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Original scientific paper

POLYPHENOLS AND THEIR ANTIOXIDANT EFFECT IN BEERS FROM THE BOSNIA AND HERZEGOVINA MARKET

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ABSTRACT
Data on the content of total polyphenols in beer is important both because of their negative effects on the stability of beer and the formation of turbidity, and because of the positive health effects. The aim of this study was to determine the content of total polyphenols and antioxidant capacity in 22 samples of light and dark beer using two methods: Folin–Ciocâlteu and DPPH method and to confirm the correlation between the total content of UP and AC beer. The content of shell polyphenols in dark beers was statistically significantly higher ($p < 0.001$) compared to the content of total polyphenols in samples of light beers from the same producer. The antioxidant capacity of dark beers is statistically significantly higher ($p < 0.001$) compared to the antioxidant capacity of light beers from the same producer.

Key words: antioxidant capacity, beer, market, total polyphenols.

INTRODUCTION
According to modern understanding, beer is a refreshing drink with a low concentration of alcohol and a characteristic aroma of hops, obtained by fermenting beer wort with brewer's yeast. Various raw materials are used in beer production, such as: water, malt, substitute raw materials, hops and yeast (Marić, 2009). Beer is rich in different groups of polyphenols, the most important of which are tannins (especially in dark beers), phenolic acids, flavones, flavonols and proanthocyanidins. Most of the polyphenolic compounds in beer are derived from malt, and about 30% come from hops. This percentage may also vary depending on the variety and method of hop cultivation (Collin et al., 2013). Polyphenolic compounds are important antioxidants, with mechanisms involving both free radical scavenging and metal chelation. During beer storage, phenolic compounds react with proteins and form high molecular weight species and hazes. Polyphenols are biologically active substances very widespread in nature and significantly present in the human diet. By structure these are aromatic compounds with multiple hydroxyl substituents. They are rarely found in free form in nature, they are mostly in esterified or conjugated form (Čović et al., 2003). Beer contains different groups of polyphenols, such as tannins, phenolic acids, flavones, flavonols and proanthocyanidins, which have a significant impact on the stability of beer. Moderate consumption of beer and wine, due to the high content of polyphenols and antioxidants, has a positive effect on cognitive abilities and reduces the possibility of dementia in old age.

Isoflavones in beer have a potential anticancer effect, but the amount of the same in beer is 20 times less than the effective dose used in treatment. Silicic acid, which is present in small amounts in beer, has a positive effect on aluminum excretion and kidney function (Bamforth, 2004). Research suggests that polyphenols play an important role in the prevention of human disease, along with vitamins and minerals (Berend & Grabarić, 2008). Phenolic components of beer are of great interest to breweries because they directly affect the quality of beer. In addition to having a positive effect on preventing oxidation, they can adversely affect colloidal stability and foam stability, thus shortening the shelf life of beer (Habschied et al., 2020). The aim of this study was to determine the UP content using Folin-Ciocalteu (FC) method, and antioxidant capacity (AC) with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical in samples of light and dark beers of the same producers represented on the market of Bosnia and Herzegovina, using UV/Vis spectrophotometry, and based on the results to confirm the correlation between the total polyphenol content and the antioxidant capacity of beer. Examine the content of polyphenolic compounds to indicate the nutritional and health significance of beer.

MATERIAL AND METHODS

The content of total phenols (UP) by the Folin-Ciocaltu method was determined in 22 samples of light and dark beer from different producers represented on the Bosnian market. The results of the analysis are expressed in mg GA / L and are shown in Tables 1-3.

a) Preparation of standards: Gallic acid weighing 0.025 g weighed on an analytical precision balance was dissolved in 200 µL of 95% ethanol. After dissolution, the solution was transferred to a 50 ml volumetric flask and made up to the mark with distilled water. This was a standard GA solution with a mass concentration of 500 mg / L. 200, 500, 1000, 2000, 3000, 4000 µL of standard GA solution were pipetted and diluted with distilled water into 10 mL vessels. Working solutions of the following concentrations of 10, 25, 50, 100, 150, 200 mg / L were obtained. 10.5 g of Na_2CO_3 was dissolved in distilled water in a 100 mL volumetric vessel. Folin-Ciocalteu reagent was diluted with distilled water in a ratio of 1: 2.

b) Preparation of gallic acid measuring solutions and drawing a calibration curve: After the GA working solutions were prepared, the GA measuring solutions with Folin-Ciocalteu (FC) reagent and sodium carbonate (Na_2CO_3) were also prepared as follows: 1.00 mL of FC reagent was added to 0.2 mL of working GA solution and after 10 min 0.80 mL Na_2CO_3. Thus prepared measuring solutions of GA which were 0.1; 0.5; 1; 2; 5; mg / L absorbances were recorded at an absorption maximum of 765 nm. The dependence of absorbance on GA concentrations is shown in Figure 1. The obtained equation of the linear calibration direction was used to calculate the UF content in light and dark beer samples.

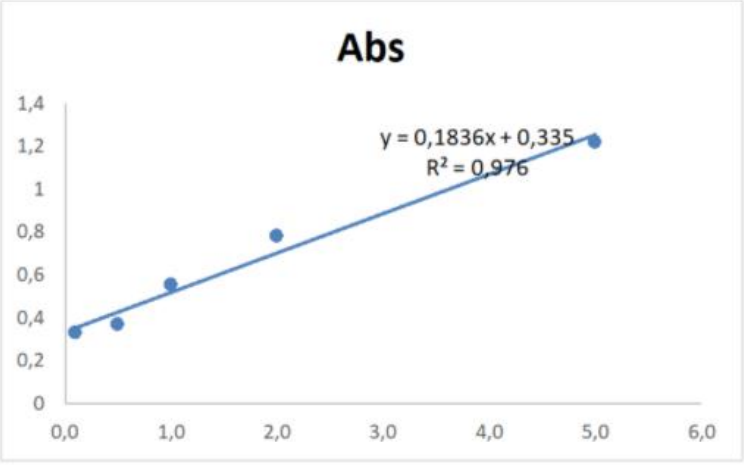

Figure 1. Calibration curve for calculating UP content in analyzed beer samples

c) Preparation of samples for measurement: Beer samples for UP measurement were prepared by diluting the beer 10 times in ependorphs (100 µL of beer + 900 (L of water). Then 0.2 mL of each prepared sample was weighed in three parallels and 1.0 mL of FC reagent was added, followed by 0.80 mL of Na_2CO_3 after 10 min. After 30 minutes, the absorbances at 765 nm were measured with the beer sample solutions thus prepared.

Determination of antioxidant capacity

a) Preparation of standards: Determination of antioxidant capacity was performed by DPPH method. Prior to determination, a solution of DPPH in ethanol was made at a concentration such that the absorbance at 517 nm was in the range of 0.8-1.0. From a working solution of GA with a concentration of 10 mg / L, solutions with a concentration of 7.5 were prepared; 5; 2.5; 1; mg / L as follows:

- 7.5 mg / L (750 µL solution + 250 µL ethanol)
- 5.0 mg / L (500 µL of solution + 500 µL of ethanol)
- 2.5 mg / L (250 µL solution + 750 µL ethanol)
- 1.0 mg / L (100 µL solution + 900 µL ethanol)

b) Drawing the calibration curve: The procedure consists of adding 1 mL of DPPH to the cuvettes and measuring the absorbances at a wavelength of 517 nm. After the measurement, 100 µL of the prepared GA solutions are added, shaken and allowed to stand for 30 minutes in the dark at room temperature. After 30 min, the absorption of the solution is measured at a wavelength of 517 nm. All concentrations were done in three replicates. Ethanol was used as a blank. Radical scavenging activity (18 RSA) was calculated as the ratio of the decrease in absorbance of DPPH solution after addition of sample solution and absorbance of DPPH solution to which no sample solution was added, according to the formula: $RSA = 1 - A_1 A_o x 100$ where: A_1 is the absorbance of DPPH solution after addition of sample solution, A_o is absorbance of DPPH solution to which no sample solution was added. The obtained equation of linear calibration direction with GA as a standard was used to calculate the AC content in light and dark beer samples (Figure 2).

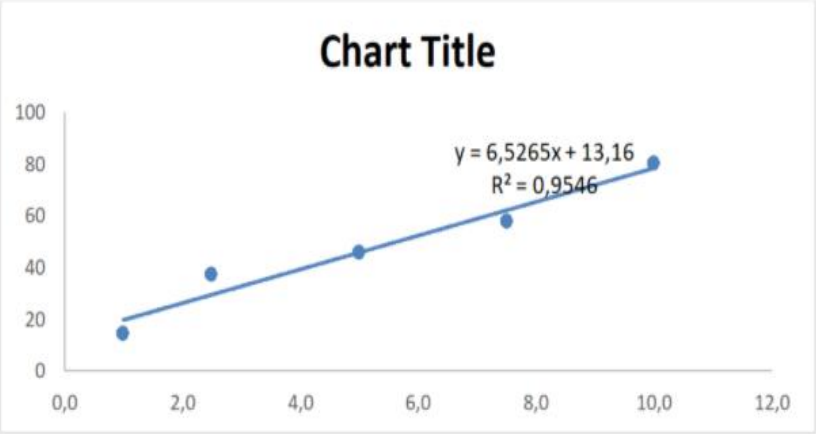

Figure 2. Calibration curve for calculating AC in analyzed beer samples

c) Preparation of samples for measurement: Beer samples for measurement were prepared by diluting the beer 10 times in ependorphs. In 3 cuvettes for each sample, 1 mL of DPPH was added, absorbances were measured and then 100 µL of sample was added with stirring and allowed to stand in the dark at room temperature for 30 min. After that, the absorption was measured at a wavelength of 517 nm. The Exel 2007 program was used for statistical

processing of the obtained results. Statistical T test was used ($p < 0.05$ was considered statistically significant).

RESULTS

The content of UP was determined by the Folin-Ciocalte method, in 22 samples of light and dark beer from different producers that are represented on the Bosnian market. The results of the analysis are expressed in mg GA / L and are shown in Tables 1 to 3.

Table 1. Content of total polyphenols in light beers

The name of the beer	Type of beer	Total polyphenols Folin-Ciocalteu γ (mg GA/L)	STDEV (±)	RSD
A1	light	54,69	162,91	29,79
A2	light	210,75	8,86	0,42
A3	light	306,24	170,42	5,57
A4	light	418,71	128,06	3,06
A5	light	683,00	57,39	0,84
A6	light	438,32	78,37	1,79
A7	light	521,08	76,64	1,47
A8	light	644,07	40,82	0,63
A9	light	463,76	15,21	0,33
A10	light	400,85	39,48	0,98
A11	light	1127,75 ***	34,85	0,31

STDEV-standard deviation, RSD relative standard deviation, ***, statistically significant difference of A11 light beer in relation to all analyzed light beers, ($p < 0,001$).

Table 2. Content of total polyphenols in dark beers

The name of the beer	Type of beer	Total polyphenols Folin-Ciocalteu γ (mg GA/L)	STDEV (±)	RSD
B1	dark	230,42	35,24	1,53
B2	dark	938,08	76,26	0,81
B3	dark	384,94	400,73	10,41
B4	dark	520,89	238,21	4,57
B5	dark	530,88	16,56	0,31
B6	dark	1220,80 ***	8,47	0,07
B7	dark	870,07	85,31	0,98
B8	dark	1059,83	196,23	1,85
B9	dark	791,78	174,85	2,21
B10	dark	723,53	82,03	1,013
B11	dark	1087,42	2,31	0,02

STDEV-standard deviation, RSD relative standard deviation, ***, statistically significant difference of B6 dark beer in relation to all other analyzed dark beers, ($p < 0,001$).

Antioxidant capacity was determined by DPPH method, in 22 samples of light and dark beer from different producers that are represented on the Bosnian market. The results of the analysis are expressed in mg GA / L and are shown in Tables 4 to 5.

Table 3. Values of AC analyzed light beer samples

The name of the beer	Type of beer	Total polyphenols Folin-Ciocalteu γ (mg GA/L)	STDEV (±)	RSD
A1	light	284,31	162,52	5,72
A2	light	599,71	112,46	1,88
A3	light	476,05	236,23	4,96
A4	light	751,25	614,70	8,18

A5	light	347,60	174,25	5,01
A6	light	--------	--------	----
A7	light	278,13	245,63	8,83
A8	light	1083,30 *,***	280,08	2,59
A9	light	935,55	140,40	1,60
A10	light	221,79	125,23	5,65
A11	light	1004,87 ***	388,31	3,86

STDEV-standard deviation, RSD- relative standard deviation, *, statistically significant difference of AC A8 light beer in relation to A11 light beer, ($p<0,05$) ***, statistically significant difference of AC A8 light beer in relation to each other analyzed beers except A11 light beer, ($p < 0.001$).

Table 4. Values of AC analyzed dark beer samples

The name of the beer	Type of beer	Total polyphenols Folin-Ciocalteu γ (mg GA/L)	STDEV (±)	RSD
B1	dark	418,12	123,10	2,94
B2	dark	752,80	383,55	5,09
B3	dark	696,93	74,02	1,06
B4	dark	706,36	33,04	4,68
B5	dark	213,64	39,54	1,85
B6	dark	1078,54**,***	147,56	1,37
B7	dark	748,40	342,05	4,57
B8	dark	382,58	14,21	3,71
B9	dark	578,87	197,17	3,41
B10	dark	--------	--------	-----
B11	dark	917,93	524,55	5,71

, statistically significant difference of AC B6 dark beer in relation to B11 dark beer ($p <0.01$) *, statistically significant difference of AC B6 dark beer in relation to all analyzed dark beers except B11 dark beer ($p < 0.001$).

Table 5. Values AC content in samples of light and dark beers from the same producer

The name of the beer	Type of beer	Total polyphenols Folin-Ciocalteu γ (mg GA/L)	p
A1-B1	light	284,30	0,0003 ***
	dark	418,12	
A2-B2	light	599,71	0,0027**
	dark	752,80	
A3-B3	light	476,05	0,0001***
	dark	696,93	
A4-B4	light	751,25	0,0001***
	dark	706,36	
A5-B5	light	347,69	0,0002***
	dark	213,64	
A6-B6	light	----------	--------
	dark	1078,54	
A7-B7	light	278,13	0,0001***
	dark	748,40	
A8-B8	light	1083,30	0,0001***
	dark	382,58	
A9-B9	light	935,55	0,0001***
	dark	578,87	
A10-B10	light	221,79	----------
	dark	--------	
A11-B11	light	1004,87	0,0823 ns
	dark	917,93	

** ($p < 0.01$); *** ($p < 0.001$); statistically significant difference between light and dark beer from the same producer; ns ($p > 0.05$), not statistically significant.

Determination of AC was performed in a total of 22 samples of light and dark beers from the same manufacturer. Due to turbidity in two samples, light A6 beer and dark B10 beer, AC could not be determined. In this regard, it was not possible to determine a statistically significant difference in AC between light and dark beer in these beer producers. AC values in samples of light and dark beers from the same producer are shown in Table 5.

DISCUSSION

The UP content, determined by the Folin-Ciocalteu method, in the analyzed 22 beer samples ranged from 54.69 to 1220.80 mg / L expressed as gallic acid equivalent (mgGA / L). The range for light beers (11 samples) ranged from 54.69 (A1 beer) to 1127.75 mgGA / L (A11 beer), while the range for dark beers (11 samples) ranged from 230.42 (B1 beer) up to 1220.80 mgGA / L (B6 beer). The highest UP content was found in A11 light beer, which is statistically significantly higher ($p < 0.001$) UP content compared to the UP content of all other analyzed light beer samples. No statistically significant difference was observed between A4 light beer with A6 and A10 light beer ($p > 0.05$). The values obtained for light beers are not consistent with the values reported by Pai et al. (2015) who determined total polyphenols in 15 samples of light (lager) beers, with a range of 150 to 620 mg / L expressed as equivalent tannic acid. Zhao et al. (2009) determined UP in 34 samples of light (lager) beers and the obtained range of UP concentrations was lower than the results obtained by this study and ranged from 152.01 to 339.12 mg GA / L. The highest UP content was found in B6 dark beer, B11 dark beer and B8 dark beer, while B1 dark beer has the lowest UP content. The UP content in B6 dark beer was statistically significantly higher compared to all other analyzed dark beers ($p < 0.001$). No statistically significant difference was observed between B4 dark beer with B5 and B3 dark beer ($p>0.05$). Also, there is no statistically significant difference between B8 and B11 dark beer ($p>0.05$). Compared to the UP content in light beer samples, the UP content in dark beers from the same producer was statistically significantly higher ($p < 0.001$). The smallest statistically significant difference in UP content in light and dark beer samples was observed in A4-B4 beer producers ($p < 0.05$). No statistically significant difference was found in the UP content between A3-B3 light and dark beer ($p > 0.05$). Granato et al. (2011) determined UP in 18 samples of light (lager) beer and 11 samples of dark (ale) beer. The concentration ranges for light (119.96 - 200.00 mg GAE / L) and dark beers (280.10 - 525.93 mg GAE / L) were lower than the results obtained in this study. Mitić et al. (2013) report high concentrations of UP determined in light and dark beers using the Folin-Ciocalteu method in the amount of 331.88 to 545.32 mg GAE / L for light beers and from 446.38 to 510.97 mg GAE / L for dark beers, but they are lower than the values obtained by this research. Studies using the Folin-Ciocalteu method have confirmed that beer is a good source of polyphenols, and that dark beers have a higher UP concentration than light beers, with concentration ranges from 100 mg / L for light beers to even more than 800 mg / L for dark beers (Piazzon et al., 2010; Granato et al., 2011; Mitić et al., 2013). Studies conducted by Lermusieau et al. (2001) on different varieties of hops, given the proportion of UP, showed that depending on the variety of hops and the method of processing, the proportion of polyphenols can vary by up to 250%. Therefore, the use of different varieties of hops and barley in beer production can lead to a difference in the final concentration of polyphenolic ingredients. Polyphenolic ingredients are important antioxidants, which can bind free radicals. During beer storage, phenolic compounds react with proteins to form high molecular weight compounds that cause product turbidity. Several parameters determine the basic quality of beer (barley and unsweetened cereals content, extract in basic wort, vol% alcohol, CO_2 content, fermentation), while two properties of the finished product cause special attention, namely colloidal and taste stability (Aron & Shellhammer, 2010). Precisely because of the presence of polyphenols in beer, preserving the colloidal stability and uniformity of taste is a major technological challenge. Consumers expect

clear and colloidal stable beer without sediment. Proteins in beer in the presence of polyphenols create the so-called. PP (polyphenol-protein) complexes that increase the turbidity of beer and must be removed by filtration or sedimentation. Eleven samples of light beer were analyzed, but due to turbidity it was not possible to determine the AC in A6 light beer. AC values in 10 samples of light beers range from 221.79 mg GA / L (A10) to 1083.30 mg GA / L (A8 beer). A8 light beer and A11 light beer have the highest AC, which is a statistically significantly higher AC compared to all analyzed light beers ($p < 0.001$). Compared to AC A11 light beer, AC A8 light beer is also statistically significantly higher ($p < 0.05$). No statistically significant difference was observed between A1 light beer and A7 light beer ($p>0.05$). Eleven samples of dark beer were analyzed, but due to turbidity it was not possible to determine the AC B10 of dark beer. AC in dark beers (10 samples) ranges from 382.58 mgGA / L (B8 beer) to 1078.54 mgGA / L (B6 beer). B6 dark beer has the highest AC, which is statistically significantly higher compared to other analyzed dark beers ($p < 0.001$), except for B11 dark beer ($p < 0.01$). There is no statistically significant difference in AC B7 with B3 and B2 beer ($p > 0.05$). Compared to AC in samples of light beers, AC in dark beers of the same producer was statistically significantly higher ($p < 0.001$). The smallest statistically significant difference in AC in light and dark beer samples was observed in A2-B2 beer producers ($p < 0.01$), while no statistically significant difference was found in AC between Arcan light and dark beer ($p > 0.05$). Due to turbidity in two samples, light A6 beer and dark B10 beer, AC could not be determined. There is a good correlation between the total polyphenol content and the antioxidant capacity of beer (Rivero et al., 2005; Tedesco et al., 2005), which is confirmed by the results of the tested samples that B6 dark beer with the highest polyphenol content has the highest AC value. The exceptions are dark beers, whose antioxidant capacity is contributed not only by polyphenols, but also by Maillard's reaction products and melanoidins, which show great potential for stopping chain reactions, reducing reactive oxygen radicals and chelating metals (Borrelli &Fogliano, 2005).

CONCLUSION

Polyphenols are very stable molecules that can be found in different concentrations and different types of beer. According to our research, the UP content in dark beers was statistically significantly higher ($p < 0.001$) compared to the UP content in samples of light beers from the same producer. Identification has shown that polyphenolic compounds can be found in dark beers in higher concentrations, which may indicate that dark beers are more beneficial to human health. A8 and A11 light beer have the highest AC, which is statistically significantly higher AC compared to all analyzed light beers ($p < 0.001$). B6 dark beer has the highest AC, which is a statistically significantly higher AC compared to all analyzed dark beers ($p < 0.001$). The AC of dark beers was statistically significantly higher ($p < 0.001$) compared to the AC of light beers from the same producer. A good correlation between the total polyphenol content and AC has been proven, which is confirmed by our results that B6 dark beer with the highest polyphenol content also has the highest AC value.

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