J Food Sci Technol (April 2017) 54(5):1158–1167
DOI 10.1007/s13197-017-2503-1

ORIGINAL ARTICLE

Folic acid content and antioxidant activity of different types of beers available in Hungarian retail

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Revised: 14 December 2016 / Accepted: 19 January 2017 / Published online: 1 March 2017

Abstract In this study 40 Hungarian retail beers were evaluated for folic acid content, antioxidant profile and physicochemical parameters. The physicochemical parameters, folic acid content and antioxidant activity of alcohol-free beers were the lowest. Folic acid content of beers aged with sour cherries showed high values, more than 0.4 mg/l and an alcohol-free beer-based mixed drink made with lemon juice contained more than 0.2 mg/l of folic acid. Dark beers and beers aged with sour cherries had the highest antioxidant activity probably owing to their high extract content, components released from the fruits and special malts. These results highlight the possibility of achieving adequate folic acid and relevant antioxidant intake without excessive alcohol and energy consumption by selecting appropriate beer types.

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Keywords Beer · Fruit beer · Alcohol free beer · Folic acid · Antioxidant activity · Principal component analysis (PCA)

Introduction

Beer is one of the most commonly consumed alcoholic beverages. There are many studies investigating raw materials and the effects of technological steps and aging on polyphenol content, antioxidant activity and other compounds of this beverage (Fogarasi et al. 2015; Fumi et al. 2011; Siqueira et al. 2011). However, there is only limited information available on the amount of health protecting compounds of commercially available beers. Lugasi (2003) found that the polyphenol content—expressed in catechin equivalent—of dark beers (380–600 mg/l) is higher than that of pale ones (270–470 mg/l). Granato et al. (2011) reported antioxidant activity of commercial beers from 424 to 10,508 μmol/l trolox equivalent by ORAC test, and also found higher values in case of dark beers. Zhao et al. (2010) found that there are significant correlations between five antioxidant measuring methods in case of beers. Beside the most familiar products, beers mixed with fruit juice and their alcohol-free alternatives are becoming more popular even among those who do not like the taste of beer or cannot consume alcohol. Many different kinds of juices are used for their production, e.g. lemon, orange, grapefruit, apple, raspberry, black-currant etc. Beside these products, craft beers are also getting more popular. Among them there are plenty of different beer types made from special ingredients. Although they are getting more and more popular, we still lack information about their potential health-positive components and qualities.

This shortcoming is regrettable as these beverages can have a positive effect on our health by their unbound components from malt, hops and in some cases from fruit juices. They are rich in vitamin B group components, minerals, some micronutrients and biologically active components etc. Folic acid is found in relatively high amounts in beers (Buiatti 2009). This compound has a pivotal role in the proper function of the human body and inadequate intake is now related not only to neural tube defects among newborns but to infertility, certain types of cancer (Aune et al. 2011) and to heart diseases (Blom and Smulders 2011) as well. The American recommended dietary allowance (RDA) for folic acid is 0.4 mg/day/capita (Whitney and Rolfes 2007). This amount is hard to be covered even by a well-balanced diet regarding to the folic acid content of foods (Whitney and Rolfes 2007), hence every folate-rich foodstuff, including beer, is an important part of the diet. Other studies suggested that beside the above mentioned compounds, beers also contain polyphenols and antioxidants—70–80% of these compounds come from malt and 20 to 30% from hops respectively (Gerhäuser 2009)—which also have proven positive effects on health (Saura-Calixto et al. 2011). The antioxidant activity of beers mainly comes from Maillard-reaction products, sulfites, phenolic compounds and added antioxidants (e.g. ascorbic acid) (Saura-Calixto et al. 2011). The most common polyphenols of beers are simple phenols, phenolic acids, benzoic acid derivatives, cinnamic acids, flavanones, flavanols, flavonols, isoflavones and flavones. These components protect from oxidative stress caused by reactive oxygen—and nitrogen species (ROS and RNS, respectively) (Arranz et al. 2012).

Indeed, a paradigm change is happening as new research insights imply that antioxidant activity as a surrogate marker for food quality and its effect on health should be discouraged since it is hard to interpret the in vitro measured values compared to the in vivo system of human physiology. This makes us reconsider our previous results and statements of many foods that were nominated as good sources for free radical eliminating antioxidants. It is different in the case of beer as in vivo studies were performed on humans which prove the metabolism and absorbtion of phenolic compounds from beers into human blood. For instance the plasma level of 4-hydroxyphenylacetic acid increased ~12% 30 min after beer consumption (Nardini et al. 2006). Alvarez et al. (2009) measured lower low-density lipoprotein oxidation and oxidative stress parameters following a 45-day long alcohol-free beer consumption period (0.5 l/day). They reported an ~18% decrease in thiobarbituric-acid reactive substances and −21% plasma carbonyl group content compared to the initial values. Taken everything into consideration, detailed results on different types of beers that are available in retail may give support information for selecting the best types of these products from a practical nutritional viewpoint.

However, as it has been previously mentioned, most of the publications on beers analyzed the changes of these compounds during the brewing process and in raw materials. There are only few studies which examined the final products that are available in the retail of some countries. Therefore, we aimed to draw a comprehensive picture of the folic acid content and the antioxidant activity of commercial beers in light of their physicochemical properties. Our findings have practical relevance for dietitians and nutritionists, who could incorporate these beverages into a well-balanced, healthy diet and could form an opinion about them knowing some of their health protecting compounds and physicochemical properties. Furthermore, our findings may also indicate some directives regarding the development of folate and antioxidant rich beer as a state-of-the-art functional beverage.

Materials and methods

Solvents, reagents and standards

HCl, iso-octane, methanol, Na_2CO_3, $FeCl_3 \cdot 6H_2O$, $CuCl_2$, NH_4Ac, NaCl, glucose, H_2O_2 were purchased from VWR International Co. (Radnor, PA, USA). Folin-Cioalteau reagent, gallic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), neocuproine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

Beer samples

Forty different beers were purchased which are available in the Hungarian commercial trade. Apart from the most common ones some special kinds of beers were included, e.g. beers mixed with fruit juices or aged with fruits, Hungarian craft beers as well as alcohol-free products. Beers were classified based on the Beer Style Guidelines of the Beer Judge Certification Program (BJCP 2008). We investigated three alcohol-free pale lagers, three alcohol-free beer-based mixed drinks, three beer-based mixed drinks, two strong pale lager, 11 European pale lagers, one American adjunct lager, two Czech pilsners, two stouts, one altbier, four hefeweizen (unfiltered weissbier), two dark lagers, one Belgian strong pale ale, one Irish ale, one dunkler bock and three specialty fruit beers. With this data selection we aimed to involve many types of beer. Our samples are presented in Table 1 with their most important physicochemical parameters. Samples were homogenized and degassed by sonication in ultrasonic bath for 5 min. Samples were stored at −18 °C until analysis.

Physicochemical properties

The alcohol content, original extract and energy values were determined by a beer analyzer (Anton Paar Alcolyzer Plus).

The bitterness value was determined according to Analytica-EBC method 9.8 (EBC 1998).

Results of these parameters were used as supportive values and for the correlation analysis.

Folic acid content

In order to determine the folic acid content sandwich ELISA-based method was used by the instructions of the producer (Immunolab GmbH, Kassel, Germany). This method is capable of measuring the total folic acid content without the separation of the different derivatives. Whereas HPLC based methods would enable us to simultaneously measure the important folate-substances, this immunosorbent assay makes it possible to determine many samples together for an affordable price.

Antioxidant activity

The antioxidant activity was determined by five commonly applied assays as there is no standard method which can objectively characterize this parameter (Huang et al. 2005).

The total polyphenol content (TPC) assay was performed based on the description of Singleton and Rossi (1965), which is also based on the reduction power of antioxidants rather than on the selective reaction of polyphenols, thus it was evaluated together with the other antioxidant activity assays (Martinez-Periñan et al. 2011). First 1250 µl tenfold diluted Folin–Cioalteau reagent and 240 µl methanol:water (4:1) solvent were pipetted in the test-tubes. Then 10 µl degassed sample was added. After homogenization and the 1-min reaction time 1 cm^3 0.7 M Na_2CO_3 was added, vortexed and before measurement the mixture was allowed to stand for 5 min at 50 °C. The absorbance was measured at $\lambda = 765$ nm and was expressed as mg/100 cm^3 gallic acid equivalent (GAE).

The ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain (1996). Samples were added to FRAP reagent that contained 10 mM TPTZ dissolved in 40 mM HCl, 300 mM pH 3.6 acetate buffer and 20 mM $FeCl_3 \cdot 6H_2O$. After 5 min of incubation time, absorbance was measured at $\lambda = 593$ nm and the results were expressed as ascorbic acid equivalent (ASAE).

The DPPH assay was performed as described by Brand-Williams et al. (1995). 6×10^{-5} M DPPH solution was prepared with methanol. 100 µl sample was added to 3.9 cm^3 DPPH solution and was incubated in dark for 20 min then it was measured at $\lambda = 517$ nm against methanol blank. Inhibition percentages (I%) were calculated and used for comparison.

The cupric reducing antioxidant capacity (CUPRAC) assay was performed according to Apak et al. (2004). 100 µl sample was added to 1 cm^3 10^{-2} M $CuCl_2$, 1 cm^3 7.5×10^{-3} M neocuproine solution (dissolved in 96% ethanol), 1 cm^3 pH 7.4 1 M NH_4Ac buffer solution and 0.9 cm^3 distilled water. It was incubated in dark for 30 min and the absorbance was measured at $\lambda = 450$ nm to a trolox calibration curve. The results were expressed as µM trolox equivalent (TE).

The ABTS assay was performed as described by Salah et al. (1995). 10 µl degassed sample was pipetted into 96 well plates. 20 µl solution was added, which contained 9% NaCl, 1% glucose, 50 mg/ml myoglobine dissolved in pH 7.4 potassium-phosphate buffer. Then 150 µl 1 mg/ml ABTS solution and 25 µl 3% H_2O_2 dissolved in 0.1 M pH 5 citric buffer was added. It was shaken for 5 min at 37 °C then absorbance was measured at $\lambda = 405$ nm. Quantification was done with a calibration curve prepared with trolox and results were also expressed as µM trolox equivalent.

Statistical analysis

To analyze the antioxidant activity values of the investigated samples obtained by the five assays simultaneously, principal component analysis (PCA) was performed. To test the correlations between the measured parameters Spearman rank correlation was executed as a test of normality (utilized according to Kolmogorov–Smirnoff) indicated non-normal distribution of data. All statistical analysis was performed on 5% of significance level with the XLSTAT software (Addinsoft, New York, NY, USA).

Results and discussion

Folic acid content

Fig. 1 illustrate the differences between the folic acid content of the samples. This is in line with the findings of Owens et al. (2007), who found that the folate content of beers were between 0 and 0.283 mg/bottle, Buiatti (2009) also reported 0.040–0.600 mg/l folate content. The majority of the samples were in a narrow range around 0.050 mg/l in terms of the folic acid content. Some dark

Table 1 Physicochemical attributes of the investigated beers of different types and styles analyzed in this study (n = 40)

Sample no.	Type	Country of origin	Ingredients	Bitterness (EBC)[a]	Original extract content (%Plato)	Ethanol (%V/V)	Energy (kJ/100 ml)
1	Alcohol-free pale lager	Hungary	Water, barley malt, hops extract, hops	14	3.96	0.18	59.99
2	Alcohol-free pale lager	Hungary	Water, barley malt, wheat malt, aroma, hops	18	6.16	0.00	87.43
3	Alcohol-free pale lager	Hungary	Water, barley malt, hops extract, hops	17	4.61	0.14	70.04
4	Alcohol-free beer-based mixed drink	Hungary	(40%) blackcurrant-lime juice: water, sugar, lime concentrate, blackcurrant concentrate, caramel, citric acid, sweeteners, ascorbic acid (60%) beer: water, barley malt, corn sleet, hops	11	5.64	0.00	86.09
5	Alcohol-free beer based mixed drink	Hungary	(63%) lemon juice: water, sugar, lemon juice concentrate, orange extract, orange juice concentrate, sweeteners, aroma, stabilizers, ascorbic acid; (37%) beer: water barley malt, corn sleet, hops	8	4.60	0.00	65.73
6	Alcohol-free beer-based mixed drink	Hungary	(63%) grapefruit juice: water, sugar, lemon juice concentrate, grapefruit juice concentrate, orange juice concentrate, citric acid, K-citric, calcium lactate, magnesium carbonate, natural grapefruit aroma, other natural aromas, stabilizer, sweetener (37%) beer: water barley malt, corn sleet, hops	5	5.08	0.00	71.33
7	Beer-based mixed drink	Hungary	The same as no. 5	6	0.00	1.78	0.00
8	Beer-based mixed drink	Hungary	The same as no. 6	5	7.31	1.57	110.32
9	Beer-based mixed drink	Hungary	The same as no. 4	8	5.54	1.58	82.32
10	Strong pale lager	Romania	Water, barley malt, barley, hops	26	14.40	6.79	218.72
11	European pale lager	Romania	Water, barley malt, hops	13	11.22	4.95	168.37
12	European pale lager	Romania	Water, barley malt, barley, hops	17	9.95	4.51	148.30
13	European pale lager	Hungary	Water, barley malt, hops	20	10.64	4.67	159.35
14	European pale lager	Hungary	Water, barley malt, corn, hops, hops extract	6	4.26	2.06	61.64
15	European pale lager	Hungary	Water, barley malt, corn sleet, hops	16	8.71	3.73	129.38
16	Czech pilsner	Czech republic	Water, barley malt, hops, hops extract	27	7.86	2.82	117.12
17	Czech pilsner	Czech republic	Water, barley malt, barley, hops	42	9.84	4.11	147.19
18	European pale lager	Hungary	Water, barley malt, corn, hops, hops extract	21	11.43	5.09	171.66
19	European pale lager	Hungary	Water, corn sleet, hops	15	9.07	4.01	134.88
20	European pale lager	Hungary	Water, barley malt, corn, hops, hops extract	17	6.77	2.81	99.91
21	Munich helles	Romania	Water, barley malt, corn sleet, hops	13	8.57	3.76	127.15
22	Wienna lager	Hungary	Water, barley malt, corn sleet, hops	15	8.21	3.86	121.27
23	American adjunct lager	Mexico	Water, barley malt, corn and/or rice, antioxidant: e300, stabilizer	10	5.95	2.27	87.75

Table 1 continued

Sample no.	Type	Country of origin	Ingredients	Bitterness (EBC)[a]	Original extract content (%Plato)	Ethanol (%V/V)	Energy (kJ/100 ml)
24	Dunkel lager	Hungary	Water, barley malt, corn, hops, hops extract	21	5.02	4.13	70.42
25	Schwarzbier	Hungary	Water, barley malt, hops, yeast	30	12.82	4.96	194.85
26	Altbier	Hungary	Water, barley malt, wheat malt, caramel malt, hops, yeast	32	12.61	4.68	191.81
27	Strong pale lager	Hungary	Water, malt, hops, yeast	17	8.93	3.58	133.14
28	Belgian strong pale ale	Belgium	Water, barley malt, hops, yeast	20	16.32	8.29	249.35
29	Hefeweizen	Austria	Water, wheat malt, barley malt, hops	–	7.13	2.82	105.61
30	Hefeweizen	Germany	Water, wheat malt, barley malt, hops, yeast	12	9.57	3.88	143.09
31	Stout	Belgium	Water, barley malt, hops, yeast	18	16.87	8.17	258.95
32	Irish red ale	Ireland	Water, malt, hops	19	8.21	3.52	121.77
33	Irish stout	Ireland	Water, barley malt, roasted barley, hops, nitrogen	17	8.05	3.43	119.23
34	Munich dunkel lager	Hungary	Water, barley malt, hops	18	6.72	2.63	99.29
35	Dunkler bock	Hungary	Water, barley malt, hops, hops extract	10	17.21	6.90	266.51
36	Hefeweizen	Hungary	Water, wheat malt, pilsen malt, yeast	21	10.08	3.78	151.54
37	Hefeweizen	Hungary	Water, barley malt (pilsner, caramel, acidulated), wheat malt, hops (magnum), yeast	8	12.45	5.77	187.59
38	Fruit beer	Belgium	Water, barley malt, hops, yeast, candy, sour cherry (10%)	9	17.31	8.34	266.38
39	Fruit beer	Belgium	Barley malt, hops, sour cherry	7	14.95	5.90	229.21
40	Fruit beer	Belgium	Water, barley malt, wheat, sour cherry, elderberry, sugar, hops, yeast	12	14.16	8.21	212.78

[a] EBC unit: bitterness unit, mean coefficient of variation = 3.9%

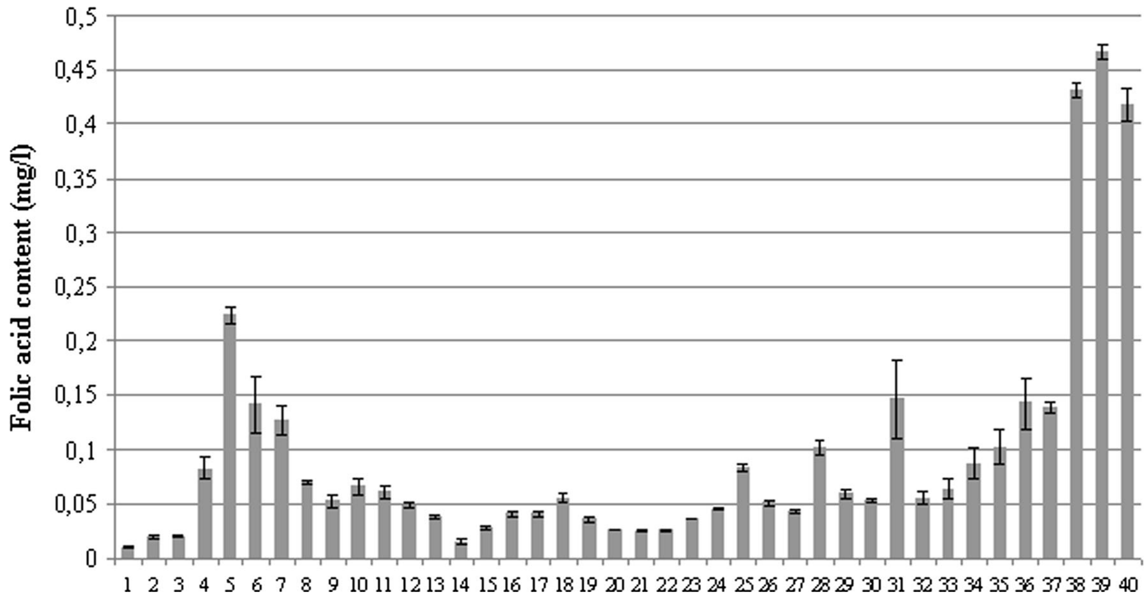

Fig. 1 Folic acid content of the investigated samples (mean, SD, N = 3)

and craft beers (no. 25, 28, 31, 34, 35, 36, 37) had a slightly higher content of this compound. It can be explained by their higher original extract content since folate originates from malt, and by fermentation with brewing yeast that significantly contributes to the folic acid content of the beer (Pietercelie et al. 2011). Alcohol-free beers (number 1–3) showed the lowest values ranging from 0.010 to 0.020 mg/l. It is the consequence of their low original extract content, the limited fermentation time and short contact with the brewing yeast. In case of the ones with added fruit juice a part of the folic acid content may have been provided by the juice (no. 6 made with grapefruit and no. 7 with lemon juice). Sample number 5, an alcohol-free beer made with added lemon juice, had more than 0.200 mg/l folic acid. It may be due to its lemon juice content but needs further investigation because the origin of the fruit can highly influence the composition of the fruit and basically citrus fruits do not contain folic acid in this high concentration (Chew et al. 2012). It has practical relevance from the viewpoint of dietetics and nutritionists because half a liter of this alcohol-free product still contains more than the quarter of folic acid recommended daily intake (RDI) in the USA and according to the EU Regulation 1169/2011 more than the half of folic acid nutrient reference value (NRV) in the EU and can be applied in a well-balanced diet or consumed by pregnant without the need to consider the harmful effects of alcohol. Samples (38, 39 and 40) aged with sour cherries or sour cherry juice contained the highest amount of folic acid, above 0.400 mg/l. It exceeds the RDI in the USA. Some types of sour cherry contained high amount of folate and this value was highly dependent on the origin of the fruit used during the production (Strålsjö et al. 2003).

Antioxidant activity

Fig. 2 indicates that dark beers and beers aged with sour cherry had higher antioxidant activity ranging from 427.91 to 1033.66 mg/l GAE by TPC method (Table 2). Similarly others samples also showed higher antioxidant activity measured using TPC method for dark beers (280–525 mg/l GAE) compared to pale beers (119–200 mg/l GAE) (Granato et al. 2011). These differences in dark and craft beers may be due to the special malts, like crystal or caramel malts, and other coloring malts used in their production. During the kilning and roasting process of such malts different compounds are generated via the Maillard-reaction which has antioxidant activity (Tubaro 2011). Furthermore, hops were also a source of antioxidant compounds, e.g. polyphenols (Krofta et al. 2008). Thus some beer types made with higher amount of hops (i.e. pilsner) had higher TPC values which partially indicated elevated polyphenol contents: samples 16 and 17 have 436.68 and 453.36 mg/l GAE, respectively. However, according to our measurements, specialty malts seemed to be increased the antioxidant activity to a much greater extent than hops. Samples containing sour cherry had higher antioxidant activity mostly due to the unbound antioxidant compounds especially anthocyanins from the fruit (Damar and Ekşi 2012). The product containing blackcurrant juice showed the highest antioxidant activity among alcohol-free beers (no. 4). It is owing to the high antioxidant activity of blackcurrant juice, containing high amount of phenolic compounds, and ascorbic acid which

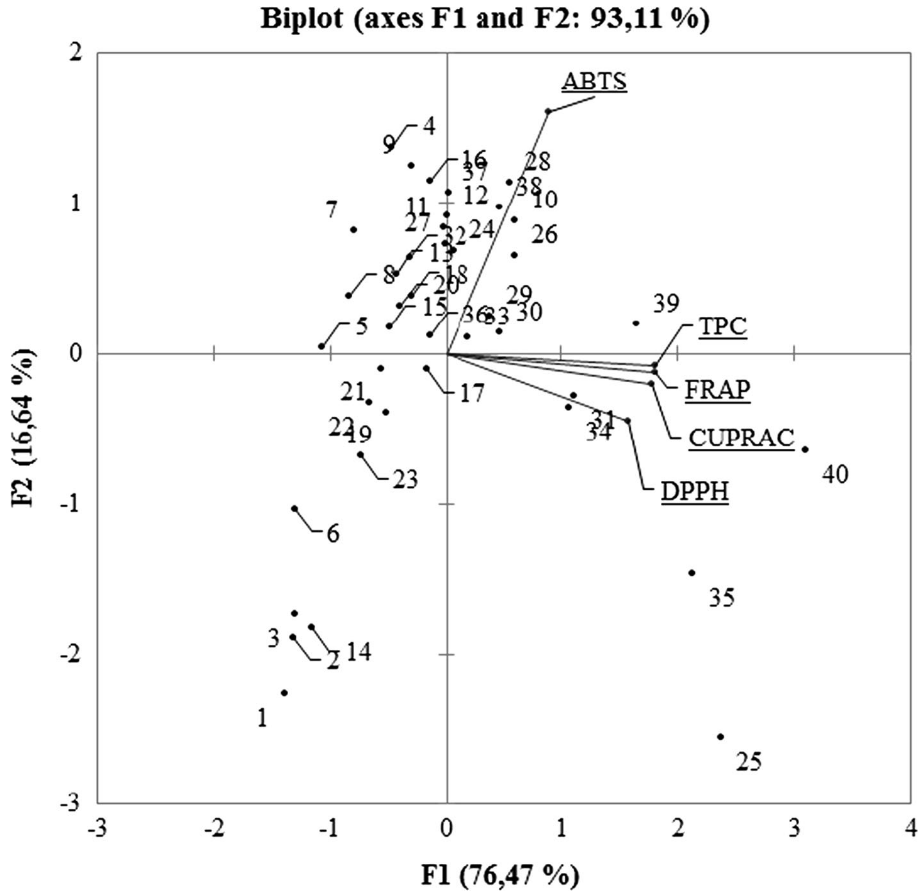

Fig. 2 PCA BiPlot of the antioxidant profile of the investigated samples (N = 5)

have antioxidant activity (Mattila et al. 2011). Alcohol-free beers and beer-based mixed drinks had the lowest antioxidant activity. It may be due to the amount and the type of raw materials: these types of beers were rarely made with special malts, has low original extract content and lightly hopped. The original extract content in Table 1 clearly indicates this difference. The ABTS values are clearly lower (127.69–399.60 µM troloxE) than the obtained by CUPRAC (322.38–3205.93 µM troloxE) (Table 2) although both are expressed in µM troloxE. It is maybe due to a higher selectivity to antioxidants of the ABTS method.

Correlation analysis

This analysis was performed to find interactions between the physicochemical parameters and the folic acid content plus antioxidant values of beers. It indicated how the alcohol, energy, original extract content and bitterness value of beers determine the measured nutritional values. Table 3 indicated the correlation of bitterness with CUPRAC, TPC and antioxidant activity measured by various methods. Bitterness indicates the amount of isomerized alpha-acids from hops, which provides the bitter taste to beers. Probably these two assays were more sensitive to these phenolic compounds (Humulone homologues), which had antioxidant activity. The antioxidant activity measuring methods significantly correlated with each other, it was consistent with the results of Zhao et al. (2010). It was due to the fact that all methods were single electron transfer (ET) reaction based assays. Folic acid content also correlated with these assays, as some studies highlight free radical scavenging behavior of folate (Joshi et al. 2001).

Folic acid content, antioxidant activity, alcohol, energy and original extract content were significantly correlated with each other. The original extract content indicated the quality and quantity of raw materials used and affected the metabolism of brewing yeast. Thus it has significant effect on the composition (i.e. folate and alcohol content) and other properties (i.e. energy content and antioxidant activity) of the final product.

Conclusion

The current study on 40 beers available in the Hungarian retail market indicated their similar antioxidant activity as those of beer of other countries. The present study demonstrated that the different types of beers had unique antioxidant and folic acid content.

Table 2 Antioxidant activity profile of the investigated samples organized based on the type of the beers

Sample no.	ABTS (μM troloxE)	TPC (mg/l GAE)	FRAP (mg/100 ml ASAE)	DPPH (I%)	CUPRAC (μM troloxE)
Alcohol-free pale lager					
1	127.69 ± 25.58	219.54 ± 7.36	2.53 ± 0.33	21.33 ± 0.84	443.63 ± 11.26
2	152.12 ± 14.74	252.84 ± 14.91	2.96 ± 0.19	15.55 ± 2.39	459.01 ± 4.93
3	163.30 ± 36.81	226.02 ± 17.47	3.44 ± 0.20	18.18 ± 1.13	499.32 ± 13.88
Alcohol-free beer-based mixed drink					
4	387.89 ± 3.15	296.06 ± 16.81	11.13 ± 0.54	37.10 ± 1.23	750.03 ± 17.97
5	285.30 ± 42.28	223.16 ± 9.046	4.43 ± 0.36	25.55 ± 0.57	384.50 ± 13.13
6	209.22 ± 27.12	202.96 ± 12.34	2.58 ± 0.39	22.11 ± 1.52	322.38 ± 8.33
Beer-based mixed drink					
7	343.88 ± 9.53	261.06 ± 21.52	6.82 ± 0.41	33.77 ± 0.60	502.40 ± 23.16
8	313.97 ± 14.75	276.65 ± 12.10	6.50 ± 0.48	31.08 ± 2.29	522.23 ± 11.22
9	386.58 ± 8.47	335.46 ± 23.01	14.11 ± 0.62	46.08 ± 1.28	855.76 ± 29.89
Strong pale lager					
10	385.94 ± 6.85	621.47 ± 57.05	26.30 ± 0.67	63.32 ± 1.22	1442.81 ± 55.33
27	358.26 ± 13.74	472.12 ± 30.28	16.15 ± 0.63	45.15 ± 2.86	1179.71 ± 22.17
European pale lager					
11	362.86 ± 21.32	463.11 ± 51.20	17.35 ± 0.89	37.29 ± 6.52	1183.79 ± 15.79
12	372.53 ± 20.22	451.11 ± 26.74	17.27 ± 1.12	49.46 ± 2.73	1148.33 ± 24.48
13	329.90 ± 16.12	381.52 ± 22.60	12.96 ± 0.44	21.25 ± 3.41	944.18 ± 34.58
14	164.53 ± 36.22	238.24 ± 14.43	5.55 ± 0.30	31.06 ± 0.59	569.24 ± 8.97
15	307.45 ± 28.06	354.36 ± 26.03	12.97 ± 0.53	29.09 ± 4.05	898.18 ± 26.04
18	328.43 ± 18.88	407.65 ± 25.59	13.49 ± 0.74	39.94 ± 2.53	966.14 ± 31.20
19	274.94 ± 46.90	343.51 ± 12.77	11.78 ± 0.42	43.39 ± 1.64	910.86 ± 19.47
20	323.82 ± 10.16	363.23 ± 29.14	12.74 ± 0.38	44.93 ± 1.52	877.39 ± 22.99
Czech pilsner					
16	379.19 ± 22.52	436.68 ± 29.76	14.95 ± 0.80	32.16 ± 10.67	1067.06 ± 24.59
17	300.68 ± 43.73	453.36 ± 22.81	15.88 ± 0.68	44.74 ± 4.34	1123.43 ± 29.01
Munich helles					
21	291.16 ± 27.47	324.20 ± 19.22	11.67 ± 0.36	37.93 ± 1.21	864.16 ± 19.10
Wienna lager					
22	273.73 ± 38.51	310.56 ± 37.22	10.36 ± 0.19	35.94 ± 1.48	825.23 ± 21.02
American adjunct lager					
23	247.18 ± 30.41	332.22 ± 12.33	9.14 ± 0.28	30.72 ± 4.99	779.69 ± 17.36
Dunkel lager					
24	359.30 ± 26.32	427.91 ± 32.83	20.04 ± 0.84	47.42 ± 3.29	1329.50 ± 26.51
Schwarzbier					
25	246.36 ± 9.09	813.43 ± 17.68	55.93 ± 1.97	207.21 ± 11.24	3055.42 ± 71.75
Altbier					
26	370.66 ± 0.86	551.64 ± 37.30	27.94 ± 1.72	52.13 ± 5.46	1841.94 ± 61.19
Belgian strong pale ale					
28	399.60 ± 0.74	503.61 ± 36.19	29.99 ± 1.11	48.77 ± 2.61	1675.66 ± 34.87
Hefeweizen					
29	364.54 ± 5.92	431.18 ± 38.46	17.01 ± 0.32	145.34 ± 14.03	1132.64 ± 28.94
30	355.54 ± 11.38	480.14 ± 25.24	19.73 ± 0.95	133.32 ± 4.09	1234.55 ± 19.71
36	332.58 ± 9.06	315.94 ± 75.30	15.18 ± 0.49	100.70 ± 2.92	921.68 ± 18.56
37	372.21 ± 5.38	500.49 ± 50.80	22.14 ± 1.33	13.63 ± 7.84	1166.98 ± 25.44
Stout					
31	337.70 ± 8.46	628.47 ± 37.22	33.21 ± 2.40	110.57 ± 5.79	2148.56 ± 39.12

Table 2 continued

Sample no.	ABTS (µM troloxE)	TPC (mg/l GAE)	FRAP (mg/100 ml ASAE)	DPPH (I%)	CUPRAC (µM troloxE)
Irish red ale					
32	344.12 ± 15.31	370.46 ± 25.27	12.67 ± 0.66	36.82 ± 3.04	1062.77 ± 18.05
Irish stout					
33	325.33 ± 6.18	461.41 ± 18.81	21.85 ± 0.97	48.53 ± 2.11	1624.24 ± 42.35
Munich dunkel lager					
34	326.63 ± 2.92	655.04 ± 52.00	34.17 ± 2.14	92.19 ± 7.46	2159.07 ± 49.53
Dunkler bock					
35	290.57 ± 10.67	943.64 ± 65.83	49.39 ± 2.66	141.14 ± 3.93	2833.08 ± 80.77
Fruit beer					
38	379.60 ± 8.09	537.06 ± 40.85	33.03 ± 1.06	24.56 ± 11.75	1580.34 ± 32.98
39	397.08 ± 3.44	779.10 ± 101.25	36.24 ± 0.61	183.38 ± 7.08	1750.02 ± 42.32
40	379.50 ± 2.31	1033.66 ± 77.92	67.11 ± 1.10	193.10 ± 3.93	3205.93 ± 24.16

Data are given in mean ± SD format. Results were obtained from five parallel measurements

Table 3 Spearman correlation matrix of the measured parameters

	p values									
	Bitterness	Original extract content	Ethanol	Energy	DPPH	CUPRAC	ABTS	FRAP	TPC	Folic acid content
Spearman r-values										
Bitterness	**1**	0.068	0.061	0.070	0.061	**0.014**	0.633	0.056	**0.041**	0.164
Original extract content	0.295	**1**	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	**0.004**	**<0.0001**	**<0.0001**	**0.005**
Ethanol	0.303	**0.918**	**1**	**<0.0001**	**0.001**	**<0.0001**	**0.002**	**<0.0001**	**<0.0001**	**0.016**
Energy	0.293	**1.000**	**0.918**	**1**	**0.001**	**<0.0001**	**0.004**	**<0.0001**	**<0.0001**	**0.005**
DPPH	0.303	**0.512**	**0.520**	**0.520**	**1**	**<0.0001**	**0.014**	**<0.0001**	**<0.0001**	**0.016**
CUPRAC	**0.392**	**0.775**	**0.813**	**0.778**	**0.752**	**1**	**0.001**	**<0.0001**	**<0.0001**	**0.005**
ABTS	0.079	**0.454**	**0.483**	**0.452**	**0.392**	**0.512**	**1**	**0.000**	**0.000**	**0.002**
FRAP	0.309	**0.785**	**0.815**	**0.788**	**0.751**	**0.977**	**0.584**	**1**	**<0.0001**	**<0.0001**
TPC	**0.329**	**0.811**	**0.822**	**0.815**	**0.702**	**0.975**	**0.540**	**0.970**	**1**	**0.004**
Folic acid content	−0.227	**0.446**	**0.385**	**0.448**	**0.385**	**0.446**	**0.495**	**0.541**	**0.456**	**1**

Values in bold are different from 0 with a significance level $\alpha = 0.05$

Fruit beers aged with sour cherry are excellent sources of these health promoting substances. While alcohol-free products are not a good source of antioxidants and folic acid, the alcohol-free beer-based mixed drinks made with lemon or grapefruit juice also contained a nutritionally relevant amount of folic acid.

Our results correlated with the most important physicochemical parameters of the samples, e.g. alcohol, energy and extract content. This fact has importance not only for nutritionists and dietitians but for R&D experts as well to develop new, functional beers rich in antioxidants and folic acid. Based on our current findings the type of the product and the information indicated on the label let us presume the antioxidant activity and folic acid content of beers.

Authors' contributions DK: performed measurements, drafted the initial manuscript. CO: obtained samples, participated in study design and manuscript preparation. NG: contributed to data analysis and language editoring. SK: contributed as an expert of laboratory measurements and participated in manuscript preparation. BV: contributed as an expert of brewing and distilling and result interpretation. GK-F participated in study design and also in measurements.

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