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# ASSESSMENT OF THE INFLUENCE OF HEALTHY FOODS ON THE ANTIOXIDANT CAPACITY AND IMMUNE RESPONSE OF THE ORGANISM

**Aldina Kesić[1], Nadira Ibrišimović Mehmedinović, Almir Šestan, Amela Avdibašić[1], Mirza Ibrišimović[2] and Mirela Grebić[3]**

[1]University in Tuzla, Faculty of Natural Science and Mathematics, Department of Chemistry
Urfeta Vejzagića 4, 75 000, Tuzla, Bosnia and Herzegovina

[2]Sarajevo School of Science and technology, Sarajevo Medical School,
Hrasnička cesta 3a Sarajevo, Bosnia and Herzegovina

[3]University in Tuzla, Faculty of Natural Science and Mathematics, Department of Biology
Urfeta Vejzagića 4, 75 000, Tuzla, Bosnia and Herzegovina

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## ABSTRACT

The analyzes carried out in this research were performed on meadow and mountain honey samples from Bosnia and Herzegovina. The geographical origin of the analyzed honey samples is the area of Mount Majevica in the Tuzla Canton and the area of Livno, Canton 10, Sarajevo, Sarajevo Canton and Vlašić, Central Bosnia Canton. Honey samples were collected from 8 different localities. During the analysis, all honey samples were up to one year old. With the aim of testing the antioxidant capacity of honey, the polyphenol content and total antioxidant capacity were determined. The total antioxidant capacity of honey was determined by the indirect FRAP (Ferric Reducing Antioxidant Power) method, and the polyphenol content by the Folin-Ciocalteu method. The results showed that there is a significant correlation between the antioxidant capacity of the analyzed honey samples and the content of polyphenols in them. In order to compare the antioxidant activity and polyphenol content of pure honey samples (without additives) with honey mixtures (additions: ginger, garlic, sweet wormwood, propolis), mixtures were prepared for all samples, namely 5 mixtures of honey and ginger; 5 mixtures of honey and garlic, 5 mixtures of honey and sweet wormwood and 3 mixtures of honey and propolis. Analyzing the results, a comparative analysis of the polyphenol content and total antioxidant capacity in honey samples before and after the addition of wormwood, ginger, garlic and propolis was performed, and the influence of the mixture's standing time on

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the tested parameters was also examined. The results of these analyzes showed that additives to honey significantly affect its antioxidant activity and polyphenol content, and that standing the prepared mixtures can lead to an additional increase or decrease of the tested parameters.

**KEYWORDS:** honey, ginger, sweet wormwood, garlic, antioxidants, poliphenols, propolis

## 1. INTRODUCTION

Contemporary trends in society bring with them great benefits for the human population, but they are certainly accompanied by a fast and unhealthy lifestyle and diet of people. Human awareness of the importance of a healthy diet is increasing every day, which is why the study of natural food products and their benefits for health is extremely important. Honey is a natural food product that is recognizable by the content of many medicinal components wich are important for the normal growth and development of the organism. It is also recognizable by its high content of polyphenolic compounds, which have one of the highest in vitro antioxidant activities among natural products. The concentration of polyphenol compounds and total antioxidants in honey depends on several parameters. Particularly important indicators are the type of honey and the geographical location where the honey was produced, that is, the composition of the flora that contributed to the creation of a certain type of honey, as well as the time of maturation of the honey. Numerous studies have proven a wide range of antibacterial, antiradical and anticancer effects of bee products, as well as a supportive effect in the prevention and treatment of many diseases. The largest number of these studies were conducted on honey and propolis precisely because of their greatest application in nutrition. The proven immunomodulatory properties that honey and propolis possess are a consequence of the content of a large number of polyphenols in their composition, which are also responsible for antioxidant activity. The topic of this paper is precisely related to the study of the antioxidant potential of honey and mixtures of honey and ginger. The research used meadow honey collected from 5 different locations in the Tuzla Canton. The research carried out in this work was with honey samples from Bosnia and Herzegovina. They cover the north-eastern part of Bosnia and Herzegovina, i.e. the area of the Tuzla Canton, Sarajevo Canton, Canton 10 and Central Bosnan Canton. With the aim of testing the antioxidant potential of honey, the polyphenol content and antioxidant capacity were determined. The total antioxidant capacity of honey was determined by the indirect FRAP (Ferric Reducing Antioxidant Power) method. In this research, the content of polyphenols was determined using the Folin-Ciocalteu method. A significant correlation was demonstrated in the total antioxidant activity and polyphenol content in honey and the tested mixtures. The sample with the highest antioxidant capacity also shows the highest polyphenol content. In order to adequately monitor and analyze the antioxidant activity and polyphenol content of pure samples of honey and a mixture of honey and ginger, time intervals of fifteen and thirty days were determined, in which analyzes of these parameters were performed. In this way, it was possible to monitor the influence of the standing time of the prepared mixtures on the examined

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parameters. The analysis determined that the sample from commercial use had the most antioxidants in the mixture with sweet wormwood, and the sample from Kalesija had the most polyphenol content. The sample from Kalesija municipality has the most antioxidants in the mixture with ginger. The polyphenol content is the highest in the same sample. The sample from commercial use has the most antioxidants in the mixture with ginger after 30 days of standing, and the sample from Kalesija has the most polyphenols.

## 2. LITERATURE REVIEW

Numerous studies that studied the effect of honey on the immune system proved its anti-inflammatory effect, i.e. effect of certain components of honey on production of immune cells.[1] According to research by Ahmad et al. from 2009, it was proven that thrombin-induced oxidative respiratory shock in human neutrophils and peritoneal macrophages is inhibited by coincubation with different types of honey. [1][2] It has been proven that chronic inflammation is also associated with the development of cancer, due to damage to the recovery mechanisms themselves caused by inflammatory processes. Various pro-inflammatory enzymes and cytokinins induce inflammatory processes. The enzyme cyclooxygenase 2 (COX-2) is involved in the process of carcinogenesis, which is proven by its increased presence in various malignant conditions. COX-2 acts as a catalyst that metabolizes arachidonic acid to prostaglandin, which is an integral reaction between carcinogenesis and the inflammatory process. [1][3][4].

The anti-inflammatory properties of honey are attributed to the phenolic compounds and flavanoids present in honey. In several conducted studies, it has been proven that these compounds have a suppressive effect on COX-2 activity and/or induce the synthesis of nitric oxide (iNOS), which results in an anti-inflammatory response. [1][4][5][6] It has also been proven that flavanoid and phenolic extracts from Malaysian honey have strong anti-inflammatory and cytoprotective effects. [1][7].

Honey is an extraordinary product from hives that bees produce from nectar and other sugary substances originating from various plants. Antioxidants in honey can be enzymatic and non-enzymatic, such as flavonoids, phenols, tocopherol, ascorbic acid, caroteonides, etc. Many phytochemicals present in honey serve as sources of non-enzymatic antioxidants, and their amount depends primarily on the botanical and geographical origin of the honey. [8][9].

Ginger (Zingiber officinale) is a perennial tuberous plant from the lily family known for its roots. It has been used for thousands of years as medicine, spice, food and for cosmetic purposes. This plant is native to China, but today it is cultivated in many countries, including Asia, India, Africa and the Caribbean islands.[10][11] Ginger is rich in essential oils, vitamin B, potassium, manganese, copper and magnesium. Its root contains numerous antioxidants such as terpenoids, alkaloids, polyphenols, beta-carotene and ascorbic acid.[12]

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Garlic (Allium sativum) is a plant with a very strong taste, known for its pronounced medicinal properties. Garlic contains a large number of bioactive organic compounds, the most abundant of which are carbohydrates. Garlic contains minerals wich are very important for the human body, especially potassium and sulfur, and it is also rich in vitamins (B1, B2, C, E, K). Its main ingredient is allicin, which has antibacterial, antiviral and antifungal effects, and also acts as a strong antioxidant. [11][12] Sweet wormwood or fragrant wormwood (Artemisia annua) is an annual herbaceous plant from the genus Artemisia. Artemisinin, which is produced in the plant, was first isolated in 1971 and has long been successfully used in traditional Chinese medicine as an anti-malarial agent and to strengthen immunity. [13][14][15]

Propolis is collected by bees from plant resinous secretions such as mucus, gummy substances, resins, leaf buds, from different plant species such as pine, beech, poplar, and numerous conifers, and after collection, they mix it with saliva and enzymatic secretions and process it in propolis. Its chemical composition contains over several hundred different substances, the most abundant substances in the composition of propolis are polyphenols (flavonoids, phenolic acids and esters), which gives it a strong antioxidant, anticancer, antiradical, antimicrobial and general immunostimulating effect. The profile of polyphenols depends directly on the botanical origin, i.e. plant source since polyphenols are secondary metabolites of plants.

Free radicals are atoms or molecules that contain one or more unpaired electrons in their outer shell. Free radicals are formed by homolytic cleavage of a covalent bond, with each electron remaining attached to the neighboring atom.[16] They are a permanent product of cellular metabolism and are very reactive. Free radical reactions are chain reactions. The causes of the formation of free radicals in the body can be endogenous and exogenous. Reactions in which free radicals are formed are redox reactions. The most important free radicals are reactive oxygen and nitrogen species (ROS and RNS).[17] Most of the oxygen radicals are generated in the mitochondria, during the creation of ATP, through a series of processes of oxidative phosphorylation, reduction of O2 to H2O. In this case, O2 should receive 4 electrons, and it may happen that O2 receives only 1 or 2 electrons, resulting in O2- or H2O2 production, which can change to another form of free radical. Lipid peroxidation is the attack of free radicals on the double bonds of fatty acids. (Figure 1.)

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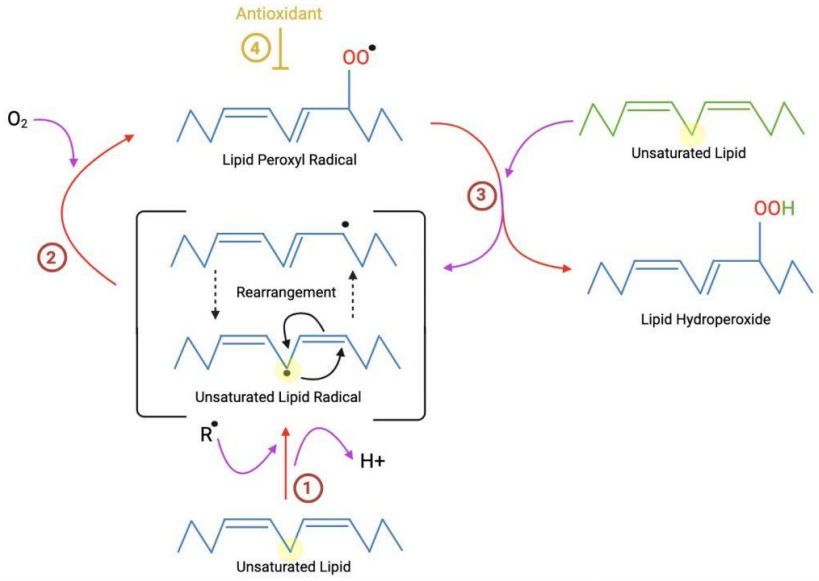


**Figure 1. Lipid peroxidation**

The state that occurs when the balance of the amount of ROS/RNS produced is disturbed is a state of oxidative stress. It is caused by the accumulation of damaged macromolecules, an increase in the amount of prooxidants and a lack of antioxidants. In a state of oxidative stress, there is an excessive formation of free radicals and their accumulation in the body, which results in the failure of the organism defense mechanism. Although there is cellular antioxidant protection in the body, with aging, the production of antioxidants decreases significantly. Because of that it is necessary to consume more and more antioxidants with food over time. [16][18]

Antioxidants are a diverse group of molecules that, if present in low concentrations compared to the concentrations of the oxidizing substrate, significantly retain or prevent the oxidation of that substrate, control the relationship between reducing or oxidizing states in a biological system. Endogenous antioxidants are produced in the cell, and exogenous antioxidants are most often introduced into the body through food or in the form of vitamin and similar supplements.

Polyphenols presented in honey, such as flavonoids and phenolic acids, can act as natural antioxidants.[19] Polyphenols come in different forms and concentrations, depending on the food, and the recommended daily intake is 3-70 mg. The biggest source of polyphenols are plants, and certainly also honey, because bees transfer these components from plants. Polyphenols, as secondary metabolites of various plant species, form a large group of chemical compounds that can be classified into several groups based on their structure and chemical properties. The largest groups of polyphenols are: flavonoids, lignans,

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phenolic acids, stilbenes and other phenols, which are divided into smaller subgroups. Scientific research has proven the positive biological effects of polyphenols: anti-inflammatory, antimicrobial, antifungal, diuretic, antihepatotoxic, antihypertensive, antiarrhythmic, anticoagulant, spasmolytic, cardiotonic, antiallergic, antiulcer, analgesic, antimalarial, hypoglycemic and antioxidant effects. Dietary polyphenols have numerous properties that are extremely beneficial for human health.[19][20][21] Polyphenols have a positive effect in the treatment of cardiovascular diseases, cancer, osteoporosis, diabetes. In a state of oxidative stress, there is excessive formation of free radicals and their accumulation in the body, which results in the failure of the defense mechanism. Although there is cellular antioxidant protection in the body, the production of antioxidants decreases significantly with aging. That is why it is necessary to consume more and more antioxidants with food over time.

The aim of this research was to examine the significant antioxidant activity of pure honey and the mixture of honey with sweet wormwood, ginger, garlic and propolis, and to analyze the influence of standing time on the antioxidant activity and polyphenol content in pure honey and in the prepared mixtures. One of the goals was also to examine the medicinal properties of natural food products from Bosnia and Herzegovina. A detailed analysis of the effect of additives on the antioxidant activity of honey and polyphenol content was performed, as well as an assessment of the effect of consumption of these foods on overall human immunity. Based on the obtained results, the bioavailability of antioxidants and polyphenols found in these food products was also assessed. It is especially important to point out that this research emphasizes the importance of natural resources of Bosnia and Herzegovina and that natural food products originating from Bosnia and Herzegovina are promoted in an extremely important area such as healthy nutrition. Given that geographical, botanical, ecological and all other environmental influences are directly reflected on the chemical composition of honey, and thus directly affect its antioxidant activity, it is extremely important to compare these characteristics of honey and honey with the above additives with similar research in more developed countries of the European Union . This is significant, especially from the aspect of food quality control, which is one of the priority tasks of the integration of Bosnia and Herzegovina into the European Union, which requires a high-quality and reliable product on its market.[19][20]

## 3. Material and Methods

Determination of antioxidant capacity and polyphenol content of meadow and mountain honey was performed on honey samples from the following locations: Priboj, Majevica, Kalesija, Kladanj, commercial sample from free sale, Livno, Sarajevo and Vlašić. During the analysis of total polyphenols and antioxidant capacity of different honey samples, a comparison was made between pure honey samples and honey samples to which sweet wormwood, ginger, garlic and propolis were added.

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3.1. Determination of the concentration of total polyphenols by the Folin-Ciocalteu (FC) method

The content of total polyphenols in honey samples was determined using the Folin-Ciocalteu (FC) method.[22] The FC method is one of the oldest indirect methods that is sensitive to phenolic and polyphenolic compounds. From the standard solution (2g/L gallic acid) by dilution, 100 ml solutions of the mentioned acid with the following concentrations were prepared in flasks: 8.0 mg GA/L, 20 mg GA/L, 32 mg GA/L, 40 mgGA/L, 80 mg GA/L, 120 mg GA/L, 250 mg GA/L and 500 mg GA/L. Absorbance is measured against a sweet analogue of honey (40% fructose, 30% glucose, 10% maltose in 20% distilled water) within 20 minutes at a wavelength of 750 nm.[23] The concentration of total polyphenols is calculated using the expression obtained from the calibrated direction.

$$X = \frac{Y-0,003}{0,003}$$

The results were recalculated according to the calibration curve for gallic acid:

$$y=0.003x + 0.003$$

where y is the absorbance at 765 nm and x is the concentration of gallic acid expressed in mg GAE/L; $R^2$=0,9983.

**3.2. Determination of antioxidant capacity by the FRAP method**

The determination of the antioxidant capacity of the tested honey samples was carried out using the method of the reducing property of iron.[23] Ten $FeSO_4$ x $7H_2O$ solutions with concentrations from 0.05 to 1.6 mM were prepared to construct the calibration curve. The concentration of antioxidants in the measured sample based on the obtained values is calculated according to the expression:

$$X = \frac{Y - 0,0295}{2,402}$$

Where: X – antioxidant concentration, Y – measured average absorbance for the sample
The absorbance of the sample is measured in relation to the blank sample. The results are expressed in μmol FeII/L 10% honey solution. FRAP values were calculated according to the calibration curve for $FeSO_4 \times 7H_2O$:

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$$y=2,402x + 0,0295$$

where y is the absorbance at 593 nm and x is the concentration of $FeSO_4 \times 7H_2O$ expressed in mmol FeII/L; $R_2$=0.9994

## 4. RESULTS AND DISCUSSION

The results of the analyzes provided a significant picture of the possible influence of honey and honey with additives on the total antioxidant potential of the organism and on its immune response. Given that the results showed that honey is rich in polyphenolic and other antioxidants, we can say that consuming honey, and honey with tested additives, can significantly improve human health, and in this way various diseases of the body can be prevented. The total content of antioxidants on the first day of analysis in pure honey samples from Tuzla Canton and in prepared honey mixtures with the addition of sweet wormwood, ginger and garlic are shown in Figure 1.

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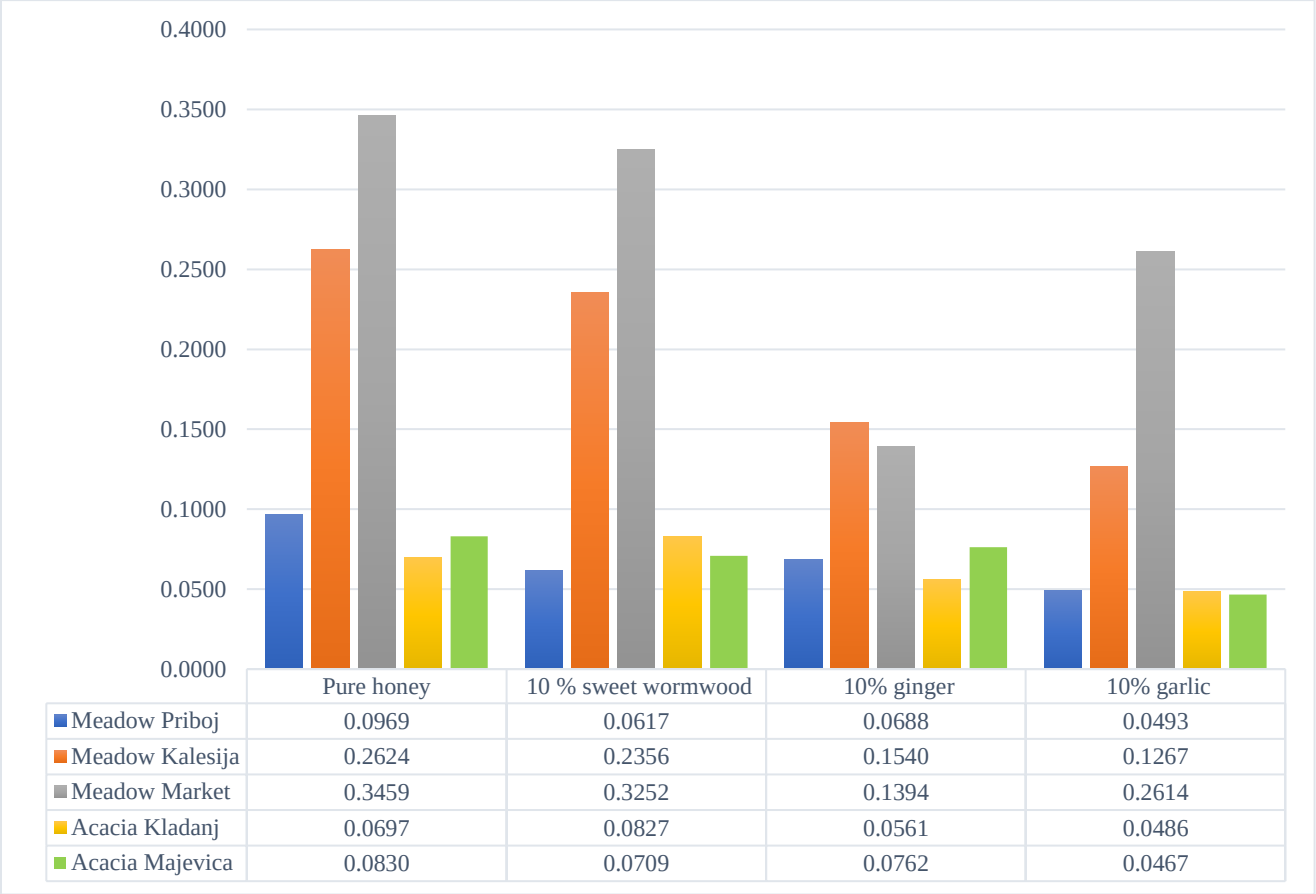


| | Pure honey | 10 % sweet wormwood | 10% ginger | 10% garlic |
|---|---|---|---|---|
| Meadow Priboj | 0.0969 | 0.0617 | 0.0688 | 0.0493 |
| Meadow Kalesija | 0.2624 | 0.2356 | 0.1540 | 0.1267 |
| Meadow Market | 0.3459 | 0.3252 | 0.1394 | 0.2614 |
| Acacia Kladanj | 0.0697 | 0.0827 | 0.0561 | 0.0486 |
| Acacia Majevica | 0.0830 | 0.0709 | 0.0762 | 0.0467 |

**Figure 2. Total antioxidants in honey and prepared mixtures – first day**

We can conclude that on the first day of analysis of the collected samples, the value of total antioxidants decreases in the mixtures compared to the pure sample of honey without additives, except for the sample of acacia honey from the Kladanj area, where the value of antioxidants is the highest in the mixture with sweet wormwood. The samples in the mixture with garlic show the lowest value of antioxidants. Except for the sample of commercial meadow honey where the lowest value of antioxidants is in the mixture with ginger. We can say that the addition of these components to honey leads to a slight decrease in antioxidant activity, because complete unification has not yet occurred and active antioxidants have not been fully released into the mixture.

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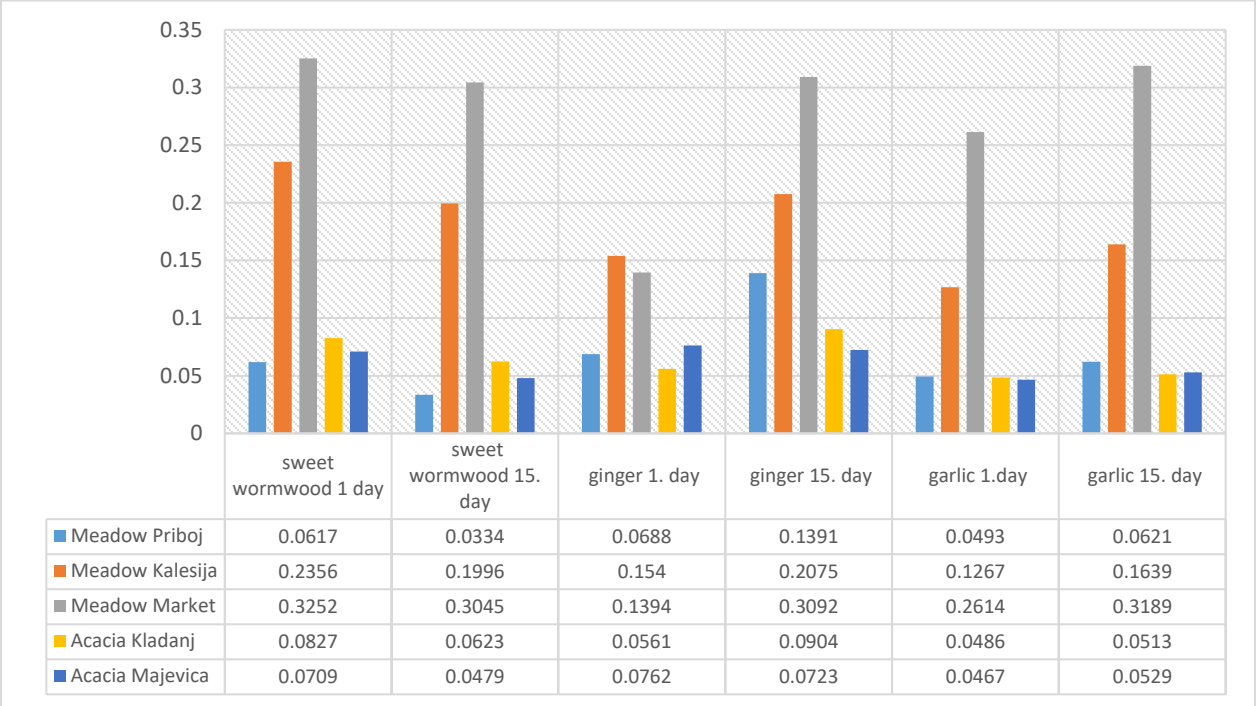


| | sweet wormwood 1 day | sweet wormwood 15. day | ginger 1. day | ginger 15. day | garlic 1.day | garlic 15. day |
|---|---|---|---|---|---|---|
| Meadow Priboj | 0.0617 | 0.0334 | 0.0688 | 0.1391 | 0.0493 | 0.0621 |
| Meadow Kalesija | 0.2356 | 0.1996 | 0.154 | 0.2075 | 0.1267 | 0.1639 |
| Meadow Market | 0.3252 | 0.3045 | 0.1394 | 0.3092 | 0.2614 | 0.3189 |
| Acacia Kladanj | 0.0827 | 0.0623 | 0.0561 | 0.0904 | 0.0486 | 0.0513 |
| Acacia Majevica | 0.0709 | 0.0479 | 0.0762 | 0.0723 | 0.0467 | 0.0529 |

**Figure 3. Total antioxidants in honey and prepared mixtures – first day and fifteenth day**

After 15 days, the value of total antioxidants in the mixtures changes, which can be seen in Figure 3. We can conclude that after 15 days of standing the mixture of honey with additives (sweet wormwood, ginger, garlic) the antioxidant value increases in almost all mixtures, which is proof that standing causes the release of antioxidant active substances in honey.

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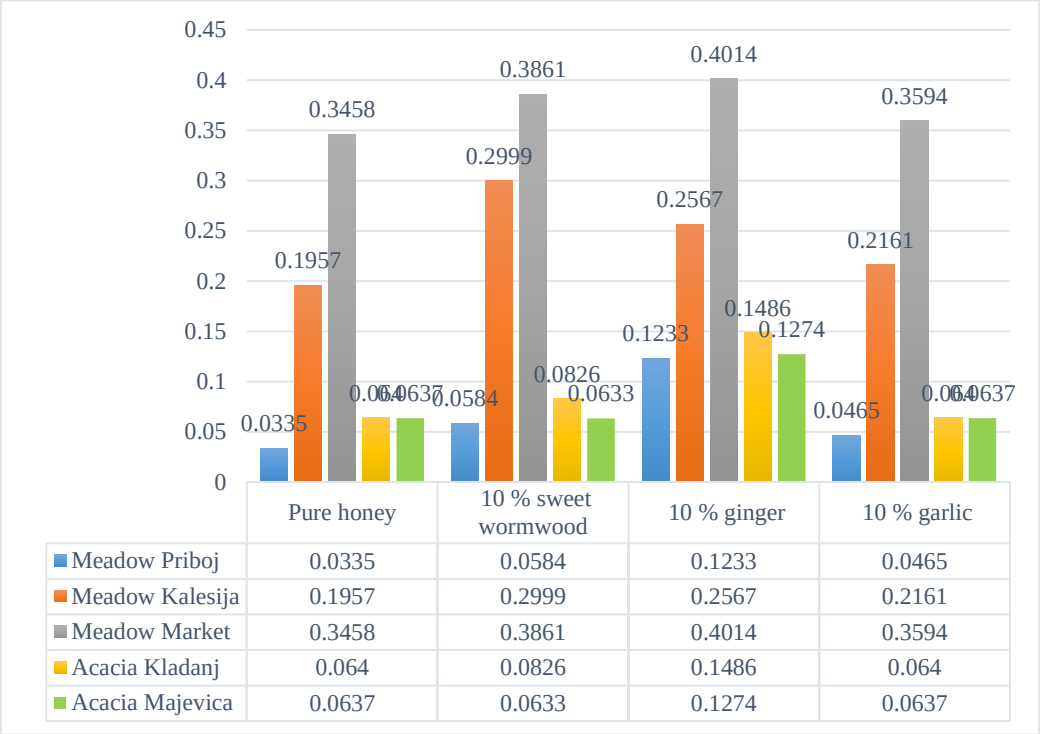


| | Pure honey | 10 % sweet wormwood | 10 % ginger | 10 % garlic |
|---|---|---|---|---|
| Meadow Priboj | 0.0335 | 0.0584 | 0.1233 | 0.0465 |
| Meadow Kalesija | 0.1957 | 0.2999 | 0.2567 | 0.2161 |
| Meadow Market | 0.3458 | 0.3861 | 0.4014 | 0.3594 |
| Acacia Kladanj | 0.064 | 0.0826 | 0.1486 | 0.064 |
| Acacia Majevica | 0.0637 | 0.0633 | 0.1274 | 0.0637 |

**Figure 4. Total antioxidants in honey and prepared mixtures – thirty days**

After a month, it can be seen that the concentration of antioxidants has increased in all the mixtures, which speaks in favor of the above statement.

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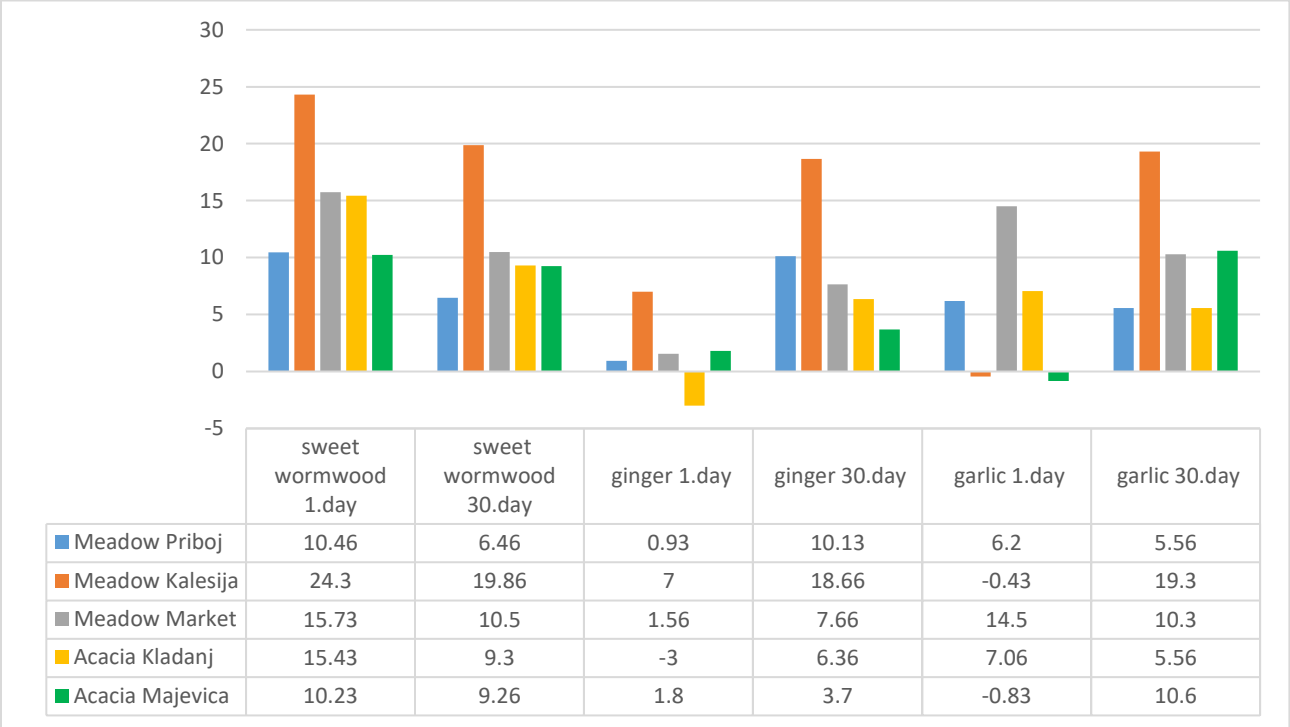


| | sweet wormwood 1.day | sweet wormwood 30.day | ginger 1.day | ginger 30.day | garlic 1.day | garlic 30.day |
|---|---|---|---|---|---|---|
| Meadow Priboj | 10.46 | 6.46 | 0.93 | 10.13 | 6.2 | 5.56 |
| Meadow Kalesija | 24.3 | 19.86 | 7 | 18.66 | -0.43 | 19.3 |
| Meadow Market | 15.73 | 10.5 | 1.56 | 7.66 | 14.5 | 10.3 |
| Acacia Kladanj | 15.43 | 9.3 | -3 | 6.36 | 7.06 | 5.56 |
| Acacia Majevica | 10.23 | 9.26 | 1.8 | 3.7 | -0.83 | 10.6 |

**Figure 5. Total poliphenols in honey and prepared mixtures – first and thirty dav**

Analysis of pure honey samples confirmed a significant concentration of total polyphenols in honey. After 30 days and re-analyzing, an increase in polyphenol content was confirmed. The sample from the Kalesija area shows the highest concentration of polyphenols in all mixtures.

In the samples from Livno, Sarajevo and Vlašić, the total antioxidant activity and polyphenol content were analyzed in pure samples and after the addition of propolis. The results of determining the total antioxidant activity before and after the addition of propolis alcoholic extract are shown in Figure 6.

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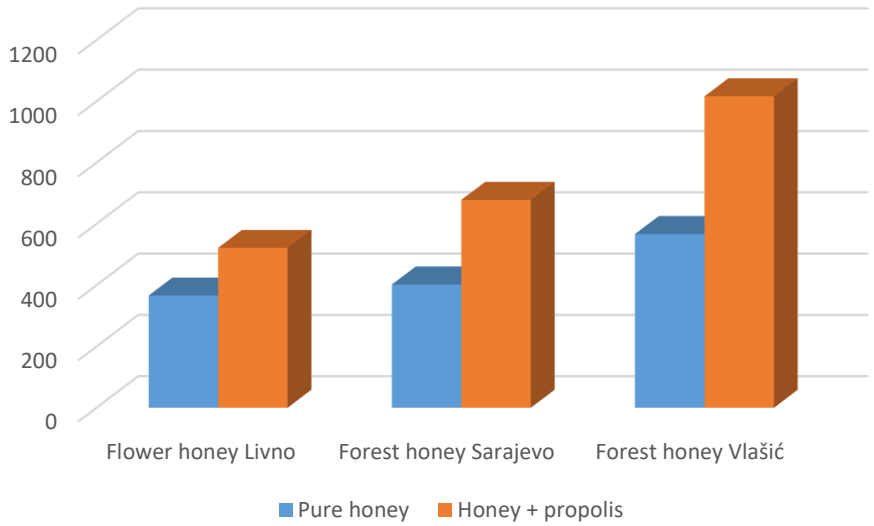


**Figure 6. Total antioxidants in pure honey and mixture honey and propolis in μmol Fe(II)/L**

Based on the obtained results, we can conclude that the addition of propolis significantly increases the antioxidant activity of the resulting mixture, up to 79%. These results indicate a significant improvement in the bioavailability of the present antioxidants and the significant benefits of consuming such mixtures in order to improve the body's immune response. Figure 7 shows the content of total polyphenols in pure honey samples and in mixtures of honey and alcoholic extract of propolis.

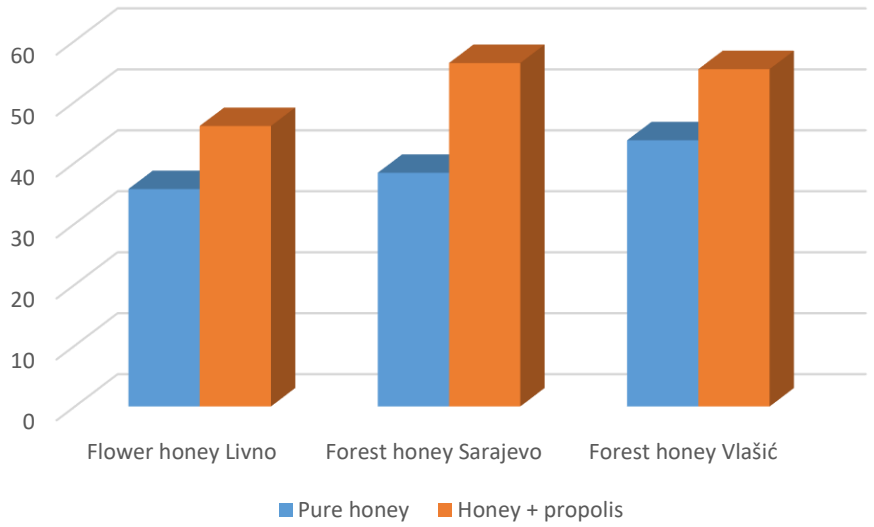


**Figure 7. Total poliphenols in pure honey and mixture honey and propolis in mg/kg**

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Based on the obtained results, it is clear that the content of polyphenols significantly correlates with the content of total antioxidants, both in pure honey samples and in prepared mixtures. The increase in the content of total polyphenols reaches 46% in the sample of honey from the Sarajevo area.

## 5. ACKNOWLEDGMENTS

Based on the obtained results, we can conclude that honey from all over Bosnia and Herzegovina, thanks to its rich botanical and geographical origin, represents a rich source of antioxidants and polyphenols in the diet, and by consuming it, we can significantly improve the body's immune response. Additions to honey significantly affect its antioxidant capacity, and by preparing different mixtures, we can introduce different bioactive substances into the body, which will be a source and support for our body to create an immune response against various diseases.

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## AUTHOR PROFILE

**Aldina Kesić** is full professor at Faculty of Natural Science and Mathematics, at University in Tuzla, Bosnia and Herzegovina. Aldina Kesić is author of 42 scientific publications and seven books and book chapters. She was a mentor for the preparation of 6 master's theses. She is the head of the chemistry department and the president of the Tuzla Canton Association of Chemists. Selected publications:

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**Nadira Ibrišimović Mehmedinović** is full professor at Department of Chemistry, Faculty of Natural Sciences and Mathematics, University of Tuzla, Tuzla, Bosnia and Herzegovina. Nadira Ibrišimović Mehmedinović is author of 23 scientific publications and four books, including two patents: 1. Ibrišimović, N., Pittner, F., Ibrišimović, M., Barth, M., Bohrn, U. (2009). Device comprising a polymer layer and a reflecting layer. EP2278300 A1, Europe and USA.

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**Almir Šestan** is associate professor at Department of Chemistry, Faculty of Natural Sciences and Mathematics, University of Tuzla, Tuzla, Bosnia and Herzegovina. Almir Šestan is author of 28 scientific publications. His research area is in general and inorganic Chemistry.

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**Mirza Ibrišimović** is associate professor at Sarajevo Medical School, at University Sarajevo School of Science and Technology in Sarajevo, Bosnia and Herzegovina, and also works as embryologist and cytoscreener-supervisor in Gynecology Polyclinc “Korak do života” in Tuzla, Bosnia and Herzegovina. Mirza Ibrišimović is author of 22 scientific publications and four books, including one patent: Patent: Ibrišimović, N., Pittner, F., Ibrišimović, M., Barth, M., Bohrn, U. (2009). Device comprising a polymer layer and a reflecting layer. EP2278300 A1, Europe and USA. Selected publications:

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Mirela Grebić is master student on the Faculty of Natural Science and Mathematics, Biology Depatment, University in Tuzla, Bosnia and Herzegovina. Her research area is in field of ecology and natural products. She is a participant in two scientific research projects that are being implemented at the University of Tuzla..