Antioxidant Effects of Water Extracts from Barley (*Hordeum vulgare* L.) Prepared under Different Roasting Temperatures

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The antioxidant effects of water extracts of roasted barley (WERB) were investigated under different roasting temperatures and compared with those of the water extracts of unroasted barley (WEUB). It was found that the Maillard reaction products increased upon increasing the roasting temperatures. Both WERB and WEUB exhibited significant antioxidant activities in linoleic acid and liposome model systems. Although WERB and WEUB afforded considerable protection against the damage of deoxyribose and proteins, the antioxidant efficiency of roasted samples was weaker than that of unroasted samples because of the reduction of antioxidant components (catechin, tocopherol, and lutein) with increasing roasting temperature. Unroasted samples were more effective in reducing power, quenching free radical, hydroxyl radical, and chelating iron than the roasted samples. The different antioxidant activity among roasted and unroasted barley samples may be partly attributed to the changes in catechin, tocopherol, and lutein contents.

Keywords: *Antioxidant effect; lipid peroxidation; barley (Hordeum vulgare L.); different roasting temperatures; oxidative damage; free radical; scavenger; catechin; tocopherol; lutein*

INTRODUCTION

Lipid peroxidation can cause deterioration of foods and make them unacceptable to consumers. Moreover, some studies (*1*) have shown that lipid peroxidation is strongly associated with aging and carcinogenisis. It is therefore necessary to suppress lipid peroxidation in food, not only to preserve the nutritive and aromatic qualities, but also to maintain the food's safety and wholesomeness. Antioxidants are defined as substances that, when present at low concentrations compared to those of an oxidizable substrate, significantly delay or prevent the oxidation of that substrate (*2*). Hence, antioxidants are widely used in food manufacturing to inhibit lipid oxidation.

Antioxidant substances, present in herbs, spices, and other plants, were extracted and systematically evaluated by Chipault et al. (*3*) and reported in 1952. Since then, many investigations have been conducted to find naturally effective antioxidants to substitute for synthetic antioxidants which have safety concerns (*4*).

Barley (*Hordeum vulgare* L.) is one of the world's most important crops. Not only is it a source of starch, the grains themselves also provide an excellent source of energy. Therefore, barley is used extensively in industrial commodity and fermentation products. As for the investigations of antioxidant activity of barely, analysis of its antioxidant components has been reported (*5*). Fermented barley was shown to scavenge superoxide anion radical (*6*). Furthermore, 2″(3″)-*o*-glycosylisovitexin, an antioxidative component, was identified and exists in barley leaves (*7*). In China, roasted barley has been used daily in beverages for centuries. Although barley and fermented barley have been proven to exhibit antioxidant activities (*5*, *6*), no attempt has been undertaken to study the antioxidant activity of roasted barley. In addition, the effects of roasting temperature on the antioxidant activity of barley are not known.

The purpose of the present study was to determine the antioxidant activity of water extracts of barley prepared under different roasting temperatures (unroasted and roasted) and to elucidate the mechanism of antioxidative action.

MATERIALS AND METHODS

Materials. The two-rowed barley (*Hordeum vulgare* L.) whose cultivar is Varley was donated by Yeuan-Yuou Enterprise Co., Ltd., Taiwan, Republic of China. Linoleic acid, ammonium thiocyanate, ferrous chloride, and butylated hydroxyanisole (BHA) were purchased from E. Merck (Darmstadt, Germany). 5,5′-Dimethyl-1-pyrroline *N*-oxide (DMPO) and protease (from *Streptomyces griseus*) were obtained from Sigma Chemical Co (St. Louis, MO). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Chemie AG (Switzerland).

Sample Preparation. To obtain barley with different roasting temperatures, the barley was unroasted or roasted at 434, 446, 458, and 465 °C (internal temperature) for 48 s in air to remove the moisture of the barley using an automatic roasting machine (named machine I). The dried samples conveyed from machine I were roasted again at 327, 332, 335, and 341 °C (internal temperature) for 60 s in air using an automatic roasting machine (named machine II). Each unroasted and roasted sample (25 g) was extracted with boiling water (300 mL) for 10 min, and the filtrate was freeze-dried. The water extracts of unroasted barley were named WEUB, and the water extracts of roasted barley prepared under different temperatures were named WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C), respectively.

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Determination of Color Value. Color values L, a, and b of unroasted barley and barley prepared under different roasting temperature were measured with a color difference meter (Model JC 801, Color Techno System Co., Ltd., Tokyo, Japan). The system provides the values of three color components: L (black−white component, luminosity), and the chromaticness coordinates, a (+red to −green component) and b (+yellow to −blue component). The instrument was standardized against beige ceramic tile. The sample was held in a cuvette with an optical glass bottom for reading the Hunter "L", "a", and "b" values, and each reported reading is the average of three replicate analyses.

Determination of Nonenzymatic Browning Index. The browning index of each sample was carried out by the method of Palombo et al. (*8*). The dried barley sample (100 mg) was mixed thoroughly with 1 mL of deionized water. A 0.2-mL aliquot of the mixture was transferred to a test tube which contained 0.2 mL of protease solution (20 mg protease/mL buffer tris, pH 7.0, with 50 mM $CaCl_2$). The test tubes were placed on a rack and incubated for 2 h at 45 °C in a water bath. The rack was cooled in ice water and 0.3 mL of trichloroacetic acid (100% TCA) was added to each tube. Centrifugation (20 min at 7000 rpm) and filtration were used for clarification. The optical density of the clear filtrates was determined by a spectrophotometer (Hitachi 2000, Japan). Samples were read in a 1 mL cuvette with 1 cm path length. Browning index, *OD*, was calculated as

$$OD = A_{420\ nm} - A_{550\ nm}$$

Sugar Analysis. Extracts (0.5 g) were added to the solvent acetonitrile/water (75: 25, v/v). The resulting mixture was filtered through a 0.45-μm Millipore filter before use. Filtrate (20 μL) was injected into the HPLC system (Hitachi, Japan). A Hypersil NH_2 column (5 mm × 250 mm, 5 μm) and RI detector (L-7490, Hitachi, Japan) were used to separate the sugar compositions. Isocratic separations were achieved with the mobile phase (acetonitrile/water, 75: 25, v/v). The flow rate was 1.2 mL/min. Measurement of peak area was used to quantify the sugar composition in each sample, and all quantitative determinations were made in duplicate.

Determination of Free Amino Acid. The determination of free amino acid in extracts was according to the method of Masuda et al. (*9*). Extract (0.1 g) was added to 30 mL of ethanol (70%, 60 °C). The mixture was homogenized and centrifuged (10 min at 14 000 rpm). The upper layer was concentrated and evaporated to dryness in vacuo, and the residue was dissolved in 5 mL of distilled water. The resultant was mixed with 20 mL of ether. After thoroughly stirring, the hydrophilic phase was evaporated to dryness in vacuo, followed by the addition of 3 mL of citrate buffer (0.1 M, pH 2.2), and finally quantified to 4 mL. Filtration was done to avoid contamination of the analyzer columns. The free amino acids were analyzed with a Beckman model 6300 analyzer. The free amino acids from each sample were compared with amino acid standards run under the same experimental conditions.

Antioxidant Activity in a Linoleic Acid System. Antioxidant activity assay was carried out by using the linoleic acid system. Linoleic acid emulsion (0.02 M) was prepared with linoleic acid (0.2804 mg) and Tween 20 (0.2804 mg) in phosphate buffer (50 mL, 0.05 M, pH 7.4). A reaction solution containing each extract (0.2 mL, 5.0 mg/mL), linoleic acid emulsion (2.5 mL), and phosphate buffer (2.3 mL, 0.2 M, pH 7.0) was mixed with a homogenizer. The reaction mixture was incubated at 37 °C in dark, and the degree of oxidation was measured according to the thiocyanate method (*10*), by sequentially adding ethanol (4.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), and sample solution (0.1 mL). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm, and the inhibition percent of linoleic acid peroxidation was calculated as (%) inhibition = [1 − (absorbance of sample at 500 nm)/(absorbance of control at 500 nm)] × 100. All tests were run in duplicate, and analysis of all samples was run in triplicate and averaged.

Determination of Antioxidant Effect on Liposome Oxidation. Lecithin (580 mg) was sonicated in an ultrasonic cleaner (Branson 8210, Branson Ultrasonic Corporation, Danbury, CT) in 58 mL of 10 mM phosphate buffer (pH 7.4) for 2 h. The sonicated solution (10 mg lecithin/mL), $FeCl_3$, ascorbic acid, and each extract (0.2 mL, 5 and 50 mg/mL) were mixed to produce a final concentration of 3.12 μM $FeCl_3$, and 125 μM ascorbic acid. The mixture was incubated for 1 h at 37 °C by the thiobarbituric acid (TBA) method (*11*). The absorbance of the sample was read at 532 nm against a blank which contained all reagents except lecithin.

Determination of the Effects on Oxidation of Deoxyribose. The determination was carried out as described by Halliwell et al. (*12*). The reaction mixture (3.5 mL), which contained each extract (0.2 mL, 5 and 50 mg/mL), deoxyribose (6 mM), H_2O_2 (3 mM), KH_2PO_4− K_2HPO_4 buffer (20 mM, pH 7.4), $FeCl_3$(400 μM), ethylenediaminetetraacetic acid (EDTA; 400 nM), and ascorbic acid (400 nM) was incubated at 37 °C for 1 h. The extent of deoxyribose degradation was tested by the TBA method. 1 mL of 1% TBA and 1 mL of 2.8% trichloroacetic acid (TCA) were added to the mixture, which was then heated in a water-bath at 90°C for 20 min. The absorbance of the mixture was read spectrophotometrically at 532 nm. All analyses were run in three replicates and averaged.

Determination of the Effects on Protein Oxidation. The effects of antioxidants on protein oxidation were carried out according to the method of Lenz et al.(*13*). The reaction mixture (1.2 mL), containing each extract (0.2 mL, 0.5 and 5 mg/mL), phosphate buffer (20 mM, pH 7.4), bovine serum albumin (20 mg/mL), $FeCl_3$(100 μm), H_2O_2 (2.0 mM), and ascorbic acid (200 μM), was incubated for 1 h at 37 °C and 1 mL of 20 mM in 2 M HCl was added to the reaction mixture. Cold TCA (1 mL, 20%, w/v) was added to the mixture and centrifuged at 3000*g* for 10 min. The protein was washed three times with 2 mL of ethanol−ethyl acetate (1: 1, v/v) and dissolved in 2 mL of 6 M guanidine−HCl (pH 6.5). The absorbance of the samples was read at 370 nm. Triplicate samples were run for each set.

Reducing Power. The reducing power of the extracts was determined according to the method of Oyaizu (*14*). Extracts (0−10 mg) in phosphate buffer (2.5 mL, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 mL, 10 mg/mL), and the mixture was incubated at 50 °C for 20 min. TCA (2.5 mL, 100.0 mg/mL) was added to the mixture, which was then centrifuged at 650*g* for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 1.0 mg/mL), and then the absorbance was read spectrophotometrically at 700 nm. Higher absorbances of the reaction mixture indicated greater reducing powers.

Determination of Effect on DPPH Radical. The effect of extracts on DPPH radical was estimated according to the method of Hatano et al. (*15*). The extracts were added to a methanolic solution (0.5 mL) of DPPH radical (final concentration of DPPH was 0.2 mmol/L). The mixture was shaken vigorously and left standing at room temperature for 30 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm. The tests were run in duplicate, and analyses of all samples were run in triplicate and averaged.

Measurement of Chelating Activity on Metal Ions. The chelating activity of sample on Fe^{2+} was measured according to the method of Carter (*16*). Briefly, each extract was incubated with 0.05 mL of $FeCl_24H_2O$ (2.0 mM). The reaction mixture was initiated by the addition of 0.2 mL ferrozine (5.0 mM), and finally quantified to 0.8 mL with methanol. After the mixture had reached equilibrium (10 min), the absorbance at 562 nm was read. EDTA served as the positive control, and an untreated sample served as the negative control. Triplicate samples were run for each set and averaged.

Scavenging Effect on the Hydroxyl Radical. The hydroxyl radical generated reacted rapidly with nitrone spin-trap DMPO. The resultant DMPO−OH adduct was detected by means of electron paramagnetic resonance (EPR) spectrometry using an EPR spectrometer (Bruker EMZ-10/12,

Karlsruhe, Germany) set under specific conditions: with a magnetic field of 3483 ± 100 G, modulation amplitude of 1.0 G, scan time of 200 s, and a time constant of 0.5 s. The EPR spectrum was recorded 2.5 min after 0.5 mL of sample (1 mg of extracts) was mixed with H_2O_2 (10.0 mM, 0.2 mL), Fe^{2+} (10.0 mM, 0.2 mL), and DMPO (0.3 M, 0.2 mL) in phosphate buffer (0.2 M, pH 7.2) solution (*17*). A smaller peak of a sample indicated a higher scavenging activity on the hydroxyl radical. The data were the mean value of duplicates.

Separation and Quantification of Catechin, Lutein, and Tocopherol. Extraction and purification of catechin, carotenoid, and tocopherol in barley was carried out according to the procedure described by Goupy et al. (*15*). In brief, 30 g of barley powder (unroasted and roasted at different temperatures) was homogenized in 100 mL of acetone (70%) containing 0.5% sodium metabisulfite for 1 min. Three successive extractions with aqueous acetone (70%) were employed at 4 °C for 30 min. After removing acetone under vacuum at 35 °C, carotenoids were separated by three successive extractions with petroleum ether (2: 1, v/v). The three organic phases were combined, filtered, and evaporated in vacuo. The residue was dissolved in 3 mL of methanol. Methanol extracts called CT extracts were used for analysis of carotenoid and tocopherol. Ammonium sulfate (20%) and metaphosphoric acid (2%) were added to the aqueous phase. Phenolic compounds were then extracted with ethyl acetate (1: 1, v/v) three times. The three organic phases were combined, filtered, and evaporated in vacuo. The residue was dissolved in 3 mL of methanol, and the extracts, called phenolic extracts, were used for analysis of catechin. The catechin in phenolic extracts was determined by HPLC performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo) consisting of a model L-7100 pump, and a model L-7420 UV−vis detector set at 280 nm. A hypersil BDS RP-18 reversed-phase column (5 μm, 250 × 4.6 mm, i.d. Hypersil) was used for analysis. The volume injected was 20 μL. The mobile phase was acetonitrile/ethyl acetate/phosphoric acid (0.1%) (0.85: 0.2: 8.95, v/v/v). The flow rate was 1 mL/min.

Analysis of the carotenoid in CT extracts was performed by HPLC system. The analytical apparatus was the same as that used for catechin separation. Carotenoids were separated along a hypersil BDS RP-18 reversed-phase column with the isocratic mobile phase methanol/ acetonitrile/ethyl acetate (75:15:10, v/v/v), and the flow rate was 1.0 mL/min. Carotenoid was determined by external standard calibration at 450 nm.

Separation of tocopherols contained in CT extracts was performed along a hypersil BDS RP-18 reversed-phase column (5 μm, 250 × 4.6 mm, i.d. Hypersil) with the isocratic mobile phase methanol (100%), and the flow rate was 1 mL/min. Peaks have been identified by comparison of retention time and spectra with standards at 290 nm.

Statistical Analysis. Statistical analysis involved use of the Statistical Analysis System (*18*) software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests (*19*), at a level of $P < 0.05$.

RESULTS AND DISCUSSION

For drinks prepared from roasted barley, it is very important to produce the desired aroma and taste by the optimum roasting process to satisfy different levels of consumers. Thus, it is necessary to evaluate the antioxidant activity of barley roasted under different temperatures.

Color Difference of Barley Prepared under Different Roasting Temperatures. Table 1 shows the color difference of barley powder prepared under different roasting temperatures. The color changed with different roasting temperatures. Barley roasted at 341 °C produced a dark brown color (Hunter *L* value as well as *a* and *b* values) are lower. The color unit value *a* of barley increased with roasting temperature up to 332 °C, and then decreased. However, the values of *L* and *b* decreased with increasing the roasting temperature. These color differences of barley resulted from different roasting temperatures. In the model tested, the color of the sample prepared under different roasting temperature changed from light brown to brown and finally to dark brown.

Table 1. Color Difference of Barley Prepared under Different Roasting Temperatures[b,c]

sample[a]	*L*	*a*	*b*
unroasted	53.98 ± 1.70[a]	1.69 ± 0.09[c]	21.05 ± 0.73[a]
roasted			
327 °C	36.29 ± 5.58[b]	2.34 ± 0.66[b]	14.68 ± 1.22[b]
332 °C	28.21 ± 1.15[c]	2.79 ± 0.58[a]	11.58 ± 0.76[c]
335 °C	20.33 ± 0.45[d]	1.21 ± 0.64[d]	5.15 ± 0.53[d]
341 °C	18.30 ± 0.21[e]	0.71 ± 0.11[e]	2.70 ± 0.61[e]

[a] Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively. [b] Values are means ± standard deviation of three replicate analyses. [c] Means with different superscript letters in the column are significantly different ($P < 0.05$).

Table 2. Nonenzymatic Browning of Barley Prepared under Different Roasting Temperatures

sample[a]	browning index[b,c] (*OD*/0.1 g of barley)
unroasted	0.048 ± 0.001[e]
roasted	
327 °C	0.230 ± 0.003[d]
332 °C	0.330 ± 0.002[c]
335 °C	0.730 ± 0.002[b]
341 °C	0.776 ± 0.001[a]

[a] Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively. [b] Values are means ± standard deviation of three replicate analyses. [c] Means with different superscript letters in the column are significantly different ($P < 0.05$).

Table 3. Sugar Analysis of Water Extracts of Unroasted Barley (WEUB) and Water Extracts of Roasted Barley (WERB) (μg/g of Extract)

		roasted[a]			
sugar	unroasted	327 °C	332 °C	335 °C	341 °C
glucose	98.87	60.33	40.46	33.42	32.63
xylose	163.36	150.43	141.59	58.81	50.27
sucrose	139.82	0	0	0	0
lactose	29.94	0	0	0	0
fructose	349.01	335.52	286.73	203.78	0
malactose	302.73	23.49	11.15	0	0
mannos	246.18	117.96	92.62	73.62	23.49
rabinose	159.44	99.32	13.24	11.15	0
arabinose	636.64	270.54	119.32	118.44	41.61
galactose	561.08	510.33	492.27	113.02	49.25

[a] Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively.

Browning Index of Barley Prepared under Different Roasting Temperatures. The nonenzymatic browning of barley prepared under different roasting temperatures is presented in Table 2. Significant differences ($P < 0.05$) in browning index were found among the samples tested. The higher the roasting temperature, the greater is the browning index. This result revealed that the color formation in barley was dependent on the roasting temperature.

Sugar Content of Barley Prepared under Different Roasting Temperatures. Table 3 shows the sugar analysis of WEUB and WERB prepared under different roasting temperatures. The amounts of sugar in extracts decreased with the increase in the roasting temperature. According to the results shown in Table 3, arabinose (636.64 μg/g of extract) is the major sugar in the WEUB followed by galactose (561.08 μg/g of

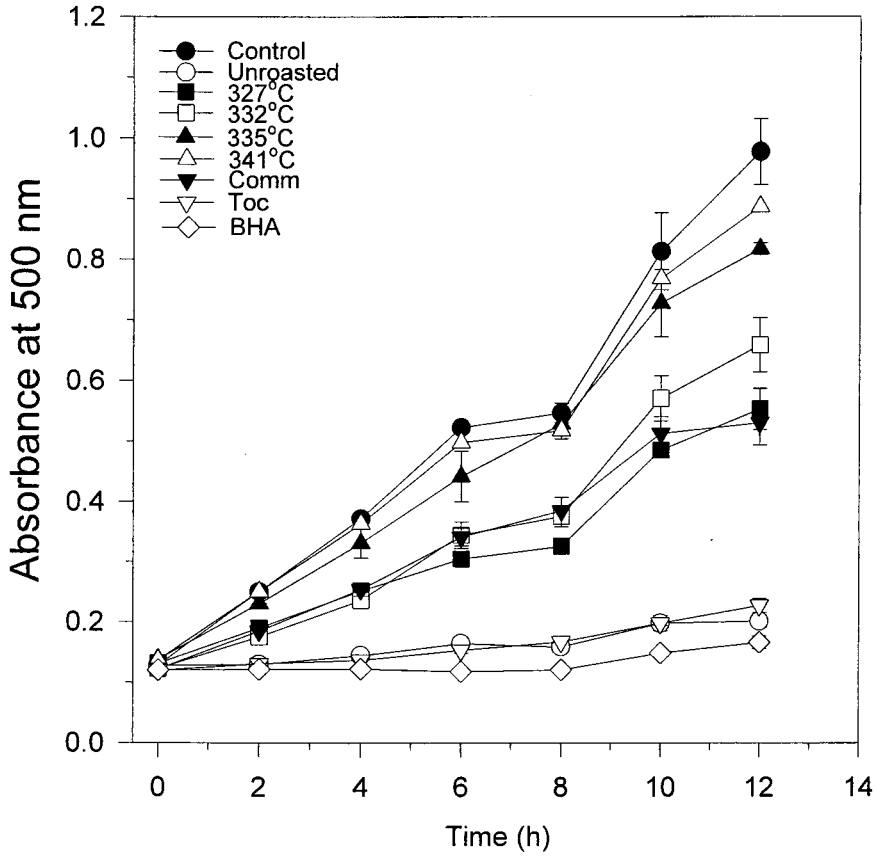

Figure 1. Effect of water extracts of unroasted barley (WEUB) and water extracts of roasted barley (WERB) on oxidative stability of linoleic acid. Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively. Comm: commercial product; Toc: tocopherol; BHA: butylated hydroxyanisole.

extract). After roasting, the amounts of arabinose and galactose in WERB (341 °C) were 41.61 and 49.25 μg/g of the extract. In other words, arabinose and galactose in the WEUB were lost by 93.5% and 91.2%, respectively, due to higher roasting temperature. Moreover, sucrose and lactose of WEUB were depleted after roasting.

Amino Acid of Barley Prepared under Different Roasting Temperatures. Table 4 presents the amino acid compositions of extracts. As compared to WEUB, the amount of amino acid in WERB decreased with the increase in the degree of roasting. The higher the roasting temperature, the greater is the decrease in amino acid. Furthermore, the methionine in WERB (332 °C), WERB (335 °C), and WERB (341 °C) was completely depleted.

As shown in Tables 3 and 4, the decrease in the contents of amino acid and sugar of roasted barley may be attributed to the formation of Maillard reaction products (browning substances). This may also explain why the nonenzymatic index increased with the increase in roasting temperature (Table 2).

Effect of Extracts on the Oxidation of Linoleic Acid. The effects of extracts on the oxidation of linoleic acid, determined by the thiocyanate method, are plotted in Figure 1. The autoxidation of linoleic acid without adding extracts and antioxidants was accompanied by a rapid increase of peroxide value. Significant differences ($P < 0.05$) in peroxide value were found between the control and the linoleic acid containing extracts (1 mg) or antioxidants (1 mg), which slowed the rate of peroxide formation. WEUB showed stronger antioxidant activity, and no significant difference ($P > 0.05$) was found among WEUB, BHA, and Toc. Although the antioxidant activity of WERB decreased with increasing the roasting temperature, WERB showed inhibitory effect on linoleic acid peroxidation. However, the higher the roasting temperature in the present model tested, the less is the antioxidant activity of the WERB. From the results obtained, WEUB and WERB exhibited marked inhibitory effect in the peroxidation of linoleic

Table 4. Free Amino Acid Analysis of Water Extracts of Unroasted Barley (WEUB) and Water Extracts of Roasted Barley (WERB) (nmol/50 μL)

amino acid	unroasted	roasted[a] 327 °C	332 °C	335 °C	341 °C
Asp	1.6731	0.8335	0.4268	0.2130	0.1337
Ser	0.6063	0.2131	0.0570	0.0593	0.0229
Thr	3.5608	1.0056	0.4977	0.4349	0.1739
Glu	5.3154	0.5058	0.2124	0.2205	0.1349
Pro	3.9774	0.4228	0.2182	0.2248	0.1082
Gly	0.8968	0.9949	0.5901	0.5085	0.3863
Ala	3.8237	2.1439	1.0700	0.5793	0.4897
Cys	0.0091	0.0072	0.0035	0.0033	0.0036
Val	0.9174	0.1775	0.0388	0.0446	0.0352
Met	0.0210	0.0105	0	0	0
Ile	0.4008	0.1008	0.0567	0.0643	0.0353
Leu	0.5816	0.1229	0.0811	0.1050	0.0552
Tyr	0.1968	0.0694	0.0382	0.0366	0.0172
Phe	0.4482	0.0828	0.0465	0.0537	0.0227
His	0.3065	0.3100	0.1386	0.1347	0.1138
Lys	0.3361	0.1743	0.0976	0.0807	0.0237
Trp	0.4420	0.1517	0.0600	0.0235	0.0220
Arg	0.5218	0.2882	0.1152	0.0529	0.0338[†]

[a] Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively.

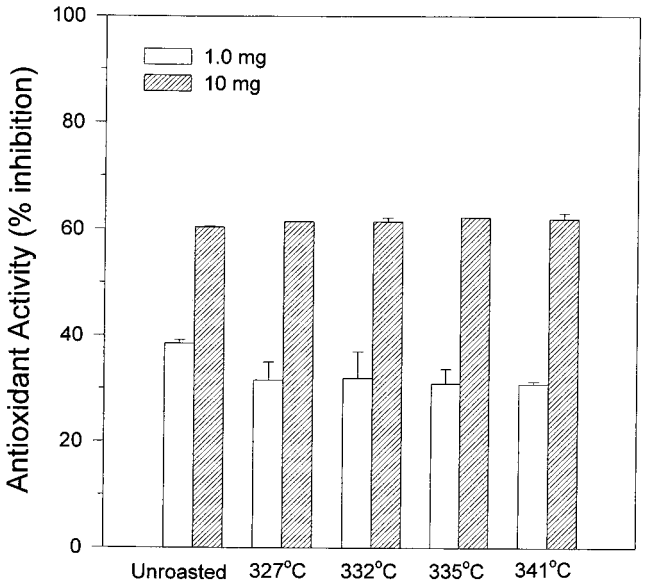

Figure 2. Effect of water extracts of unroasted barley (WEUB) and water extracts of roasted barley (WERB) on liposome peroxidation. Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively.

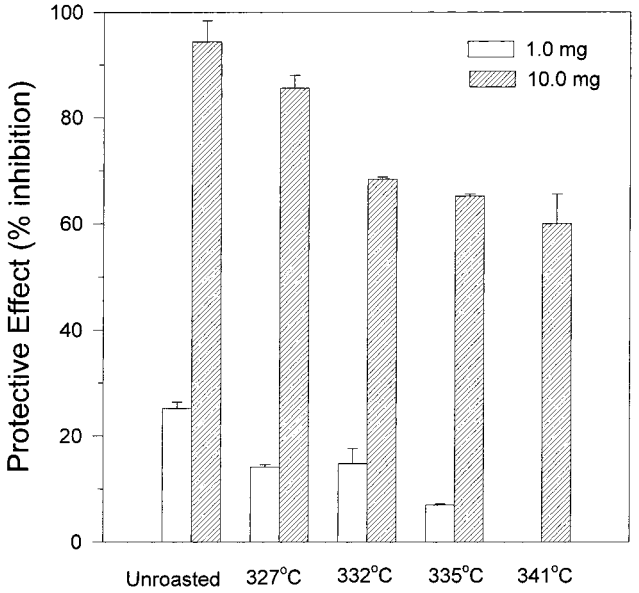

Figure 3. Effect of water extracts of unroasted barley (WEUB) and water extracts of roasted barley (WERB) on deoxyribose oxidative damage. Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively.

acid, however, the antioxidant activity of WEUB was greater than that of WERB.

Effect of Extracts on the Oxidation of Liposome. Linoleic acid was considered not to reflect complete lipid peroxidation, because of its unique physical properties in aqueous micelles (*20*). Phospholipid is generally thought to be the major fraction responsible for the oxidative deterioration and off-flavor development of foods because of its greater degree of unsaturation (*21*). Hence, phospholipid may be regarded as a better substrate for evaluating the antioxidant activity in lipid food systems. Fe^{3+}-ascorbate-dependent nonenzymatic lipid peroxidation in a phospholipid model system was assessed by estimating the formation of thiobarbituric acid reaction substances (TBARS) (Figure 2). All the samples at 1.0 mg, WEUB, WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C) showed 38.5, 31.6, 32.0, 31.0, and 30.9% inhibition of phospholipid peroxidation, respectively, indicating that WEUB showed a more significant ($P < 0.05$) inhibitory effect in liposome peroxidation than that of WERB. However, all the samples at 10 mg showed 60.4, 61.5, 61.5, 62.3, and 62.0% inhibition of phospholipid peroxidation for WEUB, WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C), respectively, and no significant difference ($P > 0.05$) was found among the samples tested.

As shown in Figure 2, barley with or without roasting generated significantly ($P < 0.05$) lower TBARS formation when compared with the control. This result implies that WEUB and WERB may protect against damage to cell membrane because they reduce the level of lecithin peroxides. Frankel et al. (*22*) reported that the antioxidant activity of natural antioxidants is more system dependent, and that a wide range of activities can be observed according to the lipid systems used as substrates. In the present model system, WEUB showed significant inhibitory effect in the formation of hydroperoxide (Figure 1) and the formation of TBARS (Figure 2) than that of WERB. In addition, WEUB and WERB can inhibit the peroxidation of linoleic acid and lecithin model systems.

Effect of Extracts on the Oxidation of Deoxyribose. Many antioxidants that protect lipids could accelerate oxidative damage to other food constituents such as carbohydrates or proteins, with the potential production of cytotoxic products (*23*). Therefore, to study barley in detail with or without roasting in biological systems, deoxyribose and bovine serum albumin were used as the substrates to evaluate barley with or without roasting against the inhibitory activity of nonlipids in cell membranes.

The effects of WEUB and WERB on deoxyribose damage induced by Fe^{3+}/H_2O_2, is plotted in Figure 3. All the samples at 10 mg showed 94.4, 85.6, 68.4, 65.2, and 60.0% inhibition of deoxyribose oxidative damage for WEUB, WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C), respectively. Obviously, WEUB at 10 mg showed almost a complete inhibitory effect (94.4%) on the oxidation of deoxyribose. Regarding the effect of WERB on oxidation of deoxyribose, WERB (327 °C) showed the greater protective effect (85.6%) from oxidative damage of deoxyribose, followed by WERB (332 °C), WERB (335 °C), and WERB (341 °C). WEUB at 1.0 mg exhibited a more significant ($P < 0.05$) inhibitory effect (25.2%) in the deoxyribose damage than that of WERB. As shown in Figure 3, no significant difference ($P > 0.05$) in the protective effect of deoxyribose oxidation was found between WERB (327 °C) and WERB (332 °C), however, no protective effect was found in WERB (341 °C). This result revealed that the higher the roasting temperature, the less the inhibitory effect of barley on deoxyribose oxidation.

Effect of Extracts on the Oxidation of Albumin. Stadman (*24*) reported that some amino acid residues are oxidized to carbonyl derivatives; consequently, it becomes evident that the carbonyl content of protein could be used as a measure of protein damage. The protective effect of barley (with or without roasting) on protein carbonyl formation in albumin, induced by $FeCl_3$, H_2O_2, and ascorbic acid, is plotted in Figure 4. All the samples at 1.0 mg exhibited a protective effect on protein carbonyl formation. The order (=, equal to; >, higher than) of protective effect of extracts was

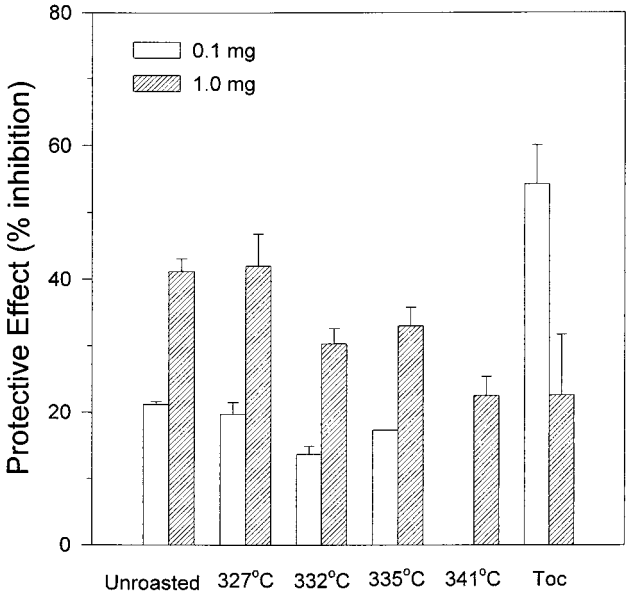

Figure 4. Effect of water extracts of unroasted barley (WEUB) and water extracts of roasted barley (WERB) on protein oxidative damage. Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively.

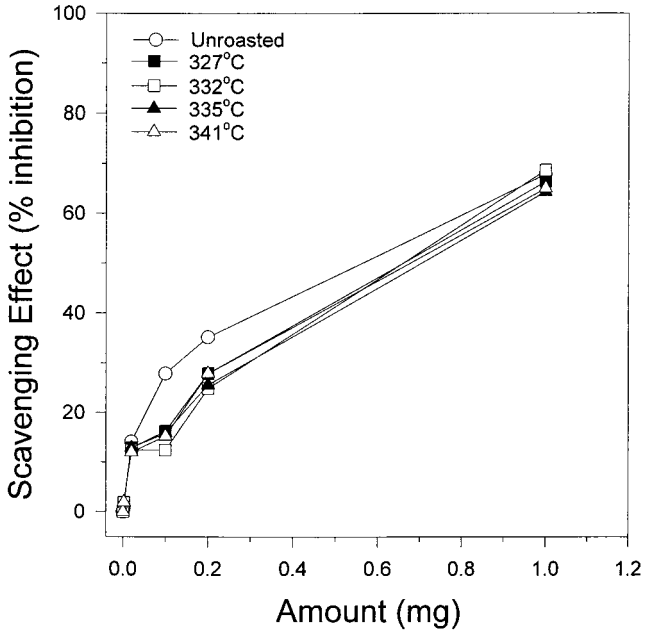

Figure 5. Scavenging effect of water extracts of unroasted barley (WEUB) and water extracts of roasted barley (WERB) on DPPH radical. Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively.

WEUB = WERB (327 °C) >WERB (332 °C) = WERB (335 °C) > WERB (341 °C). However, 0.1 mg WERB (341 °C) showed no inhibitory effect in the protein oxidation. In addition, at a dose of 1.0 mg of samples, significant differences ($P < 0.05$) were found among Toc (22.6%), WEUB (41.2%), WERB (327 °C) (42.0%), WERB (332 °C) (30.3%), and WERB (335 °C) (33.0%) in the inhibition of protein oxidation. No significant difference ($P > 0.05$) was found between 1.0 mg WERB (341 °C) (22.5%) and 1.0 mg Toc (22.6%). However, the activity of 1.0 mg Toc (22.6%) in inhibition of protein oxidation was less than that of 0.1 mg Toc (54.3%). This result is in good agreement with the report of Duh et al. (*25*) who showed that the effect of Toc on the inhibition of protein oxidation increased with increasing amounts up to 0.1 mg, and then decreased with increasing amounts.

Reducing Power. The reducing power of extracts was measured and was found to increase with increasing amounts of extracts. The reducing power (absorbance at 700 nm) of WEUB, WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C) at a dose of 1.0 mg, was 0.27 ± 0.001, 0.205 ± 0.007, 0.169 ± 0.003, 0.158 ± 0.002, and 0.148 ± 0.006, respectively. The largest reducing power was observed in the WEUB relative to other samples and significant differences ($P < 0.05$) in reducing power were found among the samples tested. Although the reducing power of WERB decreased with higher roasting temperature, WERB prepared under different roasting temperatures still retained considerable reducing power. Some reports (*17*) noted that antioxidative effect is concomitant with the development of reducing power. Therefore, the significant antioxidant activity of WEUB and WERB may be related to their reducing power.

Scavenging Effect of Extracts on DPPH Radical. DPPH• is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (*26*). The reduction in DPPH• was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Hence, DPPH• is usually used as a substrate to evaluate antioxidative activity of antioxidants (*17*). Figure 5 illustrates the scavenging effect of WEUB and WERB on DPPH radical. Samples at 0.2 mg show 35.2 ± 2.5, 27.9 ± 0.9, 24.9 ± 0.1, 25.6 ± 0.92, and 16.7 ± 2.1% scavenging effect on DPPH radical for WEUB, WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C), respectively, indicating that WEUB showed the greatest antiradical activity; however, no significant differences ($P > 0.05$) in scavenging effect on DPPH radical were found between WEUB and WERB ($P > 0.05$) at a dose of 1.0 mg. These results show that WEUB and WERB are free radical inhibitors. From the results shown in Figure 5, it can be seen that WEUB and WERB have effective activities as hydrogen donors and as primary antioxidants by reacting with the lipid radical. This may be responsible for the main cause of suppression of autoxidation, in both lipids and nonlipids model systems.

Chelating Effect of Extracts on Ferrous Ions. Figure 6 shows the chelating effect of WEUB and WERB on ferrous ions. The chelating effect increased with increasing amounts of WEUB, WERB (327 °C), and WERB (332 °C), respectively. As for the WERB (335 °C) and WERB (341 °C), the chelating effect increased with increasing amounts of samples up to 0.2 mg, and then decreased. All the samples at 1.0 mg showed 77.4 ± 3.4, 77.9 ± 2.3, 70.6 ± 3.0, 20.8 ± 3.1, and 12.3 ± 2.1% chelating effect on ferrous ions for WEUB, WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C), respectively, indicating that the higher roasting temperature results in the lower chelating effect on ferrous ions. Iron is essential for life because it is required for oxygen transport, respiration, and the activity of many enzymes. However, iron is an extremely reactive metal and will catalyze oxidative changes in lipids, proteins, and other cellular components (*27*). In addition, the liposome peroxidation, and both oxidative damage of deoxyribose and protein model systems, were induced by a Fenton reaction. Smith et al. (*28*) noted that the molecules that inhibit deoxyribose degradation are those that can chelate the iron ions from the

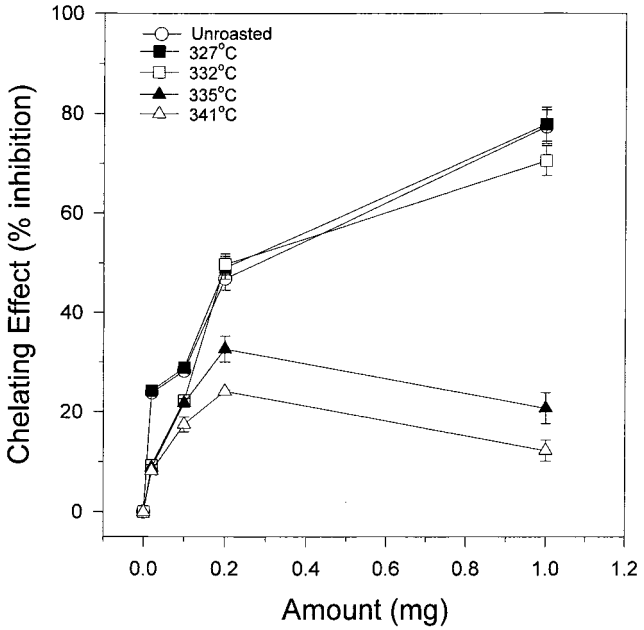

Figure 6. Chelating effect of water extracts of unroasted barley (WEUB) and water extracts of roasted barley (WERB) on iron ion. Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively.

Table 5. Scavenging Effect of Water Extracts of Unroasted Barley (WEUB) and Water Extracts of Roasted Barley (WERB) on Hydroxyl Radical

sample[a]	scavenging effect (%)[b,c]
unroasted	67.5 ± 0.07[a]
roasted	
327 °C	65.7 ± 0.05[b]
332 °C	64.5 ± 0.07[c]
335 °C	58.1 ± 0.00[d]
341 °C	45.5 ± 0.07[e]

[a] Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively. [b] Values are means ± standard deviation of three replicate analyses. [c] Means with different superscript letters in the column are significantly different ($P < 0.05$).

deoxyribose and render them inactive or poorly active in a Fenton reaction. As shown in Figure 6, WEUB and WERB exhibited chelating effect on ferrous ions, suggesting that they minimize the concentration of metal in the Fenton reaction. Consequently, WEUB and WERB as liposome-, deoxyribose-, or protein-protectors may be partially related to iron interaction.

Scavenging Effect of Extracts on Hydroxyl Radical. Table 5 shows the scavenging effect of extracts on hydroxyl radical. All the samples at 1.0 mg scavenged 67.5, 65.7, 64.5, 58.1, and 45.5% of hydroxyl radical for WEUB, WERB (327 °C), WERB (332 °C), WERB (335 °C), and WERB (341 °C), respectively, and a significant difference ($P < 0.05$) in scavenging effect was observed among the samples. Apparently, the activity to scavenge hydroxyl radical by extracts depends on the roasting temperature. The hydroxyl radical is an extremely reactive free radical formed in biological systems and reacts rapidly with molecules of almost every type found in living cells, such as sugars, amino acids, phospholipids, DNA bases, and organic acid (*29*). In addition, the hydroxyl radical is known to be capable of abstracting hydrogen atoms from membrane lipids resulting in peroxidic reaction of lipids. Gutteridge (*30*) noted that the attack of hydroxyl radical on deoxyribose produces the toxic aldehyde malondialdehyde, which is very reactive and takes part in cross-linking with DNA and protein, and also damages liver cells (*31*). Zhao and Jung (*32*) reported that any hydroxyl radical scavenger added to the reaction mixture would compete with deoxyribose for hydroxyl radical to an extent, depending on its rate constant for reaction with hydroxyl radical and its concentration relative to deoxyribose. Apparently, the rate constant of barley for reaction with hydroxyl radical was greater than that of deoxyribose; therefore WEUB and WERB can protect deoxyribose from degradation. Stadtman (*24*) reported that all amino acid residues of a protein are susceptible to oxidative damage by hydroxyl radicals. According to the data obtained for this paper, inhibition of oxidative damage of proteins was related to the scavenging of hydroxyl radicals. In addition, the ability of WEUB and WERB to scavenge hydroxyl radical seems to relate directly to the prevention of propagation of the process of liposome peroxidation. Therefore, WEUB and WERB as inhibitors of lipid peroxidation and nonlipids oxidative damage may be attributed to the scavenging of hydroxyl radicals. Moreover, WEUB and WERB are both good scavengers of active oxygen and secondary antioxidants that reduce the rate of chain initiation (*33*). Although WERB was prepared under high roasting temperatures, such as 327 °C, 332 °C, 335 °C, and 341 °C, it still exerted a marked scavenging effect on hydroxyl radicals. These results reveal that roasted barley still retains the activity of scavenging hydroxyl radicals. This observation is significantly meaningful because barley drinks prepared from roasted barley still exhibited remarkable scavenging effect on hydroxyl radicals.

Table 6. Contents of Catechin, α-Tocopherol, and Lutein of Barley Prepared under Different Roasting Temperatures (μg/g of Dried Barley)[b,c]

sample[a]	catechin	α-tocopherol	lutein
unroasted	42.6 ± 4.43[a]	3.4 ± 0.70[a]	12.7 ± 0.02[a]
roasted			
327 °C	16.3 ± 0.76[b]	0[b]	11.6 ± 0.14[b]
332 °C	12.6 ± 1.33[b]	0[b]	11.3 ± 0.10[c]
335 °C	13.5 ± 0.32[b]	0[b]	11.1 ± 0.11[c]
341 °C	14.1 ± 0.31[b]	0[b]	0[d]

[a] Barley was unroasted and roasted at 327, 332, 335, and 341 °C, respectively. [b] Values are means ± standard deviation of three replicate analyses. [c] Means with different superscript letters in the column are significantly different ($P < 0.05$).

Changes in the Antioxidative Components of Barley with Different Roasting Temperatures. Catechin and its derivatives, tocopherols, and carotenoids have been reported to be the main active components in barley (*5*). Thus, the contents of catechin, tocopherols, and carotenoids in barley with or without roasting were determined to understand their role in the mechanism of antioxidant action. From the results shown in Table 6, the content of catechin in unroasted barley was significantly ($P < 0.05$) greater than that in roasted barley, with no significant difference ($P > 0.05$) found among the roasted samples prepared under different roasting temperatures. The content of lutein in barley decreased with increasing roasting temperature. Unroasted barley contained α-tocopherol (3.4 μg/g), however, 100% was lost in the roasted barley prepared under different roasting temperatures, indicating that tocopherol was significantly affected by thermal treatment in the tested model. In addition, the roasting was performed in air which can also deplete tocopherol. Catechin derivatives are important members of the flavonoid family and can be found in fairly large

amounts in tea and in fruits. Tea has been found to possess antimutagenic activity (*34*), antioxidative activity (*35*), and anticarcinogenic effects (*36*−*38*) in several systems. The polyphenols are the most biologically active group of the tea components, especially certain catechins. α-Tocopherol is a monophenolic compound which can quench free radicals and inhibit the lipid peroxidation. Besides catechin and α-tocopherol, rutein, a member of the carotenoids, is also considered an antioxidant (*39*). Unroasted barley exhibited marked antioxidant activity attributable to the combination of catechin, tocopherol, lutein, and some minor antioxidants. However, the above result implied that catechin, tocopherols, and lutein may be degraded by thermal treatment, leading to a reduction in antioxidant activity of WERB. This result also may explain why the antioxidant activity of unroasted barley in the different model systems tested was greater than that of roasted barley.

Lingnert and Eriksson (*40*) noted that Maillard reaction products (MRP) obtained by reactions between sugars and amino acids, peptides, or enzymic protein hydrolysates were found to have a strong antioxidant activity. However, the antioxidant activity of MRP was significantly reduced by heat treatment (*41*). In addition, some amino acids have been reported to show antioxidative activity (*42*, *43*). According to the results given in Table 2, although the MRP was formed in roasted barley, the antioxidative activity of roasted barley was less than that of unroasted barley. This result implied that MRP was degraded by severe heat treatment leading to a reduction in the antioxidant activity. Moreover, based on the results given in Table 4, the content of amino acids in roasted barley was decreased, which may also explain the reduction of antioxidant activity of WERB.

CONCLUSIONS

On the basis of the results of the present study, WEUB was more effective in the protection of lipid and nonlipids against various oxidative model systems than WERB was in different thermal treatments. The mechanism of the inhibitory effects by which barley, unroasted or roasted, protects against oxidation may involve free radical scavenging, reducing ability, hydroxyl radical quenching, and metal binding. In addition, changes in catechin, tocopherol, and lutein content may explain why WEUB and WERB showed different antioxidant activities.

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