

Antibiotics and heavy metals in Georgian honey

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ABSTRACT

Georgia is considered the oldest producer of honey. Honey is a therapeutic and preventive product, so it is important to study the content of antibiotics and heavy metals, since their presence adversely affects human health. Using HPLC-UV and Vis UPLC-MS methods, we have studied 12 antibiotics in the samples of chestnut, acacia, lime, field and polypropylene (canned) honey produced in Western Georgia. The research has shown that the use of antibiotics is impractical. In the analyzed 50 samples of honey the content of heavy metals - Cd, As, Cr, Hg, Zn, Pb is below the allowable rate.

Keywords: Honey, Antibiotics, Heavy metals, Therapeutic, Mineral content, Pesticides.

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Background

Georgia is one of the historical places of bee-keeping and honey production. Honey is produced according to different vegetation (monofloric, polypropylene, lime, acacia, chestnut) and location (meadow, alpine, etc.) origin. For Georgia honey is one of the products that can be exported to the EU market. International honey standards are specified in a European Honey Directive and in the Codex Alimentarius Standard for Honey. The article deals with the present knowledge based on the different quality criteria. The standard drafts include standards and methods for the determination of the following quality factors: moisture, ash, acidity, HMF, apparent reducing sugars, apparent sucrose, diastase activity and water-insoluble matter. International honey standards for fructose/glucose content, the sucrose content and electrical conductivity are proposed. There has also been discussed the use of other quality factors, such as invertase activity, proline and specific rotation, used in many countries [1]

Honey is used in medicine as a therapeutic and prophylactic agent for coughing, stenocardia, skin ulcers, stomach ulcers and in other cases [2-4], but there may be an excess of antibiotics and heavy metals in honey, due to which this important product may become quite dangerous.

Antibiotic waste is perceived as a serious problem, because the waste products themselves can cause a toxic, allergic and other hypersensitive reaction to the consumer [5], as well as skin irritation, dermatitis, gastrointestinal irritation, very low dosage of anaphylaxis [6].

Waste adversely affects the so-called positive microorganisms, and some (nitrofurans, nitromimazole) of them can be carcinogenic and cause cancer. Even a small amount of them in honey can cause microbial resistance [7].

The main causes of contamination of honey with antibiotics are the beekeepers, who improperly use antibiotics against bacterial diseases, and the bees themselves. Many international scientific works deal with the above issues. Honey contains an excess of the norm [8,9] of Oxytetracycline and Chloramphenicol, but because of its resistance to these antibiotics, there are often used such antibiotics as Erythromycin, Lincomycin and Streptomycin. Various antibiotics were found in honey gathered in different countries. Thus, honey collected on the coast of the Marmara Sea (Turkey) often contained from 50 to 1700 ng / kg of antibiotics. At the same time, their content was maintained after 3 months as well [9]. As it was proved in Belgium, the greater the amount of Sulfonamide was in combs, the greater its content was in honey [10]. In the case

of Lincomycin, its concentration was 24 mg after 3 days after the start of treatment, 3.3 mg after 4 months and 1 mg after 1 year [11].

The content of 5 antibiotics - Tetracycline, Oxytetracycline, Doxycycline, Chlorotetracycline and Chloramphenicol, has been studied in China; the minimum amount was 10 mg / km [12]. The high content of antibiotics has also been noted in honey brought from India to the EU and the USA. Streptomycin, Tetracycline, Sulfonamide [13,14] were found in 20% of the tested samples: in Greece and France, Tetracycline metabolism wastes [15], in the UK - Oxytetracycline [16], in Switzerland - antibiotics as a degradation product when using herbicides [17], in Spain - Tylosin, Sulfamidine and Sulfachloropyridazine [18]; more than 20% of honey samples in Germany contained Streptomycin [19], in Asia - Chloramphenicol [20,21], and in Turkey - Sulfonamide and Tetracycline [22].

Many EU countries, such as Switzerland, Great Britain, Belgium, etc., prohibit the use of antibiotics in beekeeping. The minimum limit values for antibiotics are set to be from 0.01 to 0.05 mg / kg [23]. The negative effects of antibiotics, pesticides and their metabolites on human health were studied [24, 25] as well.

The mineral content of honey depends on many factors - environmental conditions, botanical and geographical origin, and others [26]. A part of heavy metals gets into honey from the environment, and a small part comes from machine installations used during technological processes. The content of heavy metals in honey is much less in a clean environment, since the way they get into honey is not only the environment, but also nectar obtained from the plant grown in it [27, 28].

Many countries study honey samples in heavy metals [29-31]. According to FAO / WHO reports, there are cases when the amount of heavy metals in honey is at the tolerable limit [32,33].

The amount of Zn is undesirable, although a small amount is a necessary component for many drugs used against human diseases. Its especially large amount is contained in Malaysian honey (4.70 and 173.77 mg / kg), much less than it is in the honey collected in Italy (3.1 mg / kg), Spain (3.9 mg / kg), Turkey (2.7 mg / kg), Ireland (5 mg / kg) and India (12.69 mg / kg) [34-38].

The Cd content in Malaysian honey is maximum 1.03 mg / kg, and in the famous Manuka honey its content is slightly lower (1.01 mg / kg), Greek and Indian honey [39] is also characterized by low Cd content; it is slightly more in Turkish honey. The

honey, collected in honeycombs in the vicinity of where the railway passes, contains a large amount of Pb, which is characterized by high toxicity [40,41]. Co concentration is rather low in Malaysian and Indian honey and relatively high in Turkish one [42].

European regulations rightly prohibit the ingestion of antibiotics into honey and limit the presence of heavy metals in it. The intensification and diversification of agriculture have radically changed the problems of beekeeping.

Improper use of drugs in agriculture causes bee disease. Antibiotics are often used in an unbiased way for medical treatment. The problem is also complicated by the fact that most of these drugs are not registered in Georgia. After the association of Georgia in the EU, the requirements of the Euro regulation were recognized, and as a result of the ingestion of antibiotics and heavy metals in honey, this useful product remains unrealized on the market, while honey, produced in Georgia, is distinguished by a large number of antioxidants [43].

The objectives and goals of the work is to study the quantitative content of west Georgian Honey Antibiotics and Heavy metals.

Objectives and Methods

Research material

There have been studied the 50 samples of various botanical origin honey gathered in Western Georgia (Adjara, Guria, Samegrelo and Imereti), among which there are 10 samples of chestnut, 10 - lime, 10 - acacia, 10 - polyflora (meadow) and also 10 samples of polyphora, collected in semi-wild conditions [44].

Sample preparation

Samples for concentrating antibiotics were processed in the following way: 3-5 ml of honey were released into the clipboard, then samples were placed in Waters Sep-Pak C18 (500 mg); All samples were filtered before analysis; the Filter Waters Acrodisc LC PVDF Filter 13 mm 0,45 µm was used for samples filtering.

Used techniques and chemical compounds

The experiment has been conducted in the Chemical Analysis and Food Security Department of Agricultural and Membrane Technologies Institute of Batumi Shota Rustaveli State University and the Chromatographic Center of Western Georgia.

The research of biochemical indicators was carried out by physico-chemical and instrumental methods. The division-identification analysis of the compounds required: UPLC-MS-PDA (Waters Acquity QDadetector), HPLC (Waters Breeze 1525, UV-Vis 2489 detectors), HPLC (Waters Breeze 1515, Conductivity detectors), PH- Meter (Mettler Toledo); Conductometer (Mettler Toledo); C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg), Chemicals –Metals cations (Merck-Germany). Metals cations analysis was conducted by ICP-MS.

HPLC-UV, Vis UPLC-MS

Antibiotics research is based on chromatographic methods, using ultra violet and mass-detectors, which allow to reveal even the smallest amount of them (0.1 ppb).

The analysis of antibiotics was conducted by HPLC, in the C18 analytical and preparatory column C18. The solution A: Acetonitrile, The solution B: Water-pH (8.5) (B) adjusted with 0.01% ammonia, the gradient (0-7 min- 20%-from 0%B, 8 min 0% B, 10-12 min 20% B, 14–20% B). UPLC-MS the analysis BEN C18, 1.7 μ m, BEN Amide 1.7 μ m, column. Flow 0,4 ml/min, column temp 50 °C, MS- scan 200–1200 da, Probe 600 °C, Positive (Negative) 0,8 kV, Capilari 1,5 kV, CV -15. The DAD detector monitoring (220-400 nm), the sample injection volume was 10 μ l.

The sample was analysed by HPLC equipped with UV detector using a Bridge C₁₈ 5 μ m (150 x 4.6 mm I.D.) column in gradient conditions in gradient conditions given below, with mobile phase - Acetonitrile (A) and Water-pH (8.5) (B) adjusted with 0.01% ammonia. The gradient (0-14 min- 20%-from 0%B, 16 min 0% B, 20-24 min 20% B, 25–80% B). Flow (1 ml/min).

Results and discussion

The following 12 antibiotics are mainly used in beekeeping in Georgia: Metronidazole (Retention Time-6,759), Ronidazole (RT-8,228), Erythromycin (RT-8.868), Nitrofurantoin RT-11,712), Lincomycinum (RT-12.185). By UPLC-MS method there have been identified: Oxytetracycline (m/z-459.14, m/z+461,14), Tetracycline (m/z-443.15, m/z+445.16), Streptomycin (m/z-580.25, m/z+582.27), Sulfadimethoxine (m/z- 309.06, m/z+311.08), Chloramphenicol (m/z- 321.00, m/z+323.01), Metroni-

dazole (m/z-170.05, m/z+ 172.07), Ronidazole (m/z- 199.04, m/z+201.06), Erythromycin (m/z- 732.45, m/z+ 734.47), Nitrofurantoin (m/z-112.00, m/z+114.01), Lincomycinum (m/z- 405.21, m/z+407.22), Tylosin (m/z-914.51, m/z+916.52), Rifampicinum (m/z- 821.39, m/z+823.41) (Fig).

The conducted analysis has shown that antibiotic-Oxytetracycline is observed in 20% of the chestnut honey samples, in 30% of lime ones, in 30% of acacia ones and in 20% of meadow honey its quantity is more than the allowable rate; Tetracycline is in 30 % of chestnut honey samples, in 20% of lime ones, in 40 % of acacia ones and in 20% of meadow ones it is more than the allowable rate; Streptomycin is in 30 % of chestnut honey samples, in 30% of lime ones, in 50 % of acacia ones and in 20% of meadow ones it is more than the allowable rate; Sulfadimethoxine is in 20 % of chestnut honey samples, in 20% of lime ones, in 30 % of acacia ones and in 10% of meadow ones it is more than the allowable rate; Chloramphenicol is in 10% of chestnut honey samples, in 10% of lime ones, in 30% of acacia ones and in 10% of meadow ones it is more than the allowable rate; Metronidazole in in 30 % of chestnut honey samples, in 30% of lime ones, in 40 % of acacia ones and in 20% of meadow ones it is more than the allowable rate; the percentage of Ronidazole is distributed in the same way as in Metronidazole; Erythromycin is in 20 % of chestnut honey samples, in 20% of lime ones, in 30 % of acacia ones and in 10% of meadow ones it is more than the allowable rate; Nitrofurantoin is in 20 % of chestnut honey samples, in 20% of lime ones, in 20 % of acacia ones and in 10% of meadow ones it is more than the allowable rate; Lincomycinum is in 30 % of chestnut honey samples, in 30% of lime ones, in 50 % of acacia ones and in 20% of meadow ones it is more than the allowable rate; Tylosin is in 20 % of chestnut honey samples, in 20% of lime ones, in 30 % of acacia ones and in 10% of meadow ones it is more than the allowable rate; Rifampicinum is in 20 % of chestnut honey samples, in 40% of lime ones, in 50 % of acacia ones and in 10% of meadow ones it is more than the allowable rate (Table 1).

In the honey production process, human intervention is virtually eliminated (only a hive – Jara is made by a man).

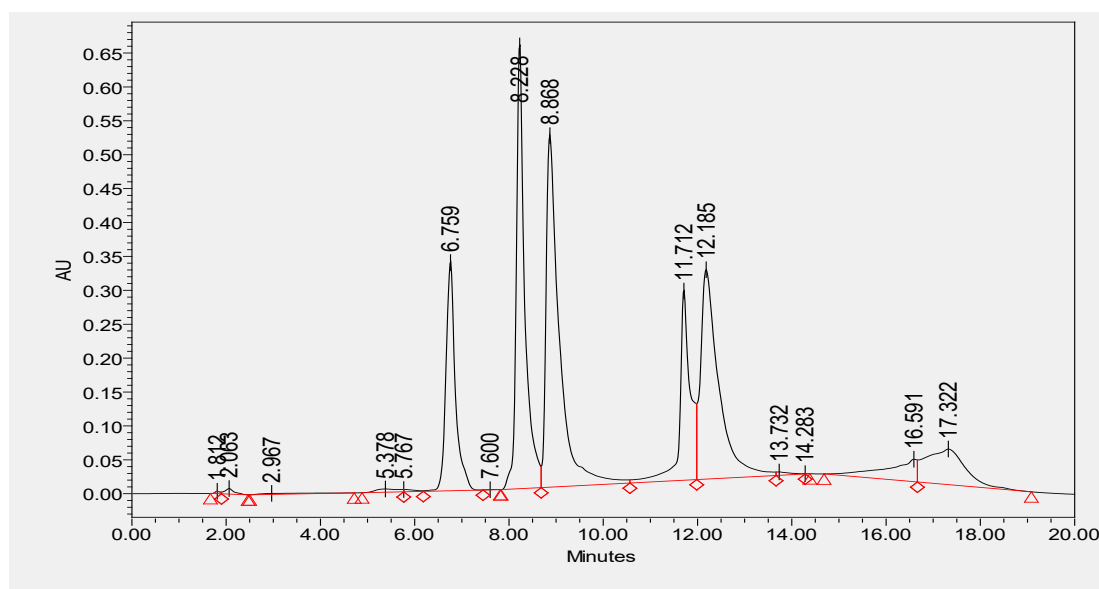


Fig. HPLC-UV detector-320 nm, Chromatogram of standart antibiotics (Metronidazole, Ronidazole, Erytromycin, Nitrofurantoin, Lincomycinum)

Table 1. Honey antibiotics M / Z specifications and the number of honey samples (%), in which the quantity of antibiotics is more than 1.5 mg / kg

Parameter	µg/kg	HoneyJ ara	Chestnut Honey	Lime Honey	Acacia Honey	Midow Honey	MS-	MS+
Oxytetracycline	5.0	n.n.*	20	30	30	20	459.14	461.14
Tetracycline	5.0	n.n.	30	20	40	20	443.15	445.16
Streptomycin	5.0	n.n.	30	30	50	20	580.25	582.27
Sulfadimethoxine	5.0	n.n.	20	20	30	10	309.06	311.08
Chloramphenicol	5.0	n.n.	10	10	30	10	321.00	323.01
Metronidazole	5.0	n.n.	30	30	40	20	170.05	172.07
Ronidazole	5.0	n.n.	30	30	40	20	199.04	201.06
Erythromycin	5.0	n.n.	20	20	30	10		734.47
Nitrofurantoin	5.0	n.n.	20	20	20	10	112.00	114.01
Lincomycinum	5.0	n.n.	30	30	50	20	405.21	407.22
Tylosin	5.0	n.n.	20	20	30	10	914.51	916.52
Rifampicinum	5.0	n.n.	10	40	50	10	821.39	823.41

n.n.* = below loq = limit of quantitation 2 ppb

The accumulation of antibiotics in honey samples is naturally caused by their use in inadequate and high doses. The studies have shown that a high percentage in spring honey (acacia and lime) is associated with the frequency of antibiotics use, since an excess amount of antibiotics and other drugs is used in spring to care for the health of bees. The honeycombs, previously used in the hive and not tested for

the content of antibiotics, are also used in spring.

We have studied heavy metals in all honey samples, regardless of its origin. No arsenic content was found in any of the samples. The Cd content in all samples ranges from 0.26 to 0.29 mg / kg, only in Jara honey it is in the range of 0.15 mg / kg. The content of Co is from 0.28 to 0.31, and in Jira honey it is much less (0.17 mg / kg).

Table 2. *The content of heavy metals in honey samples is mg / kg.*

Sample Name	Cd mg/kg	Co mg/kg	Cr mg/kg	Hg mg/kg	As mg/kg	Pb mg/kg	Zn mg/kg
Max. limit	2.0	1.0	1.0	0.01	0.2	1.0	20,0
Honey Jara	0.03±0.01	0.17±0.01	0.24±0.05	n.n.*	n.n.	n.n.	0.7±0.01
Honey Castanea	0.28±0.02	0.31±0.02	0.53±0.05	n.n.	n.n.	0.14±0.001	4.57±0.1
Honey Tilia	0.26±0.01	0.32±0.02	0.535±0.05	n.n.	n.n.	0.16±0.002	10.3±0.2
Honey Acacia	0.295±0.03	0.27±0.01	0.54±0.05	n.n.	n.n.	0.07±0.001	6.40±0.1
Honey Field	0.27±0.01	0.30±0.02	0.875±0.07	n.n.	n.n.	0.16±0.002	4.23±0.1

n.n. = below loq = limit of quantitation 1 ppb*

The content of Cr and Zn in honey is often associated with the utensils used in the production of honey, although their content in our samples is not close to the critical threshold (0.53–0.87 mg / kg and 4.23–10.3 mg / kg respectively). The copper content is quite low - from 0.3 to 1.06 mg / kg. The iron content in honey is comparatively higher, from 9.65 mg / kg to 12.7 mg / kg. It is important that the lead content is low - from 0.07 to 0.16 mg / kg. In all the analyzed samples, the content of Hg and As is less than LOQ. Particularly, it is noteworthy that the lead content in Jara honey is lower than that of LOQ determiner and the content of other heavy metals is much less than in samples of honey of other breeds. As a rule, Jara honey is collected in a relatively ecologically clean mountain region, which minimizes contamination of the plants and, consequently, the nectar and honey, obtained from them (Table 2).

The content of heavy metals in honey mainly depends on environmental factors and is not related to botanical origin.

Conclusion

The following 12 antibiotics have been detected by 50 HPLC and UPLC-MS methods in 50 local and vegetable honey samples produced in Western Georgia: Oxytetracycline, Tetracycline, Streptomycin, Sulfadimethoxine, Chloramphenicol, Metronidazole, Ronidazole, Erythromycin, Nitrofurantoin, Lincomycinum, Tylosin, Rifampicinum.

The quality characteristics of antibiotics were established. The samples of acacia and lime honey are characterized by an average level of pollution; this indicator is relatively small in samples of meadow and chestnut honey. The wild Jara Honey also does not contain antibiotics.

ow and chestnut honey. The wild Jara Honey also does not contain antibiotics.

The samples of acacia and lime honey contain the greatest amount of iron, and are relatively less, and also within the normal range of Cd, Co, Cr, Hg, As, Zn, Pb. Jara honey is characterized by a minimum content of heavy metals, due to the peculiarity of its origin.

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