. Deng, Z. G. Ru, P. Wu and J. C. Tian 2013 Quantitative trait loci controlling amino acid contents in wheat (*Triticum aestivum* L.). *Aust. J. Crop Sci*. **7**: 820–829

Kamal–Eldin A., L–Å. Appelqvist 1996 The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids*. **31**: 671–701

Knutsen S. and A. Holtekjølen 2007 Preparation and analysis of dietary fibre constituents in whole grain from hulled and hull–less barley. *Food Chem*. **102**: 707–715

Lampi A–M., R. A. Moreau, V. Piironen and K. B. Hicks 2004 Pearling barley and rye to produce phytosterol–rich fractions. *Lipids*. **39**: 783–787

Likes R., R. L. Madl, S. H. Zeisel and S. A. Craig 2007 The betaine and choline content of a whole wheat flour compared to other mill streams. *J. Cereal Sci*. **46**: 93

Mariscal–Landín G., J. Rodríguez and T. Reis de Souza 2005 Evaluation of hulless barley as feed ingredient in growing–finishing pigs diets: amino acid ileal digestibility. *Anim. Feed Sci. Techno*l. **120**: 169–176

Mustafa A., P. Åman, R. Andersson and A. Kamal–Eldin 2007 Analysis of free amino acids in cereal products. *Food Chem*. **105**: 317–324

Panfili G., A. Fratianni, T. D. Criscio and E. Marconi 2008 Tocol and β–glucan levels in barley varieties and in pearling by–products. *Food Chem*. **107**: 84–91

Parsons J. G. and P. B. Price 1974 Search for barley (*Hordeum vulgare* L.) with higher lipid content. *Lipids*. **9**: 804–808

Petti S. and C. Scully 2009 Polyphenols, oral health and disease: a review. *J. Dent*. **37**: 413–423

Ryan E., K. Galvin, T. O'Connor, A. Maguire and N. O'Brien 2007 Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. *Plant Foods Hum. Nutr*. **62**: 85–91

SPSS 2007 SPSS Statistics, Release 16.01. SPSS Inc., Chicago, IL, USA.

Van Wijk H., P. Moughan, S. Hodgkinson, P. Jansen and G. Pearson 1998 Variation in apparent and true ileal amino acid digestibility in barley using a rat model. *Anim. Feed Sci. Technol*. **76**: 9–22

Vos E. 2000 Whole grains and coronary heart disease. *Am. J. Clin. Nutr*. **71**: 1009–1009

Yang D., J. A. Shin, L. J. Gan, X. M. Zhu, S. T. Hong, C. K. Sung, J. W. Cho, J. H. Ku and K. T. Lee 2012 Comparison of nutritional compounds in premature green and mature yellow whole wheat in Korea. *Cereal Chem*. **89**: 284–289

Zeisel S. H. 2006 Choline: critical role during fetal development and dietary requirements in adults. *Annu. Rev. Nutr*. **26**: 229

Zhu X. M., M. A. Alim, J. N. Hu, A. Prakash, J. H. Lee and K. T. Lee 2009 Lipase–catalyzed production of solid fat containing conjugated linoleic acid in binary models. *Food Sci. Biotechnol*. **18**: 803–807