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Characterization of Phenolics Content and Antioxidant Activity of Different Beer Types

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Despite the wide literature describing the biological effects of polyphenols, scarce data are available on their content in the human diet. This study examined total polyphenols content, free and total phenolic acids profile, and antioxidant activity of different commercial beers types (abbey, ale, bock, wheat, lager, pilsner, and dealcoholized). Ferulic acid is by far the most abundant phenolic acid in beers, followed by sinapic, vanillic, caffeic, *p*-coumaric, and 4-hydroxyphenylacetic acids. Ferulic, caffeic, syringic, sinapic, and, to a lesser extent, vanillic acids are present in beers mainly as bound forms, whereas *p*-coumaric and 4-hydroxyphenylacetic acids are generally present equally in free and bound forms. Total polyphenols and phenolic acids contents greatly vary among different beer types (i.e., total polyphenols, from 366 μg/mL gallic acid equivalents for dealcoholized beers to 875 μg/mL gallic acid equivalents for bock beers, with higher values measured in bock, abbey, and ale beers and lower values in dealcoholized beers). Similarly, the antioxidant activity measured with the ferric reducing antioxidant power (FRAP) assay is remarkably different depending on beer type (from 1525 μM for dealcoholized beers to 4663 μM for bock beers), with higher values in bock, abbey, and ale beers and lower values in dealcoholized beers. FRAP values strictly correlate with polyphenols and phenolic acids content. The contribution of single phenolic acids to the antioxidant activity measured with FRAP assay was also studied.

KEYWORDS: Antioxidant; beer types; FRAP assay; HPLC-ECD; phenolic acids

INTRODUCTION

Oxidative stress is involved in the pathology of many diseases, such as atherosclerosis, diabetes, neurodegenerative diseases, aging, and cancer. Dietary antioxidants may afford protection against oxidative stress-related diseases (*1*).

Among dietary antioxidants, phenolics are by far the most abundant in common human diets. Epidemiological studies have suggested associations between the consumption of phenolics-rich food and the prevention of many human diseases associated with oxidative stress (*2*−*5*). On the basis of their daily intake, which greatly exceeds that of other antioxidants (i.e., vitamin E, vitamin C, β-carotene), phenolic compounds may be a major factor in assuring the antioxidant potential of the diet and may contribute to maintaining the endogeneous redox balance in humans. Phenolic acids are a major class of phenolics widely distributed in the diet, mostly in fruits, vegetables, coffee, wine, beer, and olive oil (*6*, *7*). They received special attention because of their relatively high concentration in foods and beverages, strong antioxidant activity, easy intestinal absorption, and lack of adverse effects on human health. Phenolic acids occur in food mainly in esterified forms with organic acids, sugars, and lipids (*7*). The average phenolic acids intake has been reported to be on the order of 200 mg/day within a large range, depending on nutritional habits and preferences (*2*, *6*, *7*). Beverages account for a very high proportion of dietary antioxidant intake in the Mediterranean diet (*8*). Coffee is the main contributor, followed by red wine, fruit juice, beer, tea, and milk.

For individuals regularly consuming wine, coffee, beer, and tea, these beverages will likely be the major sources of phenolics. Beer is a very popular beverage consumed in large amount all over the world and is a source of natural antioxidants, particularly phenolic acids, originating from barley and hop (*9*−*12*). The antioxidant activity of beer coupled with low ethanol content is a relevant factor in determining the nutritive characteristics of beer. Recently, phenolic acids from beer have been described as being quickly absorbed and extensively metabolized in humans (*13*, *14*). Beer drinking has been reported to increase plasma antioxidant and anticoagulant activities and to positively affect plasma lipid levels in humans (*13*−*18*). In animal models beer drinking also decreases susceptibility to oxidation of low-density lipoproteins (*19*, *20*). Moreover, beer consumption seems to have no effect or even an inverse effect on total homocysteine concentration (*21*−*23*).

The existence of many different beer types prompted us to study and characterize some of the more common beer types available on the market. To compare different beer types, total polyphenols, and phenolic acids content, phenolic acids profile and total antioxidant activity were measured in different commercial beer types (bock, abbey, ale, pilsner, wheat, lager, and dealcoholized beers).

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MATERIALS AND METHODS

Chemicals. 2,4,6-Tri(2-pyridyl)-*S*-triazine (TPTZ), Folin−Ciocalteu's reagent, iron chloride, iron sulfate heptahydrate, chlorogenic acid, catechin, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, Trolox, sinapic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid-)diammonium salt (ABTS), and potassium peroxodisulfate were from Sigma-Aldrich (St. Louis, MO). *o*-Coumaric acid, isoferulic acid, and 4-hydroxyphenylacetic acid were from Extrasynthese (Genay Cedex, France). Supelclean LC-SAX SPE cartridges (1 mL tubes) were from Supelco (Bellefonte, PA). Glacial acetic acid and all organic solvents were obtained from Carlo Erba (Milano, Italy). For HPLC analysis, ultrapure water from a Milli-Q system (Millipore, Bedford, MA) was used.

Stock solutions of standard phenolic acids were prepared in methanol (1 mg/mL), stored at −80 °C, and used within 1 week. Working standard solutions were prepared daily by dilution in sample buffer (1.25% glacial acetic acid, 7% methanol in water).

Beers. Beers were purchased at local markets, stored at 10 °C, and analyzed within a few days from the purchase. All beers used in this study were analyzed before the expiry date (at least 3−6 months before expiry date). Beer bottles were uncapped just the day of the analyses and used immediately. Seven beer types were used in this study: bock, abbey, ale, pilsner, wheat, lager, and dealcoholized beers. Five different brands were analyzed for each beer type. Beers were produced in Germany (10 beers), Belgium (9 beers), Italy (7 beers), The Netherlands (2 beers), England (2 beers), France (2 beers), Austria (1 beer), Czech Republic (1 beer), and Ireland (1 beer). The ethanol contents were in the ranges of 6.6−10.0% alc vol for abbey beers (mean = 8.1 ± 1.4), 6.3−13.0% alc vol for ale beers (mean = 8.2 ± 2.7), 4.4−4.9% alc vol for pilsner beers (mean = 4.7 ± 0.2), 3.5−5.2% alc vol for lager beers (mean = 4.4 ± 0.8), 4.9−5.5% alc vol for wheat beers (mean = 5.1 ± 0.3), 6.5−11.0% alc vol for bock beers (mean = 8.4 ± 1.9), and 0−0.5% alc vol for dealcoholized beers. Ingredients of analyzed beers were water, barley malt, hop, and yeast. In wheat and in a few other beers (three abbeys, three ales, one pilsner, and one bock) wheat malt was also used; one beer (dealcoholized) contained maize and one beer (ale), rice.

HPLC Instrumentation. Phenolic acids in beverages, food and human plasma extracts are routinely detected in our laboratory by HPLC-ECD (*24*−*26*). The HPLC consists of a Perkin-Elmer series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT) with a gradient pump, a column thermoregulator, and an autosampling injector (Gilson, Beltline, Middleton, WI) equipped with an electrochemical coulometric detector (Coulochem II, ESA, Bedford, MA). A chromatography workstation with Turbochrom software (Perkin-Elmer) was used for data processing. Operating conditions were as follows: column temperature, 30 °C; flow rate, 1 mL/min; injection volume, 100 μL; electrochemical detection at +600 mV, sensitivity range, 200 nA; filter, 2 s. Chromatographic separations were performed on a Supelcosil LC-18 C_{18} column (5.0 μm particle size, 250 × 4.6 mm i.d.) including a guard column (C_{18}, 5.0 μm particle size, 20 × 4.0 mm i.d.; both Supelco). For gradient elution mobile phases A and B were employed. Solution A was 1.25% glacial acetic acid in water; solution B was absolute methanol. The following gradient was used: 0−30 min, from 98% A, 2% B to 88% A, 12% B, linear gradient; 31−60 min, from 88% A, 12% B% to 80% A, 20% B, linear gradient; 61−85 min, from 80% A, 20% B to 74% A, 26% B, linear gradient; 86−93 min, 55% A, 45% B; 94−123 min, 98% A, 2% B. Prior to HPLC analysis, all samples were filtered using Millex-HV filters (Millipore) with 0.45 μm pore size.

Determination of Phenolic Acids Content. Beer samples were degassed by sonication, and 0.5 mL aliquots were treated for free and total (free plus conjugated) phenolic acids determination as already reported (*24*). Prior to HPLC analysis, the dried residue after ethyl acetate extraction was dissolved in 0.5 mL of bidistilled water and passed through the LC-SAX tube preconditioned with 1 mL of absolute methanol and 2 mL of water (*14*). After tube washing with 1 mL of water, phenolic acids were eluted with 1 mL of buffer containing 1 N acetic acid/MeOH (90:10). The eluant was brought to pH 3 with 6 μL of 4 N NaOH and filtered, and an aliquot (100 μL) was injected into the HPLC system after appropriate dilution. For calibration curve, appropriate volumes of the stock standard solutions were diluted with sample buffer. Three replicates of standards at four concentration levels (20, 100, 200, and 500 ng/mL) were analyzed. The calibration curve was determined on each day of analysis. For quantitative determination, peak areas in the sample chromatograms were correlated with the concentrations according to the calibration curve.

Table 1. Total Polyphenols Content of Different Beer Types[a]

beer type	polyphenols (GAE mg/L)	Student *t* test
dealcoholized	366 ± 73	a
lager	452 ± 86	bc
pilsner	484 ± 37	bc
wheat	504 ± 44	ab
ale	563 ± 52	bc
abbey	622 ± 77	bc
bock	875 ± 168	c

[a] Total polyphenols content was assayed by the Folin−Ciocalteu method using gallic acid for calibration curve and expressed as gallic acid equivalents (GAE). Values are mean ± SE, $n = 5$. Values with different letters are significantly different at $p \leq 0.05$ by Student *t* test.

Determination of Antioxidant Activity of Beers. The total antioxidant activity of beers was measured by the ferric reducing antioxidant power (FRAP) assay (*27*). This is a simple method of determining the reduction of a ferric−tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. Iron sulfate was used for the calibration curve in the range of 10−100 μM. The working FRAP reagent was prepared daily. Readings at 4 min were used for calculation of FRAP values. A blank containing FRAP reagent and water instead of beer was also taken. The difference between sample absorbance and blank absorbance was used to calculate the FRAP value. The reducing capacity of beer tested was calculated with reference to the reaction signal given by Fe^{2+}, on the basis of iron sulfate calibration curve. The antioxidant activity of beer is expressed as micromoles of Fe^{2+} per liter of beer. In a subset of beers, representative for the seven different beer types, antioxidant activity was also measured with the ABTS method on 10 μL samples (*28*).

In a separate set of experiments, standard phenolic acids (0.5 mM) were individually tested for ferric reducing antioxidant activity with the FRAP assay. Methanol, used to dissolve phenolic acids, was ≤0.5% in the final test mixture. Methanolic solutions of iron sulfate (≤0.5% MeOH in the final test mixture) were used for calibration in the range of 10−100 μM.

All measurements were made in quadruplicate.

Determination of Total Polyphenols Content of Beers. The total polyphenols content of beers was determined by using the Folin−Ciocalteu method (*29*, *30*). Gallic acid as reference compound was used for calibration curve in the range 1−20 μg. All measurements were performed in quadruplicate. Absorbance values of beer samples were converted to gallic acid equivalent (GAE) mg/L beer.

Statistical Analysis. Data presented are the mean ± standard error. Statistical analysis was performed by paired Student *t* test, using a statistical package running on a computer (Stat View 4.01, Abacus Concepts, Inc., Berkeley, CA). A probability of $p < 0.05$ was considered to be statistically significant.

RESULTS

Five different brands for each of the seven beer types (dealcoholized, lager, pilsner, wheat, ale, abbey, and bock) selected in this study were analyzed.

Table 1 shows the total polyphenols content of beers measured according to the Folin−Ciocalteu method. The total polyphenols content significantly varied depending on the beer type, ranging from 366 GAE mg/L for dealcoholized beers to 875 GAE mg/L for bock beers. The polyphenols content was significantly higher in abbey and ale beers with respect to dealcoholized beers.

The antioxidant activity of beers measured by the FRAP assay is reported in **Table 2**. Similarly to the polyphenols content, the ferric reducing antioxidant activity of beers showed significant differences depending on beer type, ranging from 1525 μmol of Fe^{2+}/L for dealcoholized beers to 4663 μmol of Fe^{2+}/L for bock beers. Again, the higher antioxidant activity was measured in bock beers, followed by abbey and ale beers. We also ran the ABTS assay on a subset of beers representative of the seven

Table 2. Ferric-Reducing Antioxidant Power (FRAP) of Different Beer Types[a]

beer type	FRAP (μmol of Fe^{2+}/L/4 min)	Student *t* test
dealcoholized	1525 ± 217	a
lager	2189 ± 199	b
pilsner	2172 ± 157	b
wheat	2403 ± 72	b
ale	3125 ± 46	c
abbey	3558 ± 301	c
bock	4663 ± 863	c

[a] FRAP was measured using iron sulfate for calibration curve. The antioxidant activity of beer is expressed as μmol of Fe^{2+}/L of beer. Values are mean ± SE, $n = 5$. Values with different letters are significantly different at $p \leq 0.05$ by Student *t* test.

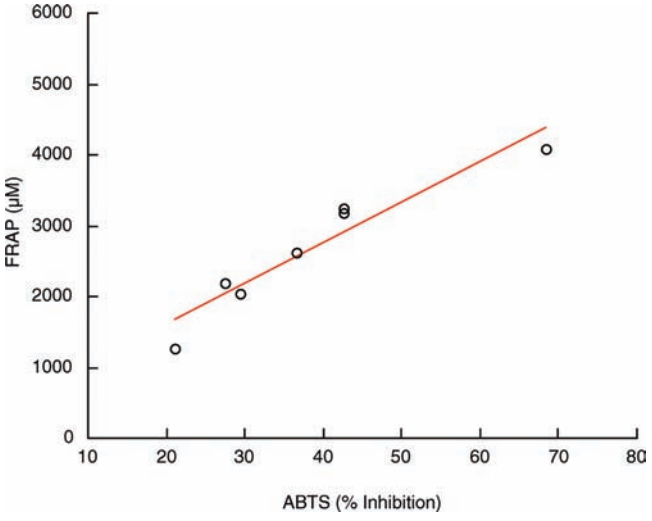

Figure 1. Correlation between antioxidant activity values measured with FRAP assay and ABTS assay. Antioxidant activity was measured on beer samples representative of the seven different types as described under Materials and Methods ($r = 0.95$, $p < 0.0001$).

different types, in parallel with the FRAP assay (**Figure 1**). A significant correlation between antioxidant activity measured with FRAP assay and ABTS assay was found ($r = 0.95$, $p < 0.0001$).

As shown in **Figure 2**, a strict correlation between total polyphenols content measured by the Folin−Ciocalteu method and antioxidant activity of beers measured by FRAP assay was found ($r = 0.92$, $p < 0.0001$).

On a subset of 21 beers (3 different brands for each of the 7 beer types) the content of both free and total (free plus conjugated) phenolic acids was determined by HPLC-ECD (**Table 3**). Ferulic acid was by far the most abundant phenolic acid in beers, followed by sinapic, vanillic, caffeic, *p*-coumaric, and 4-hydroxyphenylacetic acids. Ferulic, caffeic, syringic, and sinapic acids are present in beers mainly as bound forms, whereas *p*-coumaric and 4-hydroxyphenylacetic acids are generally present equally in free and bound forms except for a slight prevalence of the bound forms in abbey beers. For vanillic acid, there is a prevalence of the free forms in dealcoholized beers, whereas in all the other beer types a prevalence of the bound forms is evident, more pronounced in abbey beers. Significant differences in phenolic acids content were evident depending on beer type, with the lowest levels in dealcoholized beers and the highest levels in bock beers. Chlorogenic acid was detected in 7 of 21 beers at low level (< 0.2 mg/L), whereas catechin was detected in 5 of 21 beers in the range of 0.1−2.6 mg/L (data not shown).

The phenolics content of beers was compared to their antioxidant activity, measured by the FRAP assay (**Table 4**). In addition to the correlation between FRAP values and total

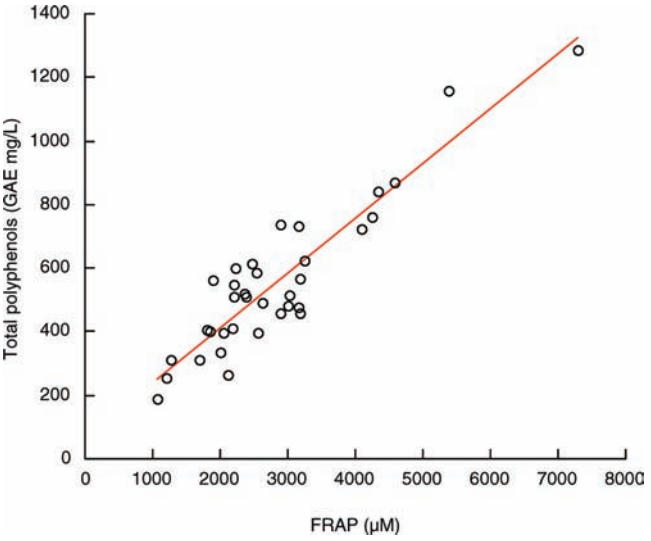

Figure 2. Relationship between antioxidant activity (FRAP values) and total polyphenols content (Folin−Ciocalteu) of beers. Five different brands were analyzed for each of the seven beer types under study. Data were analyzed for correlation by Student *t* test for paired data ($n = 35$; $r = 0.92$, $p < 0.0001$).

polyphenols content, a strong, significant correlation was evident between measured FRAP values and total phenolic acids content (free plus conjugated phenolic acids) ($p < 0.001$) (**Figure 3**), whereas no significant correlation was found between FRAP values and the content of free phenolic acids (**Table 4**), indicating that conjugated forms of phenolic acids in beer retain antioxidant activity and are responsible for most of the ferric reducing antioxidant activity of beer. Among phenolic acids, a significant, strong correlation was found between FRAP values and the content of ferulic (**Figure 4**) and vanillic acids (**Figure 5**) ($p < 0.0005$). A significant correlation was also evident between FRAP values and the content of caffeic acid, *p*-coumaric acid, 4-hydroxyphenylacetic, syringic, and sinapic acids ($p \leq 0.05$) (**Table 4**).

To study the contribution of the single phenolic acids to FRAP, standard phenolic acids were tested for their ferric reducing activity with the FRAP assay. **Table 5** shows the FRAP values obtained with the different standard phenolic acids (500 μM), expressed as micromole equivalents of Fe^{2+} per liter at 4 min of reaction, in comparison to Trolox, the water-soluble derivative of vitamin E. The higher FRAP values were obtained for syringic, sinapic, caffeic, and ferulic acids, followed by Trolox, whereas vanillic, *p*-coumaric, and 4-hydroxyphenylacetic acids exhibited lower ferric reducing activity. From these results, the number of hydroxy and methoxy groups on the aromatic ring of phenolic acids seems to affect the ferric reducing antioxidant activity of phenolic acids, with monosubstituted compounds exhibiting the low FRAP values, and syringic and sinapic acids, both three-substituted, showing the higher FRAP values. **Table 6** shows the regression equation for all of the compounds tested by FRAP assay, including iron sulfate, as well as the correlation coefficient and concentration intervals assayed. Slope values allow for a comparison of the antioxidant efficiency of the compounds tested. Again, higher slope values, denoting higher ferric reducing ability, were observed for syringic, sinapic, caffeic, and ferulic acids. These compounds are more efficient than iron sulfate itself in reducing ferric chloride in the FRAP assay. The antioxidant activity of vanillic acid is quite similar to, although somewhat lower than, the activity of iron sulfate. *p*-Coumaric acid and 4-hydroxyphenylacetic acid exhibited low antioxidant activities, on the basis of the low slope values. Our data are in

Table 3. Phenolic Acids Content of Beers (Micrograms per Milliliter)[a]

	dealcoholized	pilsner	lager	bock	wheat	abbey	ale
4-hydroxyphenylacetic acid							
free	0.32 ± 0.03 (a)	0.69 ± 0.17 (abc)	0.45 ± 0.06 (ac)	0.81 ± 0.11 (b)	0.52 ± 0.15 (abc)	0.59 ± 0.08 (bc)	0.53 ± 0.06 (bc)
total	0.68 ± 0.12 (a)	1.36 ± 0.19 (b)	1.28 ± 0.20 (ab)	1.41 ± 0.12 (b)	0.93 ± 0.20 (ab)	1.46 ± 0.19 (b)	1.13 ± 0.03 (b)
vanillic acid							
free	0.45 ± 0.10	0.56 ± 0.02	0.57 ± 0.11	0.81 ± 0.19	0.67 ± 0.10	0.65 ± 0.16	0.61 ± 0.15
total	0.75 ± 0.08 (a)	1.58 ± 0.16 (b)	1.51 ± 0.36 (ab)	2.22 ± 0.37 (b)	1.52 ± 0.10 (b)	2.43 ± 0.44 (b)	1.78 ± 0.36 (b)
caffeic acid							
free	0.14 ± 0.03	0.14 ± 0.06	0.12 ± 0.02	0.17 ± 0.05	0.19 ± 0.05	0.25 ± 0.07	0.14 ± 0.03
total	0.77 ± 0.19 (a)	1.51 ± 0.45(abc)	1.18 ± 0.07 (ab)	1.47 ± 0.11 (bc)	0.98 ± 0.08 (ab)	1.92 ± 0.26 (c)	1.30 ± 0.03 (bc)
syringic acid							
free	0.16 ± 0.07 (ab)	0.27 ± 0.08 (ab)	0.14 ± 0.03(a)	0.22 ± 0.05 (ab)	0.35 ± 0.05 (b)	0.22 ± 0.03 (ab)	0.20 ± 0.08 (ab)
total	0.60 ± 0.37 (a)	0.87 ± 0.43 (ab)	0.99 ± 0.08 (ab)	0.69 ± 0.23 (ab)	1.23 ± 0.32 (b)	0.70 ± 0.25 (ab)	0.49 ± 0.19 (ab)
p-coumaric acid							
free	0.40 ± 0.09 (ab)	0.76 ± 0.46 (ab)	0.59 ± 0.15 (ab)	0.72 ± 0.17 (a)	0.26 ± 0.02 (b)	0.23 ± 0.05 (b)	0.58 ± 0.19 (ab)
total	0.72 ± 0.01 (ab)	1.36 ± 0.60(abc)	1.20 ± 0.16 (bc)	1.55 ± 0.18 (bc)	0.55 ± 0.08 (a)	1.59 ± 0.14 (bc)	1.19 ± 0.18 (abc)
ferulic acid							
free	1.40 ± 0.45 (ab)	1.03 ± 0.03 (ab)	1.81 ± 0.30 (a)	2.00 ± 0.47 (ab)	0.34 ± 0.23 (c)	0.92 ± 0.06 (bc)	1.54 ± 0.38 (ab)
total	6.38 ± 0.73 (a)	16.6 ± 4.43(abc)	11.3 ± 1.08 (b)	17.5 ± 1.18 (c)	10.4 ± 0.34 (b)	17.5 ± 0.63 (c)	13.3 ± 2.78 (abc)
sinapic acid							
free	0.26 ± 0.04 (a)	0.44 ± 0.17 (abc)	0.53 ± 0.20 (abc)	0.45 ± 0.07 (abc)	0.55 ± 0.05 (c)	0.34 ± 0.04 (ab)	0.29 ± 0.08 (ab)
total	1.58 ± 0.03 (a)	3.14 ± 0.92 (ab)	2.62 ± 0.90 (ab)	3.53 ± 0.27 (b)	4.18 ± 0.67 (b)	3.18 ± 0.21 (b)	2.35 ± 0.59 (ab)
phenolic acids							
free	3.18 ± 0.91	5.42 ± 2.42	4.71 ± 1.08	5.49 ± 0.92	2.90 ± 0.55	3.23 ± 0.40	3.92 ± 0.90
total	11.1 ± 0.94 (a)	26.7 ± 7.21 (abc)	21.0 ± 1.40 (bd)	29.1 ± 1.45 (cd)	19.9 ± 0.85 (b)	28.7 ± 0.65 (c)	21.7 ± 3.86 (bc)

[a] Three different brands for each beer type were analyzed for free and total phenolic acids content. Values are means ± SE (n = 3). Values with different letters within rows are statistically different at $p < 0.05$ by Student t test.

Table 4. Relationships between Antioxidant Power (FRAP) and Phenolics Content of Beers[a]

	correlation coefficient	p
ferulic acid (total)	0.78	<0.0001
caffeic acid (total)	0.59	0.0046
vanillic acid (total)	0.80	<0.0001
sinapic acid (total)	0.54	0.0116
4-hydroxyphenylacetic acid (total)	0.48	0.0261
p-coumaric acid (total)	0.64	0.0025
syringic acid (total)	0.44	0.0434
phenolic acids (total)[b]	0.77	<0.0001
phenolic acids (free)[c]	0.30	ns
total polyphenols[d]	0.92	<0.0001

[a] Single phenolic acids were measured by HPLC-ECD, with (total) or without (free) alkaline hydrolysis pretreatment. [b] Total phenolic acids content represents the sum of the single phenolic acids content measured after alkaline hydrolysis. [c] Free phenolic acids content represents the sum of the single phenolic acids content in the free form (without alkaline hydrolysis). . [d] Total polyphenols were measured by the Folin−Ciocalteu method. Data were analyzed for correlation by Student t test for paired data, n = 21.

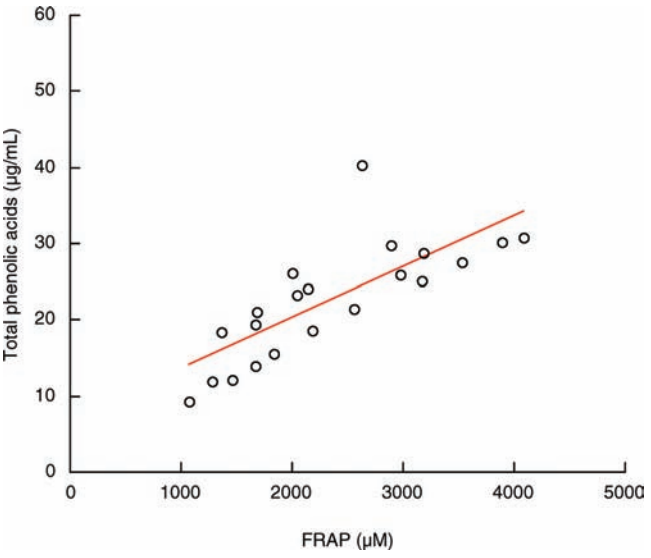

Figure 3. Relationship between antioxidant activity (FRAP values) and total phenolic acids content of beers. Total phenolic acids were measured by HPLC-ECD after alkaline hydrolysis. Data were analyzed for correlation by Student t test for paired data (n = 21; r = 0.77, $p < 0.0001$).

agreement with data present in the literature for ferulic acid, Trolox, and iron sulfate, whereas for caffeic we obtained a slightly lower value (*29*).

DISCUSSION

Beer contains an appreciable amount of phenolic compounds, originating mainly from barley (about 70%) and hop (about 30%) (*9*−*11*, *31*−*33*), that contribute to the overall antioxidant activity of the beverage. Phenolics content and antioxidant activity of beer depend on the quantity and quality of starting materials and on the industrial brewing process itself. The role of phenolic compounds in relation to the color, taste, and stability of beer is well-known (*34*). Beer rich in phenolic antioxidants shows higher quality, more stable sensory properties, such as flavor and aroma, foam stability, and longer shelf life with respect to beer with lower antioxidant activity (*9*−*11*, *35*−*38*). In the present study, significant differences in both polyphenols content and antioxidant activity were demonstrated in different commercial beer types (abbey, ale, bock, wheat, lager, pilsner, and dealcoholized beers). Polyphenols content and antioxidant activity increased in the order dealcoholized < lager < pilsner < wheat < ale < abbey < bock, so that the polyphenols content and the antioxidant activity of bock beers were found to be about 3 times higher with respect to dealcoholized beers. Vinson et al. (*39*) reported higher polyphenols content, measured with the Folin−Ciocalteu method, in ale beer with respect to lager and dealcoholized beers. Recently, both polyphenols content and antioxidant activity have been reported to be lower in alcohol-free beers

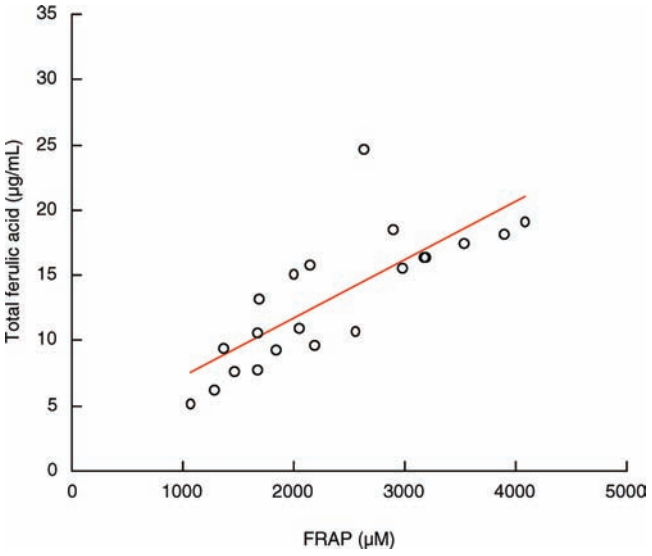

Figure 4. Relationship between antioxidant activity (FRAP values) and total ferulic acid content of beers, measured by HPLC-ECD after alkaline hydrolysis. Data were analyzed for correlation by Student *t* test for paired data ($n = 21$; $r = 0.78$, $p < 0.0001$).

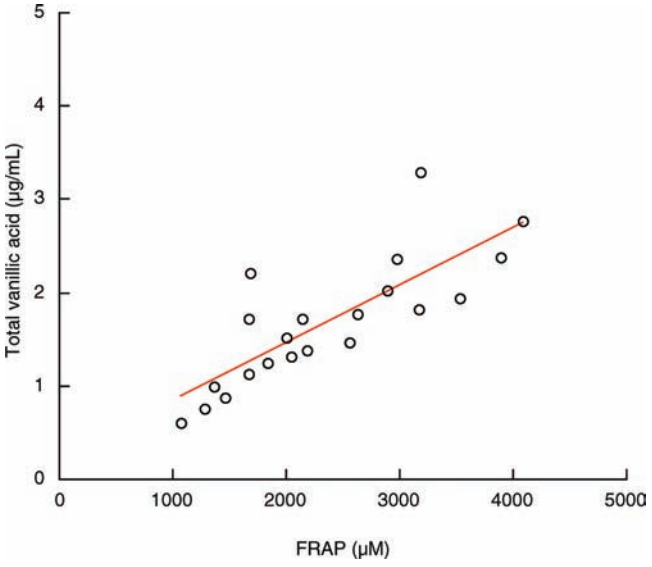

Figure 5. Relationship between antioxidant activity (FRAP values) and total vanillic acid content of beers, measured by HPLC-ECD after alkaline hydrolysis. Data were analyzed for correlation by Student *t* test for paired data ($n = 21$; $r = 0.80$, $p < 0.0001$).

with respect to strong and dark beers (*40*). Alcohol-free beers are usually brewed with lower original wort extract and inhibition of alcohol formation, or as normal alcoholic beers, with the alcohol removal at the last step, whereas dark, strong beers are brewed from wort with higher extract content. Moreover, the brewing process itself may influence the final polyphenols content and antioxidant activity of beers (*41*−*43*).

The most critical stages for changes in polyphenols content and antioxidant activity during beer production have been reported to be filtration and clarification, as well as boiling, fermentation, and maturation (*40*, *44*). During mashing, free hydroxycinnamic acids in wort are both water-extracted and enzymatically released by cinnamoyl esterase activity (*41*). Esterase activity clearly differs between different barley malt varieties. Moreover, mashing variables such as temperature, time, and pH influence the

Table 5. Contribution to FRAP Values of Standard Phenolic Acids (500 μM) in Comparison to Trolox[a]

compound	μmol of Fe^{2+}/L/4 min
4-hydroxyphenylacetic acid	14.8 ± 0.1
p-coumaric acid	216.0 ± 1.7
vanillic acid	445.3 ± 3.5
Trolox	1034.9 ± 16.3
ferulic acid	1227.5 ± 0.5
caffeic acid	1305.0 ± 10.0
sinapic acid	1412.0 ± 32.0
syringic acid	1621.5 ± 39.5

[a] FRAP was measured using iron sulfate for calibration curve. Standard Trolox and phenolic acids solutions (0.5 mM) were tested with the FRAP assay and results expressed as μmol equiv of Fe^{2+}/L at 4 min of reaction. Values are means ± SE ($n = 3$).

Table 6. Assay Concentration Intervals and Regression Equations with Correlation Coefficients of Standard Phenolic Acids in Comparison with Fe_2SO_4 and Trolox at 4 min of Reaction with the FRAP Reagent[a]

compound	concentration range (μmol/L)	slope	intercept	*r*
Fe_2SO_4	10−100	0.0208	−0.0417	0.9999
4-hydroxyphenylacetic acid	50−500	0.0006	−0.0520	0.9975
p-coumaric acid	5−50	0.0079	+0.0176	0.9977
vanillic acid	5−50	0.0178	+0.0035	0.9981
Trolox	5−50	0.0419	+0.0233	0.9997
ferulic acid	2.5−25	0.0486	+0.0387	0.9989
caffeic acid	2.5−25	0.0535	−0.0951	0.9999
sinapic acid	2.5−25	0.0564	+0.0272	0.9978
syringic acid	2.5−25	0.0668	−0.0109	0.9998

[a] Equations were calculated with at least five different concentrations assayed in triplicate ($p < 0.0001$). All equations followed a lineal regression model.

release of hydroxycinnamic acids. The release of phenolic acids during mashing also depends on the amount of ester-bound phenolic acids initially present in the wort and on its endoxylanase activity (*41*). Finally, a large variability in polyphenols content of barley and hop has been described, depending on the variety, the geographic origin, freshness, and the harvesting procedure (*10*, *42*, *45*−*47*).

Phenolic acids (hydroxybenzoic and hydroxycinnamic acids), as both free forms and glycosidic esters, are present in barley grains and hop (*10*, *48*). Phenolic acids received particular attention in the past decade because of their relatively high concentration in the human diet, their strong antioxidant activity, and demonstrated intestinal absorption in humans. In our study, the contents of both free forms and conjugated forms of phenolic acids were measured in seven beer types. The results obtained are in agreement with data from the literature (*24*, *31*, *32*, *47*, *49*−*53*), concerning the free phenolic acids content of beer, whereas the total phenolic acids content is usually not routinely assayed. Ferulic acid is by far the major phenolic acid in beer, followed by sinapic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and 4-hydroxyphenylacetic acid. The contents of bound, conjugated forms of ferulic acid, caffeic acid, sinapic acid, syringic acid, and, to a minor extent, vanillic acid largely exceed those of the respective free forms, whereas *p*-coumaric and 4-hydroxyphenylacetic acids are generally present equally in the free and bound forms. The strong correlation between FRAP values and total phenolic acids content of beers and the lack of correlation between FRAP values and the content of free phenolic acids indicate that conjugated forms of phenolic acids in beer retain antioxidant activity and actively contribute most of the ferric reducing antioxidant activity of beer. Finally, when the role of individual phenolic acids in the total antioxidant activity of beer is

considered, not only the concentration but also the structure of single phenolic acids should be taken into evaluated. From our results, syringic, sinapic, caffeic, and ferulic acids are the major contributors to the antioxidant activity of beer.

Hydroxycinnamic and hydroxybenzoic acids are known antioxidants acting as free radical acceptors and chain breakers. The antioxidant activity and biological effects of caffeic, ferulic, vanillic, and *p*-coumaric acids have been widely studied and described in the literature in the past decade. 4-Hydroxyphenylacetic acid has been described to scavenge reactive oxygen and nitrogen species both in vitro and in vivo (*54*−*56*). Phenolic acids from beer are small molecules rapidly absorbed in humans and mammals. Recently, phenolic acids from beer have been demonstrated to be absorbed in humans from the gastrointestinal tract and to circulate in blood after being largely metabolized to the form of glucuronide and sulfate conjugates (*13*, *14*).

Besides polyphenols, barley and malt extracts contained other antioxidants: carotenoids (lutein and zeaxanthin), tocopherols, ascorbic acid, and Maillard reaction products, the latter being generated during malting and wort boiling (*9*, *11*). All of these compounds, although present in beer at very low levels, might contribute to some extent to the overall antioxidant activity of beer.

Recently, a renewed interest has been focused on beer, due to its high phenolic antioxidants and low ethanol content. Mild to moderate alcohol consumption is associated with beneficial healthy effects on the cardiovascular system (*57*, *58*). Population-based studies have observed that moderate drinking in the range of one to three drinks daily is associated with a rate of coronary disease 30−40% lower compared with that in the nondrinking population. The association between alcohol consumption and cardiovascular disease is not linear but "U-shaped", with higher death rates found among those who abstain as well as those who drink an excess of six drinks a day (*57*). Moreover, beer consumption seems to have no effect or even an inverse effect on total homocysteine concentration (*21*).

In conclusion, beer may represent an important part of the overall dietary intake of antioxidants, particularly in the form of phenolic acids. The contribution of beer to antioxidants/phenolic acids intake may vary significantly on the basis of the different beer types.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; TPTZ, 2,4,6-tri(2-pyridyl)-*S*-triazine.

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