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IMMUNOMODULATORY ABILITY OF HONEY ENRICHED WITH PROPOLIS

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ABSTRACT: *A well-developed immune system of the organism, which, among other things, arises as a result of a healthy lifestyle, is a prerequisite for a healthy and quality life. Natural food products, primarily honey and other bee products, greatly contribute to the proper development of the body's immune response to the harmful effects of foreign substances. Honey and other bee products are a valuable and rich source of biologically active substances. They have been used for centuries in traditional medicine, due to their wide range of antibacterial, antiradical, antioxidant and anticancer effects, as well as their supportive effect in the prevention and treatment of many diseases. Consumption of honey contributes to the improvement of immunity and enriches the human diet with many valuable nutrients and bioactive substances. Bioactive substances, including polyphenols, are organic chemical compounds naturally present in honey. Polyphenols are important secondary metabolites of plants that are transferred to honey along with nectar, pollen or propolis. Several studies have confirmed the immunomodulatory role of the basic phenolic compounds present in honey. For the purpose of our research, a total of 21 samples of honey and 10 samples of alcoholic propolis extract were collected, mostly from the Federation of Bosnia and Herzegovina. Based on the obtained results, it is clear that honey itself is a rich source of antioxidants in the diet, but that the addition of propolis significantly increases its antioxidant power. The addition of propolis to honey significantly increased the antioxidant activity of all analyzed samples. After the addition of propolis, the highest antioxidant activity was again shown by a sample of forest honey from the area of Bihać, Una-Sana Canton (Š5) and its antioxidant activity is 1143.96 µmol Fe (II) / L. The lowest antioxidant activity after the addition of propolis was shown by the meadow sample of honey from Sanski Most, Una-Sana Canton, (L21), 462.71 µmol Fe (II) / L.Based on the presented results, it is clear that after the addition of propolis in the analyzed honey samples there was a significant increase in the concentration of polyphenols by an average of 11.96%.*

KEYWORDS: honey, propolis, antioxidant acivity, poliphenols, immunomodulatory

INTRODUCTION

Immunity means a set of reactions that mobilize various factors in the body, which by the mechanisms of their action recognize and remove foreign substances from the body. These reactions are of a specific nature and in them many humoral factors of the cell are activated, non-specific or specific for a foreign substance, which thus protects the organism from infectious agents.

In addition, the body's immune mechanisms remove spent products and damaged cells and control cell proliferation, ie recognize and destroy aberrant cells that have broken away from control mechanisms responsible for regulating growth, proliferation and differentiation of cells, thus preventing tumor growth. Immune mechanisms most often perform their defense functions without consequences for the body, but in some cases tissue damage occurs as a result of increased or altered reactivity of the immune system, which is manifested by allergies and autoimmune diseases (Milić N. et al. 2017).

The basic functions of the immune system, therefore, include: defense of the organism, maintenance of homeostasis and control, ie. immune control of cell proliferation. The body's immune system includes natural and acquired defense mechanisms. Natural or innate defense mechanisms form the first line of defense of the organism. These include anatomical and physiological barriers, effector cells of nonspecific immunity, circulating effector proteins, cytokines, inflammatory response, intraepithelial B and T lymphocytes. B and T lymphocytes, which belong to white blood cells, are important for specific or acquired immunity. B lymphocytes react by producing antibodies that bind to antigens or bacteria, and then send a signal to the phagocytes to clear the antigen-antibody structure from the body. We can significantly influence the maintenance and strengthening of immunity with a healthy lifestyle, proper diet and intake of antioxidants, especially from natural food products. (Milić N. et al. 2017)

Honey

Honey is a sweet liquid processed by honey bees (Apismellifera) (Sammugam L., et.al. 2017). It is recognized worldwide as a valuable food because of its important nutrients, which have a beneficial effect on human health and immunity (Sammugam L., et.al. 2017). Honey is also very important in the diet, as a functional food that provides energy and improves the function of vital organs in the body.

Honey and other bee products are a valuable and rich source of biologically active substances (Habryka C., 2020). They have been used for centuries in traditional medicine, due to their wide range of antibacterial, antiradical, antioxidant and anticancer effects, as well as their supportive effect in the prevention and treatment of many diseases (Habryka C., 2020). Due to all the above properties, and above all due to its significant antioxidant potential, bee products are indispensable ingredients of healthy food. Among the compounds present in honey that exhibit antioxidant properties, the most important are proteins, amino acids, carotenoids, phenolic compounds, flavonoids, ascorbic acid, organic acids and products of the Maillard reaction (Habryka C., 2020). The antioxidant profile of honey and other bee products and its biological activity depend on many factors, including plant species that are a source of nectar and their varieties, as well as seasons, climatic and environmental conditions, genetic and many other factors (Habryka C., 2020). So, we can say that honey is a very valuable preventive, health and medicinal product. Consumption of honey contributes to the improvement of immunity and enriches the human diet with many valuable nutrients and bioactive substances (Habryka C., 2020). Bioactive substances, including polyphenols, are organic chemical compounds naturally present

in honey (Habryka C., 2020). Polyphenols are important secondary metabolites of plants that are transferred to honey along with nectar, pollen or propolis. They can appear in the form of phenolic glycosides, phenolic acids, free phenols, flavonoids, catechins, anthocyanins (Habryka C., 2020). Phenolic acids commonly found in honey include: p-hydroxybenzoic, p-coumaric, cinnamic, gallic, ferulic, and caffeic acids (Habryka C., 2020). Figure 1. shows the structures of cinnamic acid and its derivatives.

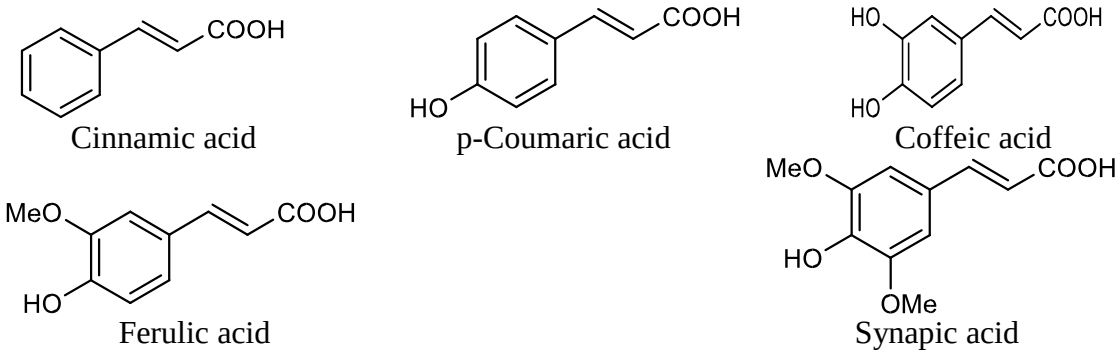

Figure 1. Cinnamic acid and its derivatives

The antimicrobial activity of honey has been known since ancient times, and thanks to this ability it is used in the treatment of bacterial infections (wounds, skin, mucous membranes, ulcers and other diseases). The antibacterial properties of honey are ensured by its high osmolarity, acidity (low pH), content of hydrogen peroxide (H_2O_2) and non-peroxide components, and the presence of phytochemical components such as methylglycosal (MGO) (Deb Mandal M., Mandal S., 2011). Numerous studies that have studied the effects of honey on the immune system have proven its anti-inflammatory effect, ie. the action of certain components of honey on immune cells. Studies have shown that thrombin-induced oxidative respiratory shock in human neutrophils and peritoneal macrophages is inhibited by co-incubation with different types of honey. (Deb Mandal M., Mandal S., 2011) Another study conducted by Gannabathula et al demonstrated a significant reduction in human neutrophil superoxide production following a therapeutic procedure with three types of New Zealand honey. (Deb Mandal M., Mandal S., 2011).

Propolis According to historical records, propolis has been used as a medicine in traditional medicine since 300 BC. The antibacterial properties of propolis were recognized in Europe in the 17th and 20th centuries. Up to 180 different substances can be found in the chemical structure of propolis and the composition itself varies from season to season. In general, propolis contains polyphenols (flavanoids, phenolic acids and esters), phenolic aldehydes and ketones, and many other compounds. The composition of propolis includes: resins with a content of 50%, beeswax 30%, pollen 5%, essential and aromatic oils with a content of 10%, and some other substances that include organic compounds (Anjum S., et. al. 2019).

The antimicrobial activity of propolis should be observed on two levels. The first level is associated with the direct action of propolis on the microorganism, and the second

level is the stimulation of the immune system, which results in the activation of the body's natural defense mechanisms. The mechanism of antimicrobial action of propolis is based on the action on the permeability of the cell membrane of the microorganism, disruption of the membrane potential and the production of adenosine triphosphate (ATP) as well as on reducing the motility of microorganisms (Przybylek I., et.al. 2019). Propolis itself has a specific smell and taste, so its consumption is sometimes not pleasant for the consumer. For this reason, and appreciating the importance of consuming such a rich natural product, finding a way to enable its consumption and intake in the form of appropriate mixtures of honey and propolis is even more important.

Influence of propolis addition on sensory characteristics and honey quality parameters

Given that both honey and propolis, as the most widely used bee products in the diet, are extremely important sources of antioxidants, in this study we tried to prove that adding small amounts of propolis to honey will significantly improve the antioxidant properties of honey without changing its sensory properties. and organoleptic properties. Habryka C. et al. investigated four sensory parameters of honey: color, odor, texture and taste and determined how propolis affects the change of sensory properties of honey. (Habryka C., 2020) The tested semi-floral honey that was not enriched with propolis was rated as very bright, uniform and clear. Sensory analysis indicated that the addition of propolis affects the reduction of clarity, brightness and uniformity. Descriptive parameters were evaluated for odor evaluation: honey, waxy, sweet, molasses, floral and foreign. Sensory analysis described the smell of polyfloral honey not enriched with propolis as floral and honey. The intensity of the impression as a sweet scent was described as barely noticeable. The smell of wax and molasses was described as barely or moderately perceptible, and the foreign odor was not perceptible. The perception of foreign odor was mostly influenced by the addition of propolis in the amount of 1%. The smell of honey without additives was rated as strong. With the increase in the amount of propolis in the tested honey samples, the sense of smell decreased to barely noticeable. With the increase in the concentration of propolis, the impression of molasses and the smell of wax was hardly perceptible. The sweet smell is described as moderately sensitive to honey with the addition of propolis of 0.7% (Habryka C., 2020).

The water content in honey is an important parameter that determines its quality. Too much water can negatively affect the stability of honey, including its sensitivity to fermentation processes, the activity of osmophilic yeasts, certain physicochemical properties. The maximum permissible water content in honey is up to 20%, except for heather honey. In studies by Habryk C. et al. the tested polyfloral honey had a water content of 17 g / 100 g of honey, and the addition of propolis did not significantly affect this parameter. (Habryka C., 2020; Journal of Lows of the Republic of Poland, 2003) Another parameter that characterizes the commercial quality of honey is the content of insoluble substances in water, including traces: pollen, propolis, fragments of bees, other insects, bacterial spores, algae and fungal cells. The analyzed polyfloral honey contained 0.06 g of insoluble matter (Habryka C., 2020; Journal of Lows of the Republic of Poland, 2003). Juszczaj et al. found that the addition of bee products honey,

including: pollen, propolis or royal jelly due to their low solubility in water, significantly affect the increase in the content of substances insoluble in water. The mentioned authors claim that the addition of other bee products to polyfloral honey can lead to an increase in the content of water-insoluble substances up to four times. (Habryka C., 2020; Juszczak L. et.al. 2018).

Honey also contains mineral compounds that affect the electrical conductivity of honey solution. The quality requirements related to the electrical conductivity of honey determine the maximum conductivity up to 0.8 mS / cm (Habryka C., 2020; Journal of Lows of the Republic of Poland, 2003). The determined electrical conductivity of the tested polyfloral honey was 0.50 mS / cm. Additions of propolis extract of different concentrations did not significantly result in a change in the specific value of electrical conductivity. Propolis supplements in higher percentages also had little effect on increasing free acidity. The addition of 0.1% propolis extract led to an increase in free acidity at a concentration of 24.63 mval / kg, while the addition of 1% led to an increase in free acidity at a concentration of 30.93 mval / kg. (Habryka C., 2020; Juszczak L. et.al. 2018) The total fructose and glucose content of honey must not be less than 60g / 100g of honey, while the sucrose content must not exceed 5g / 100g. (Habryka C., 2020; Journal of Lows of the Republic of Poland, 2003) In the tested polyfloral honey, the content of glucose, fructose and sucrose was determined in the amount of 25.53 g / 100 g, 39.62 g / 100 g and 1.8 g / 100 g, which is in accordance with the legal parameters. (Habryka C., 2020; Journal of Lows of the Republic of Poland, 2003) Another factor that determines the quality of honey is the presence of 5-hydroxymethylfurfural (5-HMF) and its increased content indicates that the honey was stored in inappropriate conditions. The determined HMF content in the tested polyfloral honey was 10.76 mg / kg, which is in accordance with the legal regulations (Habryka C., 2020). The addition of propolis extract to polyfloral honey did not significantly affect the HMF content. The effect of the addition of propolis to honey does not significantly affect its commercial quality, as it has been found that the additive has an effect only on the increase in the amount of water-insoluble substances (Habryka C., 2020).

Immunomodulatory effect of major phenolic compounds in honey

Polyphenols are chemical compounds that are formed as secondary metabolites of plants. These compounds play a very important role in the reproduction and development of plants, as well as in their protection against UV radiation, mechanical and microbiological damage. Based on their chemical structure, polyphenols can be divided into flavanoid and non-flavonoid (phenolic acids) compounds. Flavanoids are water-soluble chemical compounds with low molecular weight. They are formed from two benzene rings connected by three carbon atoms and a minimum of two phenolic groups (OH). Flavanoids are divided into flavanols, flavones, flavanones, isoflavones, anthocyanins and anthocyanins. Phenolic acids (phenolcarboxylic acids) contain a phenolic ring and at least one organic carboxylic acid in their structure. According to their structure, they can be divided into coumaric, ferulic, caffeic, acetophenonic, phenylacetic, syringic, vanillic and gallic acids. The phenolic composition of honey depends on the geographical and botanical origin of the honey itself. Table 1 shows the most common phenolic compounds present in different types of honey. (Kurtagić H. 2017)

Table 1. The most common phenolic compounds present in honey

No	Number of C atoms	C-scelet	Phenol classes
1.	6	C6	Simple phenols
2.	7	C6-C1	Hydroxybenzoate
3.	8	C6-C2	Acetophenomas and phenylacetates
4.	9	C6-C3	Hydroxycinnamates, phenylpropenes
5.	10	C6-C4	Coumarins and hormones
6.	13	C6-C1-C6	Naftokinomi
7.	14	C6-C2-C6	Xanthoni
8.	15	C6-C3-C6	Stilbeni and anthraquinones
9.	18	(C6-C3)2	Flavonoids
10.	30	(C6-C3-C6)2	Liganins
11.	n	(C6)n	Bioflavonoids and catechol melanin
12.	n	(C6-C3)n	Lignins
13.	n	(C6-C3-C6)n	Condensed tannins

Several studies have confirmed the immunomodulatory role of the basic phenolic compounds present in honey. In a study by Cho et al., For example, quercetin inhibited lipopylsaccharide-induced release of TNF-α, IL-1β, and IL-6 by macrophages by suppressing and activating extracellular signal-regulated kinase and mitogen-activated protein kinase (Razan J. et.al. 2021; ChoS Y. et.al. 2003; Wadsworth T.L. et. al. 2001) Also, a study conducted by Huang and co-workers on experimental animals proved that quercetin treatment results in reduced production of propulsion cytokines and chemokines. (Razan J. et.al. 2021; Huang R.Y. et.al. 2010) Quercetin has the ability to inhibit the release of IL-6 by human mast cells by triggering the Fc epsilon RI (FcεRI) signal or a non-allergic IL-6 dependent pathway. Quercetin treatment of human peripheral blood mononuclear cells has also been shown to primarily induce interferon gamma (IFN-γ) expression and synthesis and inhibit IL-4 production. It has also been shown that quercetin can activate Th 1 cells in various ways, thus ensuring its possible potential antitumor mechanism of action. (Nair, M.P.et.al. 2002).

The immunomodulatory effect of quercetin in various autoimmune diseases has also been investigated. In a study conducted by Sternberg et al., A study was performed on human peripheral blood mononuclear cells isolated from patients with multiple sclerosis, the treatment showed a reduction in the proliferation of these cells, which was also accompanied by reduced release of TNF-α and IL-1β cytokinin (Razan J. et.al.

2021; Sternberg Z. et.al. 2008). Quercetin has also been shown to suppress TNF-α secretion and interfere with the onset of inflammation in intestinal disease. In acute colitis, quercetin also showed an anti-inflammatory effect mediated by a weakened dendritic cell response. The effect of quercetin has also been experimentally proven in autoimmune encephalitis, where quercetin blocked Th 1 differentiation (Razan J. et.al. 2021; Muthian G. et.al. 2004). Based on available research, it can be concluded that quercetin has a wide range of biological activities, including its anti-inflammatory, antiviral, antioxidant and anticancer effects. Other polyphenols have also been shown to have immunomodulatory activity. Luteolin has been shown to inhibit the gene expression of proinflammatory cytokines as well as the release of TNF-α in lipolysaccharide-induced macrophages by p38 activation (Razan J. et.al. 2021; Muthian G. et.al. 2004; Garcia-Lafuente, A. et.al. 2009). Similarly, luteolin inhibited the secretion of TNF-α and IFN-γ by mediated signal transduction activation and transcriptional activator 1, signal transduction and transcriptional activator 3 and cyclooxygenase 2 COX-2 in macrophages. Luteolin also suppressed both NFkB activation and TNF-α secretion in cultured intestinal epithelial cells RAW 264.7. (Razan J. et.al. 2021; Xagorari A. 2002; Xia N et.al. 2016; Nishitani. Y. et.al. 2013)

METHODOLOGY

For the purpose of our research, a total of 21 samples of honey and 10 samples of alcoholic propolis extract were collected, mostly from the Federation of Bosnia and Herzegovina. Samples were collected systematically in a period of one year from September 2020 to September 2021, and their physico-chemical analyzes were performed in parallel. Sampling of mountain and meadow honey and samples of alcoholic / ethanolic propolis extract from various locations in the area of 10 cantons from the territory of the Federation of Bosnia and Herzegovina was planned and performed. Samples were collected from local beekeepers. When collecting samples, data on the geographical and botanical origin of honey were taken from the producers. Honey samples were collected and stored until the moment of analysis in dark glass jars, and propolis samples were collected in original packaging, glass bottles with a volume of 10-20 mL.

Determination of total antioxidant capacity of honey by FRAP method

Direct methods (ORAC method, for determining antioxidant capacity with β-carotene) and indirect methods (DPPH, ABTS +, FRAP) are used to determine antioxidant capacity. In our study, the FRAP method of investigating antioxidant activity was used. The FRAP method is a simple, fast and automated method based on the reduction of iron from ferrous Fe^{3+} to ferrous Fe^{2+} form in the presence of antioxidants where at low pH an intense blue colored ferro tripyridyltriazine complex with an absorption maximum at 593 nm develops. The reaction is not specific and the results are expressed as μmol Fe^{2+} equivalent (Fe) / mL sample. Figure 2 shows the reduction reaction of 2,4,6-tripyridyl-s-triazine iron.

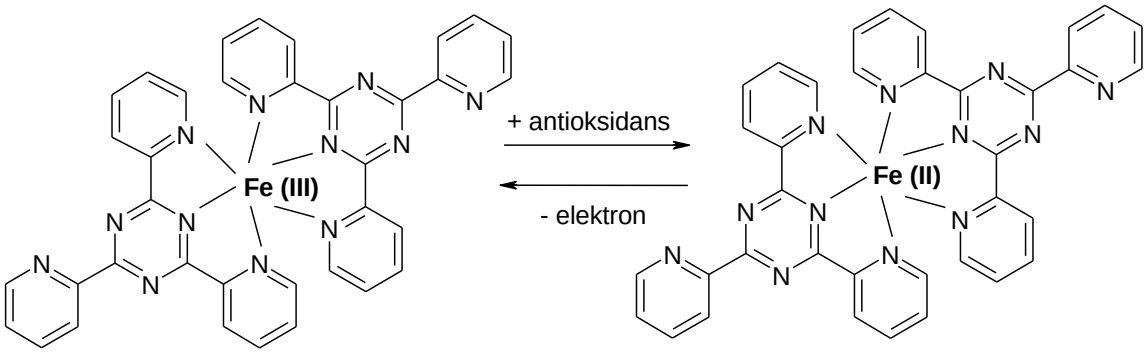

Figure 2. Iron reduction reaction of 2,4,6-tripyridyl-s-triazine (TPTZ)

Ten $FeSO_4 \cdot 7H_2O$ solutions with a concentration of 0.05 to 1.6 mM were prepared for the construction of the calibration curve. The concentration of antioxidants in the measured sample based on the obtained values is calculated according to the expression:

$$x = \frac{y - 0{,}0243}{0{,}0011}$$

Where is: x - concentration of antioxidants; y - measured average absorption for the sample. Honey samples were prepared by weighing 5.00 +/- 0.001 g of the sample in a glass beaker. 20 mL of distilled water was added to the beaker and the honey was dissolved with stirring. The sample was transferred to a 50 mL flask made up to the mark with distilled water. The contents of the flask were homogenized by stirring. After mixing, 200 µl of sample was transferred to a test tube with an automatic pipette, to which 1.8 mL of freshly prepared FRAP reagent was then added. It is allowed to react for 10 minutes, and a colored solution is obtained, as a result of the reaction of the antioxidants present in the sample with the FRAP reagent. The absorbance is then measured on a spectrophotometer at a wavelength of 593 nm. Measurement of the absorbance of the sample is performed in relation to the blank. The results are expressed in µmol FeII / L 10% honey solution.

Determination of total polyphenol content by Folin-Ciocalteu (FC) method Determination of total polyphenols was done using the Folin-Ciocalteu method, which is a standardized and widely used method. The basic mixture for analysis consists of tungsten and molybdate in the base medium (7.5% Na_2CO_3). Due to the base environment, phenol oxidation and O2 formation occur. It reacts with molybdate to form molybdenum (IV) oxide, which it absorbs at a wavelength of 765 nm. The polyphenols determined by this method are expressed in mg of gallic acid per L solution. Preparation of the calibration curve for Folin-Ciocaltea was performed by preparing standard gallic acid solutions. From the standard solution (2 g / L of gallic acid), 50 mL of gallic acid solutions of the following concentrations were prepared by

dilution in flasks: 2.5 mg GA / L, 10 mg GA / L, 20 mg GA / L, 40 mg GA / L and 50 mg GA / L. After preparation of the solutions, the absorbance was measured on a spectrophotometer. (Doctoral thesis of Prof. Aldin) For analysis, 5.00 ± 0.001 g of honey sample was weighed into a glass beaker and dissolved in 20 mL of distilled water. The sample was transferred to a 50 mL flask and made up to the mark with water. The contents of the flask were homogenized by stirring. Pipette 200 µl of the sample into a test tube with an automatic pipette, to which 2 ml of Folin - Ciocalte reagent solution is then added. The sample is then homogenized by stirring for 2 minutes. Absorbance was measured relative to the sweet analogue of honey (40% fructose, 30% glucose, 10% maltose in 20% distilled water) within 20 minutes at a wavelength of 750 nm. The concentration of total polyphenols is calculated using an expression obtained from the calibrated direction. (Kesić A.2018)

$$x = \frac{y + 0{,}005}{0{,}022}$$

RESULTS/FINDINGS

The results obtained by detailed spectrophotometric analyzes of honey samples without propolis addition and after propolis addition are shown in the table. Table 2 shows the results of the total antioxidant activity of honey before the addition of propolis and after the addition of propolis to honey.

Table 2. Total antioxidant activity of analyzed honey samples before and after propolis addition and percentage of its increase

No.	Sample mark	Honey samples	Honey samples + propolis	Percentage of antioxidant activity increase
		µmol Fe(II)/L	µmol Fe(II)/L	%
1.	B1	256,8750	643,12	150,36
2.	P2	401,8750	678,12	68,74
3.	L3	366,0416	521,45	42,46
4.	Š4	502,2916	854,37	70,09
5.	Š5	1064,3750	1143,95	7,477
6.	Š6	498,1250	671,04	34,71
7.	L7	435,6250	770,21	76,81
8.	Š8	566,8750	1016,04	79,23
9.	Š9	386,0416	565,62	46,52
10.	L10	199,3750	603,54	202,72
11.	L11	387,7083	746,46	92,53
12.	L12	351,4583	616,87	75,52
13.	L13	242,2916	509,37	110,23
14.	L14	392,7083	627,29	59,73
15.	L15	246,0416	763,12	210,16
16.	M16	538,5416	751,04	39,46
17.	L17	293,125	549,79	87,56
18.	L18	374,7916	528,54	41,02
19.	L19	450,6250	697,71	54,83
20.	Š20	298,9583	851,04	184,67
21.	L21	275,6250	462,71	67,88
22.	P22	726,4583	839,79	15,60

We have proven that there is a significant correlation between the total antioxidant activity of honey and the polyphenol content in honey. We performed an analysis of polyphenol content before the addition of propolis to honey and after the addition of propolis. These results are shown in Table 3.

Table 3. Results of polyphenol content in honey before the addition of propolis and after the addition of propolis to honey and the percentage increase in their concentration

No.	Sample mark	Honey samples	Honey samples + propolis	Percentage of polyphenol concentration increase
		mg/L GA	mg/L GA	%
1.	B1	50,66	56	5,34
2.	P2	38,33	56,33	18
3.	L3	35,66	46	10,34
4.	Š4	32,66	53	20,34
5.	Š5	68,33	78	9,67
6.	Š6	39,66	42,66	3
7.	L7	32	57,33	25,33
8.	Š8	43,66	55,33	11,67
9.	Š9	51	55,33	4,3333
10.	L10	29,33	52	22,67
11.	L11	37,66	52,66	15
12.	L12	41	47	6
13.	L13	38	42	4
14.	L14	42	51	9
15.	L15	31	43,66	12,66
16.	M16	38	59,66	21,66
17.	L17	31	43,60	12,6
18.	L18	36	46	10
19.	L19	41,66	51	9,34
20.	Š20	24	43	19
21.	L21	27	34	7
22.	P22	39,66	46	6,34

DISSCUSION

Honey is a natural food product that has many healing and therapeutic properties. The antioxidant activity of honey is an important parameter for assessing its quality. As honey is an important food product, it is a rich source of antioxidants for the body. The overall antioxidant protection of the body from the harmful effects of free radicals largely depends on the intake of antioxidants through the diet. When we take all this into consideration, the immunomodulatory effect of honey and propolis is very

significant. The total antioxidant activity of the analyzed honey samples before propolis addition and after propolis addition is shown in Figure 3.

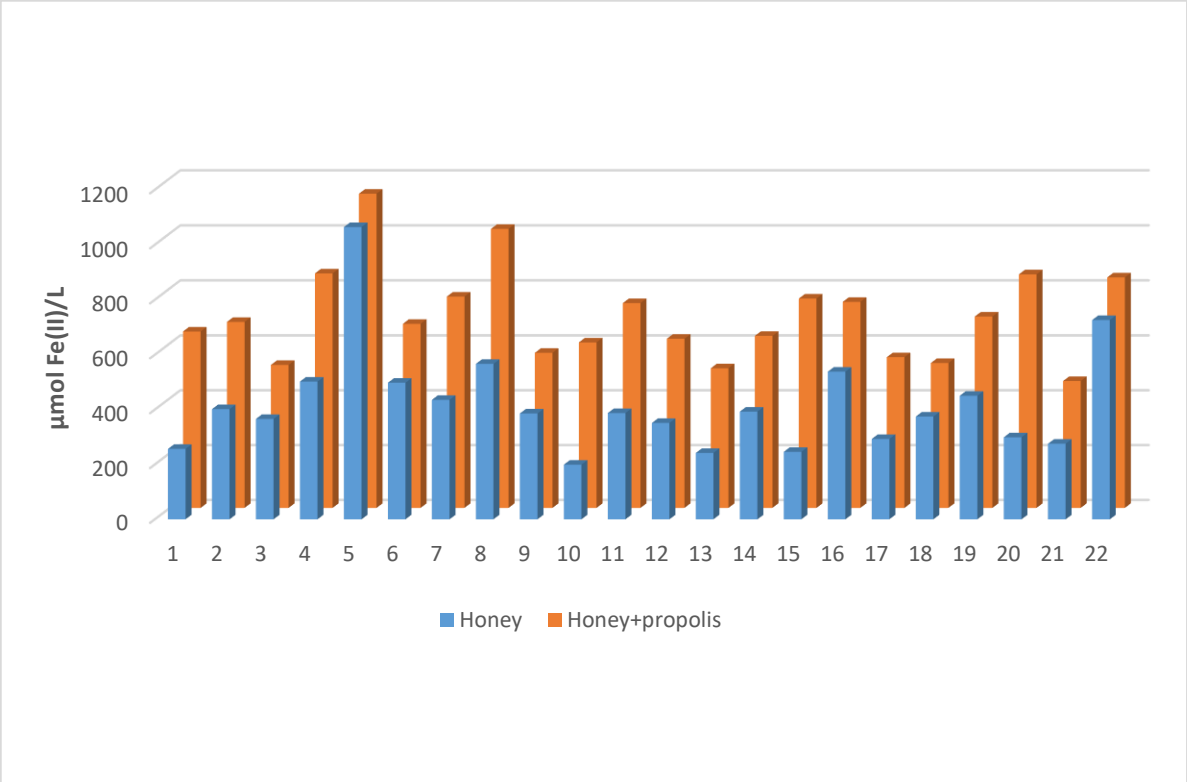

Figure 3. Influence of propolis addition on the total antioxidant activity of honey

Based on the obtained results, it is clear that honey itself is a rich source of antioxidants in the diet, but that the addition of propolis significantly increases its antioxidant power. The results showed that the richest in antioxidants is forest honey from the area of Bihać, Una-Sana Canton (Š5) and its antioxidant activity is 1064.37 µmol Fe (II) / L. The least antioxidants were contained in a commercial sample of honey from the area of Vlašić, Central Bosnia Canton, (L10) 199.37 µmol Fe (II) / L. Croatian scientists investigated the antioxidant activity of thirty samples of acacia honey and the obtained results showed values ranging from 6.95 to 142.43 µMFeII / L.(Al-Mamary M.et.al. 2002)

The results of A. Kesic (Kesić A. 2018; Kesić A. Zaimović I. et.al. 2018; Kesić A. Stipe Č. et.al. 2020) research showed a slightly larger range of values of antioxidant activity of acacia honey samples from BiH, which ranged from 4.7 to 893.82 µMFeII / L, while our research showed a range of 199.37 µmol Fe (II) / L to 1064.37 µmol Fe (II) / L, which we expected considering that the analyzed honey samples were mainly from mountainous areas. The only sample of acacia honey that we analyzed showed antioxidant activity in the amount of 256.87 µMFeII / L, which is consistent with the results of the above researchers.

The addition of propolis to honey significantly increased the antioxidant activity of all analyzed samples. After the addition of propolis, the highest antioxidant activity was again shown by a sample of forest honey from the area of Bihać, Una-Sana Canton (Š5) and its antioxidant activity is 1143.96 µmol Fe (II) / L. The lowest antioxidant activity after the addition of propolis was shown by the meadow sample of honey from Sanski Most, Una-Sana Canton, (L21), 462.71 µmol Fe (II) / L.

The average antioxidant activity of the analyzed honey samples before the addition of propolis was 420,72 µmol Fe (II) / L, and after the addition of propolis 700.51 µmol Fe (II) / L, which is shown in Figure 4.

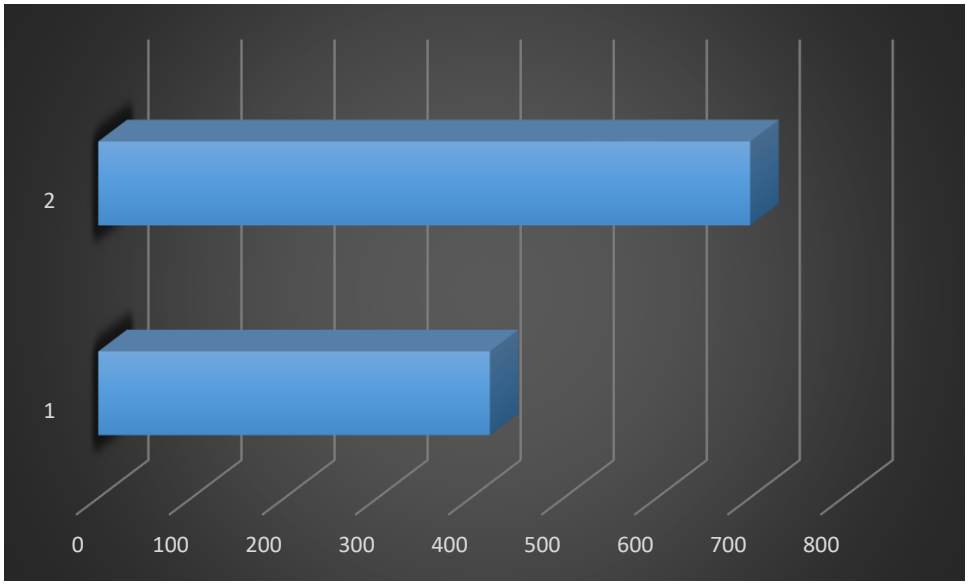

Figure 4. Average value of total antioxidant activity of analyzed honey samples without propolis addition (1) and after propolis addition (2)

The highest percentage change was in the antioxidant activity of meadow honey from the Tuzla area, Tuzla Canton (L15) in the amount of 210.16% and the commercial honey sample from the Vlašić area (L10) in the amount of 202.7%. If we take into account these results we can say that these samples of honey after the addition of propolis are a much richer source of antioxidants in the diet than before the addition of propolis. Antioxidant activity in all samples increased by an average of 82.65%. The percentage increase in antioxidant activity of the analyzed honey samples after the addition of propolis is shown in Figure 5.

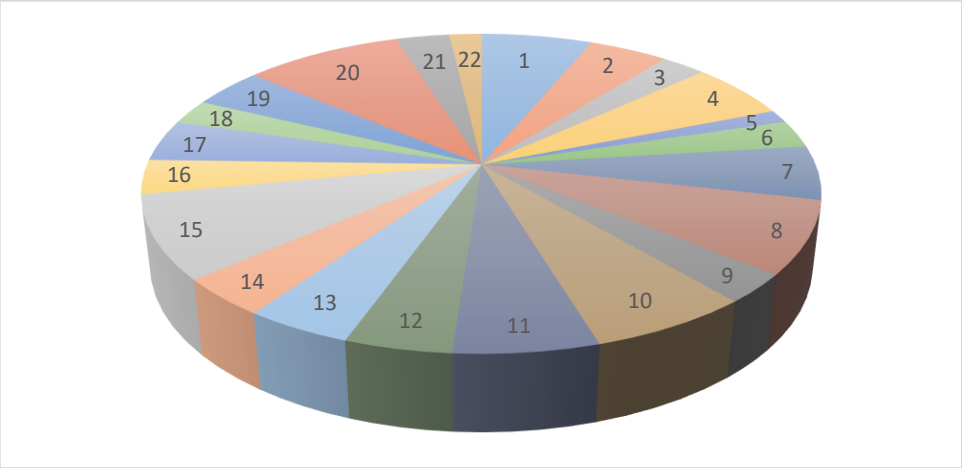

Figure 5. Percentage increase in total antioxidant activity of honey after propolis addition

The results of polyphenol content in analyzed samples of honey and honey with added propolis are shown in Figure 6. The content of polyphenols in analyzed samples of honey ranged from 24 mg / 100 g GA in a sample of forest honey from Sanski Most, Una-Sana Canton, (W20), to 68.33 mg / 100 g GA in forest honey from the area of Bihać, Una-Sana Canton (Š5). If we compare the results with the research of honey from Bosnia and Herzegovina that was realized within the doctoral dissertation of A. Kesić (Kesić A. 2018; Kesić A. Zaimović I. et.al. 2018; Kesić A. Stipe Č. et.al. 2020) in the period from 2005 to 2009, in which the polyphenol content ranged from 5.16 mg / 100g to 32.71 mg / 100g with our research we can see that the polyphenol content in our samples is slightly higher. Considering that these researches mainly included samples of mountain and forest honey, which on average show higher concentrations of polyphenols, we can explain this as a consequence of the influence of geographical and botanical origin. Compared to studies of honey from Turkey (Duran Ö. And Sibel S. 2017) which showed that the concentration of polyphenols in honey samples from these areas in the range of 57.59–261.71 mg GA / 100 g, our samples showed slightly lower concentration. This is also a consequence of geographical and botanical influence. The lowest concentration of polyphenols in honey samples after propolis addition was proven in the sample of meadow honey from Sanski Most, Una-Sana Canton (L21) in the amount of 34 mg / 100 g GA, and the highest in the sample of forest honey from Bihać, Una Sana Canton worth 78 mg / 100 g GA (Š5).

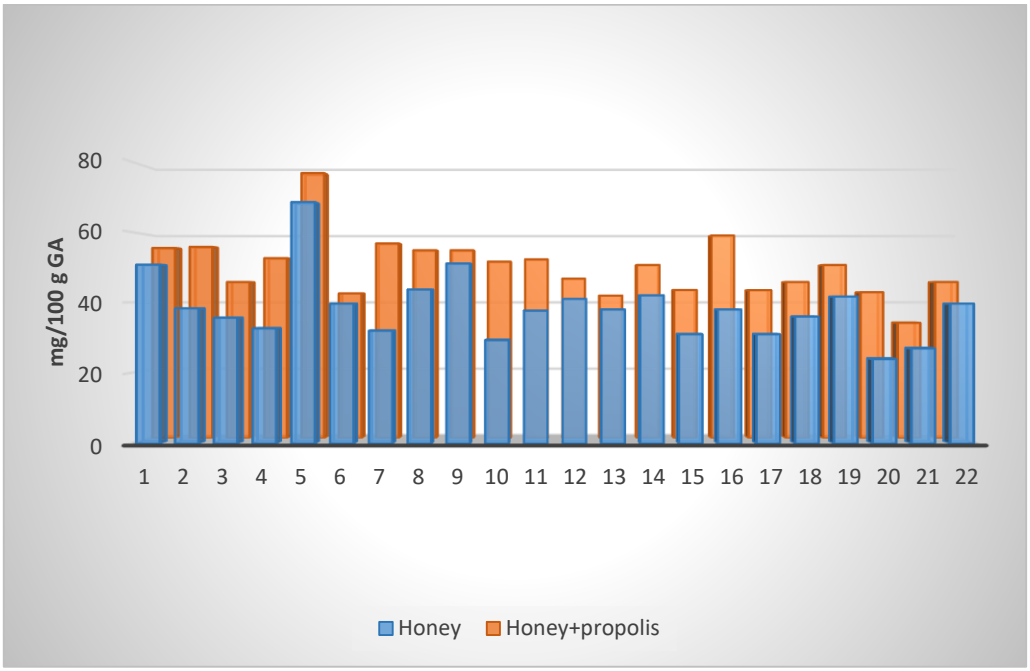

Figure 6. Influence of propolis addition on polyphenol content in honey

Based on the presented results, it is clear that after the addition of propolis in the analyzed honey samples there was a significant increase in the concentration of polyphenols by an average of 11.96%.

The average value of polyphenol concentration in the analyzed honey samples before the addition of propolis was 38.55 mg / 100 g GA and after the addition of propolis 50.53 mg / 100 g GA (Figure 7).

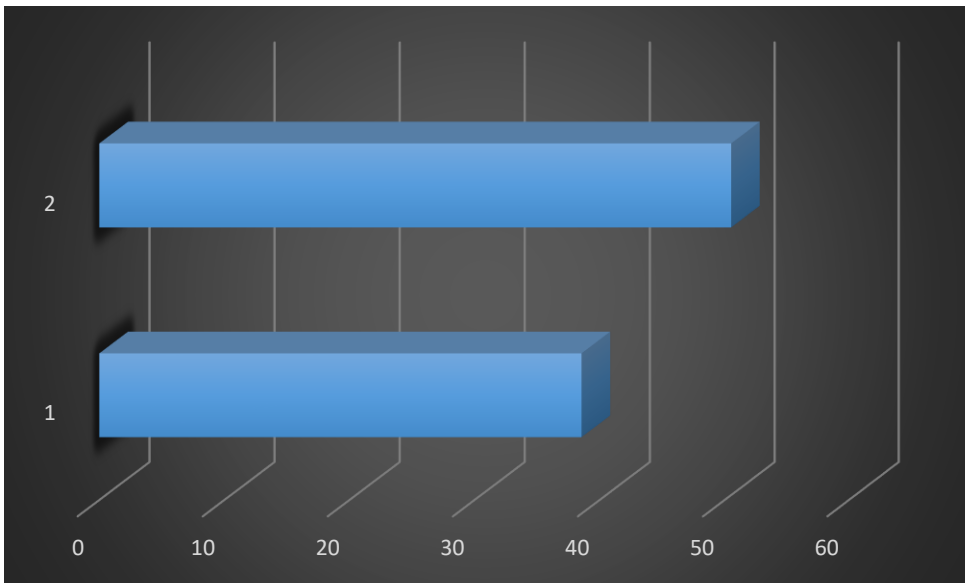

Figure 7. Average value of polyphenol content in analyzed honey samples without propolis addition (1) and after propolis addition (2)

If we analyze the percentage increase in polyphenol concentration in honey samples after propolis addition, we see that the largest increase occurred in samples of meadow honey from Bihać, Una-Sana Canton in the amount of 25.33% and meadow honey from Vlašić, Central Bosnia Canton, in amounting to 22.67%. These results are shown in Figure 8.

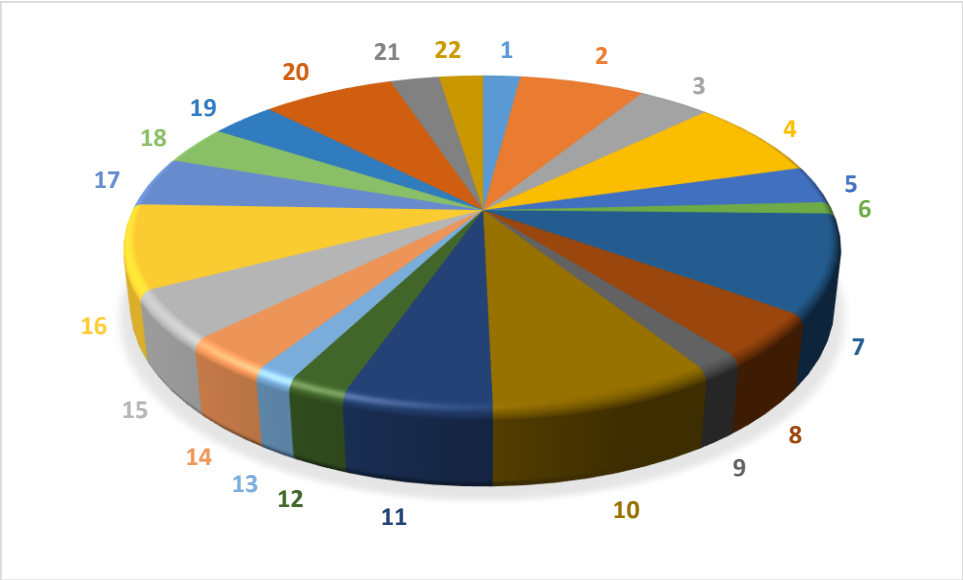

Figure 8. Percentage increase in total polyphenols in honey samples after propolis addition

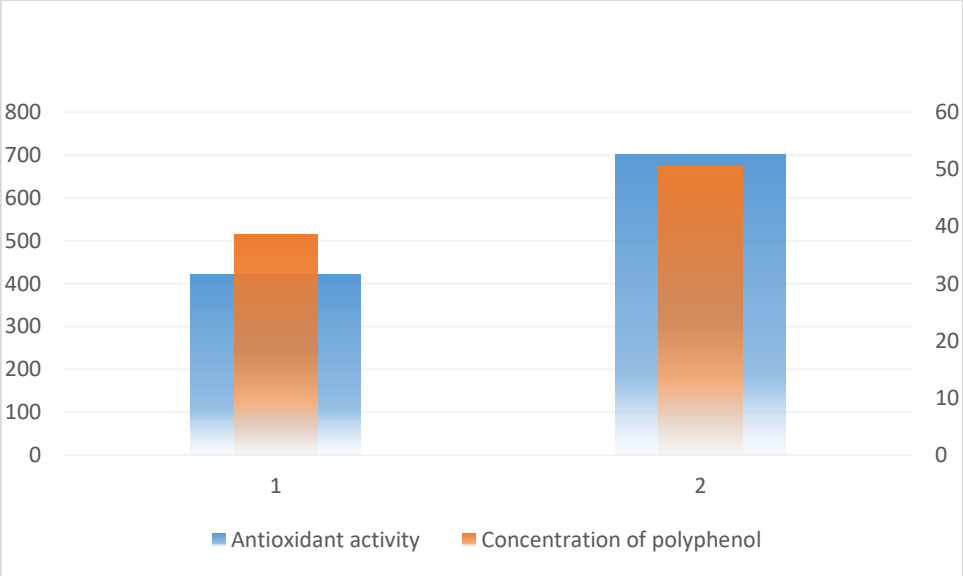

Figure 9. Correlation between total antioxidant activity and polyphenol concentration in honey samples before propolis addition (1) and after propolis addition (2)

If we compare the concentration of polyphenols and the total antioxidant activity in the analyzed samples of honey before the addition and after the addition of propolis, we can see that, increasing the total antioxidant activity increases the concentration of polyphenols. We can say that there is a significant correlation between these two parameters, which is shown in Figures 10 and 11.

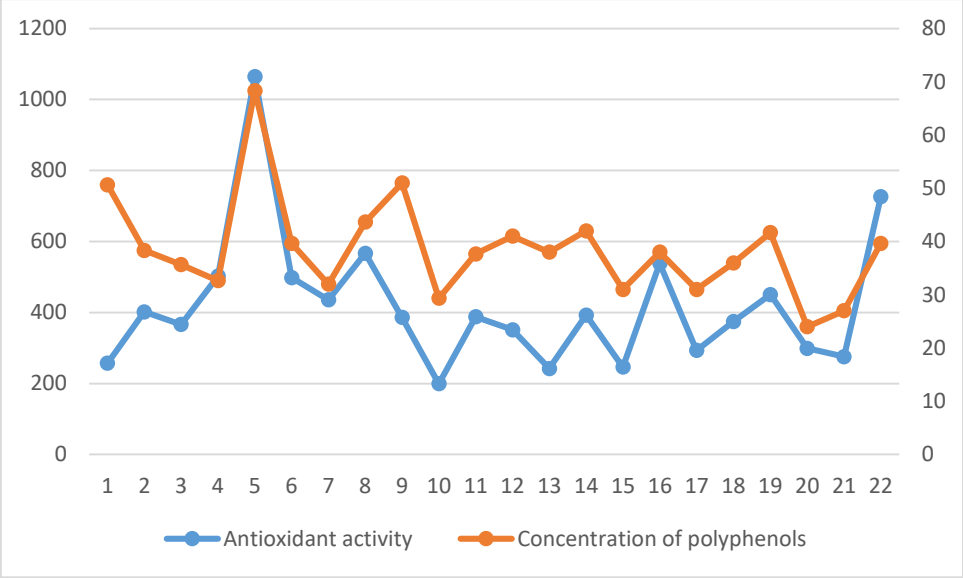

Figure 10. Correlation between total antioxidant activity and polyphenol content in analyzed honey samples

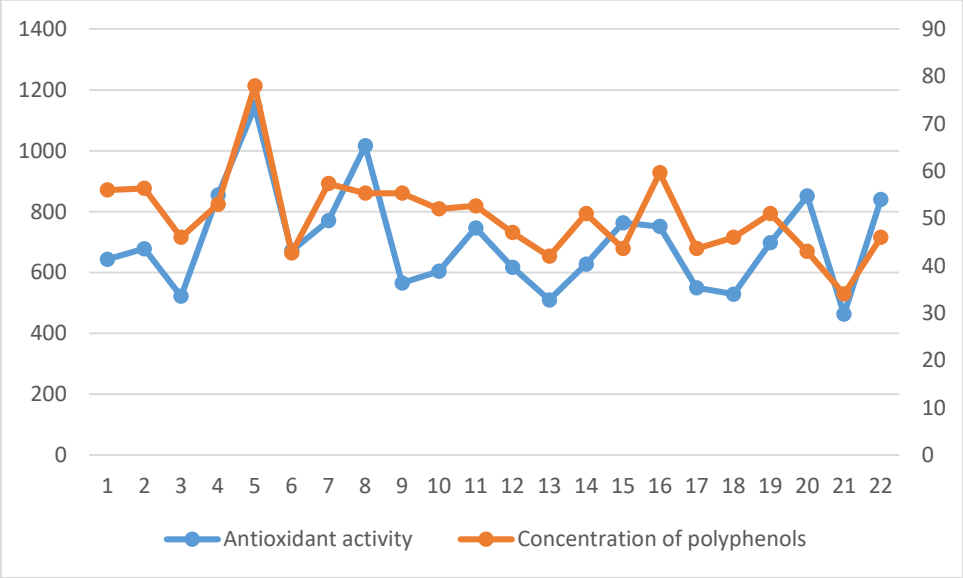

Figure 11. Correlation between total antioxidant activity and polyphenol content in analyzed honey samples after propolis addition

CONCLUSION

The immunomodulatory effect of honey and propolis is very significant. The antioxidant activity of honey is an important parameter for assessing its quality. Based on the obtained results, it is clear that honey itself is a rich source of antioxidants in the diet, but the addition of propolis significantly increases its antioxidant power. The addition of propolis to honey significantly increased the antioxidant activity of all analyzed samples. Based on the presented results, it is clear that after the addition of propolis in the analyzed honey samples there was a significant increase also in the concentration of polyphenols by an average of 11.96%.

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