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ANTIOXIDANT ACTIVITY AND POLYPHENOL CONTENT OF MALT BEVERAGES ENRICHED WITH BEE POLLEN

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ABSTRACT

In food industry, especially among the brewers, using of natural ingredients is increasingly growing demand. Beer is one of the most popular beverages 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. Bee pollen is considered to be one of the possible sources of active ingredients for that purpose. Activity of phenolic and flavonoid compounds in bee pollen can contribute to the antioxidant potential of beer. The objective of this study was to examine the influence of different types and content of bee pollen on the antioxidant properties of malt beverages and to compare phenolic and flavonoid profiles. The technological process of malt beverages preparation with addition of bee pollen was also verified. It was found out that all beverages enriched with bee pollen had higher polyphenol, flavonoid content and antioxidant potential than control sample – pure wort. The higher antioxidant activities of all extracts was measured in sample R2 - wort with 0.6% of dry rapeseed pollen and sample R4 - wort with 0.6% of frozen rapeseed pollen. The higher phenolic content than in other samples was measured in sample M4 - wort with 0.6% of frozen poppy pollen and sample M1 - wort with 0.256% of dry poppy pollen. Higher total flavonoid content was found out in sample M2 - wort with 0.6% of dry poppy pollen and M4 - wort with 0.6% of frozen poppy pollen. In conclusion, the most noticeable results of antioxidant activity, phenolic and flavonoid content were achieved in samples with higher 0.6% addition of bee pollen, mostly poppy (*Papaver somniferum* L.) pollen.

Keywords: malt wort, beer, bee pollen, antioxidant activity, phenolic content, flavonoid content

INTRODUCTION

The increased variety of malt beverages available from foreign and domestic breweries has brought a new challenge to the brewing market. Beer is a worldwide traditional natural beverage, with low calories and no fat, with organic acids and vitamins (coming from malt), proteins, hop and water. Beers raw materials are water, malt, non-malted cereals, hops and yeast. Barley malt can contribute components to wort and beer (**Coghe *et al.* 2004**). Wort components are fermentable sugars, proteins and phenolic compounds (**Szwajgier, 2011**). From the technological point of view these wort components are considered to be crucial for the overall cost-effectiveness of beer production and beer quality. The content of fermentable sugars is positively correlated with alcohol production by yeast, whereas proteins/polypeptides and phenolic compounds can cause problems with the colloidal stability of beer if present at elevated concentrations (**Ishibashi *et al.* 1996, Delvaux *et al.* 2001**). On the other hand, polyphenols are antioxidants capable of scavenging free radicals and it is generally assumed that consumption of polyphenols results in a decreased number of cases of coronary heart disease (**Ghiselli *et al.* 2000**). Beer has a higher nutritional value than other alcoholic beverages, because of its minerals and essential nutrients such as potassium, magnesium, calcium and sodium (**Ribeiro–Tafulo *et al.* 2010**). The use of cereals and malt to produce beer may also contribute for the ingestion of naturally occurring antioxidant compounds, such as polyphenols. Therefore, a possible benefit from beer consumption, not yet studied, may derive from its antioxidant properties (**Ghiselli *et al.* 2000**; **Wei *et al.* 2001**; **Girotti *et al.* 2002**). Antioxidants are any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate (**Halliwell, 2007**). Antioxidants act in various ways, which include complexation of redox-catalytic metal ions, scavenging of free radicals, and decomposition of peroxides. The intensity of this effect depends on the chemical structure and concentration of the antioxidant present. The antioxidant capacity is the measurement of moles of a given free radical scavenged by a test solution, independently of the antioxidant present in the mixture (**Mello and Kubota, 2007**). Phenolic compounds identified in beer include flavonoids, phenolic acids, proanthocyanidins, tannins, and amino phenolic compounds (**Montanari *et al.* 1999**; **Gorinstein *et al.* 2000**). All of them have been reported to possess antiradical and antioxidant properties as well as other biological effects (**Gaulejac *et al.* 1998**).

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. Bee pollen is a natural food product well known for its high nutritional and medicinal value. Bee pollen is an apicultural product which is used for its nutritional value in the human diet. It is made up of natural flower pollen mixed with nectar and bee secretions, and is rich in sugars, proteins, lipids, vitamins and flavonoids (3–5% dry weight) (**Tomas-Lorente *et al.* 1992**). Many chemical, biochemical and microbiological studies have been carried out with a wide variety of compounds from pollen, but only recently has the attention focused on a special group, namely the phenolic compounds (**Tomas-Lorente *et al.* 1992; Markham and Campos, 1996; Campos *et al.* 1997, 2002, 2003; Fatrcová-Šramková *et al.*, 2008, 2010, 2012**). Active oxygen free radicals have been implicated as causative agents in conditions such as cancer, atherosclerosis, cerebral and cardiac ischemia, Parkinson's disease, gastrointestinal disturbances and aging, among others (**Ames *et al.* 1993**). Pollen grains have specific characteristics according to the floral species or cultivation methods, but the quality depends on the collection process, cleanness, drying and storage applied by beekeepers with the objective to increase the product shelf-life.

The objective of this study was to examine the influence of different types and content of bee pollen on the antioxidant properties of malt beverages and to compare phenolic and flavonoid profiles. The technological process of malt beverages preparation with addition of bee pollen was also verified.

MATERIAL AND METHODS

Material

Biological material

Pilsner type of malt to produce sweet wort was purchased from Malt house Trnava (Slovakia), hop was purchased from Bohemia hop (Czech Republic) and yeast were purchased from biochemical laboratory Nitra (Slovakia).
Bee pollen samples were obtained directly from beekeepers from Slovakia in vegetation year 2012 and fresh were immediately frozen in -18 °C. We used monofloral bee pollen of different plant species origin. We analyzed poppy (*Papaver somniferum* L.), rape (*Brassica napus* var. *napus*), and sunflower (*Heliathus annuus* L.) bee pollen. The species purity of our samples was checked visually. According to color unity the samples contained more than 80% of proclaimed plant species, and it is still considered as monofloral bee pollen (**Campos *et al*., 2008**). For preparation of malt beverages we used frozen (stored at -18 °C until processing) and dried bee pollen. Homogenized bee pollen was dried at the temperature 35 °C until it reached moisture 5 to 10%. Then it was grinded and sieved to get powdered material.

Sample preparation

Sweet non-hopped malt wort was produced in mini brewery of Department of storage and food products processing using lager malt (for bottom fermented pilsner-style beer). Sweet wort was lautered using the grain bed as a filtration medium followed by sparging of the grains with water (76–78°C). After production of sweet wort 15 variants of worts with different addition of bee pollen were prepared. Two wort samples were prepared as control samples. Variants were prepared as follows: K1- control sample 1, pure wort; K2- control sample 2, wort with hop; R1- wort with 0.256% of dry rapeseed pollen; R2- wort with 0.6% of dry rapeseed pollen; R3- wort with 0.256% frozen rapeseed pollen; R4- wort with 0.6% of frozen rapeseed pollen; R5- wort with dry rapeseed pollen and hop 50%:50%, M1- wort with 0.256% of dry poppy pollen; M2- wort with 0.6% of dry poppy pollen; M3- wort with 0.256% frozen poppy pollen; M4- wort with 0.6% of frozen poppy pollen; M5- wort with dry poppy pollen and hop 50:50; S1- wort with 0.256% of dry sunflower pollen; S2- wort with 0.6% of dry sunflower pollen; S3- wort with 0.256% frozen sunflower pollen; S4- wort with 0.6% of frozen sunflower pollen; S5- wort with dry sunflower pollen and hop 50%:50%.
Variants were prepared according to traditional brewery technology. All samples with bee pollen and hop, and also control samples were boiled for 1.5 hour. After this time all samples were filtered and brewer's yeast were added to start fermentation process. After 5 days of fermentation at app. 5 °C samples were evaluated for antioxidant activity and total phenolic and flavonoid content.

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Methods

Free radical scavenging activity

Free radical scavenging activity of samples was measured using the 2,2-difenyl-1-picrylhydrazyl (DPPH) according to the procedures described by **Sánchés-Moreno *et al.* (1998)**. The sample (0.3 mL) was reacted with 3.7 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). Absorbance of the sample was determined using Jenway spectrophotomer (6405 UV/Vis, UK) at 515 nm. Free radical scavenging activity of the samples was expressed as mg Trolox equivalent antioxidant capacity (R^2=0.995) per L of sample (mg TEAC.L^{-1}). All analyses were performed in triplicates.

Reducing power

Reducing power of samples was determined according to the procedure by **Prieto *et al.* (1999)**. The mixture of sample (0.5 mL), 1 M H_2SO_4 (6 mL), 0.1 M KH_2PO_4 (2.8 mL), 0.1 M $(NH_4)_6Mo_7O_{24}$ (0.4 mL) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then cooled, and absorbance at 700 nm using Jenway spectrophotomer (6405 UV/Vis, UK) was detected. Reducing power of the samples was expressed as mg Trolox equivalent antioxidant capacity (R^2= 0.9909) per L of sample. All analyses were performed in triplicates.

Total phenolic content

Total phenolic content of samples was measured spectrophotometrically, using the modified Folin-Ciocalteu method as described by **Singleton and Rossi (1965)**. 0.2 mL of sample was mixed with 0.05 mL of the Folin-Ciocalteau reagent , 0.5 mL of 20% sodium carbonate, and 4.25 mL of distilled water. The mixture was allowed to stand at room temperature for 30 min. in the dark. The absorbance was read at 700 nm using spectrophotometer Jenway (6405 UV/Vis, UK). The total phenolics content was expressed as mg gallic acid equivalent (GAE, R^2= 0.9912) per L of sample. All analyses were performed in triplicates.

Total flavonoid content

Total flavonoid content was determined using the modified method by **Quettier-Deleu *et al.* (1998)** 2 mL of sample was mixed with 0.8 mL of 5% ethanolic solution of aluminium chloride and centrifugated at 10 000 g (Neofuge VS – 100 BN) for 10 min. The supernatant was used to measure the absorbance at 405 nm on a spectrophotometer Jenway (6405 UV/Vis, UK). The total flavonoid content (R^2= 0.999) was expressed as mg quercetin equivalent (QE) per L of sample. All analyses were performed in triplicates.

RESULTS AND DISCUSSION

Free radical scavenging activity

DPPH$^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reductions capability of DPPH$^{\bullet}$ is determined by the decrease in its absorbance induced by antioxidant **(Liu and Yao, 2007)**. The scavenging effect of samples on DPPH radical expressed as mg TEAC.L^{-1} is presented in Figure 1. These results indicated that all the extracts had a noticeable effect on scavenging free radical. The higher activities of all extracts were measured in samples R2 - wort with 0.6% of dry rapeseed pollen and R4 - wort with 0.6% of frozen rapeseed pollen (Fig. 1) compared to pure wort - K1.

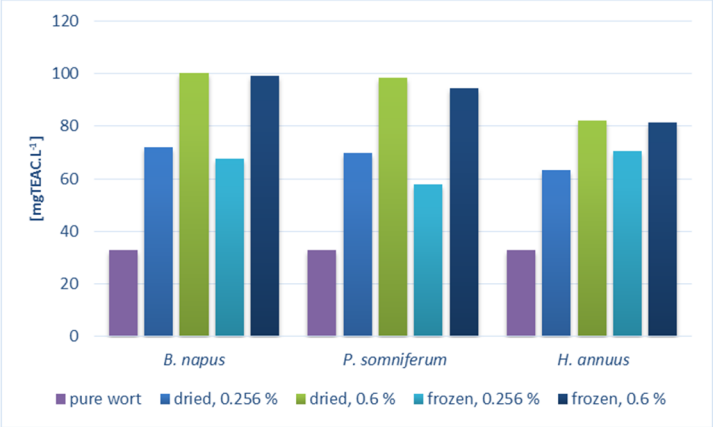

Figure 1 Free radical scavenging activity of samples (addition of pollen to wort) expressed as mg Trolox equivalent antioxidant capacity per L of samples (mg TEAC.L^{-1})

In samples where there was added to pure wort both pollen and hop, the higher activities of all extracts were measured in sample M5 - wort with dried poppy pollen and hop 50:50 and in sample S5 - wort with dried sunflower pollen and hop 50:50 in comparison to control sample K2 – wort with hop (Fig. 2).

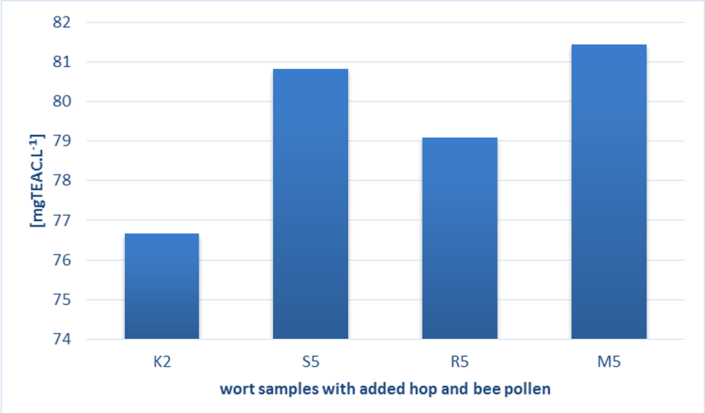

Figure 2 Free radical scavenging activity of samples (addition of pollen and hop to wort – 50%:50%) expressed as mg Trolox equivalent antioxidant capacity per L of sample (mg TEAC.L^{-1}) K2 – control sample – wort with 0.256 % hop; S5 – wort with dried sunflower pollen and hop 50%:50%; R5 - wort with dried rape pollen and hop 50:50; M5 - wort with dried poppy pollen and hop 50%:50%)

Reducing Power

For measurement of the reductive ability, the Mo^{6+} - Mo^{5+} transformation in the presence of sample was investigated. Reductive capabilities of sample extracts are shown in Fig. 3. The higher reductive ability of all extracts were measured in samples R2 - wort with 0.6% of dry rapeseed pollen and M4 - wort with 0.6% of frozen poppy pollen (Fig. 3) compared to pure wort – K1. Increase in absorbance of the reaction mixture indicated the reducing power of the samples. The reducing capacity of a compound may serve a significant indicator of its potential antioxidant activity **(Liu and Yao, 2007).** The reducing properties are generally associated with the presence of reductones **(Pin-Der, 1998)**. It is reported that the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom, or reacting with certain precursors of peroxide to prevent peroxide formation. It is presented that the phenolic compounds in samples may act in a similar fashion as reductones by donating electrons and reacting with free radicals to convert them to more stable products and terminating the free radical chain reaction **(Liu and Yao, 2007).**

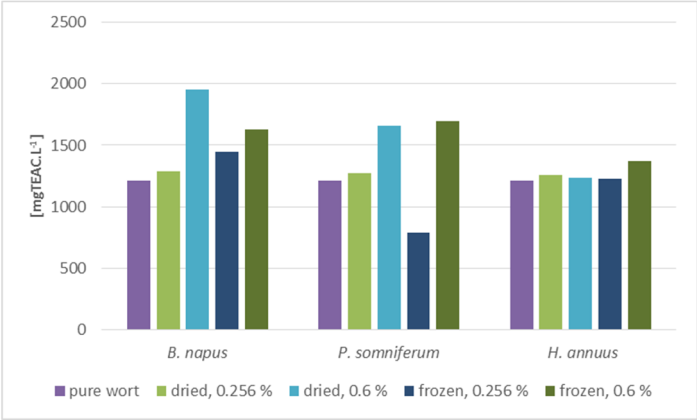

Figure 3 Reducing power of samples (addition of pollen and hop to wort) expressed as mg Trolox equivalent antioxidant capacity per L of samples (mg TEAC.L^{-1})

In samples where there was added to pure wort both pollen and hop, the highest reductive ability of all extracts was measured in sample M5 - wort with dried poppy pollen and hop 50:50 in comparison to control sample wort with hop - K2 (Fig. 4).

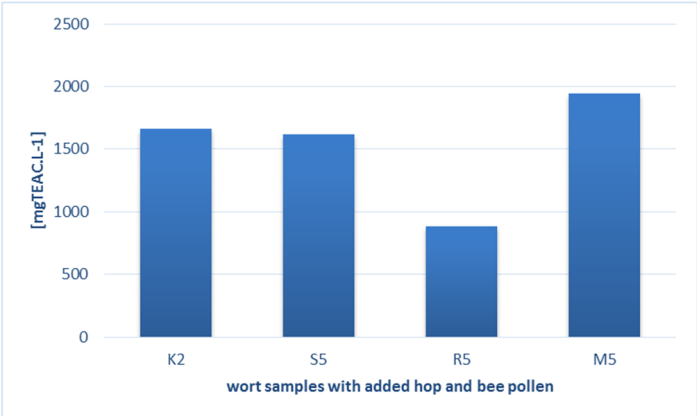

Figure 4 Reducing power of samples (addition of pollen to wort – 50%:50%) expressed as mg Trolox equivalent antioxidant capacity per L of samples (mg TEAC.L^{-1})
(K2 – control sample – wort with 0.256 % hop; S5 – wort with dried sunflower pollen and hop 50%:50%; R5 - wort with dried rape pollen and hop 50%:50%; M5 - wort with dried poppy pollen and hop 50%:50%)

Total phenolic content

Phenolics are compounds with one or more aromatic ring and one or more hydroxyl groups (**Liu, 2003**). Structurally, phenolics can be subdivided into acids derived from either benzoic acid or cinnamic acid. The phenolic composition of pollen principally consists of flavonol glycosides **(Wiermann and Vieth, 1983)** and of hydroxycinnamic acids **(Almaraz-Abarca *et al.* 2004)**. This composition tends to be species-specific and has been related to the therapeutic properties (antibiotic, antineoplasic, antidiarrhoeic and antioxidant) of pollen **(Almaraz-Abarca *et al.* 2004)**.
The total phenolic content was determined by the Folin-Ciocalteau assay. The results are presented in Tab. 1. The higher phenolic content than in other samples was measured in sample M4 - wort with 0.6% of frozen poppy pollen and sample M1 - wort with 0.256% of dry poppy pollen compared to control sample K1- pure wort (Tab. 1). All samples with addition of bee pollen had higher phenolic content in comparison to pure wort - K1 sample.

Table 1 Total phenolic and flavonoid content of sample

Sample	Total phenolic content (mg GAE.L^{-1})	Total flavonoid content (mg QE.L^{-1})
K1	83.14 ±0.48	5.52 ±0.03
K2	104.09 ±3.43	6.95 ±0.04
R1	102.19 ±1.43	8.89 ±0.12
R2	103.94 ±2.15	9.17 ±0.03
R3	100.60 ±0.99	9.51 ±0.01
R4	117.11 ±2.62	9.85 ±0.06
R5	90.13 ±1.53	8.02 ±0.01
M1	119.65 ±0.99	12.04 ±1.23
M2	96.16 ±1.92	17.20 ±0.35
M3	112.24 ±2.18	8.38 ±0.08
M4	121.23 ±2.18	14.94 ±0.08
M5	85.68 ±1.20	12.97 ±0.05
S1	93.78 ±1.45	7.45 ±0.02
S2	97.27 ±0.99	8.71 ±0.01
S3	95.37 ±0.99	9.50 ±0.02
S4	101.71 ±0.48	9.71 ±0.02
S5	85.84 ±1.45	14.07 ±0.03

(K1- control sample 1, pure wort; K2- control sample 2, wort with hop; R1- wort with 0.256% of dry rapeseed pollen; R2- wort with 0.6% of dry rapeseed pollen; R3- wort with 0.256% frozen rapeseed pollen; R4- wort with 0.6% of frozen rapeseed pollen; R5- wort with dry rapeseed pollen and hop 50%:50%; M1- wort with 0.256% of dry poppy pollen; M2- wort with 0.6% of dry poppy pollen; M3- wort with 0.256% frozen poppy pollen; M4- wort with 0.6% of frozen poppy pollen; M5- wort with dry poppy pollen and hop 50%:50%; S1- wort with 0.256% of dry sunflower pollen; S2- wort with 0.6% of dry sunflower pollen; S3- wort with 0.256% frozen sunflower pollen; S4- wort with 0.6% of frozen sunflower pollen; S5- wort with dry sunflower pollen and hop 50%:50%)

Total flavonoid content

Flavonoids are one group of phenolics, which consists of two aromatic rings linked by 3 carbons that usually in an oxygenated heterocycle ring **(Liu, 2004)**. **Ferreres *et al.* (2010)** determined in bee pollen twelve non-coloured flavonoids - kaempferol-3-O-neohesperidoside was the major compound, besides others in trace amounts. These include quercetin, kaempferol and isorhamnetin glycosides, with several of them being isomers.
The total flavonoid content of the samples is shown in Table 1. Higher concentration was found in sample M2 - wort with 0.6% of dry poppy pollen and M4 - wort with 0.6% of frozen poppy pollen. All samples with addition of bee pollen had higher flavonoid content in comparison to pure wort - K1 sample (Tab. 1).

CONCLUSION

Antioxidant activity and total flavonoid and phenol content of malt beverages enriched with different types of bee pollen was evidently improved. The obtained results indicate that the bee pollen can be used as a very interesting raw material for brewing industry. Malt beverages enriched with bee pollen had higher phenolic and flavonoid content as well as antioxidant potential than control sample – pure wort. The most noticeable results of antioxidant activity, phenolic and flavonoid content were achieved in samples with higher 0.6% addition of bee pollen, mostly poppy (*Papaver somniferum* L.) pollen. The obtained results are very promising indicating that malt beverages with defined functional and sensory properties can be produced. Such beverages can be created for special purposes and certain groups of potential consumers.

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