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Evaluation of Antioxidant Activity and Electronic Taste and Aroma Properties of Antho-Beers from Purple Wheat Grain

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Moderate consumption of beer is known to be beneficial for health. Thus, antioxidant, likely taste, and aroma properties of antho-beers made from purple wheat grain (antho-grain) were evaluated. The 2,2-diphenyl-1-picryhydrazyl free radical (DPPH•) scavenging activity, total phenolic content (TPC), oxygen radical absorbance capacity (ORAC), and phenolic acid compositions of antho-bran were also investigated. DPPH• scavenging activity at 60 min was 50.6–59.9% for control and antho-beer extracts, 15.0–54.1% for antho-bran extracts and hydrolysates. The TPC ranged from 410 to 609 mg/L, from 84 to 95 mg/L, and from 2473 to 7634 mg/kg for control (from barley malt) and antho-beer original samples, control and antho-beer extracts, and antho-bran extracts and hydrolysates, respectively. The corresponding ORAC values were 3050–4181 mg/L, 2961–3184 mg/L, and 74–213 g/kg, respectively. The major known phenolic acids comprised four types in control beer, five types in antho-beers, and seven types in antho-bran hydrolysates. Total anthocyanin content of antho-bran was up to 1160 mg/kg. Differences in likely taste and aroma were found between control and antho-beers by using electronic tongue and nose methods. Brewing materials had an effect on the antioxidant, likely taste, and aroma properties of beers; however, antho-grain may have potential as a novel brewing material.

KEYWORDS: Antioxidant activity; taste; aroma; antho-beer; antho-bran

INTRODUCTION

Beer is one of the most popular beverages in the world, and its popularity as a beverage is second only to that of soft drinks (*1*). Beer has a functional role similar to that of red wine, which can be considered as the protective beverage of choice against chronic and degenerative pathologies (*2*). Beers are known to be good sources of antioxidant compounds, such as volatile maltol and 2-furanmethanol (*1*), polyphenols (*3*), and ferulic acid (*4*). Beer has other beneficial functions that include improving digestion, promoting a healthy heart, enhancing the potency of vitamin E and preventing scurvy through the possible mechanisms of stimulating gastrin, gastric acid, cholecystokinin and pancreatic enzyme secretion; reducing serum cholesterol, triglycerides, and lipid peroxides; and elevating high-density lipoprotein (HDL) cholesterol (healthy cholesterol) levels (*5*). There are a number of digestive, antioxidant, and cardioprotective components in beer; hence the nutritional value of beer can contribute substantially to the diet (*6*). A single beer consumed daily raised HDL cholesterol levels by 4.4%, or 2 mg/dL (*7*) and had a greater capacity to prevent low-density lipoprotein (LDL) oxidation than white wine (*8*). Evidence has been mounting that moderate consumption of alcohol is beneficial for reducing cardiovascular risk (*9, 10*), and moderation is defined as no more than 1 drink per day for normal adult women and no more than 2 drinks per day for normal adult men (*11*). Gasbarrini et al. reported that a moderate consumption of beer in a well-balanced diet did not appear to cause oxidative stress in rats and beer could attenuate the oxidative action of ethanol by itself (*12*). Beer may reduce the production of neopterin and the degradation of tryptophan, and its immunosuppressive capacity may relate to the anti-inflammatory nature of beer (*8*). Beer also may protect the organism from oxidative stress and counteract carcinogenesis and osteoporosis (*13, 14*). It has the capability of decreasing the oxidizability of lower density lipoproteins in addition to inhibiting atherosclerosis and diminishing cholesterol and triglycerides (*15*). Beer was found to impair lymphokine-activated killer cell activity (*16*) and to have an antithrombotic effect (*17*). Moderate consumption of beer leads to some favorable biochemical changes in the blood of patients with coronary artery disease (*18*) and also increases plasma antioxidant capacity (*19, 20*). Other researchers also indicated that moderate consumption of red wine, beer, and spirits had counteracting effects on plasma antioxidant components, finally resulting in no significant effect on overall antioxidant status (*21*).

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Beer has been produced for more than 4000 years by a simple process. Barley is the most common grain for brewing traditional beer. Antho-beer is brewed using antho-grain, a variety of purple wheat grain. Wheat is rich in antioxidative components such as phenolic acids (*22*, *23*). Colored wheat grain, such as black or purple wheat grain, also has high antioxidant activity (*24*). Compared to common wheats, purple- and blue-colored wheats contain significant levels of anthocyanins, which contribute to antioxidant activity (*25*, *26*). Antho-beer is a potential value-added product derived from purple wheats.

Beer quality is affected by many factors, such as raw materials used, foam maintenance, physical and chemical stability, and shelf life (*27*, *28*). Phenolic acids in packaged beer have strong antioxidant activity and are essential in determining its taste and in maintaining its foam, and contribute to its physical and chemical stability (*12*). Control and evaluation of beer quality are important for beer brewing process and market sale. In recent years, the electronic tongue and nose have become useful means for assessing taste, smell, and flavor, such as predicting the taste and aroma characteristics of apple juices (*29*), bottled nutritive drinks (*30*), beer (*31*), aged Cheddar cheese (*32*), and pharmaceuticals (*33*). While the electronic nose is very effective for measuring volatile chemical components, the electronic tongue is especially useful for measuring nonvolatile components (*31*). The objectives of the study were to evaluate antioxidant properties of antho-beer and its likely taste and aroma quality. The results will be used to understand the added-value potential of antho-grain for further enlarging its utilization and to provide evidence needed for improvement in the technology of antho-beer production.

MATERIALS AND METHODS

Materials. Three samples were kindly supplied from InfraReady Products (1998) Limited (Saskatoon, SK). One was an antho-bran sample and the other two were antho-beer samples, with and without addition of dried hops in the beer. Antho-beers were produced from antho-grain, a variety of purple wheat grain as brewing material. Antho-bran was also from the same antho-grain as the antho-beers. Control beer (Fort Garry Brewery), a kind of Manitoba local brew, was purchased from the market. Control beer (original brew) contains 5.0% alcohol by volume, belongs to the pale ale class, and is brewed from malt barley through fermentation of exclusive Fort Garry Brewery ale yeast. Hops are added during production of control beer. 2,2-Diphenyl-1-picryhydrazyl free radical (DPPH•), 2,2′-azobis(2-methylpropionamide) dihydrochloride (AAPH), and 14 phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, MO). Phenolic acid standards used for HPLC analysis were as follows: gallic, gentisic, *p*-coumaric, *m*-coumaric, caffeic, sinapic, ferulic, syringic, *o*-coumaric, vanillic, protocatechuic, chlorogenic, *trans*-cinnamic, and *p*-hydroxybenzoic acids. Trolox and fluorescein were purchased from Fisher Acros Organics (New Jersey, USA) for use with the oxygen radical absorbance capacity assay. All other chemicals and solvents were of the highest commercial grade and were used without further purification.

Sample Preparation. The coarse antho-bran was further ground into fine bran through a 1-mm mesh sieve using a sample mill (Krups 50, Germany). Extraction of antioxidative compounds in antho-bran was carried out according to methods previously described (*24*). Ethanol (95%) and 1 N HCl/95% ethanol (v/v, 15/85) were used as solvents for extraction of the finely ground antho-bran. The extraction procedure involved adding 20 mL of solvent to 2.0 g of ground bran in 50-mL brown bottles and shaking the bran for 3 h at ambient temperature in a rotary shaker (Fermentation Design Inc., Allentown, PA) set at 300 rpm. The mixture of solvent and bran was then centrifuged at 7,800 × *g* (10,000 rpm, SS-34 Rotors, RC5C Sorvall Instruments) at 5 °C for 15 min. The extracts of supernatant fluid were kept at −20 °C in the dark until further analysis for DPPH• scavenging activity, total phenolic content (TPC), and oxygen radical absorbance capacity (ORAC).

Original samples of antho-beers and control beer were first degassed by vacuum, diluted to appropriate concentrations, and then directly analyzed for TPC and ORAC. Extraction of phenolic compounds in beer was carried out according to Nardini and Ghiselli (*34*), but with some modifications. Beer samples were extracted three times with ethyl acetate (×3 vol) by vortexing for 5 min. After each extraction, the organic phase was collected. The combined organic phase was dehydrated by adding 2 g of anhydrous Na_2SO_4 and then evaporated to dryness at 35 °C by using a rotary vacuum evaporator (RE III Rotavapor, Büchi, Switzerland). The residue was redissolved in 50% methanol, diluted to the appropriate concentration, and filtered through a 0.45-μm nylon filter. The filtrate (beer extract) was stored in the dark at −20 °C and subsequently analyzed by HPLC to obtain phenolic acid composition of beer samples. Antioxidant properties of beer extracts were also evaluated, including DPPH• scavenging activity, TPC, and ORAC.

Alkaline hydrolysates of antho-bran were prepared according to the methods of Mpofu et al. (*35*) and Li et al. (*24*). Briefly, ground antho-bran (2g) was hydrolyzed by using 4 M NaOH (60 mL) for 4 h under nitrogen at ambient temperature. The hydrolyzed mixture was adjusted to pH 1.5–2.5 by using ice-cold 6 M HCl and then centrifuged at 7,800 × *g* (10,000 rpm, RC5C, Sorvall Instruments, DuPont, Wilmington, DE) at 5 °C for 20 min. The supernatant was extracted three times with ethyl acetate (70 mL). The combined organic phase was first dehydrated by adding 2 g of anhydrous Na_2SO_4 and then evaporated to dryness at 35 °C by using a rotary vacuum evaporator (RE III Rotavapor, Büchi, Switzerland). The residue was redissolved in 5 mL of 50% methanol and filtered through a 0.45-μm nylon filter. The filtrate (alkaline hydrolysate) was stored in the dark at −20 °C and subsequently analyzed by HPLC to obtain phenolic acid composition of the antho-bran sample. Antioxidant properties of alkaline hydrolysates were also determined, including DPPH• scavenging activity, TPC, and ORAC.

DPPH• Scavenging Activity. The DPPH method was used according to Brand-Williams et al. (*36*) and Li el al. (*24*) with some modification. The method involves the reaction of the antioxidants with the stable DPPH• in 95% ethanol solution. Briefly, a 60 μmol/L DPPH• solution was freshly made in 95% ethanol solution. The alkaline hydrolysate of antho-bran, and extracts of beers or antho-bran (200 μL) were reacted with 3.8 mL of the DPPH• solution for 60 min. The absorbance (*A*) at 515 nm was measured against a blank of pure 95% ethanol at $t = 0$, 5, 10, 20, 30, 40, 50, and 60 min. The chemical kinetics of antioxidant activity of beer extracts was also recorded. Antioxidant activity was calculated as follows: % DPPH• scavenging activity $= (1 - [A_{\text{sample},t}/A_{\text{control},t=0}]) \times 100$. DPPH tests were all carried out in duplicate.

Determination of TPC. TPCs of original beer samples, extracts of beers and antho-bran, and hydrolysate of antho-bran were determined by using modified procedures of the Folin-Ciocalteau method (*37*, *38*). A sample (200 μL) was added to 1.9 mL of freshly 10-fold diluted Folin-Ciocalteau reagent (BDH Inc., Toronto, ON). Sodium carbonate solution (1.9 mL) (60 g/L) was then added to the mixture. After 120 min of reaction at ambient temperature, the absorbance of the mixture was measured at 725 nm against a blank of distilled water. Ferulic acid was used as a standard, and results are expressed as ferulic acid equivalents. All analyses were performed in duplicate.

ORAC Assay. The ORAC assay, first developed by Cao et al. (*39*), was used in this study according to Huang et al. (*40*), Li et al. (*41*, *42*), and Dávalos et al. (*43*). An FLx800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. The plate reader was controlled by KC4 3.0 software (version 29). Dilution of sample, rutin control, and Trolox standard was done manually. The quantity of 300 μL each of buffer solution (blank) and diluted sample solution, rutin control, and Trolox standard was transferred to a 96-well flat bottom polystyrene microplate (Corning Incorporated, Corning, NY, USA) by hand according to their designated positions. A full automation of plate-to-plate liquid transfer was programmed by using a Precision 2000 automated microplate pipetting system (Bio-Tek Instruments, Inc., Winooski, VT). Specifically, 120 μL of fluorescence working solution was transferred from the reagent holder to each well of a second 96-well microplate. Then 20 μL each

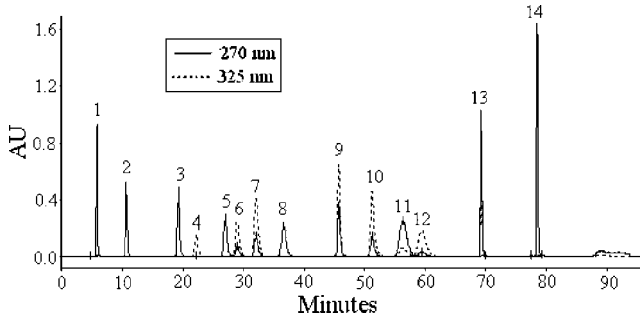

Figure 1. HPLC profile for 14 standard phenolic acids. Numbers show the following standard chemicals: 1, gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acids; 4, gentisic acid; 5, vanillic acid; 6, chlorogenic acid; 7, caffeic acid; 8, syringic acid; 9, *p*-coumaric acid; 10, ferulic acid; 11, *m*-coumaric acid; 12, sinapic acid; 13, *o*-coumaric acid; 14, *trans*-cinnamic acid.

of buffer solution (blank), Trolox standard, diluted samples, and 20 μM rutin control from designated wells of the first 96-well microplate was transferred to designated wells of the second 96-well microplate. The latter was quickly covered with an adhesive sealing film, shaken for 3 min at 37 °C in the incubator, and incubated in the preheated (37 °C) FLx800 microplate reader for a total period of 20 min. The second 96-well microplate was transferred back to its original station in the Precision 2000 automated microplate pipetting system, followed by automatically transferring 60 μL of AAPH solution from the reagent holder to designated wells. Thus, the total volume for each well was 200 μL. The second 96-well microplate was quickly covered again with an adhesive sealing film and immediately transferred to the FLx800 microplate reader, and the fluorescence was measured every minute for 50 min at 37 °C. Peroxyl radical was generated by AAPH during measurement, and fluorescein was used as the substrate (*44*). All reaction mixtures were prepared in the measured plate in duplicate, and at least three independent assays were performed for each sample.

Data was processed according to Cao et al. (*45*) and Huang et al. (*40*). The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescence decay curve. Area under curve (AUC) was calculated as follows:

$$\mathrm{AUC} = 0.5 + \frac{f_1}{f_0} + \ldots + \frac{f_i}{f_0} + \ldots + \frac{f_{49}}{f_0} + 0.5\frac{f_{50}}{f_0} \qquad (1)$$

where f_0 = initial fluorescence reading at *0* min and f_i = fluorescence reading at time *i* min.

The net AUC was obtained by subtracting the AUC of the blank from that of the sample. ORAC values were expressed as Trolox equivalents by using the standard curve.

Determination of Total Anthocyanin Content (TAC). Extraction of anthocyanins was according to the method of Abdel-Aal and Hucl (*26*) with some modification. Total anthocyanins were determined according to the pH-differential method (*46, 47*). Briefly, samples were extracted by using ethanol (95%)/1 N HCl (85:15, v/v) in a brown bottle, to avoid light-induced changes. Clear extract (1 mL) was placed into a 25-mL volumetric flask, made up to final volume with pH 1.0 buffer (1.49 g of KCl/100 mL water and 0.2 N HCl, with a ratio of 25:67), and mixed. Another 1 mL of extract was also placed into a 25-mL volumetric flask, made up to final volume with pH 4.5 buffer (1.64 g of sodium acetate/100 mL of water), and mixed. Absorbance was measured by using a UV–vis spectrophotometer (Ultrospec 2000, Pharmacia Biotech) at 510 and at 700 nm. Absorbance was calculated as $\Delta A = (A_{510\mathrm{nm}} - A_{700\mathrm{nm}})_{\mathrm{pH1.0}} - (A_{510\mathrm{nm}} - A_{700\mathrm{nm}})_{\mathrm{pH4.5}}$. Results were calculated using the following equation and expressed as milligrams of cyanidin 3-glucoside equivalents per kilogram of dry basis weight: total anthocyanins (mg/kg) = $(\Delta A/eL) \times \mathrm{MW} \times D \times (V/G) \times 1{,}000$, where ΔA is absorbance, *e* is cyanidin 3-glucoside molar absorbance (26900), *L* is cell path length (1 cm), MW is the molecular weight of anthocyanins (449.2), *D* is a dilution factor, *V* is the final volume (mL), *G* is the sample weight (g), and 1,000 is a conversion factor from gram to kilogram. All determinations were carried out at least in duplicate.

Table 1. DPPH Radical Scavenging Activity of Beer and Bran Extracts, and Bran Hydrolysate (at 60 min)[a]

name	DPPH• scavenging %	name	DPPH• scavenging %
EAEC-beer	53.23 b	EA-bran-in-E/HCl	54.12 a
EAEA-beer	50.62 b	EA-bran-in-E	14.98 c
EAEA-beer-WADP	59.88 a	HA-bran	47.16 b
LSD	5.36	LSD	4.84

[a] LSD, least significant difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. EAEC-beer, ethyl acetate extract of control beer; EAEA-beer, ethyl acetate extract of antho-beer; EAEA-beer-WADP, ethyl acetate extract of antho-beer without adding dried hops; EA-bran-in-E/HCl, extract of antho-bran in ethanol/HCl (ethanol 95%, HCl 1 N, v/v, 85/15); EA-bran-in-E, extract of antho-bran in 95% ethanol; HA-bran, hydrolysate of antho-bran.

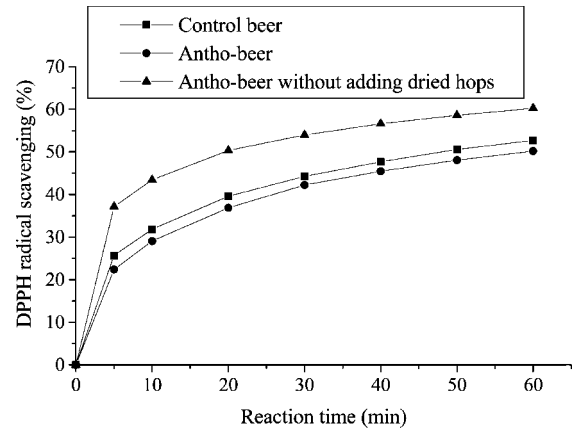

Figure 2. Antioxidant activity kinetics of antho-beer and control beer ethyl acetate extracts using DPPH free radical.

Determination of Phenolic Acid Composition. Phenolic acid compositions of beer extracts and antho-bran hydrolysate were determined using an HPLC method. HPLC analysis was performed on a Waters model 2695 chromatograph instrument (Waters, Mississauga, ON, Canada) equipped with a Waters 2996 photodiode array detector. Phenolic acids were separated on a reverse-phase Waters μBondapak RP-C18 column (300 mm × 3.9 mm) with a gradient of solvent A (water containing 1% (v/v) formic acid) and solvent B (100% methanol) for 97 min at a flow rate of 0.7 mL/min. The solvent gradient was programmed as follows: at 0 min 15% B, 10–22 min 20% B, 23–35 min 17% B, 36–54 min 24% B, 55–65 min 19% B, 66–85 40% B, 86–91 min 100% B, and 92–97 min 15% B. Phenolic acids in the eluants were monitored at 270 and 325 nm synchronously. Identification of the phenolic acids was accomplished by comparing the retention times of peaks in samples to those of phenolic acid standards. HPLC chromatograms of 14 standard phenolic acids are shown in **Figure 1**. *m*-Coumaric acid was used as an external standard. The HPLC analyses were carried out in duplicate.

Beer Taste Analysis using Electronic Tongue (E-tongue). Comparative analysis of antho-beer and control beer taste was done by using a "α-ASTREE" Liquid and Taste Analyzer (Electronic tongue, Alpha M.O.S., Toulouse, France). The e-tongue is composed of a Ω Metrohm 759 Swing Head, SC Controller, 48-position autosampler, array of liquid sensors, and an advanced chemometric software package. The array consists of seven different liquid cross-sensitive sensors (ZZ, BA, BB, CA, GA, HA, JB). These chemical sensors are made from silicon transistors with an organic coating that determines sensitivity and selectivity of each individual sensor (*31*). An electrical potential difference during measurement appears between each individually coated sensor and the Ag/AgCl reference electrode in the equilibrium state at room temperature (*30*). Taste and flavor compounds in solution

Table 2. TPC of Beer Original Solution, Beer and Bran Extracts, and Bran Hydrolysate[a]

name	equiv of FA (mg/L)	name	equiv of FA (mg/L)	name	equiv of FA (mg/kg)
C-beer-OS	609 a	EAEC-beer	84 c	EA-bran-in-E/HCl	7634 a
A-beer-OS	424 b	EAEA-beer	87 b	EA-bran-in-E	2473 c
A-beer-OSWADP	410 c	EAEA-beer-WADP	95 a	HA-bran	7554 b
LSD	10.94	LSD	1.73	LSD	52.68

[a] LSD, least significant difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. C-beer-OS, control beer original solution; A-beer-OS, antho-beer original solution; A-beer-OSWADP, antho-beer original solution without adding dried hops; EAEC-beer, ethyl acetate extract of control beer; EAEA-beer, ethyl acetate extract of antho-beer; EAEA-beer-WADP, ethyl acetate extract of antho-beer without adding dried hops; EA-bran-in-E/HCl, extract of antho-bran in ethanol/HCl (ethanol 95%, HCl 1 N, v/v, 85/15); EA-bran-in-E, extract of antho-bran in 95% ethanol; HA-bran, hydrolysate of antho-bran; equiv, equivalent; FA, ferulic acid.

Table 3. Oxygen Radical Absorbance Capacity of Beer Original Solution, Beer and Bran Extracts, and Bran Hydrolysate[a]

name	equiv of Trolox (g/L)	name	equiv of Trolox (g/L)	name	equiv of Trolox (g/kg)
C-beer-OS	4.18 a	EAEC-beer	2.96 a	EA-bran-in-E/HCl	213.45 a
A-beer-OS	3.05 b	EAEA-beer	3.16 a	EA-bran-in-E	73.80 c
A-beer-OSWADP	3.36 b	EAEA-beer-WADP	3.18 a	HA-bran	186.49 b
LSD	0.49	LSD	0.33	LSD	21.80

[a] LSD, least significant difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. C-beer-OS, control beer original solution; A-beer-OS, antho-beer original solution; A-beer-OSWADP, antho-beer original solution without adding dried hops; EAEC-beer, ethyl acetate extract of control beer; EAEA-beer, ethyl acetate extract of antho-beer; EAEA-beer-WADP, ethyl acetate extract of antho-beer without adding dried hops; EA-bran-in-E/HCl, extract of antho-bran in ethanol/HCl (ethanol 95%, HCl 1 N, v/v, 85/15); EA-bran-in-E, extract of antho-bran in 95% ethanol; HA-bran, hydrolysate of antho-bran; equiv, equivalent.

are directly measured and effectively discriminated by sensors of the e-tongue. The integral signal during measurement of each sample comprised a vector with seven individual sensor measurements. The entire process of measurement (auto-sampling, data collection, and data treatment) is automated and controlled by the chemometric software package loaded on the PC (*29*). The pattern-recognition analysis module principle component analysis (PCA) was utilized in this study. PCA is used for assessing the discrimination and similarities between samples and groups in qualitative analysis and performing product matching. Test conditions were as follows: sample volume, 20 mL; time per analysis, 3 min; acquisition time, 2 min; sample temperature, at room temperature; and sensor cleaning solution, 5% ethanol. After sensory measurement of each sample solution, a wash cycle followed to ensure there was no carryover of sample to the next analysis and also for good reproducibility. Four replicate measurements were made for each sample.

Beer Aroma Analysis using Electronic Nose (E-nose). There are two types of electronic noses (Alpha, M.O.S., France France), a sensor array system (SAS) such as the α-Fox 3000 SAS electronic nose and a fingerprint mass spectrometer (FMS) such as theα- Kronos FMS electronic nose. The SAS e-nose comprises an array of metal oxide or conducting polymer sensors with a broad and partly overlapping selectivity for the measurement of odor molecules (*48*). However, the high ethanol concentration in beer will affect the sensor sensitivity of SAS e-nose, and it was also difficult to determine the chemical difference between the samples according to the change in the sensor response of SAS e-nose (*49*). A mass selective detector is used in FMS e-nose in place of sensors in the SAS system. Evidence indicated that FMS electronic nose could exclude ethanol's effect and were useful for obtaining information about volatile compounds, which was impossible by SAS e-noses (*49*). Hence, beer samples were analysed by α- Kronos FMS e-nose in this study. α- Kronos FMS e-nose is one part of α-Prometheus equipment (Alpha M.O.S., France), which combines the gas sensor module coming from the α-Fox instrument as well as a FMS module coming from α-Kronos instrument, equipped with a headspace autosampler (HS100) to the α-Prometheus system. The measurement procedure and parameter option of FMS e-nose were according to Bleibaum et al. (*29*) and Supriyadi et al. (*50*) with some modifications. Briefly, for analysis using α-Kronos FMS electronic nose, the beer sample (1 mL) was placed into a 10-mL glass vial and sealed with septa crimped onto the top. The vial was heated for 15 min at 70 °C in order to produce an equilibrium headspace. The vial sample was agitated at 500 rpm during heating. The headspace gas (3000 μL) was automatically taken and injected into the FMS at 100 μL/s using a syringe preheated to 75 °C. The equipment was continuously purged with dry air set at 150 mL/min. The acquisition time and *m/z* range were 120 s and 48–200, respectively. Data from the FMS were analyzed using PCA. Software package for PCA was from Alpha M.O.S. Several principal *m/z* peaks were selected by computer in data treatment by PCA done in order to obtain the best discrimination among samples. Four replicate measurements are made for each sample.

Table 4. Total Anthocyanins of Antho-bran[a]

extracting time (min)	equiv of cyanidin 3-glucoside (mg/kg)
10	864 d
30	938 c
60	1022 b
180	1043 b
360	1160 a
540	1029 b
LSD	36.77

[a] LSD, least significant difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different; equiv, equivalent.

Statistical Analysis. Data were reported as means of measurements and subjected to analysis of variance. Least significant difference (LSD) was calculated using Fisher's protected LSD test at $P = 0.05$. Quantitative results were generally expressed on a dry weight basis (dwb) for antho-bran.

RESULSTS AND DISCUSSION

DPPH Radical Scavenging Activity. Free radical scavenging activities of beer and bran extracts and bran hydrolysate are shown in **Table 1**. DPPH• scavenging activities of beer extracts ranged from 50.62% to 59.88% at 60 min. The results indicated that antho-beers from antho-grain had antioxidant activity similar to that of control beer from barley malting. The reaction rate of beer extracts with DPPH• was rapid in the first 20 min, but after 20 min, it became progressively slow and stable (**Figure 2**). There were significant differences in scavenging activity between antho-beer without hops (59.88%), antho-beer (50.62%), and control beer extract (53.23%). Antho-beer without hops

Table 5. Phenolic Acid Composition in Beers and Antho-Bran Hydrolysate[a]

phenolic acids	EAEC-beer (μg/L)	EAEA-beer (μg/L)	EAEA-beer-WADP (μg/L)	LSD	HA-bran (mg/kg)
p-HA	nd	nd	nd		32
VA	672	nd	nd		208
CA	nd	326 a	347 a	30.4	37
SYA	nd	nd	nd		46
p-CA	813 a	641 b	574 b	79.6	110
FA	2391 a	1040 b	1012 b	212.3	3145
SIA	332 b	381b a	436 a	76.3	84
o-CA	nd	399 a	308 a	248.8	nd

[a] LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same row are not significantly different. *p*-HA, *p*-hydroxybenzoic acid. VA, vanillic acid. CA, caffeic acid. SYA, syringic acid. *p*-CA, *p*-coumaric acid. FA, ferulic acid. SIA, Sinapinic acid. *o*-CA, *o*-coumaric acid. nd, not detectable. EAEC-beer, ethyl acetate extract of control beer; EAEA-beer, ethyl acetate extract of antho-beer; EAEA-beer-WADP, ethyl acetate extract of antho-beer without adding dried hops; HA-bran, hydrolysate of antho-bran.

showed the highest DPPH• scavenging activity during the reaction process when compared to antho-beer and control beer extracts.

The scavenging activities of antho-bran extracts and hydrolysate were also evaluated (**Table 1**). Scavenging activity (54.12%) of antho-bran in 95% ethanol/1 N HCl (v/v, 85/15) was significantly high in comparison with that (14.98%) of antho-bran extract in 95% ethanol. Thus, there were marked effects of solvent system on evaluation of antioxidant activity. Pérez-Jiménez and Saura-calixto (*51*) also reported that there were significant differences in antioxidant capacity values obtained by the same method in the different solvents. The main difference between solvent extracts and alkaline hydrolysates is attributed to the nature of phenolics present. The bound phenolics were released in free phenolic acid forms after alkaline hydrolysis of antho-bran. The scavenging activity (47.2%) of antho-bran alkaline hydrolysate was significantly high when compared to 95% ethanol extract (15.0%) of antho-bran. However, scavenging activity was more enhanced in the 95% ethanol/HCl (1 N) extract (54.12%) of antho-bran. Polyphenols were the main contributors to the total antioxidant activity of antho-bran solvent extracts, whereas phenolic acids were responsible for the free radical scavenging activity of antho-bran alkaline hydrolysates. The presence of phenolic acids in alkaline hydrolysates can easily be identified by HPLC analysis (*24*).

TPC. The TPCs, expressed as FA equivalents, of beer original sample, beer and bran extracts, and bran hydrolysate are shown in **Table 2**. TPC (609 mg/L) of control beer original sample was the highest when compared to antho-beers (410−424 mg/L). Significant differences in TPC were found between control beer and antho-beers. TPCs in lager and dark beers were 376 and 473 mg/L, respectively, in previous reports (*3*). However, TPCs (84–95 mg/L) in beer extracts were obviously decreased when compared to original samples (410–609 mg/L) (**Table 2**). TPCs in the extracts of antho-beer samples were higher than in control beer extract. There were significant differences in TPCs among the extracts of the beer samples. Beer extracts should be composed mainly of lipid-soluble components because water-soluble phenolic compounds were removed during the ethyl acetate extraction process. Since the original beer samples contain water-soluble and lipid-soluble phenolic compounds, the high TPCs in original samples, unlike in the extracts, are attributed to contribution of both water-soluble and lipid-soluble phenolic compounds.

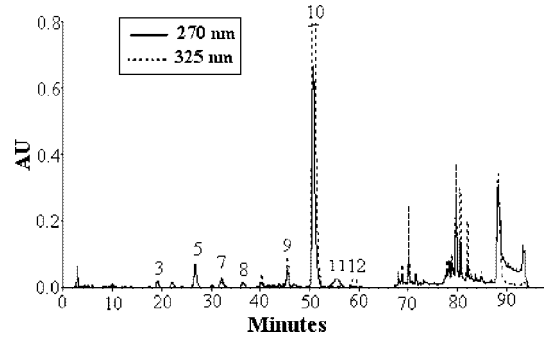

Figure 3. HPLC profile for hydrolysate of antho-bran. Numbers indicate the same chemicals as in **Figure 1**. *m*-Coumaric acid (11) as external standard.

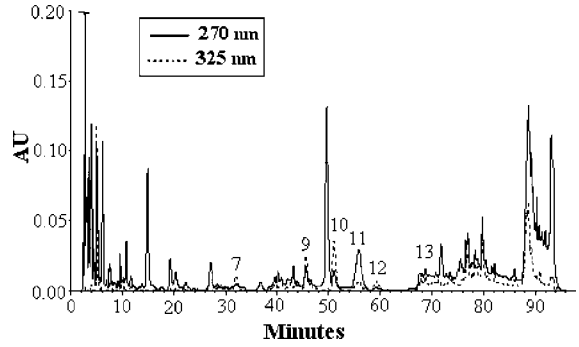

Figure 4. HPLC profile for ethyl acetate extract of antho-beer. Numbers indicate the same chemicals as in **Figure 1**. *m*-Coumaric acid (11) as external standard.

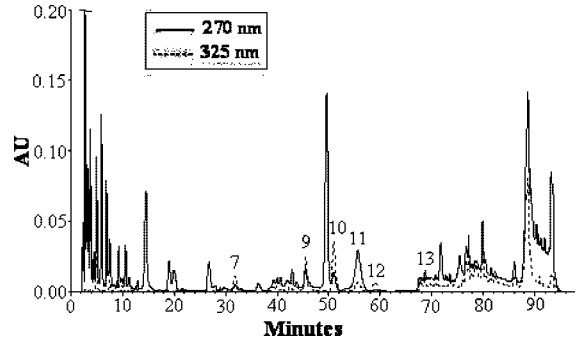

Figure 5. HPLC profile for ethyl acetate extract of antho-beer without adding dried hops. Numbers indicate the same chemicals as in **Figure 1**. *m*-Coumaric acid (11) as external standard.

Phenolic compounds in antho-beer mainly come from the outer layers (bran) of antho-grain. TPCs of antho-bran extract in 95% ethanol/HCl (1 N) and hydrolysate were up to 7634 and 7554 mg/kg, respectively (**Table 2**). However, the TPC (2473 mg/kg) of antho-bran extract in 95% ethanol was significantly lower than that in 95% ethanol/HCl (1 N) because antioxidant capacity was affected by using different extracting solvents (*51*). Significant differences in TPC were found among antho-bran extracts and between hydrolysate and each extract. The phenolic compounds in the hydrolysate are mainly phenolic acids liberated by alkaline hydrolysis of antho-bran. However, antho-bran extracts consist mainly of polyphenols and partial hydrolysis will occur in acidic solvent because soluble phenolic esters and insolubly bound phenolic acids still exist in grains prior to alkaline hydrolysis of grain (*52*).

ORAC Assay. The ORAC values, expressed as Trolox equivalents, of original beer samples, beer and bran extracts, and bran hydrolysate are shown in **Table 3**. Significant

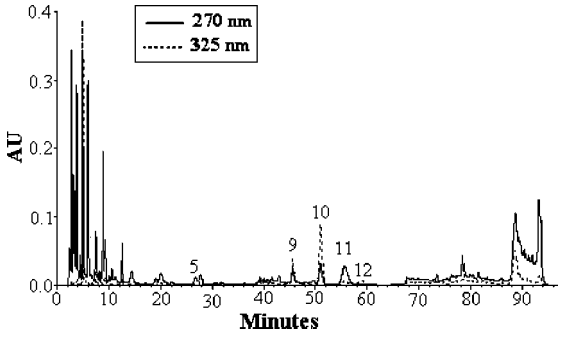

Figure 6. HPLC profile for ethyl acetate extract of control beer. Numbers indicate the same chemicals as in **Figure 1**. *m*-Coumaric acid (11) as external standard.

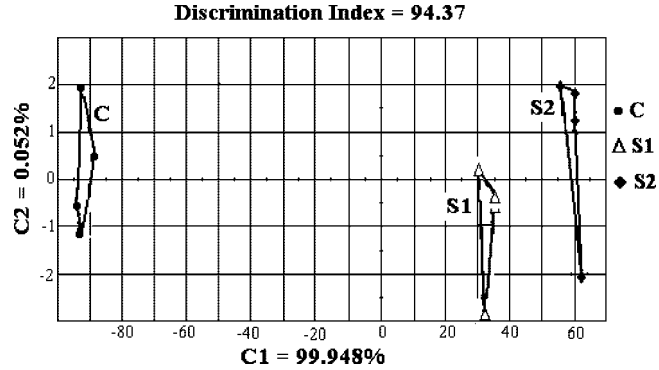

Figure 7. PCA map of control beer and antho-beers from sensor analysis of electronic tongue: C, control beer; S1, antho-beer without adding dried hops; and S2, antho-beer.

differences were found between the control and antho-beer samples. However, there was no difference in ORAC values between antho-beers with or without hops. ORAC value (4.18 g/L) of control beer original solution was higher when compared to antho-beer original solution (3.05 g/L) and antho-beer original solution without adding dried hops (3.36 g/L) (**Table 3**). There was no difference found in ORAC values (2.96–3.18 g/L) of the beer extracts (**Table 3**). The results indicated that there were similar ORAC values for lipid soluble antioxidants of control beer and antho-beer extracts.

The ORAC value (213.45 g/kg) of antho-bran extract in 95% ethanol/HCl (1 N) was significantly higher than that in 95% ethanol (73.80 g/kg) (**Table 3**), further confirming the effects of extraction–solvents on the estimation of antioxidant properties (*53*). Significant differences were also found among antho-bran extracts and between hydrolysate and extracts. The ORAC value of antho-bran extract in 95% ethanol/HCl (1 N) was also significantly higher than that of the hydrolysate (186.49 g/kg) (**Table 3**). The main difference between antho-bran extract and antho-bran alkaline hydrolysate was the nature of the phenolic structure present. The higher TPC (7634 mg/kg) of antho-bran extract in 95% ethanol/HCl (1 N) could possibly explain its higher ORAC value when compared to the hydrolysate (TPC 7554 mg/kg). The antioxidant activity of beers was likely enhanced by the phenolics transferred from the brewing materials.

The high ORAC value for antho-bran in 95% ethanol/HCl (1 N) indicated that 95% ethanol/HCl (1 N) system is a suitable solvent for extracting antioxidants. It is important to understand that a selected solvent system, such as a change in solvent polarity, may alter the overall estimation of ORAC value of the same sample and affect the efficiency of extracting antioxidant compounds. The ORAC value of extracts in a 50% acetone system was 3- to 20-fold greater than in ethanol system (*54*).

Total Anthocyanin. Total anthocyanins, expressed as cyanidin 3-glucoside equivalent, of antho-bran extracts at different extraction times are shown in **Table 4**. The color of extracts was red in 95% ethanol/HCl (1 N). The red color of antho-bran in acid media clearly indicates the presence of anthocyanin compounds. The highest anthocyanin content was 1160 mg/kg after extracting antho-bran for 360 min (6 h). The efficiency of anthocyanin extraction was significantly affected by the length of extraction time, increasing from 10 to 60 min. However, when the extraction time increased from 360 min to 540 min (9 h), the total anthocyanin content decreased to 1029 mg/kg. No anthocyanins were detected in antho-beers possibly due to degradation of anthocyanins in antho-grain during beer brewing.

Studies have indicated that anthocyanins have strong antioxidant capacity (*55*). Anthocyanins in grape juice had the ability to reduce in vitro oxidation of human low-density lipoprotein (*56*). However, many factors such as heat, light, temperature, and storage affect the stability of anthocyanins and are responsible for the degradation of anthocyanins during food drying, processing, and storage. It was reported that there were anthocyanin losses of up to 92.5% during the thermal production of pekmez (*57*), and all anthocyanins of black currant juice disappeared during 9 weeks of storage at 37 °C, whereas 60% remained after 6 months of storage at 20 °C (*58*). The brewing process to produce antho-beer led to complete disappearance of anthocyanins in antho-grain. It is anticipated that if anthocyanins could be retained after brewing, the advantage of antho-grain containing anthocyanins will be more fully utilized and antioxidant capacity of antho-beers will be further enhanced. Antioxidant properties of residual structures or adducts after anthocyanin degradation will need to be studied in future work.

Phenolic Acid Composition. Phenolic acid composition of control beer, antho-beers, and antho-bran hydrolysate is shown in **Table 5**. Seven types of phenolic acids in the hydrolysate of antho-bran (HA-bran) (**Figure 3**), five types of phenolic acids in the ethyl acetate extract of antho-beer (EAEA-beer) (**Figure 4**) and the ethyl acetate extract of antho-beer without adding dried hops (EAEA-beer-WADP) (**Figure 5**), and four types of phenolic acids in the ethyl acetate extract of control beer (EAEC-beer) (**Figure 6**) were detected. The phenolic acids included *p*-hydroxybenzoic acid (*p*-HA), vanillic acid (VA), caffeic acid (CA), syringic acid (SYA), *p*-coumaric acid (*p*-CA), ferulic acid (FA), sinapinic acid (SIA), and *o*-coumaric acid (*o*-CA). FA was the predominant phenolic acid present in EAEC-beer (2391 μg/L), EAEA-beer (1040 μg/L), EAEA-beer-WADP (1012 μg/L), and HA-bran (3145 mg/kg). No difference between EAEA-beer and EAEA-beer-WADP were found for each phenolic acid present, and this result and the HPLC profile in **Figures 4** and **5** clearly indicated no effect of adding hops on phenolic acid composition and level in antho-beer. However, there were significant differences between EAEC-beer and EAEA-beers for each phenolic acid present. Although present in antho-bran, *p*-PA and SYA were not detectable in all beer extracts. VA and CA were not detectable in antho-beer and control beer extracts, respectively. *o*-CA was not detectable in EAEC-beer and HA-bran. FA level in control beer was twice as high compared to that in antho-beers. Differences in phenolic acid composition were also found between antho-beers and the control and antho-bran hydrolysate. It is possible that the original forms of *p*-HA, VA ,and SYA were altered during antho-bran

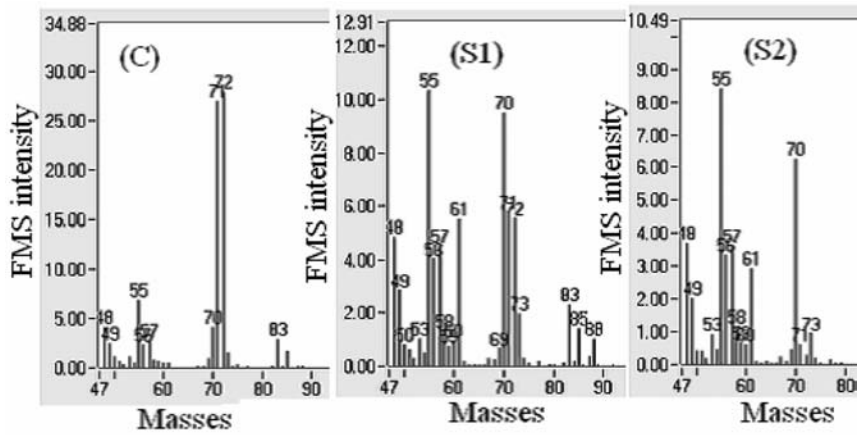

Figure 8. Fingerprint mass spectrometry signals of control beer and antho-beers: C, control beer; S1, antho-beer without adding dried hops; and S2, antho-beer.

hydrolysis. Although *o*-CA was not detectable in the antho-bran hydrolysate possibly due to its instability during alkaline hydrolysis, it was detectable in antho-beers. Standard phenolic acids decreased in antioxidant activity in the following order: protocatechuic > chlorogenic acid > CA > *p*-HA > gentisic acid > FA > VA > *p*-CA (*22*). Most phenolic acids in beer are present as bound forms, and only a small portion can be detected as free compounds (*34*). So bound phenolic acids in beer also contribute to their total antioxidant activity.

Beer Taste Analysis. The sensors ZZ and JB of e-tongue were selected for beer taste analysis. PCA map of control and antho-beers is shown in **Figure 7**. The principal components, C1 on the *x*-axis and C2 on the *y*-axis, were 99.948% and 0.052%, respectively, and the discrimination index was 94.37. Based on the PCA map, the three beer samples were clearly divided into three groups on the *x*-axis and could be distinguished from each other (**Figure 7**). Therefore, differences in likely taste among the beer samples could be easily evaluated by discriminative analysis using selected sensors of the e-tongue. The similarity distance for taste was 124.66 between control (C) and antho-beer without hops (S1), 151.90 between C and antho-beer (S2), and 27.29 between S1 and S2. Wide distances indicated large differences in taste between beer samples. It was obvious that brewing materials had an effect on beer taste. Therefore, information on beer taste was useful for quality control purposes. When dried hops were added, the distance of S2 further moved towards the right side of the *x*-axis as compared to S1. It was reported that dimethyl sulfide (DMS)-contaminated beers also had the same moving trend in comparison with original noncontaminated beer (*59*). So the effect of additives or contaminated chemicals on beer taste could be effectively discriminated by using the electronic tongue.

Beer Aroma Analysis. Fingerprint mass spectrometry (FMS) signals of aroma in three beer samples are shown in **Figure 8**. The ions at *m/z* 55, 56, 57, 61, 70, 71, and 72 were selected for PCA map. These selected ions had strong signal intensity (high peak height), by which the aroma of beer was mainly affected. The selected ions also corresponded to the following possible groups, $C_4H_7^+$ (ion 55), $C_4H_8^+$ (ion 56), $C_4H_9^+$ (ion 57), $CH_3CO\ (OH_2)^+$ (ion 61), $C_5H_{10}^+$ (ion 70), and $C_5H_{11}^+$ (ion 71) (*49*). However, ion 72 could not be assigned to a possible group. Differences in fragment ions between beer samples were also found (**Figure 8**). The PCA map from FMS analysis of the e-nose is shown in **Figure 9**. The principal components, C1 on the *x*-axis and C2 on the *y*-axis were 80.10% and 19.22%, respectively. The discrimination index was 94. The three beer samples were clearly separated in the PCA map and differences

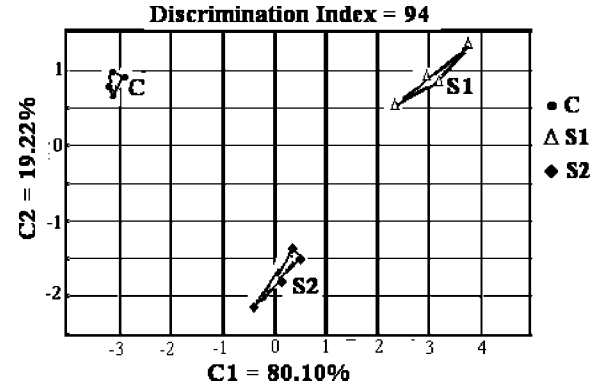

Figure 9. PCA map of control beer and antho-beers from FMS analysis of electronic nose: C, control beer; S1, antho-beer without adding dried hops; and S2, antho-beer.

in aroma were also found (**Figure 9**). Thus the FMS-type e-nose could discriminate the aroma in different types of beers. Therefore, it was useful for quality control, because its PCA data could represent one feature of the aroma, i.e., the balance of volatile compounds, and could visually exhibit the difference of one aroma feature, especially the ratio of the amount of higher alcohols to esters (*49*). The similarity distance for aroma was 31.52 between C and S1, 37.90 between C and S2, and 9.16 between S1 and S2. A wide distance also indicated a big difference in aroma between beer samples. It was also obvious that brewing materials had an effect on beer aroma.

In conclusion, the study reported the antioxidant properties of antho-beers and antho-bran, as well as the likely taste and aroma evaluation of antho-beers. Differences in antioxidant properties and in likely taste and aroma properties were found between control beer and antho-beers and between two antho-beer samples. Brewing materials had an effect on the antioxidant properties of beers from barley malt and antho-grain. Taste and aroma properties of beers also were closely related to their brewing materials. Although the TPC and ORAC values of the control were obviously higher as compared to the two antho-beer samples, DPPH• scavenging activity, TPCs, and ORAC values of extracts of antho-beer were similar or higher when compared to control beer extract. Anthocyanin compounds were the advantage of antho-grain brewing material; however, the colored groups of anthocyanins were completely degraded during antho-grain brewing. The antioxidant activity of residual structures after anthocyanin degradation needs to be studied further. Although solvents affected evaluation of antioxidant properties of antho-bran, antho-bran extract in ethanol/HCl

indicated high antioxidant activity. We evaluated antho-grain bran since the antioxidant activity of antho-grain comes mostly from the phenolic compounds in the bran. Antho-grain may have potential as a novel material for beer brewing. Future work will include the possible relationship between antioxidant activity and sensory (quality) properties of antho-beers as well as in vivo assays on the health benefits of antho-grain products.

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