

THE INFLUENCE OF TEMPERATURE AND DAYLIGHT EFFECT ON CONTENTS L-ASCORBIC ACID IN HONEY FROM BOSNIA AND HERCEGOVINA

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ABSTRACT

Vitamins found in honey have bigger curative activity comparing the synthetically produced. L-ascorbic acid is unstable in the air, in day light and during heating. During the longer storage and food processing L-ascorbic acid declines. L-ascorbic acid is specific because it participates in almost all important biochemical reaction in organism. The work comprise the testing of honey- natural nutritional product which shows healthy effect. L-ascorbic acid was defined by spectrophotometric and volumetric method by Tilsman. The aim of these work is to test thermic stability of L-ascorbic acid in samples of honey and its decomposition under the effect daylight.

KEYWORDS:

Honey, L-ascorbic acid, temperature influence, daylight effect

1. INTRODUCTIONS

Honey contains important biological substances: enzymes, hormones, vitamins and others.[1,17] Honey also contains huge number of vitamins in different quantities.[10] It has been proved that honey contains vitamin B (riboflavin), niacin, folic acid, pantothenic acid and vitamin B₆ in traces and also L-ascorbic acid (vitamin C).[7] Much plants that bees use for their nutrition contains vitamin C. Quantity and kind of vitamins in honey depends on nature of plants where bees collected nectar. The figure 1 shows average percentage of honey composition:

Honey is an excellent media for vitamins, that is not case with the fruits and vegetables.[11] Some researchers have shown that contents of vitamins in honey mostly doesn't change.[7] In honey there are also found some antioxidants. The most important

are flavonoids from which pinocembrin is specific for honey and propolis. L-ascorbic acid, catalasis and selenium also operate as antioxidants. It has been detected that darker kinds of honey have very distinctive antioxidant properties.[7]

Vitamins found in honey have bigger curative activity comparing the synthetically produced. Their effect in organism depends, not only on their quantity but also, on their complex bond with other vitamins and enzymes. Despite the quantity of vitamins in honey, there is no danger of overdose during the treatment of some hypoavitaminosis.

L-ascorbic acid dissolves in the water therefore, it easily absorbed in organism but doesn't store.[14] L-ascorbic acid partial dissolves in alcohol.[6] L-ascorbic acid which is found

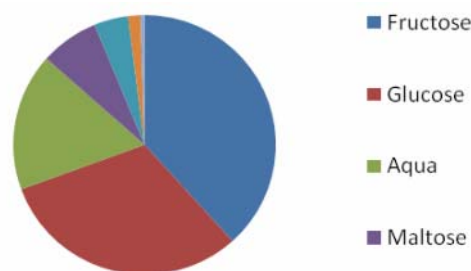


Figure 1. Average percentage of honey composition[7]

in food easily absorbs through active transport in intestinum. Optimal daily doses of L-ascorbic acid for human amounts about 70 mg, twice more than for other vitamins. For cold protection it is recommended daily dose of L-ascorbic acid from 1 to 3 grams. Daily doses from 20 to 30 grams L-ascorbic acid is also used as addition in treatment of malignant diseases.[13] About 80-90% of L-ascorbic acid absorbs in organism from 100 mg of daily usage.[14]

L-ascorbic acid is unstable in the air, in day light and during heating. During the longer storage and food processing L-ascorbic acid declines. L-ascorbic acid is specific because it participates in almost all important biochemical reaction in organism.[15]

Lack of vitamin C has the negative effect on organism, more than lack of any other nutritive ingredient.[15] Biological effect of L-ascorbic acid is based on its redox reactions. It participates in number of reactions of hydroxylation in organism. It has been proved, that L-ascorbic acid participates in hydroxylation of proline and lysine in procollagen. This is important for orderly networking of tropocollagen in collagen fibrils.

L-ascorbic acid is important for maintaining of connective tissue also for healing wounds. Bone tissue also needs L-ascorbic acid because it participates in bone formation. As L-ascorbic acid concentrates in adrenal glands (particularly during the stress) it is considered, as needed in hydroxylation process of corticosteroid.[12] It can serve as reducing agents in nonenzymic reactions. It helps resorption of iron, in that reduces from iron III to iron II in duodenum. L-ascorbic acid prevents oxidation of A, E vitamins and some group B vitamins. It provides the exploiting of folic acid. The shortage of L-ascorbic acid in organism leads to illness-scurvy.

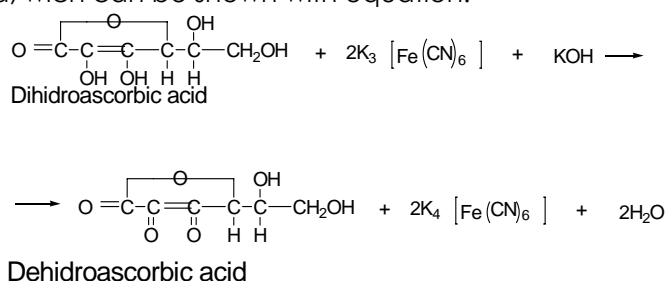
The aim of this work are:

- ✚ To collect the latest cognitions about honey as natural product and its healing effects, therefore about the contents of L-ascorbic acid in different kinds of honey in our area and, its effect on human organism.
- ✚ To choose and check quantitative methods of determining L-ascorbic acid in honey
- ✚ To examine the content of L-ascorbic acid in different kinds of honey produced in the wide area of Bosnia and Herzegovina.
- ✚ To test thermic stability of L-ascorbic acid in samples of honey and its decomposition under the effect of daylight.

2. METHODOLOGY

The work comprises the testing of honey- natural nutritive product which shows healthy effect. Tests include samples of honey from area of Tuzla's canton and wide area of Bosnia and Herzegovina. [8]

Samples of honey are selected in cooperation with The Bee Association of Tuzla Canton. The location of bee's pasture is stated for all samples. L-ascorbic acid was defined by spectrophotometric and volumetric method by Tilsman. Spectro-photometric determination of unknown concentration of L-ascorbic acid is possible only in coloured solution. Therefore it is necessary to do oxidation-reduction reaction of L-ascorbic acid with potassiumhexacyanoferrate (III) in basic media, which can be shown with equation:



The series of standard solution of L-ascorbic acid has been made. On the basis of measured values of spectrophotometric absorption is constructed diagram of absorption-concentration of L-ascorbic acid.

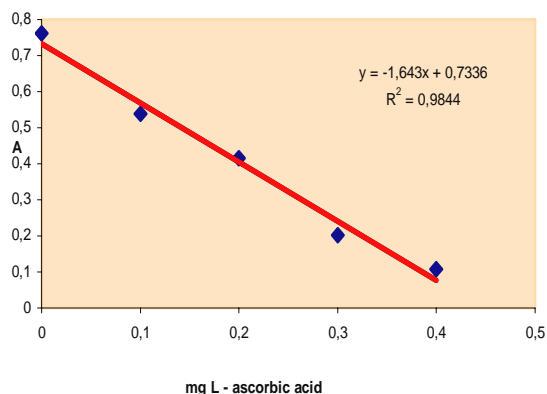
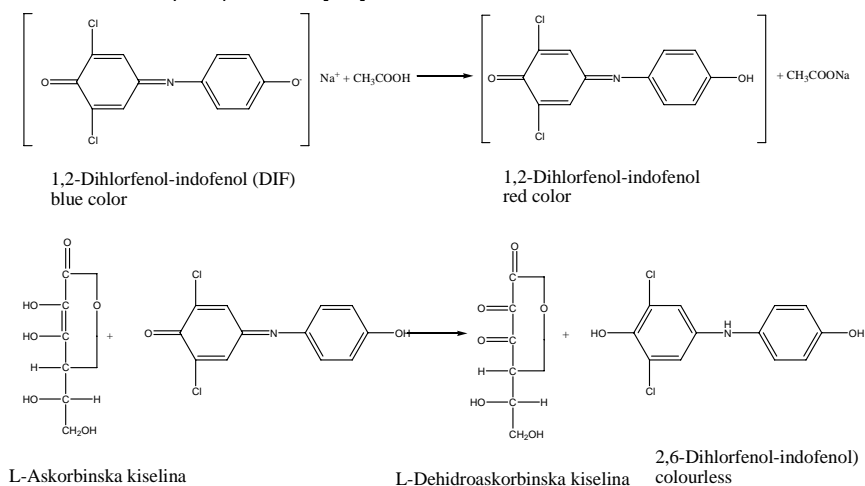


Figure 3. Diagram of absorption-concentration of L-ascorbic acid

Spectrophotometric analysis of randomly chosen samples it is determined that concentration of L-ascorbic acid in honey is important and it reaches detection borders for successful Tilman's determination method.

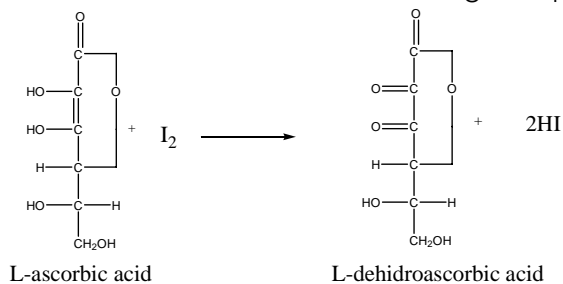
Tilman's method is based on oxidation of L-ascorbic acid to dehydroascorbic acid which can be shown by equations[16]:



Tilman's method is used for determination of L-ascorbic acid in food-stuffs, where its content less than 5000 mg/kg.[16]

Honey samples for analysis, were prepared by weighing 25 grams of honey soluted in 50 ml 0,2 % acetic acid. After that solution is filtrated through filter-paper (glue ribbon) and quantitatively transferred in 250 ml task. In 50 ml of aliquot is added 2-3 drops of concentrated acetic acid and then titrated with the solution of DIF aproximate concentration 5×10^{-4} mol/l until the appearance of pink colour, that remains at least 30 sec.[16]

Accurate concentration of DIF, that is used in this titration, is determined with the help of standard solution of L-ascorbic acid. Its accurate concentration is determined by the titration of standard iodine, according to equation:



Content of L-ascorbic acid in honey is calculated by the following term:[16]

$$X(\text{mg/kg}) = \frac{v_{\text{DIF}}(\text{mL}) \cdot 0,088(\text{mg} \cdot \text{mL}^{-1}) \cdot f_1 \cdot V(\text{mL})}{m(\text{g}) \cdot v_1(\text{mL})} \cdot 1000(\text{g/kg})$$

Where is:

v – volume of used DIF

f₁ – corective factor

V–volume of honey solution (250 mL)

m – mass of honey sample (25 g)

v₁ – aliquot volume honey sample for titration

1 mL 2,6 – dihlorfenolindofenola ($C=5 \cdot 10^{-4}$ mol/L) teoretical is equale with 0,088 mg
L – ascorbic acid (L – a.a)

$$\left[\frac{176g/mol \cdot 5 \cdot 10^{-4} mol}{1000mL} = 0,088mg/mL \right] (L - a.a)$$

3. FINAL RESULTS

In Table 1. are noted physical-chemical characteristic of tested samples of honey which are labeled with the numbers from 1 to 25.

Table 1. Physical-chemical characteristics of analised honey samples

No. sample	Honey sort	Location	Colour	Conzistention	Crystallisation
1	Meadow	Tuzla I	Dark	Dense	Two layer
2	Acacia	Tuzla II	Transcendentally yellow	Liquid	One layer
3	Meadow	Tuzla III	Dark	Liquid	One layer
4	Meadow	Tuzla Plane	Dark	Dense	Two layer
5	Medley	Tuzla Krojčica	Dark	Liquid	One layer
6	Acacia	Tuzla Krojčica	Light yellow	Liquid	One layer
7	Forest	Kladanj Plahovići	Dark	High density	One layer
8	Forest	Kladanj Drinjača	Dark	High density	Two layer
9	Meadow	Kladanj	Transcendentally dark	Dense	One layer
10	Meadow	Miričina	Transcendentally yellow	Dense	One layer
11	Meadow	Mramor	Light yellow	Dense	Two layer
12	Meadow	Fana Srebrenik	Light yellow	High density	One layer
13	Meadow	Gradačac I	Transcendentally dark	Liquid	One layer
14	Acacia	Gradačac II	Light yellow	Liquid	One layer
15	Meadow	Čelić	Light yellow	High density	One layer
16	Acacia	Čelić	Light yellow	Liquid	Two layer
17	Raspberry Linden	Živinice Suha	Transcendentally dark	Dense	One layer
18	Acacia	Zvornik Drinjača	Light yellow	Liquid	One layer
19	Meadow Acacia Linden	Zvornik Glumnina	Light yellow	Liquid	One layer
20	Linden Blackberry	Koraj	Transcendentally dark	Liquid	One layer
21	Acacia	Koraj	Light yellow	Liquid	One layer
22	Acacia	Lopare	Light yellow	Liquid	One layer
23	Meadow	Romanija	Transcendentally dark	Liquid	One layer
24	Acacia	Laništa Posavski kanton	Light yellow	High density	One layer
25	Meadow	Laništa Posavski kanton	Dark	High density	One layer

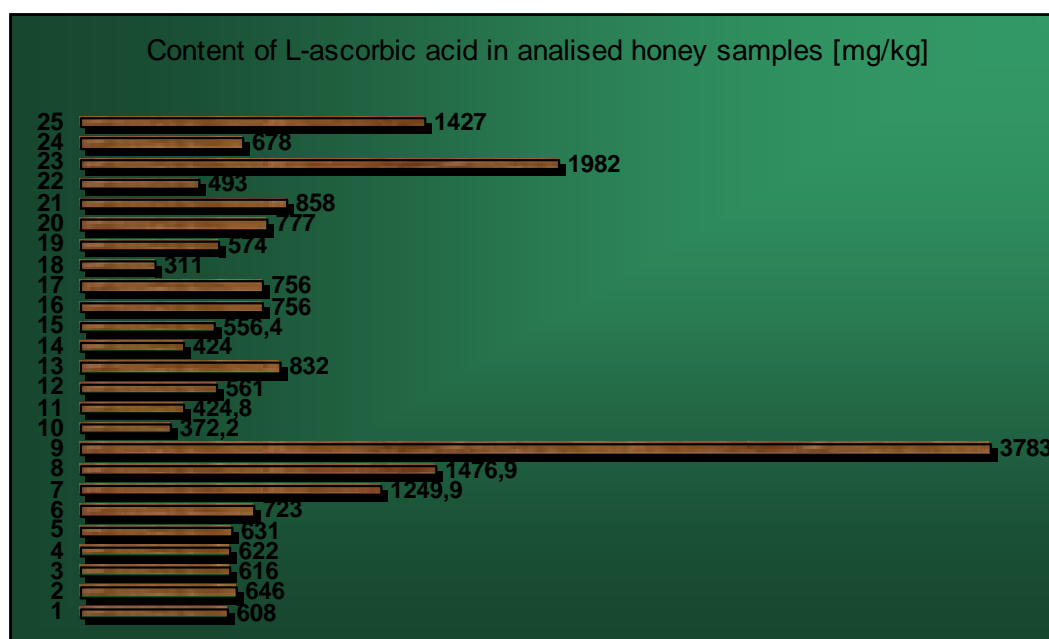


Figure 4. Results of determining the content of L-ascorbic acid in tested honey samples [mg/kg]

Tested honey samples colour is different no matter what kind honey is. Our reaserches show that some honey samples stays one-layered after one year stagnation. The other form two layers with different size of crystals. According to reaserches crystallization of two layers honey is characteristic for samples where content of glucose is less that content of fructosis. Only five honey samples tested formed two layers.

In the Figure 4. are shown the results of deterring the content of L-ascorbic acid in tested honey samples.

The testing results shows that L-ascorbic acid content ranges from 311,0 to 3783 mg/kg. The smalest content is in the acacia honey (Zvornik-Drinjača) and, the biggest is in meadow honey (Kladanj).

Our reaserches are compatibile with the reaserches of scientist Nikoleta M. Et associators.

L-ascorbic acid is water solubly vitamin and it has significant antioxidant effect. This is only vitamin that cannot overdose in nutrition. L-ascorbic acid is inputed in human organisam through food because human organisam cannot synthetisize L-ascorbic acid its own. L-ascorbic acid is photosensitive substance, and the effect of daylight was tested on its decomposition in honey. The results of honey samples (linden, forest, meadow, mixed and floral) show that decomposition degree of L-ascorbic acid for a year period makes from 0,21 to 2,59%.

In Table 2. are shown L – ascorbic acid in honey solution honey samples with the effect of daylight during the period of ten days.

Table 2. Results of determination of L-ascorbic acid concentration in randomly choosen honey samples after ten days daylight effects

No. samples	Content of L – ascorbic acid in prepared honey samples solutions [mg/kg]									
	Degree of L – ascorbic acid degradation [%]									
	I day	II day	III day	IV day	V day	VI day	VII day	VIII day	IX day	X day
7	1249.9	767.2	732.7	724.1	439.6	258.6	249.9	241.4	241.4	241.4
	-	38.6	41.4	42.1	64.8	79.3	80.0	80.7	80.7	80.7
8	1476.9	336.2	336.2	320.7	320.7	318.8	318.8	314.9	241.4	241.4
	-	77.2	77.2	78.3	78.3	78.4	78.4	78.7	83.7	83.7
10	372.2	310.1	261.2	253.7	186.1	180.4	152.2	140.9	140.9	109
	-	16.7	29.8	31.8	50.0	51.5	52.1	62.1	62.1	70.7
11	424.8	208.6	148.5	140.9	124	112.7	112.7	109	107	107
	-	50.9	65.0	66.8	70.8	73.5	73.5	74.3	74.8	74.8
15	556.4	225.6	202.9	202.9	197.3	154.1	150.4	135.3	129.7	112.7
	-	59.5	63.5	63.5	64.5	72.3	72.9	75.7	76.7	79.7

In five selected honey samples solutions, concentration of 100 g/L, the concentration of L-ascorbic acid was tested everyday in 10 day period. In all samples decrease L-ascorbic acid concentration was noted. Decomposition degree ranges from 12,02 to 86,37%. The results show that L-ascorbic acid, in honey solution, is very unstable, and it oxidates in the air easily, transforming into biologically inactive form. Comparing the contents of L-ascorbic acid in honey samples and solutions of the samples, it can be concluded that decomposition degree is bigger in honey samples solutions than in the honey itself. In ten days only honey solutions decomposition degree of L-ascorbic acid in honey samples amounts 74,64%, and a year amounts is 1,40%.

The biggest decrease of L-ascorbic acid concentrations in honey solutions, is between the 1st and second day (50,35%). After second day L-ascorbic acid concentration almost doesn't change, so there was no need for further mesurments didn't make sense.

The Figure 5. shows the testing results of L-ascorbic acid under the effect of daylight in other honey solutions.

The Figure 6. shows average decrease of L-ascorbic acid concentration in tested honey solutions, after the solutions were exposed to daylight.

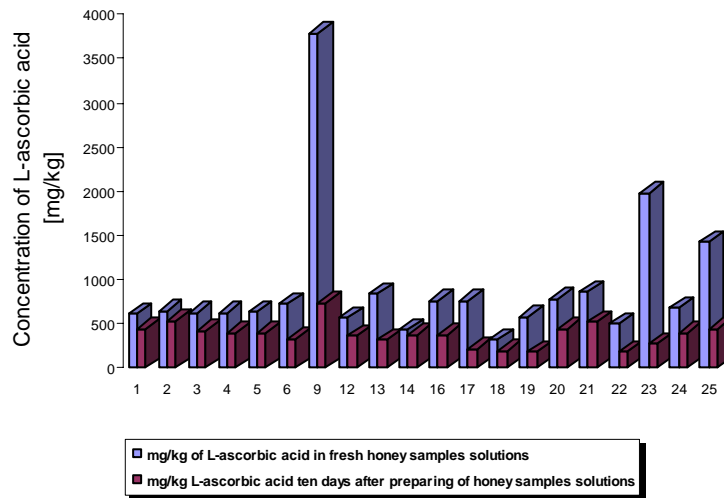


Figure 5. Change of concentration of L-ascorbic acid in honey sample solutions (100 g/L) with effect of daylight during the period of ten days

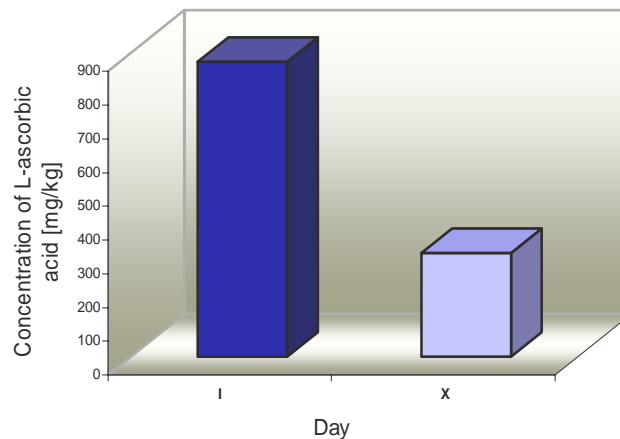


Figure 6. Average content of L – ascorbic acid in fresh state of honey samples (I) and ten days after of daylight effects (II)

According to the results we can conclude that average decrease of L-ascorbic acid concentration after the daylight effect in ten days period amounts 68,36%. Parallel with the content analysis of L-ascorbic acid in honey samples, also the change of L-ascorbic acid in its standard concentration solution about 1000 mg/L is observed. Figure 7 shows this.

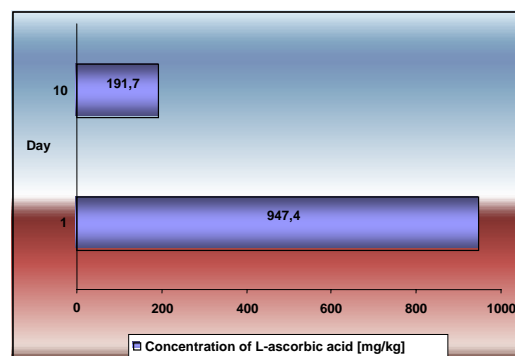


Figure 7. Decrease of L – ascorbic acid concentration in standard solution (C=947,4 mg/L)

In ten days period concentration of L-ascorbic acid in its standard solution decreased 57 times more than in analysed honey samples. It is evident that concentration decrease of L-ascorbic acid in its standard solution is bigger than in tested honey solutions. This indicates higher stability of L-ascorbic acid in honey than in its standard solution. L-ascorbic acid (vitamin C) count oneself as termolable vitamins. Temperature rising leads to its decomposition.[2,5] Because of this termolability of L-ascorbic acid is tested in honey solutions with concentration of 100 g/L. Results are shown in the Figure 10.[2]

Table 3. Results of L-ascorbic acid decomposition in honey samples with the effect of boiling

No. sample	Content of L – ascorbic acid in prepared honey samples solutions before boiling [mg/kg]	Content of L – ascorbic acid in prepared honey samples solutions after boiling [mg/kg]	Degree of L – ascorbic acid degradation [%]
1	608,0	481,0	20,9
2	646,0	494,0	23,5
3	616,0	459,0	25,5
4	622,0	454,0	27,0
5	631,0	418,0	33,8
6	723,0	439,0	39,3
7	1249,9	1017,2	18,6
8	1476,9	994,2	32,7
9	3783,0	706,0	81,3
10	372,2	259,0	30,4
11	424,8	173,0	59,3
12	561,0	392,0	30,1
13	832,0	597,0	28,2
14	424,0	373,0	12,0
15	556,4	203,0	63,5
16	756,0	589,0	22,1
17	756,0	371,0	50,9
18	311,0	209,0	32,8
19	574,0	358,0	37,6
20	777,0	493,0	36,6
21	858,0	527,0	38,6
22	493,0	236,0	52,1
23	1982,0	1486,0	25,0
24	678,0	362,0	46,6
25	1427,0	378,0	73,5

The aim of this work is testing the content of L-ascorbic acid in honey as natural product which has healing effect. This research conducted 25 samples of honey, 17 of them comes from rthe area of Tuzla's Canton and 8 of them from wide area of BIH.

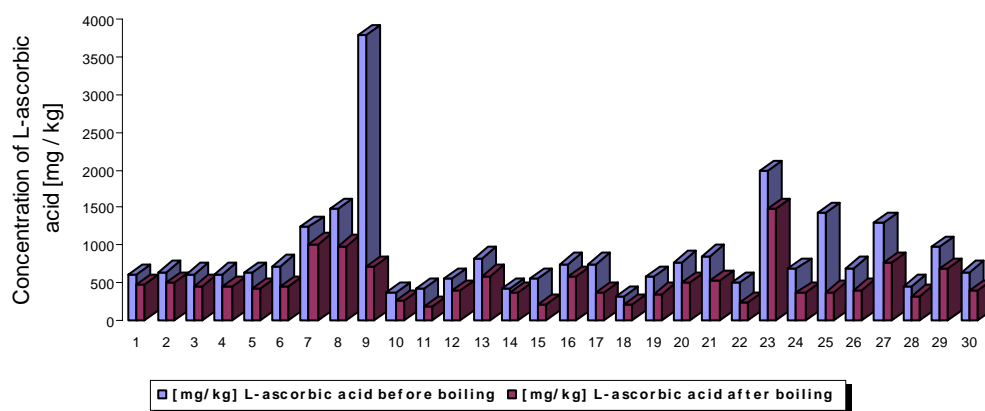


Figure 8. L – ascorbic acid concentration in prepared honey sample solutions before and after boiling

Concentration of L-ascorbic acid in tested honey solutions, after cooking, decreased for 42,64 %. These results show that L-ascorbic acid present in honey is decomposed in hot potion. Our results correspond with the results of Nikoleta Matei, Samaghiul Birghila, Simona Dobrinas i Patre Capota.[3]

4. CONCLUSIONS

Our researchers show that content of L-ascorbic acid in tested honey samples ranges from 311,0 to 3783,0 mg/kg, and it is bigger in dark samples of honey for 48,8 %. L-ascorbic acid is very stables in honey samples. After the exposure of daylight in a year period only 1,42 % of L-ascorbic acid was decomposed. Content of L-ascorbic acid in honey solutions after the exposure of ten days decrease in average for 68,36 %. In standard solution of L-ascorbic acid it decreased for 79,76 %. By cooking fresh made honey solutions, content of L-ascorbic acid decreases, in average for 42,64 %; and comes to its decomposition. Honey is remarkably significant nutritient that can provide needed quantity of L-ascorbic acid, especially when it is about dark types of honey.

REFERENCES:

- [1] Acta Chimica Slovenica, 2004; 51: 169-175.
- [2] Hariss C D. Lahrbuch der Quantitativen Analyse. Friedr.Vieweg & Sohn Verlagsgesellschaft mbH , Braunschweig/Wiesbaden, 1998.
- [3] Kesić Aldina „Identifikacija i kvantifikacija L – askorbinske kiseline i biogenih mikroelemenata (Fe, Cu i Zn) u medu; Magistarski rad, 2007
- [4] Matei N, Birghila S, Dobrinas S and Capota P. Determination of C Vitamin and Some Essential Trace Elements (Ni, Mn, Fe, Cr) in Bee Products.
- [5] Mazalović M. Medicinska hemija II. Tuzla. 1998;
- [6] Mikota K S and Plumb D C. Ascorbic Acid. Elephant Care International. 2003;
- [7] Mladenov S. Apiterapija i osnovi pčelarstva. Beograd: 1999;
- [8] Mučibašić B. Geografski atlas Bosne i Hercegovine. Sarajevo 1998.
- [9] Passmore R. The Complete Book of Vitamins. Brana Š Borislav B i sar. 1987; 209-261.
- [10] Qui P Y, Ding H B, Tang Y K and Xu R J. Determination of Chemical Composition of Commercial Honey by Near Infrared Spectroscopy. J Agri Food Chem. 1999; 47: 2760-2765.
- [11] Radtke J and Hadtke C. Gehalt an Ameisensäure und Freien Säuren in Honig nach Sommerbehandlung mit Ameisensäure. German Bee Research Institutes Seminar In: Apidologie 1998; 29: 404-405.
- [12] Stojić R V. Fiziologija životinja. Beograd. 1995;
- [13] Štraus B. Medicinska biokemija 2. Zagreb. 1992; 778-808.
- [14] The Chemistry of Bees file:///A:/The%20Chenistry%20Bees%202.htm
- [15] Vitamin C in Human Health is Still a Mystery. Nutrition Journal. 2003;
- [16] Vračar Lj. Priručnik za kontrolu svježeg i prerađenog voća, povrća i pečurki i osvježavajućih bezalkoholnih pića. Novi Sad. 2001;
- [17] Yilmaz H and Yavuz Ö. Content of Some Trace Metal in Honey from South-Eastern Anatolia. Food Chem. 1999; 65: 475-476