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OPTICAL ACTIVITY, TOTAL PHENOLIC CONTENT AND COLOR INTENSITY OF NECTARIAN HONEY AND HONEYDEW

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ABSTRACT: *Honey is an extraordinary product from beehives that produce bees from nectar and other sugary substances originating from different plants.[43] Worldwide honey is considered to be part of traditional medicine. [44,45,46] Nectar honey bees are made from nectar, a sweet solution secreted by the plant glands of nectarine. They can be monofloral and polyfloral. Honeydew most often originates from coniferous (fir, spruce, pine, larch) and deciduous (oak, beech, linden) trees. In this study, the analysis of optical activity, total polyphenol content, antioxidant activity and color of nectar honey and honeydew species was performed. The results show that due to the higher content of fructose, nectar honey rotates the plane of polarized light to the left, it shows negative optical activity, whereas because of the higher content of oligosaccharides, mainly melecitosis and erlose, the honeydew turns light to the right, ie. shows positive optical activity. These studies confirmed the high correlation coefficient between antioxidant activity of honey, color and total polyphenols content. The analyzed honeydew honey samples have on average a higher content of polyphenols, and an overall antioxidant capacity than nectar honey samples.*
KEYWORDS: honey, phenolic content, color, optical activity, nectarian, honeydew

INTRODUCTION

Epidemiological studies have shown that nutrition plays an important role in the prevention of chronic diseases: heart disease, diabetes, Alzheimer's disease, etc. Consumption of fruits and vegetables, as well as natural antioxidant-rich foods such as honey, has been associated with a reduction in the risk of chronic diseases. Daily consumption of honey has an important impact on optimal health. Certain studies have shown that a complex mixture of phytochemicals in natural food products provides better health protection than single phytochemicals through a combination of additives and / or synergistic effects. About 5,000 phytochemicals present in plants have been identified and a large percentage are still unknown. Different plants have different composition of phytochemicals that have different protective functions. Honey produced by bees using nectar and other parts of different types of plants is a significant source of antioxidants and other phytochemicals for the human body. The polyphenolic compounds present in honey have antioxidant properties very important for the protection of human health. Flavanoids have potential antioxidant and anticancer activities.[4]

LITERATURE/THEORETICAL UNDERPINNING

Free radicals and oxidative stress

Oxidative stress (OS) in the body occurs when the balance of oxidation-reduction processes is disturbed by excessive formation of free oxygen radicals that are not able to be neutralized by cellular homeostatic mechanisms. The molecules that cause oxidative damage to the body are called free radicals. A free radical is any chemical species capable of living independently, however briefly, which possesses one or more unpaired electrons in the outer orbit. All free radicals are characterized by extremely high reactivity, which is the result of their efforts to fill the valence orbit. In this way, they pair the unpaired electron and thus achieve a stable electronic configuration. For the human body, reactive oxygen species (ROS) are the most significant, which is the common name for oxygen radicals (superoxide, hydroxyl, peroxyl…) as well as reactive non-radical oxygen derivatives (hydrogen peroxide, hypochloric acid, singlet oxygen,…) and reactive nitrogen species (RNS), which include nitrogens (nitrogen (II) -oxide, nitrogen (IV) -oxide), and compounds and molecules such as peroxynitrite, nitrosyl cation.

Antioxidants

The controlled level of free oxygen radicals in the body maintains several mechanisms that are based on antioxidant reactions. Antioxidants are a diverse group of molecules that, when present at low concentrations compared to concentrations of an oxidative substrate, significantly retain or prevent oxidation of that substrate, controlling the relationship between reduction or oxidation states in the biological system. Antioxidants can be of endogenous and exogenous origin, and can be divided into natural and synthetic compounds. Natural include flavonoids, selenium, ascorbic acid (vitamin C), tocopherol (vitamin E) and beta-carotene, while synthetic compounds include probucol and stobadin and ebselen.[1] Endogenous antioxidants are produced in the cell, and the exogenous are most often taken into the body by food or in the form of vitamins and similar supplements. Honey produced by bees using nectar and other parts of different types of plants is a significant source of antioxidants and other phytochemicals for the human body. The polyphenolic compounds present in honey have antioxidant properties very important for the protection of human health. Flavanoids have potential antioxidant and anticancer activities.[4]
In recent years, research has increasingly focused on natural sources of antioxidants that have a protective effect against oxidative damage.24,25 Natural antioxidants may be phenolic components (tocopherol, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines) or carotenoids, as well as ascorbic acid.26,27 Natural antioxidants are classified as "bioactive substances" and play an important role in cellular metabolism.

Med

Honey is an extraordinary product from beehives that produce bees from nectar and other sugary substances originating from different plants.[43] Worldwide honey is considered to be part of traditional medicine. [44,45,46] However, its therapeutic activity in modern medicine has been neglected for a very long time due to the lack of systematic scientific research in proving its medical characteristics. Today, scientific research is based on a number of publications discussing the various therapeutic effects of honey.47 Honey has been shown to be effective in the treatment of wounds and burns, [48.49] as well as an effective agent with pronounced antimicrobial activity[50.51], and is also used in the prevention and treatment acute and chronic gastric lesions.[52,53] Codex standard[54] defines honey as a natural sweet substance produced by honey bees (Apis mellifera) from the nectar of plants or the excretion of living parts of plants, that is, the excretion of insects that drink the juices on living parts of plants. They collect, process, add to their own specific substances, dispose of, dry, store and leave the honeycomb to ripen.[54]

This definition determines the double origin of honey as a foodstuff: vegetable and animal. It also results in a basic division by origin into nectar or flower honey originating from the nectar of honey plants of different species and honeydew derived mainly from honey dew (secretion of insects that collect juices from living parts of plants or excretion of living parts of plants). Nectar honey can be monofloral and polyfloral. Monofloral honey is one that contains at least 45% of pollen grains of the same plant species in insoluble sediment. Polyfloral honey is a mixture of honey of different types. There is also the so-called mixed honey which is a mixture of nectar honey and honeydew.[55]

Bees produce nectar honey from nectar, a sweet solution secreted by the plant glands of nectar. Depending on where they are, we divide nectaries into floral and extra-floral. During pollination, the flower secretes the most nectar, which is logical, since one of the main roles of nectar is to attract insect pollinators.[56,57] By chemical composition, nectar is an aqueous solution of various sugars with the highest concentration of sucrose, glucose and fructose. Some types of honey contain in small quantities certain oligosaccharides such as raffinose, melebiosis, melecitosis, etc. The ratio of individual sugars in nectar depends on the type of plant, but also on climatic, soil and other conditions. In addition to sugar, nectar also contains certain amounts of nitrogen and phosphorous compounds, organic acids, vitamins (especially vitamin C), pigments, aromatic compounds, minerals, enzymes (invertase, acid phosphatase) and amino acids.[58] Honeysuckle or honeydew is a sweet substance that occurs on the leaves and other parts of coniferous and deciduous trees. It is a product ie. excretion of insects from the equine wings (Homoptera), of which the aphids are the most important for beekeeping. On the plants, they drink the juices through the thin and long rill that they pull through the plant tissue. The silo goes all the way down to the tubular tubes that move the vegetable juices they exploit, and are more or less processed to be excreted in the form of tiny droplets of honeydew that pile on the plant or fall to the ground or grass.[60]

By origin, the honeydew is most often derived from coniferous (fir, spruce, pine, larch) and deciduous (oak, beech, linden) trees. According to microscopic analysis, honeydew honey is characterized by low pollen content and honeydew elements (spores, fungi and algae). Compared to nectar honey, honeydew has a higher color, a higher mineral

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content, and a higher amount of oligosaccharides, especially melecitosis. It is also less sweet than nectar honey, has a lower acid content and has a higher pH value.[57]

Optical activity of honey

The aqueous bee honey solution is optically active, i.e. has the ability to rotate a plane of polarized light. Optical activity is a function of the proportion of individual carbohydrates in honey. Fructose turns the plane of polarized light to the left, and glucose, all disaccharides, trisaccharides and higher oligosaccharides to the right. Due to the higher content of fructose, nectar honey turns the light to the left, that is, it shows negative optical activity, whereas due to the higher content of oligosaccharides, especially melecitosis and erlose, the honeydew turns the light to the right, ie. shows positive optical activity. Therefore, measuring the specific rotation angle in some countries (Greece, Italy and the United Kingdom) is used to distinguish nectar honey from honeydew.[74]

The color of honey

The color of the honey depends on its botanical origin and ranges from light yellow, yellow, brown to dark brown. Extremely light, almost white-greenish in color is acacia and dark brown chestnut honey. The color of other types of honey ranges between these two extremes. In addition to acacia honey, light meadow honey and clover honey, reddish linden, dark yellow husk, amber-yellow sunflower honey and rapeseed, sage is yellowish brown and jelly and buckwheat honey are dark. The honey becomes lighter after crystallization (because the glucose crystals are white) but darkens during storage. Darken more intensively if stored at a higher temperature. Also, there is a relationship between the color and the chemical composition of the honey. Color is also determined by the content of carotenoids (carotene and xanthophyll - yellow color), flavonoids, chlorophyll, anthocyanin (pink color), tannin (dark color) and sugar (175) Honey becomes darker when condensing proteins and amino acids with reducing sugars (Maillard reactions) , which produces melanoids as well as due to the presence of fructose degradation products. In addition, Maillard reactions may also include polyphenols, ascorbic acid and other carbonyl compounds that can be found in honey depending on its botanical origin. The color of honey is also related to the amount of ash, especially iron, copper and manganese.77 Transparency and clarity depend on the amount of particles such as pollen. Spring honey is brighter and late summer is darker. Honey color is most commonly expressed in millimeters of Pfund scale (optical density readings used in international honey trade), or according to the classification of the U.S. Department of Agriculture (Table 1). [79,80,81]

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Table 1.
Pfund scale values, color standards and optical density

mm Pfund	USDA color standard	Optical density
<8	water white	0,0945
9-17	extra white	0,189
19-34	white	0,378
35-50	extra light white	0,595
51-85	light amber	1,389
86-114	amber	3,008
>114	dark amber	-

**Optical density (absorbance) λ=593 nm*

Antioxidants in honey

Honey antioxidants can be enzymatic (catalase, glucose oxidase) and non-enzymatic (organic acids, Maillard reaction products, amino acids, proteins, flavonoids, phenols, tocopherol, ascorbic acid, carotenoids).[101] Many phytochemicals present in honey serve as sources of non-enzymatic antioxidants, and their amount depends primarily on the botanical origin of the honey.[99] Honey contains phenolic and non-phenolic substances with antioxidant potential. Polyphenols present in honey, such as flavonoids and phenolic acids, can act as natural antioxidants.[21] Honey treated with high temperatures can easily lose some of the antioxidants present, which are easily compensated by the product of Maillard reactions.[102] In addition to polyphenols, honey also contains ascorbic acid, which also has antioxidant properties, such as antioxidant and co-antioxidant, but also microbiogenic elements copper, zinc and manganese, which are part of the body's enzymes that act as antioxidants. The antioxidants present in natural foods show higher antioxidant activity than synthetic ones.103 In addition, antioxidants in honey, as well as in other foods, prevent spoilage caused by oxidative changes due to the action of light, heat and some metals. Since honey is a complex mixture of over seventy different substances, which in each particular type of honey are in different proportions and therefore have different degrees of activity, it is impossible to synthetically obtain a product that would correspond to the chemical composition of honey.

Polyphenols in honey

Polyphenols represent a very broad group of compounds that can be found in foods of plant origin. There are over one million compounds of this group in nature, which are also secondary metabolites of plants. There is a great variety of polyphenols in that they are essentially glycosidic in nature, but they can bind different types of sugars that can take different positions. Many polyphenols have a positive effect on health. The bioactivity of the compounds of this group comes from the structure of the aglycones and not from the sugars attached to them. Research shows that the content of polyphenols is important for the antioxidant activity of fruits, vegetables, juices and other food products, including honey.[19].[20] More than 8000 phenolic compounds are known[104] of which almost 2/3 belong to the flavonoid family. For this reason, most research into the nutritional and health value of polyphenols has focused mainly on

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them.[105] Flavonoids include compounds such as catechins, anthocyanidins, proanthocyanidins, flavones, and flavonols.[106] Flavonoids commonly found in honey are pinocembrin, apigenin, campherol, quercetin, galangin, krisin, pinobanksin, luteolin, and hesperitin.[107,108] Amount of flavonoids in honey is up to 6000 μg / kg, while their proportion is much higher in pollen (0.5%) and in propolis (10%). The structural formulas of some flavonoids present in honey are shown in Figure 1:

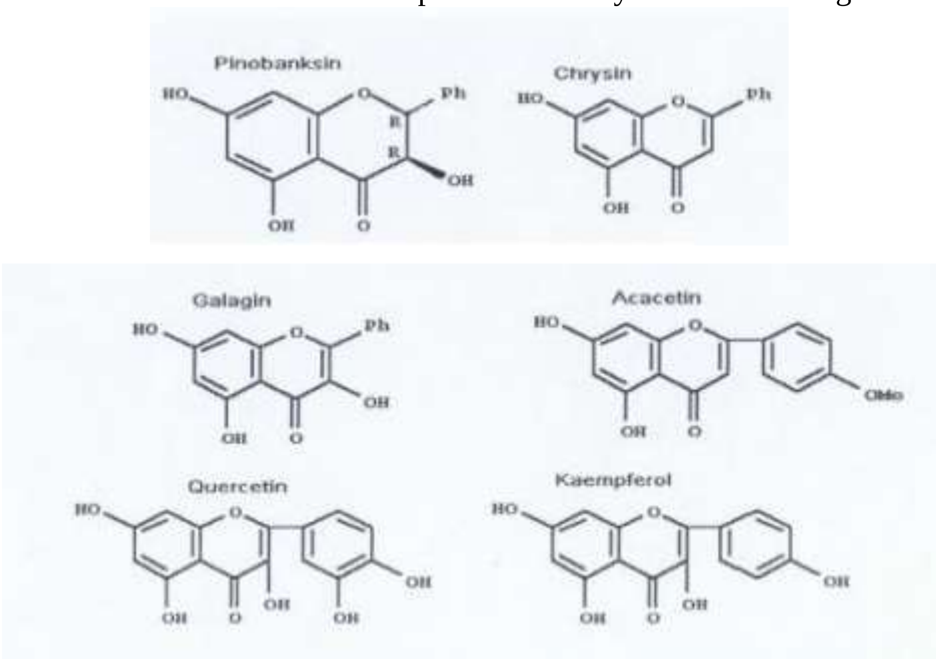

Figure 1. Structural formulas of some flavonoids present in honey[109]

METHODOLOGY

This research included 60 honey samples from Bosnia and Herzegovina and the surrounding countries. The content of total antioxidants was correlated with the content of total polyphenols and the sugar content (glucose, fructose and sucrose). In addition, the honey color as well as its optical activity were analyzed. The content of total antioxidants in honey samples was determined by the FRAP (Ferric Reducing Antioxidant Power) method. FRAP is a method described by Benzie and Strain in 1996, but further modified in 1999. [203,209] This is a simple, fast and automated method based on the reduction of iron from ferric Fe^{3+} to ferro Fe^{2+} form in the presence of antioxidants, where at low pH an intense blue colored ferro tripyridyltriazine complex develops, having an absorption maximum at 593 nm (Figure 2.). The reaction is not specific.[210,211]

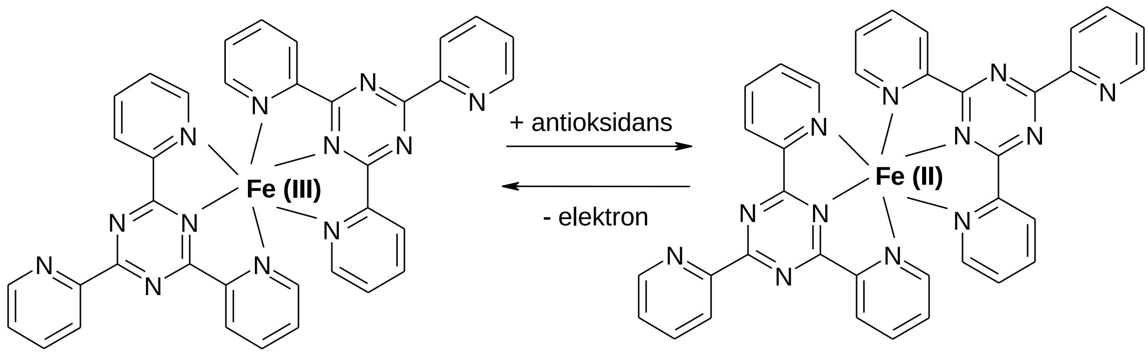

Figure 2. Reduction reaction of iron-2,4,6-tripyridyl-s-triazine (TPTZ)

Concentrations were calculated based on a calibration curve (Figure 3) and the results were expressed as µmol Fe equivalent (Fe) / mL of sample.

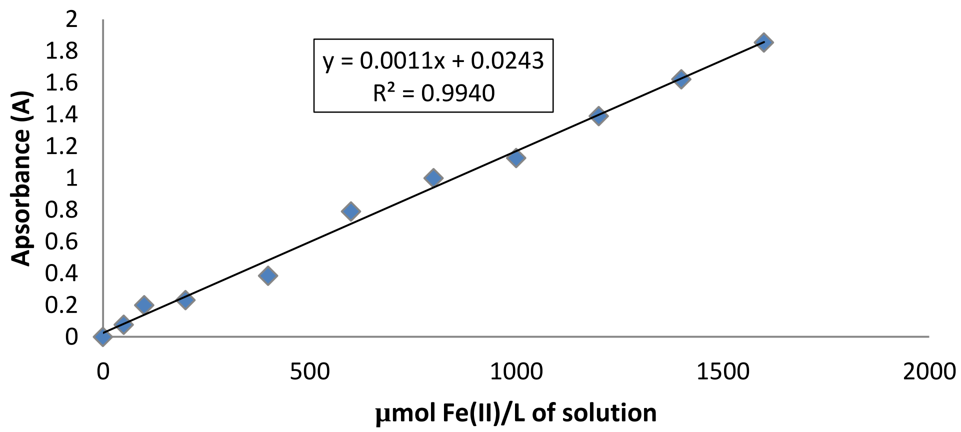

Figure 3. Calibrated curve for determining the content of total antioxidants

Total polyphenols in honey samples were determined using the Folin-Ciocalteu (FC) method.[204] The FC method is one of the oldest indirect methods that is sensitive to phenolic and polyphenolic compounds. This method is standardized and widely used in the determination of total polyphenols.The basic mixture for analysis consists of volframate and molybdate in the base medium (7.5% Na_2CO_3). Due to the base environment, the oxidation of phenol and the formation of O_2 •. It reacts with molybdate to form molybdenum (IV) oxide, which absorbs at a wavelength of 765 nm. The polyphenols determined by this method are expressed in mg of gallic acid per L solution. This method has great advantages over others.

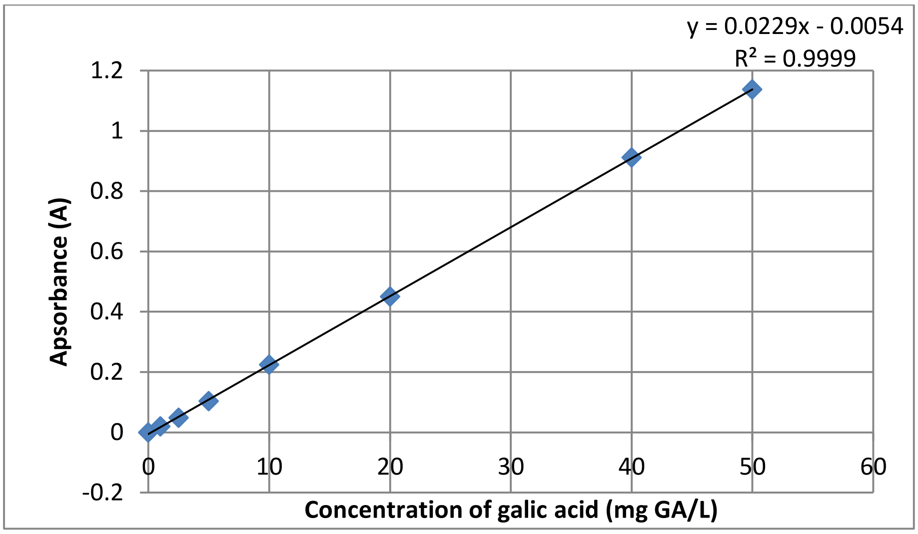

Figure 4. Calibration curve for determining the content of total polyphenols

The analysis of glucose, fructose and sucrose content in honey samples was performed by HPLC method on a high performance Agilent 1100 liquid chromatogram with a lichrospher 100 NH_2 5µm, 250 x 4.0 column. The parameters for determining glucose, fructose and sucrose content in the tested honey samples are given in Table 2:

Tabela 2.
Parameters of glucose, fructose and sucrose content in analysed honey samples[208]

Parameter	Values
Mobile phase	Acetonitril (Merk 99,8 %)/ aqua (75:25),
Flow	1,3 ml
Wavelength	192 nm
Temperature	30^0 C
Sample volume	20 µL

The determination of the specific angle of rotation of polarized light was performed polarimetrically on the polarimeter OPTECH model PL 1. The angle at which the optically active substance rotates the plane of polarized light is proportional to the number of optically active molecules in the unit of volume and the length of the path of light. The proportionality constant is called the specific angle of rotation and at a given wavelength and a certain temperature is a characteristic magnitude for each optically active substance.

$$\alpha = [\alpha]_D \cdot l \cdot c$$

The specific rotation angle is the angle by which 1 g of optically active substances dissolved in 1dL of solution rotates a plane of polarized light at an optical path of 1 dm.

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Honey color analysis was performed by spectrophotometric method[208] in 10% honey solution at 593 nm.

RESULTS/FINDINGS

The results of spectrophotometric determination of honey color, antioxidant activity and content of total polyphenols are shown in Table 3:

Table 3.
Color of analised honey samples

Sample No.	Optical density (λ = 593 nm)	Antioxidation activity (μMFe^{II}/L)	Total phenols mg/100g meda	Sample No.	Optical density (λ = 593 nm)	Antioxidation activity (μMFe^{II}/L)	Total phenols mg/100g meda
1.	0,141	73,36	11,09	31	0,090	22,9	5,19
2.	0,146	118,81	15,45	32	0,103	49,27	5,16
3.	0,293	284,72	24,19	33	0,084	86,54	5,17
4.	0,174	11,09	11,75	34	0,115	194,27	7,82
5.	0,077	233,82	5,16	35	0,083	90,64	11,31
6.	0,146	247,9	5,41	36	0,088	4,7	5,17
7.	0,114	122,9	15,45	37	0,185	189,27	31,39
8.	0,166	852,91	22,44	38	0,246	168,36	32,71
9.	0,172	469,72	19,82	39	0,137	748,82	18,95
10.	0,166	816,55	25,06	40	0,161	1071,09	29,65
11.	0,125	114,73	7,82	41	0,156	888,82	23,97
12.	0.149	173,36	11,09	42	0,174	915,18	26,81
13.	0,164	261,54	6,94	43	0,136	702,45	24,41
14.	0,245	522	19,82	44	0,201	506,55	24,85
15.	0,102	406,54	9,34	45	0,105	244,27	5,63
16.	0,106	290,64	22,44	46	0,230	1094,27	26,81
17.	0,276	457,0	19,83	47	0,084	17,9	8,69
18.	0,184	452,0	10,65	48	0,170	1606,54	17,64
19.	0,186	900,64	25,94	49	0,158	581,54	18,30
20.	0,200	740,64	26,81	50	0,156	71,09	18,73
21.	0,141	278,82	16,11	51	0,102	243,82	5,16
22.	0,185	263,36	16,98	52	0,150	627,0	13,27
23.	0,142	352,45	10,22	53	0,121	477,45	23,54
24.	0,107	249,72	5,85	54	0,177	355,64	14,58
25.	0,196	275,18	13,49	55	0,233	1029,73	30,52
26.	0,110	59,72	5,85	56	0,103	576,09	16,76
27.	0,108	893,82	6,07	57	0,231	98,36	30,52
28.	0.113	189,27	5,19	58	0,135	745,18	23,54
29.	0,116	77,45	5,19	59	0,131	678,82	25,06
30.	0,083	5,18	5,19	60	0,184	1110,18	28,56

The sugar content of glucose, fructose and sucrose in the analyzed honey samples is shown in Table 4:

Table 4.
The content of glucose, fructose and sucrose in analysed honey samples

Sample No.	Glucose [%]	Fructose [%]	Sucrose [%]
3	27,03	50,43	0
4	32,45	45,70	0
5	29,06	47,98	0
6	26,55	42,58	0
7	31,42	48,14	0
9	31,18	49,22	0
11	24,6	50,76	0
12	29,51	48,95	0
13	28,01	52,17	0
21	25,63	44,12	1,25
22	23,77	42,46	0
23	25,08	44,29	0
24	25,19	50,93	0
29	30,27	40,43	0
33	27,02	48,24	0
39	25,89	45,10	0
40	30,07	46,08	0
43	31,91	45,30	0
44	31,79	45,85	0
50	27,61	45,85	0,92
51	26,44	49,95	0
52	25,93	54,40	0
53	21,73	47,91	0
57	30,75	43,83	2,04
58	23,46	49,94	0

Rezultati analize optičke aktivnosti uzoraka meda prikazani su u Tabeli 5.:

Table 5.
Optical activity of analysed honey samples

Sample No.	Specific rotation angle $[\alpha]_{589,3nm}^{20^o C} = \frac{Q}{LC} x100$
2	-15
7	-12
9	-5
26	-10
29	-19
30	-11
44	-15
47	-19
53	2
55	11

DISCUSSION

According to the chemical composition of honey, it is mostly dominated by carbohydrates and water, but honey contains small amounts of substances that are largely responsible for its specific properties. Characterization of honey helps to understand its antioxidant properties and thus its use as a natural food product, that is, as a source of antioxidants in the human diet. FRAP analysis of the tested honey samples showed that the total antioxidant activity of honey ranges from 4.7 µMFeII / L in the acacia honey sample from Banovići municipality to 1606.54 µMFeII / L in the mixed honey sample from Tuzla municipality. Nectar honey samples are a weaker source of antioxidants than honeydew, so acacia honey samples have on average significantly lower antioxidant activity than forest and mountain honey samples. These results are in line with the results of Spanish scientists Lucía Vela et al. By analyzing honey samples from the Spanish area, they also concluded that the antioxidant activity of honeydew is on average higher than the antioxidant activity of nectar honey.[225]

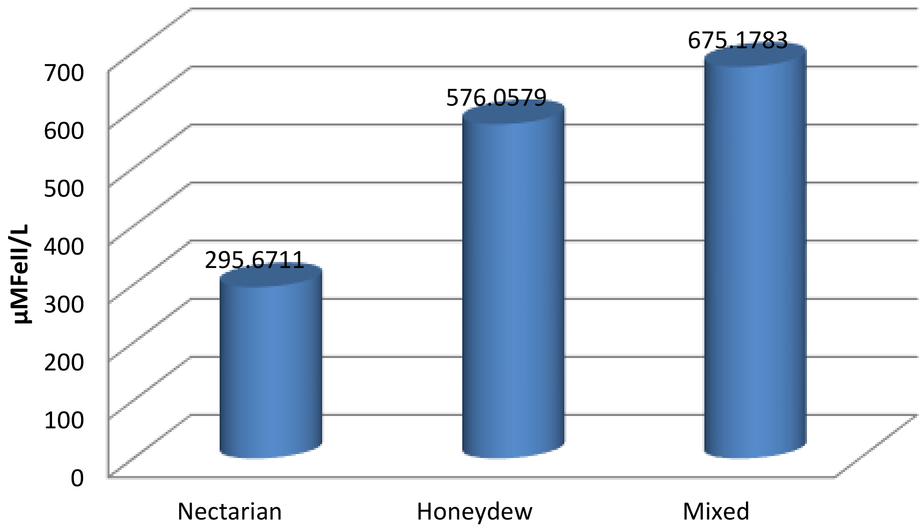
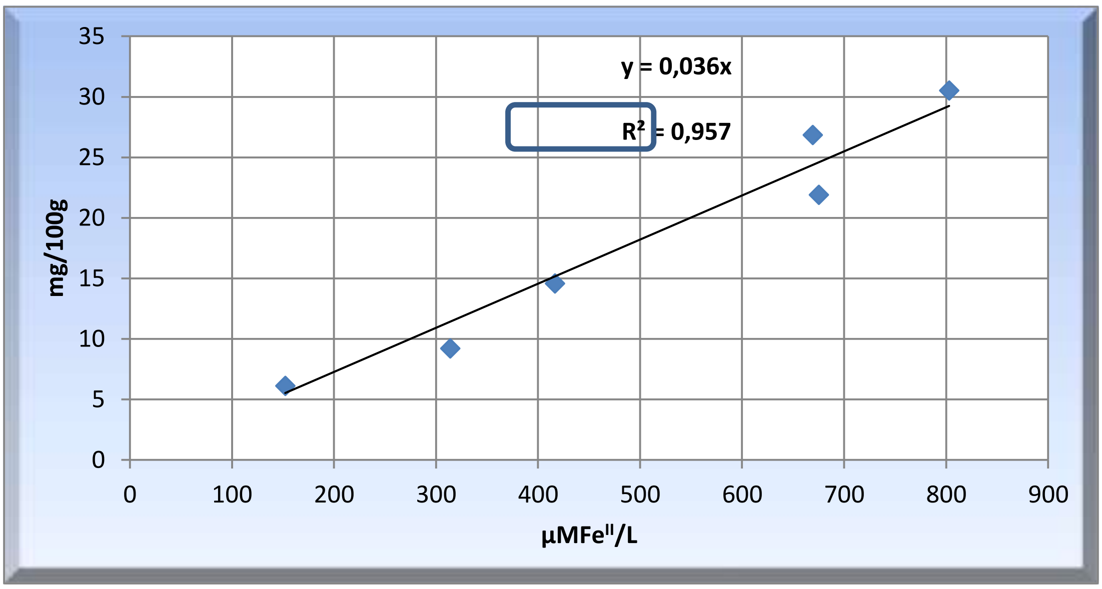


Figure 5. The difference in the total antioxidant activity of nectar honey, honeydew and mixed honey

According to our research, the content of polyphenols in the analyzed honey samples ranged from 5.16 mg / 100g to 32.71 mg / 100g. According to research by Spanish scientists[225], the total antioxidant activity of honey is correlated with the content of polyphenols in honey.Our studies confirmed the high correlation coefficient between the antioxidant activity of honey and the content of total polyphenols.



Slika 6: Corelation between total antioxidant activity i total phenolic content in honey

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The Pearson correlation coefficient of 0.957 was calculated by statistical processing of the obtained results. This means that in 95.7 % of cases, honey samples with higher antioxidant activity also have a higher polyphenol content. The analyzed honeydew samples have on average a higher content of polyphenols than nectar honey samples. Mixing nectar honey and honeydew increases the content of polyphenols relative to the nectar honey itself.

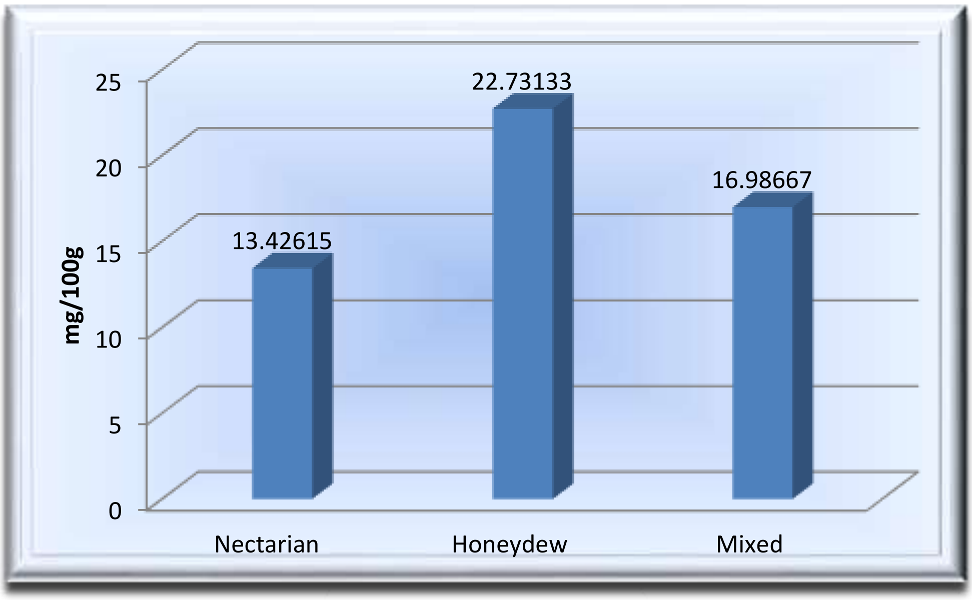

Figure 7. Difference in polyphenols content of nectar honey, honeydew and mixed honey

The average content of polyphenols in nectar honey samples is 13.43mg / 100g, in honeydew honey samples 22.73 mg / 100g, and in mixed samples 16.99 mg / 100g. These results are in line with the research of Spanish researchers.[225] Based on the obtained results, we can conclude that nectar honey samples are on average lighter in color than honeydew samples, which is in accordance with the results of Spanish and Slovenian researchers.[225,247] The relationship between honey color and total antioxidant activity is shown in Figure 8.

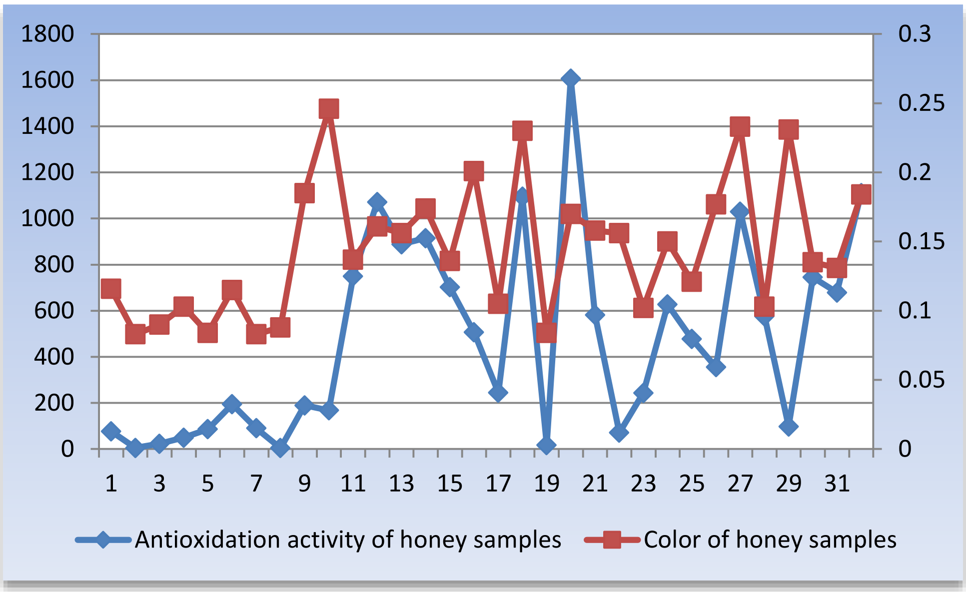

Figure 8. Color ratio and total antioxidant activity of honey

Based on the results obtained, it is clear that honey color is significantly correlated with total antioxidant activity. Darker honey samples also showed stronger antioxidant activity. These results are in line with those of Jasna Bartoncelj and colleagues who have done research on Slovenian honey samples.[204] The results of investigation the relationship between color and antioxidant activity of honey were statistically analyzed, and Pearson's correlation coefficient for this ratio is 0.870. Honey color is also significantly correlated with polyphenol content of honey.

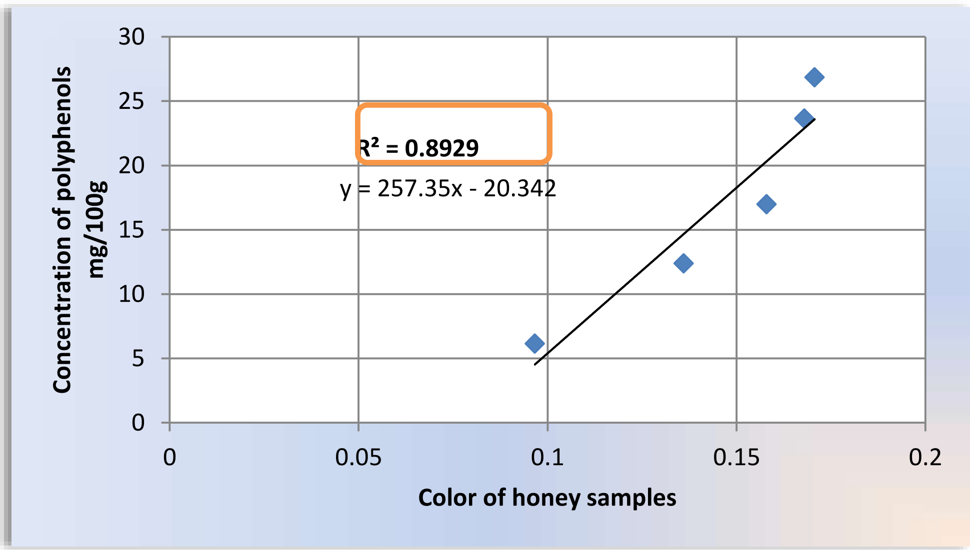

Figure 9. Coefficient of correlation between total polyphenols content and honey color

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The Pearson correlation coefficient was calculated by statistical processing of the results, which is 0.892 for this relation. Thus, darker honey samples, on average, also have a higher polyphenol content. These results are in line with those of Jasna Bartoncelj and colleagues who have done research on Slovenian honey samples.[204] The profile of carbohydrates in honey has been the subject of much research. Qualitative and quantitative determination of carbohydrates in honey is carried out in routine quality assessments, in determining the forgery of honey or in determining the botanical and / or geographical origin of honey. Nectar honey according to the quality regulation of honey must contain a minimum of 65% of reducing sugars calculated as invert sugar and minnow at least 60% of reducing sugars.[54] In this study, glucose, fructose, and sucrose content in honey samples were performed by HPLC using an Agilent 1100 high performance liquid chromatogram (Appendix 1. HPLC analysis diagrams). Investigations were carried out on 25 honey samples classified by botanical origin into 8 categories: meadow honey, acacia honey, forest honey, linden honey, mixed honey, chestnut honey, hawthorn honey and heather honey. The percentage of invert sugar (fructose + glucose) in the analyzed honey samples is shown in Figure 10.

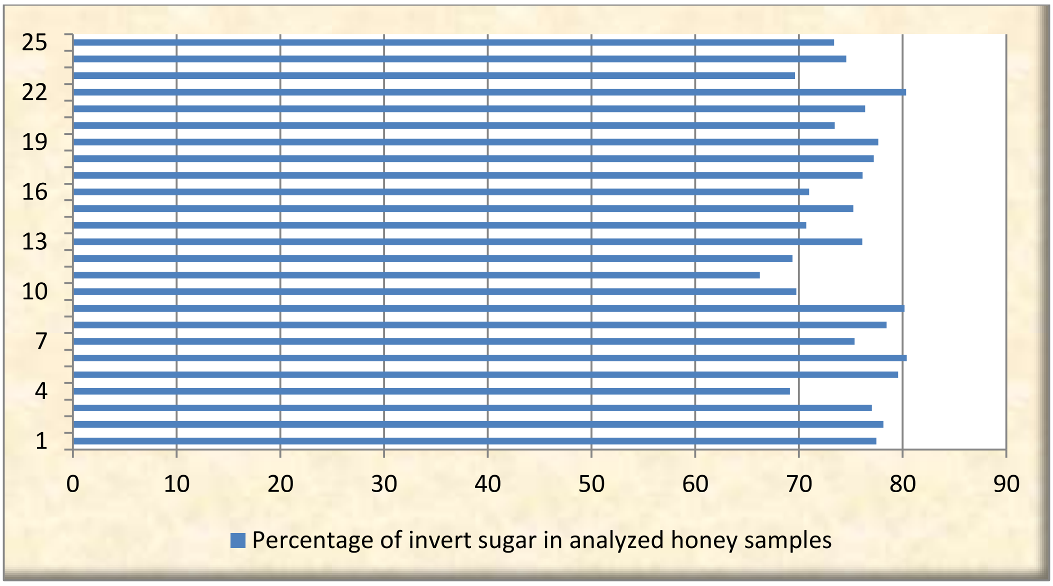

Figure 10. Percentage of invert sugar in analyzed honey samples

The proportion of invert sugar in the analyzed honey samples ranges from 66.23% to 80.40%, which is in accordance with the rules on honey quality. Researchers Golob and Plestenjak determined the content of invert sugar (fructose + glucose) in Slovenian honey, and a range of average values was obtained from 61.92% to 72.90%. and reducing oligosaccharides. The proportion of reducing sugars in honey reaches 75-80%. Somewhat lower values for the proportion of reducing sugars were obtained by Brazilian scientists led by Azered in a study conducted on 12 honey samples, ranging from 67.6-73.5% .[198] The main sugars that give honey sweetness are fructose, glucose, sucrose and maltose.[103] Since fructose is the largest sugar content in

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honey by volume, honey is, on average, 1.5 times sweeter than drinking sugar. About 95% of the sugar present in honey is fermentable, which is important in the production of mead and bakery products. Honey with a sugar content greater than 83% and a water content below 17.1% will not be fermented if stored properly.[103] Figure 11 shows the ratio of average glucose, fructose and sucrose content in honey samples of different botanical origin.

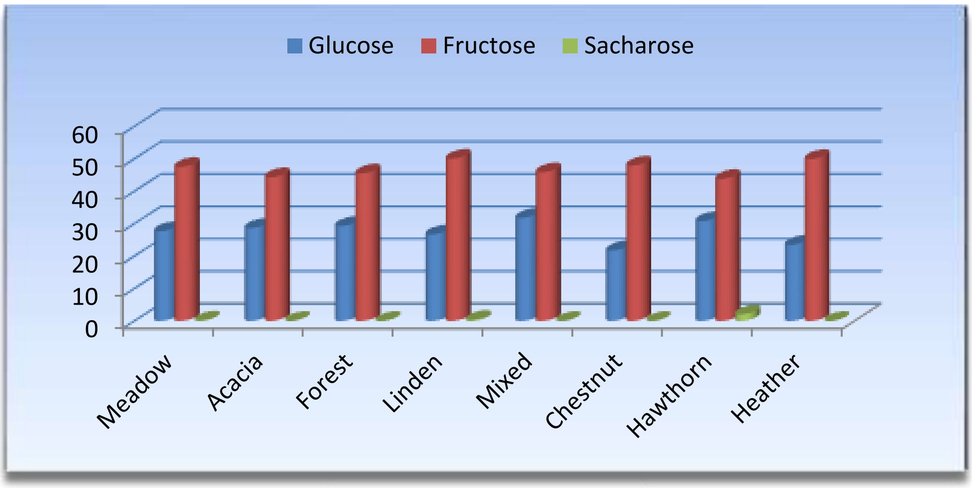

Figure 11. Average glucose, fructose and sucrose content in samples of honey of different botanical origin

The ratio of fructose to glucose (F / G) is characteristic for certain types of honey and in most cases is greater than 1.0.[79] The results of the analysis of the F / G ratio of the studied honey samples are shown in Figure 12:

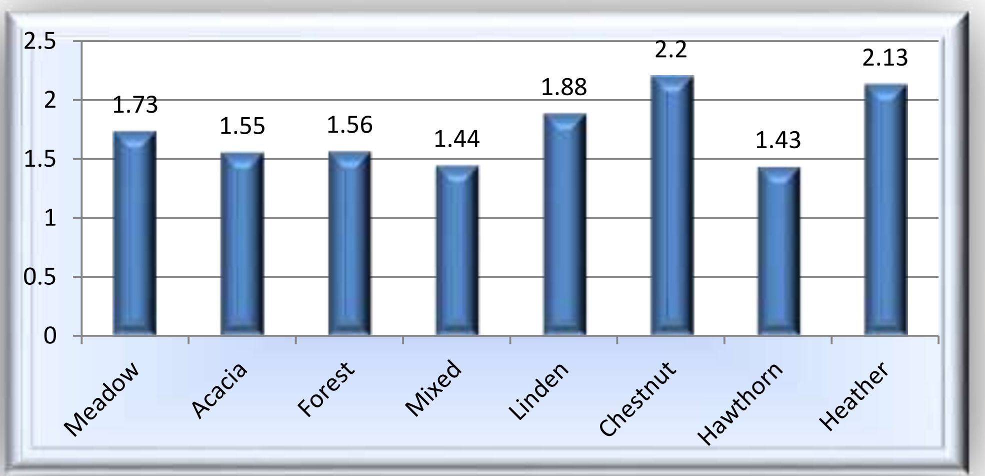

Figure 12. Average values of F / G ratio of analyzed honey samples

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According to the literature, acacia and chestnut honey (F / G 1.5-1.7) stand out as fructose-rich, while some of the rare species with higher glucose content are rapeseed and dandelion honey. [85]

In this study, sucrose was detected by HPLC analysis in only three honey samples, namely meadow honey from the Mayjevica area of 1.25%, linden honey from the Bosanski Šamac area of 0.92% and hawthorn honey from the Bosanski Šamac area. worth 2.04. The detected sucrose values in honey samples do not exceed the limit values so that, based on this parameter, all analyzed samples can be characterized as true natural honey samples to which no artificial sugars have been added. Due to the higher content of fructose, nectar honey rotates the plane of polarized light to the left, ie it exhibits negative optical activity, whereas due to the higher content of oligosaccharides, mainly melecitosis and erlose, the medulla turns light to the right, ie. shows positive optical activity. According to the results obtained in our study, all types of nectar honey showed negative optical activity, while honeydew honey samples showed positive optical activity. These results are in line with the results of honey research by other scientists (Greece, Italy and the United Kingdom) in which the measurement of a specific rotation angle is used to distinguish nectar honey from jellyfish.[73]

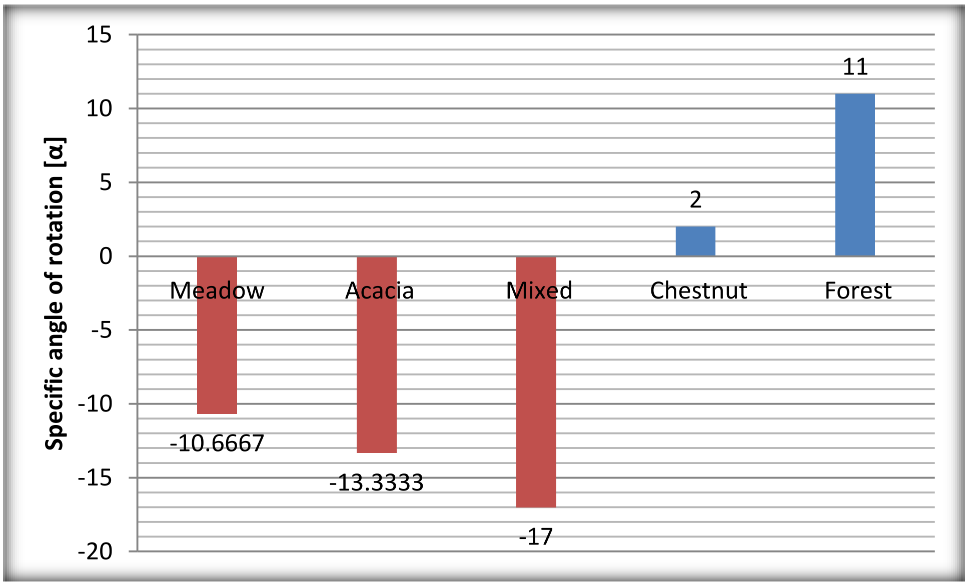

Figure 13. Optical activity of nectar honey and honeydew

Figure 14 shows the effect of the F / G ratio on the optical activity of honey:

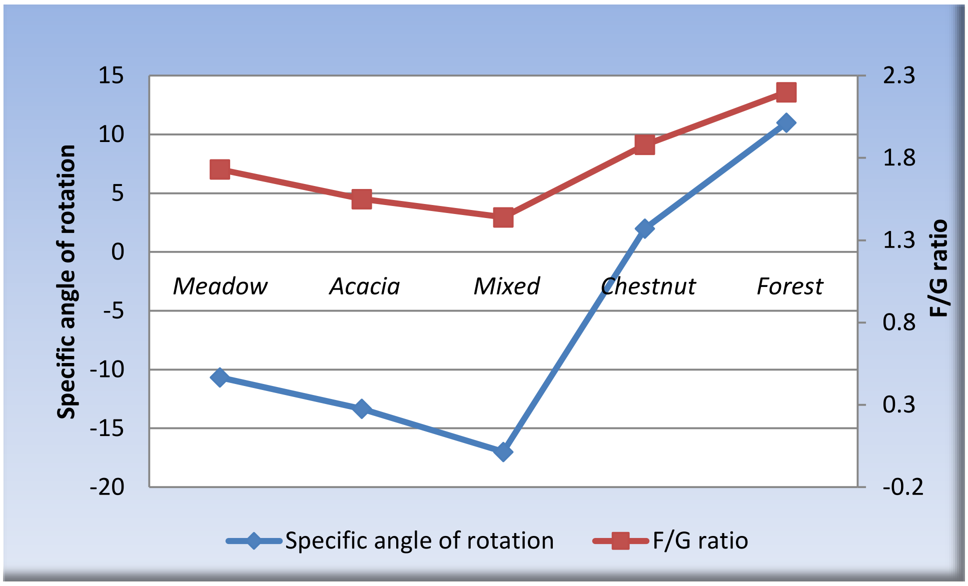

Figure 14. The influence of the fructose-glucose (F / G) ratio in honey on its optical activity

The results clearly show the correlation of the F / G ratio with the optical activity of the analyzed honey samples. The smaller the specific rotation angle, the lower the F / G ratio, the higher the fructose content in the honey. Specific rotation values of the analyzed meadow honey samples ranged from -15 to -5°, acacia honey samples from -19 to -10°, mixed honey samples from -19 to - 15 ° and honeycomb from 2-11°.

IMPLICATION TO RESEARCH AND PRACTICE

This study confirmed that nectar honey and honeydew honey showed differences in both total antioxidant content and optical activity, sugar content, and total polyphenol content. This knowledge significantly contributes to the proper guidance and use of honey as a dietary supplement, but also as an aid in the treatment of certain medical conditions.

CONCLUSION

Honey, as a natural food product, is a significant source of antioxidants for the human body. The overall antioxidant activity of honey is the result of its complex chemical composition and complex interactions of different substances. The color of the analyzed honey samples from the territory of Bosnia and Herzegovina is different and ranges from almost colorless to dark brown. Nectar honey samples are, on average, lighter in color than honeydew samples. Honey color is significantly correlated with total antioxidant activity and polyphenol content of honey. Darker honey samples also showed stronger antioxidant activity. All types of nectar honey showed negative optical activity, that is, they rotate the plane of polarized light to the left (higher fructose content), while honeydew honey samples showed positive optical activity, that is, they rotate the plane of polarized light to the right (higher glucose content). There is a

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significant correlation between the F / G ratio and the optical activity of honey. The smaller the specific rotation angle, the smaller the F / G ratio, the higher the fructose content of honey.

FUTURE RESEARCH

Future research can certainly be directed towards the identification of phenolic compounds in nectar and honeydew, and their differentiation based on this parameter. Analysis of the content and profile of minerals is also one of the directions of further research, since the content of mineral substances can significantly affect the medicinal properties of honey, as well as its physico-chemical composition.

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