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Biochemistry

Antioxidant Activity of Chestnut Honey Produced in Western Georgia

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ABSTRACT. The paper deals with the study of chemical composition, recommended by Euro Regulation, diastase and antioxidant activities and electroconductivity of chestnut honey produced in the regions of Western Georgia. As a result of the research, it was found that the titanium acidity of chestnut honey is 20.8-44.6 ml / 100 g, and pH is 4.5-4.97. Dry substance content is not less than 80%, correspondingly, the water content is not more than 20%. The electro conductivity is 1.34-1.98 ms/cm, and the correlation of ash is 1 - 2.5%. HPLC was determined as the dominant carbohydrate fructose, the content of which exceeds 40%, while the content of glucose is up to 30 %. The sucrose content does not exceed 2%, the diastase activity in all the honey is not less than 8 Shade units, antioxidants (dilution 1-100) exceed 50%. *© 2018 Bull. Georg. Natl. Acad. Sci.*

Key words: honey**,** euro regulation, chemical composition, biological activity

Honey is a natural product that is obtained as a result of the collection of flower nectar or pollen by bees (*Apis mellifera*), its transformation and ripening of honeycombs [1]. Its composition, quality, color, aroma and taste mainly depend on the honey plants, their geographical location, climate, bee type, processing, packing and storage conditions [2,3].

Honey consists of sugars (fructose, glucose, sucrose, maltose, turannosa, isomalotase and others) [4-6], enzymes (invertase, amylase, glucosoxide, catalase), amino acids, carotenoids, vitamins (vitamin B6, thiamine, riboflavin, pantotenis acid, etc.), elements ions (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), as well as aromatic compounds. Honey is also rich in biologically active phenolic compounds - flavonoids and phenolcarbic acids, which manifest high antioxidant activity [1,3].

Honey is characterized by high price and growing popularity both all around the world and in Georgia. Therefore, the cases of honey adulteration are quite frequent. Therefore it is necessary to monitor the standards and norms for identifying the honey quality and ensuring its safety in the internal and external markets [4]. The identity and quality of honey are internationally regulated by the Codex

Table 1. A sample of chestnut honey taken for analysis

Samplers	Samplers code	Sampling Time	Sampling Place
Chestnut Honey 1	CH- 1	2011	Guria, vil.Atsana
Chestnut Honey2	CH – 2	2011	Keda, vil. Otaladzeebi
Chestnut Honey3	CH – 3	2012	Keda, vil. Jalabashvilebi
Chestnut Honey4	CH - 4	2012	Keda, vil.Kharaula
Chestnut Honey5	CH - 5	2013	Kobuleti, vil. Chakvi
Chestnut Honey6	CH - 6	2016	Keda, vil.Kharaula
Chestnut Honey7	CH - 7	2016	Keda, vil. Akho
Chestnut Honey8	CH - 8	2016	Keda, vil. Jalabashvilebi
Chestnut Honey9	CH - 9	2016	Keda, vil. Abuketa
Chestnut Honey10	CH - 10	2017	Keda, vil.Tskhmorisi
Chestnut Honey11	CH - 11	2017	Keda, vil. Simoneti
Chestnut Honey12	CH - 12	2017	Batumi, Botanical Garden
Chestnut Honey13	CH - 13	2017	Tsageri, vil. Dekhviri
Chestnut Honey14	CH - 14	2017	Lechkhumi

Alimentarius (Codex Standard for Honey, 2001), which define the maturity and purity of honey. The honey maturity is measured by sugar and water content, acidity, diastase activity and hydroxymethyl furupurate [7,8], while its purity is determined by sludge content, electrical conductivity and water insoluble solids. [9] It is important to determine the honey plant origin, the content of various bioactive compounds and the antioxidant activity caused by these compounds [10-13].

The goal of the research is to study and analyze the quality characteristics of honey (color, taste, aroma, quantity and amount of carbohydrate, water mass, electric conductivity, gray, active and titrated acidity, diastase activity, common phenols and antioxidant activity) produced on the territory of Western Georgia.

Materials and Methods

14 samples of chestnut honey (ChH) were harvested in different regions of western Georgia - Adjara, Guria, Imereti (Table 1). The samples of 2011, 2012, 2013, 2014, 2015, 2016 and 2017 were analyzed. The honey specimens were kept in containers made of sealed polyethylene at 4-5 ° C. Liquid or crystallized honey without impurities was homogened for 3 minutes. The honey with impurities was filtered in a stainless steel grating with a diameter of 0.5 mm before its homogenization [4].

The moisture was measured by (Misco) Refractometer pH and titrated acidity with a potentiometer method (Metller Toledo), for which the sample of 10 gr of honey was dissolved in 75 ml of water free of CO_2 (6,7).

Electro conductivity was determined by a conductometer (**Mettler Toledo**), for which 80 ml of deionized water was added to the sample of 20 g, while the sludge was determined by a gravimetric method (550^0C in Muffel stove). The spectral method **(Mettler Toledo UV5A**) was used for determination of diastase activity. 10 g honey sample was completely dissolved in 15 ml of water and 5 ml acetate buffer, and added to 3 ml NaCl and then filled up to 25 ml. The starch solvent was used as a reaction substrate, and the optical density is 660 nm [4].

Common phenolic compounds were defined by a spectral method using Folin-Ciocalteu reagent (**Mettler Toledo UV5A**). 1ml of water extract, obtained from 10 g of honey, was added to 1 ml of

Table 2. Chestnut honey characteristics of water, dry substances, free acids, diastase activity and pH in western Georgia

	Name	Total Carbohydrate, g/100 g	Moisture (%)	Free acidity, me./100g	pH	Diastase activity (Shade)
1	CH – 1	81.1	18.9	27.88	4.52	19
2	CH – 2	80.7	19.3	30.34	4.83	20
3	CH – 3	81.0	19.0	22.96	4.87	20
4	CH – 4	81.1	18.9	43.36	4.71	>8
5	CH – 5	79.8	20.2	26	4.53	>8
6	CH – 6	79.4	20.6	32.8	4.84	>8
7	CH – 7	80.6	19.4	27.88	4.9	20
8	CH – 8	81.8	18.2	32.8	4.97	23
9	CH – 9	80.7	19.3	29.52	4.65	12
10	CH – 10	80.6	19.4	31.98	4.8	20
11	CH – 11	81.0	19.0	37	4.5	18
12	CH – 12	80.8	19.2	44.6	4.53	25.0
13	CH – 13	79.1	20.9	21.2	4.94	11
14	CH – 14	80.6	19.4	20.8	4.53	18

Table 3. Quality and quantity of chestnut honey carbohydrates in Western Georgia

	Name	Fructose	Glucose	Sucrose	Maltose	Total
1	CH - 1	40.53	34.39	1.50	2.96	79.38
2	CH - 2	40.78	30.96	1.81	5.43	78.98
3	CH - 3	41.17	31.51	2.28	3.92	78.88
4	CH - 4	41.77	32.11	2.33	2.49	78.69
5	CH - 5	41.49	32.15	1.93	3.41	78.98
6	CH - 6	41.68	25.90	2.02	7.80	77.40
7	CH - 7	41.52	31.67	2.37	3.20	78.76
8	CH - 8	42.04	32.68	2.21	2.68	79.62
9	CH - 9	43.65	29.51	2.23	3.48	78.87
10	CH - 10	42.96	31.81	1.50	2.59	78.86
11	CH - 11	42.55	29.88	1.65	5.43	79.50
12	CH - 12	40.73	32.43	1.41	4.63	79.20
13	CH - 13	40.85	30.45	1.55	4.16	77.01
14	CH - 14	41.58	30.13	2.75	4.42	78.88

Folin-Ciocalteu reagent. After 3 minutes, 10% $Na_2 CO_3$ 1 ml was added to the mixture and the volume was increased to 10 ml by adding water, and after 90 minutes, the optical density was determined at 725 nm. The obtained result was displayed by recalculation of glycerin – (mg / kg) in honey [10].

Antioxidant activity was determined by DPPH (2,2-Diphenyl-1-picrylhydrazil free radical) (Free Radical-Scavenging Activity) using 50% inhibition method. After a 15-minute exposure of honey extract and a mixture of radicals, the optical density was determined at 517 nm. The received results were expressed in % [11, 12].

Quality and quantitative analysis of carbohydrates in honey was carried out with high pressure liquid chromatography. - (HPLC-Waters Breeze RI Detector 2414) Column - Carbohydrate, Elutin - 75% AcCN.

Materials and Methods

The research of biochemical indicators was carried out by physico-chemical and instrumental methods. The division and identification of compounds and their quantitative analysis were carried out using the following equipment: HPLC (Waters Brceze 1525, RI 2414 detectors), pH-meter (Mettler

Table 4. General phenols. antioxidant activity. sludge and electrical conductivity of chestnut honey

	Name	Total phenols mg/kg Folin-Ciocalteu	Antioxidant activity %. DPPH (50% inhibition)	Ash. %	Conductivity (μs/sm)
1	CH - 1	148.60	39.30	2.0	1.6133
2	CH - 2	271.26	47.30	1.79	1.4135
3	CH - 3	276.01	50.56	1.85	1.371
4	CH - 4	387.44	52.87	0.89	1.036
5	CH - 5	350.01	51.20	2.16	1.6456
6	CH - 6	352.80	51.96	1.07	1.1143
7	CH - 7	302.20	41.02	2.45	1.6665
8	CH - 8	461.50	66.17	2.71	1.9162
9	CH - 9	278.87	54.11	1.55	1.243
10	CH - 10	201.50	44.49	1.62	1.306
11	CH - 11	408.80	52.50	1.55	1.3876
12	CH - 12	503.50	58.70	2.07	1.6236
13	CH - 13	378.80	55.20	1.75	1.243
14	CH - 14	80.60	19.40	1.86	1.548

Toledo), Refractometer -Misco, Spectrometer – Cuvette Changer (Mettler Toledo UV5A), Reactive – stability radical- 2,2-Diphenil-1-picrilhydrazyl (Aldrich-germany), AlCl3, Folin Ciocalteu reagent (preparation), standards –Callic acid, quercetin, rutin. C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg).

Results and Discussion

All 14 samples of chestnut honey taken for the analysis are dark in colour, have a bitter-sweetish taste, weak aroma and very specific smell of a chestnut flower, peculiar only to this honey.

The water content in all samples was 17.8 to 20.0%, which is within ≤ 20% of the established international norms. The highest indicator was in honey obtained in Kobuleti (Chakvi), what can be due to a high relative humidity of the region. Accordingly, the total content of sugar in the chestnut honey is 80.0-82.3% (the average indicator 81.15%), what also corresponds to the requirements of the Regualtion. The content of free acids varies between 20.8-44.6 m/100 g, pH is approximately 4.5-4.97 (Table 2), which is within the norm.

The diastase activity is an important feature during determining the quality of honey. This indicator can often significantly change under the influence of many factors (smoking space, storage time and conditions). It should be noted that the diastase activity of all samples of the chestnut honey (according to Shade) exceeds 8 units, which is one of the most important proofs that the analyzed honey is natural.

High-pressure liquid chromatography method was used to study a qualitative and quantitative composition of honey carbohydrates. The dominant 4 carbohydrates-glucose. fructose. sucrose. maltoza were identified. Among them the dominant was sugar fructose. which represents 50 - 55% of the total number of carbohydrates in all samples. while 35 - 42% is glucose, sucrose is from 1.4% - 2.75% and malosa 2.4-7. 5%. It should be noted that the sucrose does not exceed 2.75% in any sample, what also confirms the naturalness of all samples (Table 3).

Besides the qualitative characteristics, the total number of phenolic compounds and their antioxidant activity were also studied in the samples. These data are slightly different in the region. The honey obtained in the Botanic Garden in Batumi (ChH 12) is distinguished by comparatively high phenolic compounds. Next, there is the harvest of 2016 in the Keda District (ChH 8). while the lowest content was fixed in the Lechkhumi region in 2017 harvest (ChH 14). However, the sample ChH 8 is characterized by the antioxidant activity in comparison with the rest 13

samples. In other cases the antioxidant activity is correlated with the number of phenolic compounds (Table 4), what in some sense indicates that their overall quantitative content does not fully determine its antioxidant activity, which also depends on the qualitative composition. There is a certain correlation between electro conductivity (1.34-1.98 μS / cm) and the content of sludge in honey (1-2.5%). The increase in the amount of sludge leads to the increase of electro conductivity (Table 4).

Conclusion

14 specimens of chestnut honey, harvested in 12 villages of four regions of Western Georgia, were studied and analyzed. The samples of chestnut honey are characterized by the aroma and taste peculiar to chestnut. All samples satisfy the standard indicators drawn according to the recommendations of the Euroregulation: the free acidity of honey is 20.8-44.6 mm/100g, pH is 4.5-4.97. The electrical conductivity ranges from 1.34-1.98 μS/cm; the content of dry substance is no less than 80%; the diastase (Shade) number is at least 8 units. 4 Carbohydrates – Glucose, Fructose, Sucrose, Maltose were identified by HPLC method. The quantitative ratio in each sample was also studied. The identification of sludge, common phenols and antioxidant activity confirms the naturalness of the samples.

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ბიოქიმია

დასავლეთ საქართველოს წაბლის თაფლის ანტიოქსიდანტური აქტივობა

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შესწავლილია დასავლეთ საქართველოს მეფუტკრეობის რეგიონებში წარმოებული წაბლის თაფლის ქიმიური შედგენილობის ევრორეგულაციით რეკომენდებული მაჩვენებლები, დიასტაზური და ანტიოქსიდანტური აქტივობები და ელექტროგამტარებლობა. დადგენილია წაბლის თაფლის ტიტრული მჟავიანობა 20,8-44,6 მლ./100გ, pH კი 4,5-4,97. მშრალი ნივთიერების შემცველობაა არანაკლებ 80%, შესაბამისად წყლის შემცველობა არა უმეტეს 20%-სა. ელექტროგამტარებლობის მახასიათებელი 1,34-1,98 მილისიმენსი/სმ, ხოლო მასთან კორელაციაში მყოფი ნაცარი 1-დან-2,5%-მდეა. HPLC იდენტიფიცირებულ იქნა როგორც დომინანტი ნახშირწყალი ფრუქტოზა, რომლის შემცველობა 40%-ს აღემატებოდა, ხოლო გლუკოზა 30%-მდეა.საქაროზა 2%-ს არ აღემატება, დიასტაზური აქტივობა ყველა თაფლში არანაკლებ 8 შადეს ერთეულია, ანტიოქსიდანტობა (1-100 გაზავებით) 50%-ს აღემატება.

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