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Changes of antioxidant activity in honey as a result of Haber-Wais reaction

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Abstract: The antioxidants present in natural food products show a higher antioxidant activity than synthetic one [1]. Polyphenolic compounds are the most important antioxidants in bee honey. In addition to the polyphenols there are non-phenolic compounds with antioxidant potential, such as proteins, gluconic acid, L-ascorbic acid, hydrogen peroxide, hydroxyl methyl furfural (HMF), Maillard reaction products, etc. The antioxidant activity of honey largely depends on the content of micro biogenic elements. Cu acts as antioxidant because it is an integral part of some enzymes involved in the antioxidant activity of the organism. Cu in honey also acts as a pro-oxidant through Haber-weis reaction with L-ascorbic acid also present in honey. In addition to being an essential micro-nutrient Cu is potentially very hazardous because of the capacity change in oxidation state leading to the initiation of the reaction in which free radicals are formed. Therefore, the balanced intake of copper and other trace elements in the human body is very important. Therefore, changes in the antioxidant activity of food are the result of chemical changes in antioxidant active compounds present in the food product.

Keywords: Metals, UV/Vis Spectroscopy, Vitamins, Antioxidants, Haber-Weis Reaction, Cuprum

1. Introduction

As a result of the worldwide trend to minimize or totally eliminate the use of synthetic food additives because of their potential adverse effects, there is increasing interest in natural antioxidants. Antioxidants present in natural foodstuffs exhibit greater antioxidant activity than synthetically produced. In addition, the antioxidants in honey, but also in other foods, prevent spoilage caused by oxidative changes due to the effects of light, heat and some metals.[1]

The antioxidant activity of different substances in the body is very important in mechanism of protecting the human body from the damaging effects of free radicals. In situations in which the body cannot resist the action of free radicals, it is necessary to enter through diet sufficient amount of antioxidants to maintain balance antioxidant / free radical. Antioxidants are nutritional and innutritious substances that can stop the biologically destructive chemical reactions in food and living organisms [2]. They represent protection from oxidative damage caused by free radicals (reactive oxygen species - ROS) [3]. One of the most hydrophilic low molecular weight antioxidants in human plasma is L-ascorbic acid. L-ascorbic acid acts as an antioxidant in plasma and also expresses synergistic effects with other antioxidants. So, L-ascorbic acid is antioxidant and co-antioxidant. L-ascorbic acid is synthesized in plants and animals. In animals, L-ascorbic acid is formed from D - glucose. It is the enzymatic reaction that takes place in four levels (Figure 1) [4]:

Human organisms and the organisms of some animals do not possess enzyme gulonolactone oxidase which catalyzes the final stage of synthesis of L-ascorbic acid. Therefore, the human body is fully dependent on food inputted amount of L-ascorbic acid. L-ascorbic acid is a vitamin that participates in almost all major biochemical reactions in the body [5].

L-ascorbic acid has good antioxidant capacity due to its oxidation product stabilized by resonance structures (Figure 2.).

According to research, Golumbic and Mattill [6], ascorbic acid (vitamin C) has little impact on the prevention of fatty acid oxidation, while the combination of ascorbic acid with tocopherol (*-TOH) grows into a strong synergistic antioxidant effect. It led to the conclusion that one of the roles of L-ascorbic acid that “keeps” TOH. This action of

L-ascorbic acid is indicated as co-antioxidant effect [7]. So tocopherol and L-ascorbic acid act as antioxidants in different micellar colloidal systems together, and possibly synergistic.

In addition to the polyphenols as the most important antioxidants present in honey there are non-phenolic compounds with antioxidant potential, such as proteins, gluconic acid, L-ascorbic acid, hydrogen peroxide, hydroxymethylfurfural (HMF), Maillard reaction products, etc. The antioxidants that are present in natural food products show a higher antioxidant activity than synthetic one [1].

Micro biogenic elements (eg, Cu, Zn, Mn), contained in honey, also affect its antioxidant activity. In addition, as an integral part of some enzymes involved in the antioxidant activity of the organism, micro biogenic elements can act as pro-oxidants. Cu is an essential micro biogenic element, but is potentially very hazardous because of the capacity change in oxidation state leading to the initiation of the reaction in which free radicals are formed. Therefore, the balanced intake of copper and other trace elements in the human body is very important.

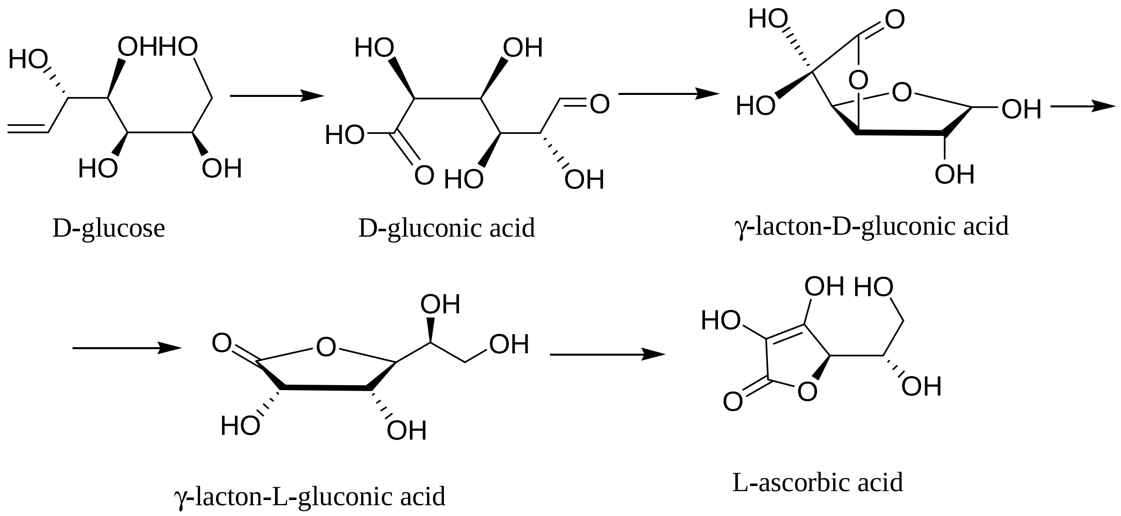

Figure 1. *Biosynthesis of L-ascorbic acid from D-glucose in the body of animals [4].*

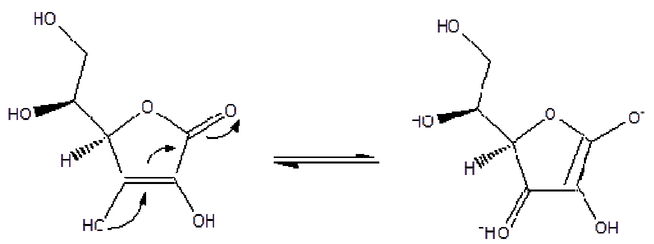

Figure 2. *Resonance structures of L-ascorbic acid.*

Copper is a micro biogenic element which is part of the reactive centers of many enzymes. It is required for the formation of red blood cells, it enters into the composition of hemocyanin and has a positive effect on the cell membrane of nerve cells. The minimum daily requirement for this micro biogenic element is 0.5 ppm. Excess copper is absorbed in the intestinal tract, leading to intestinal diseases, as well as reduced sensitivity to infection [9]. Honey is a natural food product that can be a significant source of copper.

Copper acts as an antioxidant in the human body, but also as a pro-oxidant. As an antioxidant it finds free radicals in the body and stops them. As a pro-oxidant, copper accelerates free radical reactions. Copper may participate in the development of Alzheimer's disease and possibly cervical dysplasia. It is therefore very important to take into account a balanced input of micro elements in the body.

Copper deficiency in plants and honey appears as a result of its binding to the primary and secondary soil minerals.

Changes in the antioxidant activity of food are the result of chemical changes in antioxidant active compounds present in the food product. These changes can affect differently the total antioxidant activity of a food product, as shown in Table 1:

Table 1. *Changes in antioxidant activity of food during the process of production, processing and storage.*

Processes in which the resistance to oxidation of food increases or decreases	**Changes in food products which affect its antioxidant activity**
Processes in which the resistance to oxidation increases	• Transformation of antioxidants in active compounds (eg. glycosides exceed the aglycone, Maillard reaction products); • The destruction of the pro-oxidant (eg substances that cause photosensitive effect with heavy metals); • Preventing access to oxygen (eg, encapsulation).
Processes in which the resistance to oxidation decreases	• Antioxidants destruction by oxidation or interaction with other food components; • Loss of antioxidants evaporation; • Improved access to oxygen (eg during drying); • Creation of a pro-oxidant or their release from inactive complexes.

The most significant changes of antioxidant activity of food are present as a result of rapid heating or during prolonged standing. Chemical changes that occur are oxidation reactions, especially oxidation reactions with heavy metals (metal ions in higher oxidation states).

Although the mineral substances in honey quantitatively poorly represented (average 0.1-0.2% in nectarean honey and to 1.5% in Honeydew expressed as ash), honey contains a number of minerals of which are very important for proper operation of the human body. Predominantly potassium, sodium, calcium, phosphorus, sulfur, chlorine, magnesium, ferrum and aluminum, and small amounts of copper, manganese, chromium, zinc, lead, arsenic, titanium, selenium, etc. [10].

The complex chemical composition of honey and the synergistic and antagonistic relationships of certain substances present in honey minimize the processes which reduce resistance to oxidation. These studies provide relevant evidence of the correlation of antioxidant activity of bee honey and its chemical composition. This study also demonstrated a significant correlation of copper and L-ascorbic acid, which is reflected in the overall antioxidant activity of analyzed samples of honey.

2. Materials and Methods

All chemicals and reagents were of analytical grade and were purchased from: (Fluka-Switzerland: 2,4,6 – tri [2-pyridyl]-s-triazine), (Semikem-Sarajevo: chloric acid 37 % p.a.; ferrous sulphate heptahydrate; ferric chloride hexahydrate; acetic acid-conc.), (Panreac Quimica-Espana: 2,6-dichlorophenol-indophenol Sodium salt 2-hydrate), (L-ascorbic acid: Sigma Chemical Co.). Some honey samples are collected in cooperation with Associations of Bee-keepers from Bosnia and Herzegovina. Other honey samples were purchased at the local market in Tuzla, Bosnia and Herzegovina. The antioxidant activity of honey samples measurements were performed by Shimadzu UV Visible spectrophotometer UVmini-1240 using the FRAP (Ferric Reducing Antioxidant Power) assay [11,12]. The changes that are observed during the FRAP experiment reflected in the fact that the reaction of the electron donating colorless Fe^{III} form produces a blue Fe^{II} - tripyridyltriazin which has a maximum absorption at 593 nm. Standard curve was prepared using different concentrations (50-1600 µmol/L) $FeSO_4 \times 7H_2O$. All solutions were used on the day of preparation. In the FRAP assay the antioxidant efficiency of the antioxidant under the test was calculated with reference to the reaction signal given by an Fe^{II}-solution of known concentration, this representing a one electron exchange reaction. The results were expressed in µmol Fe^{II}/L of extract. Presented data are average of three replications.

Concentration of L-ascorbic acid was determined using volumetric Tilmans assay. Tilmans assay is based on oxidation reaction of L-ascorbic acid to dehydroascorbic acid [13]. Volumetric analysis was performed using a 2.4-dichlorphenol-indophenola to appear pink color that retains at least 30 seconds. Standardization of prepared solution 2.4 - dichlorphenol-indophenola was performed in standard solution of iodine with accurately known concentration.

The concentration of copper was measured on atomic absorption spectrophotometer (AAS) "Perkin Elmer" 3110. Determination of copper was carried out according to standard ASTM E 1024-84 and ASTM E 1812 - 96 Bright (non-luminous) flame air / acetylene at a temperature of 2450 ^{0}C best flame to determine a large number of elements: magnesium, calcium, ferrum, transition metals. Therefore, in determining cuprum in the samples, a mixture of gases were used, air/acetylene. Parameters for the determination of cuprum are given in Table 2:

Table 2. *Parameters for the determination of copper.*

Analized element	Wavelenght [nm]	Slit	Linearity [mg/L]
Cu	324,8	0,7	0,077-5,00

3. Discussion

Great number of biological microelements are found in honey such as iron (ferrum), copper and zinc [14]. These microelements are essential components of metabolic processes where their existance in foodstuff is very important. The aim of the paper is to examine the copper and L-ascorbic acid content in honey samples from Tuzla Canton. The examined samples are of different types of honey (meadow honey, acacia honey, mixed honey, forest honey, raspberry-lime honey). The colour of samples examined is different. Four samples are brown, three samples are dark brown, two are light yellow and one is dark yellow. According to the researches, honey crystallisation (two layers) is very characteristic because the content of glucose is lower than the content of fructose [15]. As for the crystallization, only three of the taken samples have formed two layers in comparison to the other two that even after staying for a year had only one layer. When it comes to consistency, four samples are in liquid state, four are in semiliquid state and two are in high density. Four samples are taken from Tuzla region and other samples are from Tuzla Canton.

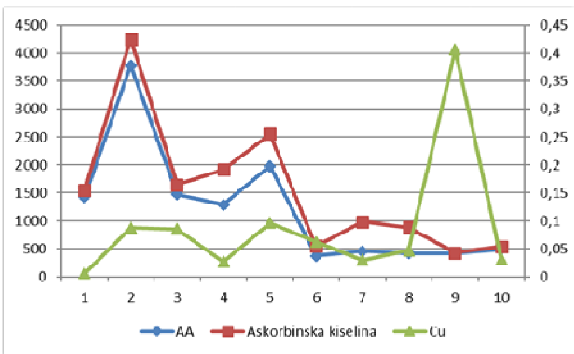

Figure 3. *The correlation between total antioxidant activity, the concentration of ascorbic acid and copper in honey.*

Figure 3. presents an influence relationship between L-ascorbic acid and copper ions on total antioxidant activity of honey.

Figure 3 clearly shows a positive correlation between antioxidant activity and the content of L-ascorbic acid in honey. L-ascorbic acid is a substance with antioxidant activity so it is logical that the concentration of this vitamin increases the total antioxidant activity of honey.

We performed a correlation analysis of copper concentration in the analyzed samples of honey with a concentration of L-ascorbic acid, but also with the total antioxidant activity.

Copper is an essential micro-nutrient which not only enters into the structure of the antioxidative active enzymes but also exerts strong pro-oxidant effect. One of the ways of this pro-oxidative action of copper is via the Haber-Weiss reaction.

The results showed that there is a negative correlation between copper content and total antioxidant activity of honey, and that there is a negative correlation between the copper content and the content of L-ascorbic acid.

In the samples in which the total antioxidant activity was larger, the content of L-ascorbic acid also was larger. Based on this, we can say that the concentration of L-ascorbic acid is in a direct correlation with the total antioxidant capacity of the analyzed honey samples. In contrast, samples with high concentration of L-ascorbic acid have lower copper concentration.

Increasing the concentration of copper in the samples reduces the concentration of L-ascorbic acid, which is directly reflected in the decrease of the total antioxidant activity.

This relationship can be explained by the Haber-Weiss reaction which describes the relationship between L-ascorbic acid and copper. These reactions cause the formation of free radicals, which significantly affects the overall antioxidant activity of honey:

$$2O^{-}_{2} + 2H^{+} \rightarrow H_2O_2 + O_2$$

$$O^{-}_{2} + H_2O_2 \rightarrow OH^{-} + HO \cdot + O^{-}_{2}$$

$$2AH + 2O_2 \rightarrow (Cu/Fe)\ 2A + 2H_2O$$

$$AH_2 + 2Cu^{2+} \rightarrow A + 2Cu^{+} + 2H^{+}$$

$$2Cu^{+} + 2H_2O \rightarrow 2Cu^{2+} + 2 \cdot OH + 2OH^{-}$$

$$3AH_2 + 2O_2 \rightarrow 3A + 2H_2O + 2 \cdot OH$$

Small amounts of soluble copper in the presence of reducing agents (A), such as ascorbate, can catalyze the formation of hydroxyl radicals from superoxide by metal-catalyzed Haber-Weiss reaction. With these reactions, decreases the concentration very significant antioxidant - L-ascorbic acid, and thus the total antioxidant activity of analyzed honey samples. This is example of the complex interactions of substances that influence the chemical composition of honey and which are at the same time antioxidants and pro-oxidant.

4. Conclusion

There is a positive correlation between antioxidant activity and the content of L-ascorbic acid in honey. Negative correlation was demonstrated between copper content and total antioxidant activity of honey. Negative correlation also demonstrated between copper and content of L-ascorbic acid.

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