The antioxidant properties of honey beer

Ana Kalušević, Gordana Uzelac, Mile Veljović, Saša Despotović, Mirjana Milutinović, Ida Leskošek-Čukalović, Viktor Nedović

Department of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade-Zemun, Serbia
(anakalusevic@gmail.com)

ABSTRACT

For centuries, honey represented a universal sweetener and it has been used for a variety of alcoholic beverages, including honey beer. In food industry, especially among the brewers, using of natural ingredients is increasingly growing demand. Honey is a natural, sweet and viscous substance, which is characterized by highly valued nutritional and physiological properties. Beer is one of the most popular beverage in the world with evident positive effects on the overall health condition. It can be used as a base for developing a variety of products with specific physiological activity. Honey is standing out as one of the possible sources of active ingredients for that purpose. Significant pharmacological activity of phenolic compounds in honey, even in such small quantities, can contribute to the antioxidant potential of beer.
The aim of this study was to investigate the influence of different types and content of honey on the antioxidant properties of beer.
The industrial wort, bottom-fermenting yeast strain and two types of monofloral honey (sunflower and linden honey) were used. Honey was added in wort immediately before fermentation in a concentration of 5 and 10%. Honey beers were analyzed for their total polyphenol content by two methods - by the Folin-Ciocalteu reagent method and by EBC method. Their antiradical capacity was tested by DPPH method and FRAP assay.
The results indicate that obtained beers have higher polyphenol content and antioxidant potential than control beer. The most significant results are achieved with 10% of linden honey.
Combination of these two fully natural products, honey and beer, are giving the product that is characterized with, not only with higher antioxidant potential, but with satisfactory sensory properties as well.

***Keywords**: honey; beer; antioxidant properties; polyphenol content.*

INTRODUCTION

Beer is a worldwide traditional natural drink which has a higher nutritional value than other alcoholic beverages. Raw materials for beer production are water, yeast, malt, non-malted cereals and hop. It contains minerals and vitamins, proteins, organic acids and antioxidant compounds, such as polyphenols. Among these antioxidants, phenolic compounds are of particular interest to brewers because they play a key role in the brewing process by delaying, retarding or preventing oxidation processes [1]. Phenolic compounds identified in beer include flavonoids, phenolic acids, proanthocyanidins, tannins, and amino phenolic compounds [2, 3]. All of them have been reported to possess antiradical and antioxidant properties as well as other biological effects [4].
There is a growing demand of natural products in human diet, due to increased consumer perception of natural nutraceuticals in recent years and the possible negative effects of synthetic food additives on human health.
Honey is a natural food product well known for its high nutritional and prophylactic-medicinal value. It has a wide range of different constituents, including polyphenols, with significantly antioxidant properties. It has been demonstrated that honey, measured by the assay of absorbance capacity of oxygen radicals on a fresh-weight basis shows similar antioxidant capacity to many fruits and vegetables on a fresh-weight basis, as measured by the assay of absorbance capacity of oxygen radicals [5].
The awareness of the therapeutic potential of honey is gradually growing and scientific evidences of its effectiveness in several experimental and clinical conditions are beginning to emerge.
The composition of active components in honey depends on various factors: floral source used to collect nectar seasonal and environmental factors, particularly plant bio and chemotype, climatic conditions, as well as its processing [5,6,7].
The objective of this study was to examine and compare phenolic profiles and antioxidant activities of two different types of honey beers.

MATERIALS & METHODS

The wort and bottom-fermenting yeast used in this study were obtained from a local brewery. Two types of honey, sunflower and linden honey, were purchased from local market. Control beer was produced by fermenting pure wort without adding of honey.

Gallic acid, Folin-Ciocalteu's phenol reagent, ammonium hydroxide, hydrochloric acid, sodium acetate trihydrate, glacial acetic acid, ammonium ferric citrate and sodium carbonate, carboxylmethylcellulose (CMC) and sodium ethylendiamintetraacetate (EDTA) were purchased from Merck (Germany). Ascorbic acid, 2,4,6-trypyridyl-s-triazine (TPTZ), ferric chloride hexahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium chloride, potassium persulfate were purchased from Sigma-Aldrich (Germany).

Determination of total phenolics:

Folin-Ciocalteau method: For the determination of total polyphenols the adjusted method with Folin-Ciocalteau reagent was used [8]. Degassed samples of beer (0.05 mL) were mixed with 0.45 mL of distilled water and 2,5 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 min. Two milliliters of saturated sodium carbonate (75 g/L) was added to the mixture and then shaken. After 2 h of reaction at room temperature, the absorbance at 760 nm was determined. The measurement was compared to a calibration line of prepared gallic acid (GA) solution, and the results were expressed as milligrams of gallic acid equivalents per liter of beer (mg GAE/L). All determinations were performed in triplicate.

EBC method [9]: Into 25 mL volumetric flask to 10 mL of beer sample, 8 mL of carboxylmethylcellulose/sodium ethylendiamintetraacetate (CMC/EDTA) and 0.5 mL 3.5% ammonium ferriccitrate solutions were added. After thorough agitation 0.5 mL dilute ammonia solution was added and after agitation the flasks were adjusted with distilled water till the mark. After 10 minutes standing at laboratory temperature absorbance of samples was measured on the spectrophotometer (Jenway 6400) at wavelength λ = 600 nm. Ammonium ferric citrate solution: 3.5 g ammonium ferric citrate was diluted in water in 100 ml volumetric flask. Ammonia solution: 1 part of concentrated ammonia solution was diluted in 2 parts of distilled water. TP was calculated as TP = $A_{600} \times 820$, where TP are total phenolics (mg/L) and A_{600} is measured absorbance. Average results were obtained from triplicate determinations.

Antioxidant capacity:

DPPH· method: Antiradical activity was measured after the reaction with free stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH・) according to Brand-Williams [10]. Fresh solution of DPPH in the concentration of 25 mg DPPH in 1L of methanol should be prepared before the determination. 3 mL of violet DPPH solution is pipette into plastic cuvettes of 10 mm length and absorbance is measured at wavelength λ = 515 nm on the spectrophotometer (Jenway 6400). Then 5 µL of sample is added and after stir with the hand stirrer in cuvettes the reaction mixture is left to stand for 30 min. The measurements were performed in triplicate. The radical scavenging activity was calculated by the formula $I = [(A_B - A_A)/A_B] \times 100$; where I = DPPH inhibition, %; A_B = absorption of a blank sample (t = 0 min); A_A = absorption of a tested honey beers at the chosen point of the reaction (t = 30 min).

FRAP assay: On FRAP assay, 25.0 mL of acetate buffer (pH= 3.65) were mixed with 2.5 mL of TPTZ, 2.5 mL of $FeCl_3 \cdot 6H_2O$. TPTZ solution was dissolved in HCl at 1900 µL of the previously described reagent was mixed with 100 µL of sample, and 100 µL of deionised water in a cuvette cell. This mixture was kept at 25 °C. Absorbance readings were made at 593 nm every 4 min. For determination of antioxidant activity of samples calibration curve was used. Results were expressed as mM $Fe(II)SO_4$ x $7H_2O$ [11].

RESULTS & DISCUSSION

Table 1. Total polyphenol content of the samples of beer and honey beers determined by Folin-Ciocalteu method

Sample	TPC[a]	p	t
B	379.50 ± 4,05	-	-
5% S	391.23 ± 4,06	0.78	-0.32
5% L	418.43 ± 5,00	0.23	-1.68
10% S	425.66 ± 6,90	0.22	-1.73
10% L	**443.30 ± 2,56***	**0.02**	**-6.37**

a- The values is expressed as means ± standard deviation of total polyphenol content total (mg GAE/L)
B - Control beer
S - Beer with sunflower honey
L - Beer with linden honey
* - Statistically significant difference
p - Level of significance, ($p<0.05$)
t- Sample value applied test

Beers contain numerous phenolics compounds which are derived from hops (30 %) and malt (60%). The content of total phenolic compounds determined by the Folin-Ciocalteu's method for the analyzed samples is shown in Table 1. In the beer with 10% of linden honey, the total amount of the phenolic compounds was significantly higher than in the other samples. As expected, the honey beers had higher amounts of total phenols than control beer. The content of total phenolic compounds decreased in the following order: beer with 10% of linden honey, 5% of sunflower honey, 5% of linden honey, 10% of sunflower honey and the lowest values were found in commercial beer.

Table 2. Total polyphenol content of the samples of beer and honey beers determined by EBC method

Sample	TPC[b]	p	t
B	138.33 ± 4.05	-	-
5% S	**179.66 ± 3.51***	**0.03**	**-5.65**
5% L	171.00 ± 5.19	0.09	-3.00
10% S	**215.33 ± 4.23***	**0.04**	**-4.85**
10% L	**181.33 ± 4.61***	**0.05**	**-4.06**

b- The values is expressed as means ± standard deviation of total polyphenol content (mg/L)
B - Control beer
S - Beer with sunflower honey
L - Beer with linden honey
* - Statistically significant difference
p - Level of significance, ($p<0.05$)
t- Sample value applied test

The results of the polyphenol content of the beer samples which was determined using the EBC method are shown in Table 2. Total polyphenol content ranged from 138.33 to 215.33 mg/l. The total amounts of the phenolic compounds were significantly higher in the beer with 10% and 5% of sunflower and 10% of linden honey than in other samples. The results obtained by this method are not completely in agreement with those obtained by Folin-Ciocalteu's method. However, the content of phenolic compounds determined by Folin-Ciocalteu's assay is correlated with antioxidant capacity of analyzed beers.

Table 3. The results of DPPH assay expressed as % of inhibition of DPPH radicals after 30 minutes

Sample	DPPH (%)[c]	p	t
B	45.94 ± 0,94	-	-
5% S	53.98 ± 1,02	0.73	0.39
5% L	**59.11 ± 0,75***	**0.02**	**6.83**
10% S	**64.25 ± 1,59***	**0.01**	**8.34**
10% L	**70.14 ± 1,09***	**0.04**	**4.82**

c-The values as expressed as means ± standard deviation
B - Control beer
S - Beer with sunflower honey
L - Beer with linden honey
*- Statistically significant difference
p- Level of significance, ($p<0.05$)
t- Sample value applied test

A number of studies have shown that phenolic compounds possess considerable antioxidant activity, even more than some natural antioxidants, such as vitamin E and vitamin C. The antioxidant capacity of beer samples was determined using DPPH (Table 3.) and FRAP method (Table 4.). The honey beers showed higher antioxidant activity compared with control beer, with the highest value in beer with 10 % of linden honey. The only this sample had statistically significantly higher antioxidant capacity in comparison with standard lager beer. There was no statistical difference between the antioxidant capacity of control beer and beer with sunflower honey. The results obtained by these two methods are mutually consistent.

Table 4. The results of FRAP assay expressed as mM $Fe(II)SO_4 \times 7H_2O$

Sample	FRAP[d]	p	t
B	1.29 ± 0.22	-	-
5% S	1.36 ± 0.28	0.80	-0.29
5% L	1.50 ± 0.04	0.20	-1.89
10% S	2.33 ± 0.76	0.19	-1.96
10% L	**2.61 ± 0.31***	**0.01**	**-6.22**

d- The values as expressed as means ± standard deviation (mM $Fe(II)SO_4 \times 7H_2O$)
B - Control beer
S - Beer with sunflower honey
L - Beer with linden honey
*- Statistically significant difference
p- Level of significance, ($p<0.05$)
t- Sample value applied test

Student's t - test were conducted to determined statistically significant difference between samples. The statistical tests were performed at the significance level $\alpha = 0.05$

CONCLUSIONS

Antioxidant capacity of the beers enriched with different types of honey was evidently improved. The obtained results indicate that the honey with long tradition in folk medicine can be very interesting raw materials for brewing industry. The results indicate that obtained honey beers have higher polyphenol content and antioxidant potential than standard beer. The most significant results are achieved in samples with 10% of linden honey. The results obtained are very promising indicating that a variety of different beers with defined functional and sensory properties can be produced. A variety of different beers with defined functional properties can be produced. Products such these could meet several goals: developing novel beer products, developing products with health-promoting properties that fulfill the market needs and eventually gain new beer consumers. They can be created for special purposes and certain groups of potential consumers.

ACKNOWLEDGEMENTS

The research is part of the project No. 20049 supported by the Ministry of Science and Technological Development, Republic of Serbia.

REFERENCES

[1] Guido L.F., Boivin P., Benismail N., Gonçalves C.R. & Barros A.A. 2005. An early development of the nonenal potential in the malting process. European Food Research and Technology, 220, 200–206.
[2] Gorinstein S., Caspi A., Zemser M. & Trakhtenberg S. 2000. Comparative contents of some phenolics in beer, red and white wines. Nutrition Research, 20, 131–139.
[3] Montanari L., Perretti G., Natella F., Guidi A. & Fantozzi P. 1999. Organic and phenolic acids in beer. Lebensmittel-Wissenschaft Und-Technoogie, 32, 535–539.
[4] Gaulejac N.S.-C, Provost C. & Vivas N. 1998. Comparative study of polyphenol scavenging activities assessed by different methods. Journal of Agricultural and Food Chemistry, 47, 425–431.
[5] Gheldof N., Wang X.H. & Engeseth N.J. 2002. Journal of Agricultural and Food Chemistry, 50, 5870.
[6] Al-Mamary M., Al-Meeri A. & Al-Habori M. 2002. Antioxidant activities and total phenolics of different types of honey. Nutrition Research 22, 1041–1047.
[7] Frankel S., Robinson G.E. & Berenbaum M.R. 1998. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. Apicultural Research 37 (1), 27–31.
[8] Singleton V.L. & Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. American Journal of Enology and Viticulture, 16, 144-158.
[9] Basařova G., Čepička J., Doležalova A., Kahler M., Kubiček J., Polednikova M. & Voborsky J. 1993. Pivovarsko-sladařska analytika. Praha, Merkanta: 594–597.
[10]Brand-Williams W., Cuvelier M.E. & Berset C. 1995. Use of a free radical method to evaluate antioxidant activity, Lebensmittel-Wissenschaft Und-Technoogie 28, 25–30.
[11]Benzie I.F.F. & Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ”antioxidant power”: The FRAP assay. Analytical Biochemistry, 239(1), 70-76.