INFLUENCE OF SEMPERVIVUM TECTORUM ON THE PHENOLIC CONTENT OF MEADOW HONEY

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Keywords: honey, phenols, *Sempervivum tectorum*

ABSTRACT:

Phenolic compounds are compounds with an aromatic ring of one or more hydroxyl groups in the structure, which may vary from a simple to complex molecule of high molecular weight phenolic polymers. These compounds act as antioxidants, remove free radicals and inhibit lipid oxidation, but they are also used in honey, as an indicator of plant origin of honey. The main functional components of honey flavonoids which contribute significantly to the total antioxidant activity of honey, which is also beneficial to human health. The Sempervivum tectorum plant extract contains about 20 different flavones and flavonols, mainly quercetin and kempferol glycosides, polyphenolic compounds, polysaccharides and micronutrients.Investigations have shown correlation between the proportion of phenolic components (primarily flavonoids and phenolic acids) and antioxidant capacity. The content of total phenols was determined by spectrophotometric method with the Folin-Ciocalte reagent. The Folin-Ciocalteu reagent is composed of phospho-tungsten and phosphomolybdic acid. Said acids, in reaction with phenolic components, are reduced to blue colored oxides. The blue dye intensity is proportional to the concentration of phenolic compounds.In our research the average value of total phenol content for the sample of honey was 257.92 mg GAE/kg, but for the sample of honey, with the addition of plant leaf Sempervivum tectorum, the value of total phenol content was considerably higher and was 1344.69 mg GAE/kg.

1. INTRODUCTION

Sempervivum tectorum (guardian) is a fleshy, succulent plant, with thick, oval leaves arranged in a rosette, rich in antibacterial substances and polysaccharides that have a beneficial effect on the immune system. The freshly squeezed juice of the *Sempervivum tectorum* plant in Europe is often used as an therapy for ear inflammation, having a beneficial effect on the warts, sores, wounds, burns, burning eyes [1, 2]. *Sempervivum tectorum* plant extract contains approximately 20 different flavones and flavonols, mainly quercetin and kempferol glycosides, polyphenolic compounds, polysaccharides and micronutrients, mainly Ca (76.52 mg/g), K (40.47 mg/g), Mg (17.85 mg/g). The characteristic monosaccharides are rhamnose, arabinose, xylose, mannose, galactose and uronic acids. [3-6]. The composition and antioxidant activity of plants depends on various factors such as the method of cultivation or collection, the quality of the processed plant raw material, geographical origin, climatic conditions, treatment processes and optimization of technological procedures for isolating the active components that have antioxidant properties. In addition, the final result of the analysis of the

determination of antioxidant activity is influenced by a number of factors such as the mode of extraction, the type of solvent, and the mode of data pretreatment [7]

"Honey is a natural, sweet substance produced by honey bees (Apis mellifera) by processing the nectar of plants, or from the juices from living parts of plants, or by collecting insect excretions that feed by sucking juices from living parts of plants, which the bees collect, process and add their own specific substances. , dehydrate and deposite in the honeycomb cell until maturation [8]. Honey is extremely rich in antioxidants and can be used as a functional food for its beneficial effects on the human body. Flavonoids in honey have antioxidant effects because they can directly capture free radicals, and they also act indirectly by protecting vitamin C from oxidation [9]. Water is one of the most important ingredients in honey. Primarily it affects its quality, sustainability and granulation. The amount of water in the honey is from 15 to 23% and thus makes it the second element of honey [10]. The antioxidant capacity of honey is the result of the synergistic action of various components such as phenolic components, peptides, amino acids, organic acids, enzymes, products of Maillard reactions, carotenoid derivatives, ascorbic acid and other trace components. Studies have shown that the antioxidant capacity of honey is correlated with the content of phenolic components (primarily flavonoids and phenolic acids) in honey [11-14]. Antioxidants are compounds that can act to capture and neutralize reactive oxygen and nitrogen species and thus stop chain reactions, in which case they are called primary antioxidants. Phenolic components are good free radical scavengers. Another way antioxidants work is to prevent the initiation of free radical formation or to reduce the rate at which a further chain reaction is carried out, which is then called preventive antioxidants [11].

The total phenol content was determined spectrophotometrically and performed with a Folin-Ciocalteu reagent. This method was primarily used to determine proteins, and after modification, it was used to determine total phenols. The folin-Ciocalteu reagent consists of phosphovolframic acid and phosphomolybdenic acid. Said acids, in reaction with the phenolic components, are reduced to blue colored oxides. The intensity of the blue coloration is in proportion to the concentration of the phenolic components. The change in color is monitored at wavelengths from 745 nm to 765 nm [15, 11]. Despite the drawbacks, the method is often used because of its speed and simplicity [16, 15, 11]. to the growing interest in finding functional foods, honey-based products containing nuts, herbs or spices have appeared on the European market. These products could combine the properties of honey and added substances [17]. The aim of this study was to determine the physicochemical parameters and the proportion of total phenols of meadow honey and honey samples with the addition of the preservative leaf Sempervivum tectorum, since there is no data on the content of total phenols in the honey samples with the addition of the preserve leaf.

2. MATERIAL AND METHOD OF WORK

To analyze this work, we needed 500 g of quality home-made meadow honey and 300 g of full preserves. The guardian had to be washed, dried and then wrapped in a clean, dry cloth to absorb the remaining water so that it could be ground in a blender. Mix the wooden spoon with honey to form a uniform mixture. Already at the beginning of mixing, the guardian's leaves begin to release their juice into the honey, so that the total liquid is less frequent than honey. After mixing, pour the mixture into glass jars, cover the lids, wrap them with foil or dark paper to protect them from light and allow them to stand in the refrigerator for about two weeks. During this time, it is desirable to stir the mixture 3-4 times, since it will thicken slightly at the top and less often in the lower layers. After 14 days the mixture was analyzed.

All analyzes were performed according to the Rulebook on methods for control of honey and other bee products [18]. The analysis was performed on six samples of 2018 meadow honey and six samples of the same honey with the addition of the leaf preservative Sempervivum tectorum. There were tested: total sugars, reducing sugars, sucrose, ash content, acidity, electrical conductivity, solutes and total phenols.

Total phenol content in prepared samples was determined with Folin-Ciocalteu method [19], and the results were calculated from the calibration curve of gallic acid. Total phenol content is expressed as

mg/g gallic acid equivalents (GAE)/L of sample. 0,2 mL of sample, 1,8 mL of distilled water, 10 mL of Folin-Ciocalteu reagent and 8 mL of sodium carbonate solution was transferred with a pipette into a test tube. After the sample had been allowed to stand for 2 hours at room temperature, photoLlab 6600 UV-VIS WTW Spectrophotometer was used at 765 nm to measure the absorbance.

3. RESULTS AND DISCUSSION

Twelve samples (six samples of meadow honey and six samples of the same honey with the addition of guardian's leaves) were analyzed in order to determine the effect of the guardian Sempervivum tectorum on the phenol content of meadow honey. The results of physicochemical analyzes of honey samples show that all values of tested parameters are within the limits prescribed by the Rulebook [18].

Table 1. Content of sugar in honey

Sample (n=6)	Reducing sugars%	Total sugars %	Saccharose %
HS 1	66.50	68.43	1.69
HS 2	67.24	69.35	2.00
HS 3	66.93	69.02	1.99
HS 4	68.70	70.83	2.02
HS 5	69.78	72.13	2.23
HS 6	68.54	70.83	2.18
AV	**67.97**	**70.09**	**2.01**

n – number of samples, HS –honey sample, AV – average value

Table 2. Content of sugar in honey with the addition of guardian's leaves (*Sempervivum tectorum*)

Uzorak (n=6)	Reducing sugars %	Total sugars %	Saccharose %
HGS 1	43.37	45.87	2.38
HGS 2	46.23	48.19	1.86
HGS 3	46.76	48.34	1.05
HGS 4	46.01	48.19	2.07
HGS 5	46.69	48.21	1.44
HGS 6	44.21	45.99	1.69
AV	**45.54**	**47.46**	**1.74**

n – number of samples, HGS - honey sample with the addition of guardian's leaves (*Sempervivum tectorum*) AV – average value

Table 3. Content of ash, total phenols, acidity in honey and honey with the addition of guardian's leaves (*Sempervivum tectorum*)

Sample(n=6)	Ash %		Total phenols mg GAE/kg		Acidity mmol/kg	
MČ 1	**HS**	**HGS**	**HS**	**HGS**	**HS**	**HGS**
1	0.74	1.26	305.16	1338.30	38.04	49.06
2	0.26	0.93	202.28	1262.52	37.02	50.02
3	0.84	0.09	233.02	1309.97	40.60	51.64
4	0.44	1.14	207.27	1346.62	29.94	48.14
5	0.67	0.96	306.27	1299.97	35.03	49.01
6	0.59	1.05	293.63	1510.80	38.85	52.08
AV	**0.59**	**1.07**	**257.92**	**1344.69**	**36.58**	**49.99**

n – number of samples, HS –honey sample, HGS - honey sample with the addition of guardian's leaves (*Sempervivum tectorum*) AV – average value

Table 4. The content of soluble solids, electrical conductivity in honey and honey with the addition of guardian's leaves (*Sempervivum tectorum*)

Sample (n=6)	Insoluble substances %		Electrical conductivity mS/cm	
	HS	HGS	HS	HGS
1	0.0124	3.59	0.701	1.372
2	0.0149	2.49	0,720	1.373
3	0.0122	2.91	0.715	1.407
4	0.0128	3.05	0.731	1.390
5	0.0137	2.50	0.729	1.446
6	0.0140	2.91	0.650	1.472
SV	**0.0133**	**2.90**	**0.707**	**1.414**

n – number of samples, HS –honey sample, HGS - honey sample with the addition of guardian's leaves,, (*Sempervivum tectorum*) AV – average value

In accordance with the Rulebook [18], the content of fructose and glucose for flower honey may not be less than 60 g/100 g of honey, while for jellyfish and mixed honey it mustn't be less than 45 g/100 g of honey. In our studies, the mean content of reducing sugars in meadow honey was 67.97%, which met the prescribed criteria. The proportion of sucrose disaccharides in honey ranges from 5 g to 15 g per 100 g of honey depending on the type of honey. Sucrose content is an important indicator of counterfeiting of honey, with the addition of sugar syrups it can be both direct and indirect. Direct counterfeiting of honey involves the addition of foreign components, most commonly sugar syrups, to honey. Indirect counterfeiting involves feeding bees for the purpose of improving yield and is extremely difficult to detect [20, 21]. The mean sucrose content of meadow honey was 2.01 %, which is in accordance with the Regulations. The electrical conductivity of honey is a property that depends primarily on the amount of mineral salts, organic acids and proteins present. The higher the mineral salt content, the higher the electrical conductivity of the honey is. However, with a higher percentage of moisture, the electrical conductivity of the honey is lower, so the two parameters are negatively correlated. In our studies, the mean electrical conductivity for meadow honey was 0.707 mS/cm.

The total values of phenol content obtained in the honey samples analyzed in this study are significantly higher than those obtained in other studies. According to Lachman et al. [22] the proportion of total phenols was in the range 94.3 - 119.2 mg of gallic acid/kg of honey, Meda et al. [23] reported in their studies total values of 32.59 to 114.75 mg with mean from 74.38 ± 20.54 mg. However, in some cases lower values of total phenol content were also found [24]. In the studies of Krpan et al. [24] the phenol content ranged from 31.72 mg/kg to 80.11 mg/kg. The same authors observed that there is a positive correlation between total phenol content and antioxidant activity, indicating that phenolic compounds are mainly responsible for the antioxidant power of honey. Piljac-Žegarac et al. [25] obtained values of 44.17 ± 2.24 mg of gallic acid/100 g of honey, and according to the results of Bobis et al. [26] where the proportion was 896.4 ± 34.8 mg of gallic acid/kg of honey, which is significantly higher than the values obtained in our studies. In our studies, the mean content of total phenols for the honey sample was 257.92 mg GAE/kg, however for the honey sample with the addition of preservatives the value of the total phenol content was much higher and amounted to 1344.69 mg GAE/kg.

In their research Wang et.al. [27], made with samples of the six most prevalent types of honey in Poland (acacia, rapeseed, linden, goldenrod, heather, buckwheat), they found that the antioxidant activity of the lighter types of honey (acacia, rapeseed, linden). Bertoncelj et.al. [13] investigated the content of total phenols of the seven most represented honey species in Slovenia by colorimetric modified Folin-Ciocolt reaction and their antioxidant activity by DPPH assay. Their results showed that the total phenol content and antioxidant value of honey varied significantly depending on the type

of honey in question. The content of total phenols was the lowest in acacia honey, expressed as gallic acid equivalent, and was 44.8 mg / kg, whereas, by comparison, this value for fir honey was 241.4 mg/kg. The same authors state that acacia honey had the lowest antioxidant activity. Research Čanandović-Brunet et.al. [28] has shown that the antioxidant activity of honey is enhanced by the addition of dried fruit fruits (prunes). The study tested the antioxidant effect, as well as the total phenol content of two types of honey originating in Serbia (lipin and homolytic), as well as samples of these types of honey with the addition of dried plums. In their research Wilczyńska et al. [17] made on honey samples with the addition of spices, they found that the type and amount of spice added had a significant effect on antioxidant activity: the ability to purify free radicals and the total content of phenolic acids. In their studies, the highest antioxidant activity was shown by cinnamon honey and the lowest by cardamom honey.

4. CONCLUSION

All honey samples were analyzed in accordance with the regulations of respect to the physicochemical parameters that were done. The mean content of total phenols of meadow honey samples was 257.92 mg GAE/kg of honey, and the samples of the same honey with leaf preservative content were 1344.69 mg GAE/kg. Overall, our studies confirm that the tested meadow honey samples are good sources of total phenols, and in particular samples containing the leaf preserver *Sempervivum tectorum* that showed increased total phenol content, and which have good antioxidant potential. Therefore, daily consumption of any type of honey can be helpful in protecting the body from diseases associated with oxidative stress. However, further studies on a larger number of samples and determination of total flavonoid content, proline content and other phenolic components, and radical activity are required by a method such as DPPH or another test.

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