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QUALITY OF NATURAL HONEY AFTER TREATMENT OF HONEY BEE COLONIES WITH NEOMYCIN

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Abstract. *Our country occupies first place in Europe and the third in the world in terms of honey production. However, there are problems with the honey export in Ukraine associated with the detection of antibiotics. In addition to compliance with safety indicators in accordance with the standards of Ukraine and the European Union, the quality control of honey is carried out by physicochemical parameters.*

The aim of the study was to determine the quality of linden honey after treatment of honey bee colonies with 1% solution of neomycin, an antibiotic that is often used by beekeepers in the treatment and prevention of infectious diseases in honey bees.

25 groups of honey bee colonies, which were kept under natural conditions in the apiary of the Laboratory of technological and special measures for honey bee diseases prevention at the National Science Centre "Institute of beekeeping named P. I. Prokopovich", were used in the experimental study.

It was found that most of the physical and chemical parameters met the requirements of DSTU 4497:2005 "Natural honey. Technical conditions", except for water and sucrose mass fractions when feeding bees by the syrup with a 0.1% neomycin solution.

Indicators of water and sucrose mass fractions when feeding syrup with 0.1% neomycin solution did not meet the requirements of the current national standard and constituted: water – 24.16 ± 0.08% on the 10th day, 24.13 ± 0.06% on the 30th day, 24.00 ± 0.11% on the 120th day; sucrose – 8.23 ± 0.09% on the 10th day, 6.08 ± 0.11% on the 30th day, 5.93 ± 0.07% on the 120th day.

Thus, the dynamics of physicochemical parameters of linden honey on the 10th day of the experiment and after 30 and 120 days of storage depended on the treatment of honey bee colonies with 1% neomycin solution.

Keywords: *linden honey, neomycin, indicators of honey quality, processing methods*

Introduction

Council Directive 2001/110/EC states that honey is a natural sweet substance produced by *Apis mellifera* bees from plant nectar or from the secretion of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (Council Directive, 2002; Sohaimy et al., 2015).

It is known that honey is a unique product of beekeeping and is characterized by the content of active substances, valuable and necessary for the vital functions of the human body. Due to its beneficial properties, honey is used as a high-quality food product and an effective means for the treatment in human medicine. Consumer requirements have always been put forward for the quality of honey – taste, color, composition, the possibility for long-term storage without losing properties of the product. At the present stage of the advancement of science, new scientific methods for evaluating the organoleptic, physicochemical, and biological properties of honey have been developed, which have become the quality criteria. The most important of them, indicative, are included in state standards, which determine the suitability of honey for use as a natural quality product or sweets (Amin et al., 2018; Reybroeck, 2018).

Currently, the world community raises questions about the need for the approval of national rules due to the lack of requirements for the characteristics of monofloral honey, the declaration of the geographical origin of the product, the natural difference between types of honey. Also, there are differences between European legislation and Codex Alimentarius standards, mainly in terms of moisture content, hydroxymethylfurfural content, diastase activity, electrical conductivity, sugar content, and the provision of microscopic confirmation (Thrasyvoulou et al., 2018). These issues are relevant in Ukraine (Pambuk et al., 2019).

We followed the requirements of the current national standard (DSTU 4497:2005) to determine the physicochemical parameters in linden honey. The Council Directive 2001/110/EC states that the color of honey may vary from almost colorless to dark brown (Council Directive, 2002). Its consistency can be liquid, viscous, partially, or completely crystallized. The flavor and aroma may vary but are derived from the plant origin. At the same time, the dynamics of indicators of honey quality after treatment of honey bee colonies with antibiotics remains unresolved.

Analysis of recent researches and publications

It is known from the scientific literature that the formation of honey aroma ends by the third or fifth month of its storage. Calcium oxalate crystals are found in linden honey, the identification of which can serve as an additional sign of the botanical origin of honey. Linden honey does not have the amino acids lysine and histidine, contains 39.27% fructose and 34.96% glucose, pH = 3.7. It has well-defined nutritional and medicinal properties, has antibacterial action against gram-positive and gram-negative microorganisms, as well as against ciliates, Amoebae, and Trichomonas. Also, linden honey contains volatile and non-volatile antimicrobial substances. It has expectorant, mild laxative, and anti-inflammatory effects. It is customary to use linden honey for sore

throat, runny nose, laryngitis, bronchitis, bronchial asthma, as a heart tonic, for inflammation of the gastrointestinal tract, kidney, and biliary diseases. At the same time, linden honey has a local healing effect on purulent wounds and burns (Polishchuk et al., 2013).

Bogdanov (Swiss Confederation), speaking at the Apimondia Forum 2010 in Bulgaria, stressed that the main contaminants of honey are antibiotics used in various countries to control American and European rot: aminoglycosides, tetracyclines, amphenicols, macrolides, beta-lactams. They contaminate royal jelly, propolis, and especially wax (Ponomariv & Faramazian, 2010).

Aminoglycosides are antibiotics that are often used in animal husbandry. If antibiotics are used in violation of the instructions, their residues are determined in food products of animal origin and the environment. Effective detection of their residues is an important part of human health protection (Yi-Fang et al., 2015).

Thus, the group of first-generation aminoglycosides (neomycin A, neomycin B, neomycin C), formed during the functioning of the ray fungus actinomycete (*Streptomyces fradie*) or related microorganisms, is neomycin.

Purpose – to determine the quality of linden honey after treatment of honey bee colonies with 1% neomycin solution.

Materials and methods of research

For the experimental study, 25 groups of honey bee families were formed: 1 control and 24 experimental, which were divided according to the methods for the treatment: 1st control group – treatment was not carried out; 2nd group – aerosol treatment by spraying (0.1% neomycin solution) and 3rd group – feeding syrup (0.1 g/0.1 L of syrup corresponding to a concentration of 0.1% – 0.5 L of neomycin per honey bee colony).

During the experiment, a certified substance of neomycin was used under the condition of a single treatment of honey bee colonies. The choice of 0.1% antibiotic concentration was justified by the scientific literature, which shows the results of their inhibitory effect on pathogens of bee rot in the laboratory conditions and to determine the antiseptic effect in the treatment of honey bee colonies affected by European and American rot in the wild nature.

The probability of the experimental results was ensured by conducting them in triplicate. All obtained digital data were processed statistically using a computer software package "Microsoft Excel" with the calculation of the arithmetic mean and its error ($M \pm m$), the level of probability (P) according to the Student's table ($P < 0.05$, $P < 0.001$, $P < 0.0001$).

Results of the research and their discussion

To conduct this experimental study, we relied on the results of scientific research that in recent years the interest of consumers and researchers in honey as a food product, which a person consumes, and ways it could help to maintain good health. Honey production is now commercialized and may now run a high risk of being falsified not only with syrups from other plants, which are difficult to detect, but also contain antibiotic residues. The dynamics of physicochemical (qualitative) parameters in natural honey, in particular from linden, after application of various methods for the treatment of honey bee colonies with 1% neomycin solution, is of scientific interest.

Table 1 shows the physicochemical parameters of linden honey under different treatment methods of honey bee colonies with neomycin.

According to the indicators shown in Table 1, it follows that the water mass fraction in honey after aerosol treatment of honey bee colonies with neomycin was significantly lower by 0.5% ($P < 0.01$), and after feeding bees with syrup – significantly higher by 7.13% ($P < 0.001$) compared with the control. When feeding bees with neomycin syrup, this figure was significantly higher by 7.63% ($P < 0.001$) compared to aerosol treatment.

The diastase number both under aerosol treatment with neomycin and feeding bees with syrup was significantly lower by 15.9 and 38.6% ($P < 0.05$; $P < 0.001$) than in the control. Also, the diastase number of honey after feeding syrup with neomycin was significantly lower by 27.0% ($P < 0.01$) than after aerosol treatment.

There was no statistically significant difference in the hydroxymethylfurfural content. There was only a tendency to reduce the hydroxymethylfurfural content after feeding bees syrup with neomycin compared to the control and to increase – after aerosol treatment of honey bee colonies. The hydroxymethylfurfural content in linden honey when feeding bees syrup with neomycin was slightly lower compared to aerosol treatment.

The acidity of linden honey depends on the method for the treatment of bees with a neomycin solution. Thus, both after feeding syrup to bees and aerosol treatment with this antibiotic, the acidity was significantly lower by 37.4 ($P < 0.001$) and 29.8% ($P < 0.001$), respectively, compared with that in the control.

At the same time, the acidity of linden honey after feeding bees syrup with neomycin was significantly lower by 10.8% ($P < 0.05$) than after the aerosol treatment.

The mass fraction of reducing sugars after feeding syrup with neomycin was significantly higher by 2.6% ($P < 0.05$) than in the control. This indicator shows a tendency to increase the mass fraction of reducing sugars in linden honey after feeding syrup with neomycin compared

1. Physicochemical parameters of linden honey on 10th day after treatment of honey bee colonies with neomycin (M ± m, n = 3)

Parameter	1st (control)	Experimental groups	
		2nd (aerosol treatment)	3rd (syrup feeding)
Water mass fraction, %	17.03 ± 0.03	16.53 ± 0.08●●	24.16 ± 0.08**▲
Diastase number, Gothe units	12.97 ± 0.49	10.90 ± 0.20●●●	7.96 ± 0.37*▲▲
Hydroxymethylfurfural content, mg/kg	0.73 ± 0.13	1.03 ± 0.14	0.80 ± 0.11
Acidity, mEq NaOH per 1 kg	8.83 ± 0.17	6.20 ± 0.17●	5.53 ± 0.03**▲▲▲
Mass fraction of reducing sugars, %	86.63 ± 0.30	88.03 ± 1.19	89.23 ± 0.77***
Sucrose mass fraction, %	9.17 ± 0.13	8.00 ± 0.15●●	8.23 ± 0.09*

Note: * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$ – 3rd experimental group compared with the control; ▲ $P < 0.001$, ▲▲ $P < 0.01$, ▲▲▲ $P < 0.05$ – 3rd experimental group compared with the 2nd experimental; ● $P < 0.001$; ●● $P < 0.01$; ●●● $P < 0.05$ – 2nd experimental group compared with the control.

with the indicator after aerosol treatment of bees and in the control.

The sucrose mass fraction after feeding bees syrup with neomycin and aerosol treatment was significantly lower by 0.94 and 1.17% ($P < 0.01$), respectively, than in the control. Also, it remains just a trend of a slight increase in the sucrose mass fraction when feeding bees syrup with neomycin compared with aerosol treatment.

This percentage of sucrose (from 8.00 ± 0.15 to 9.17 ± 0.13%) in the studied samples of linden honey might be explained based on evidence from other authors that linden, apple, and some other types of honey may contain a significant amount of sucrose in the first period after pumping because the flower nectar from these plants contains it in a large quantity. The rate of sucrose hydrolysis in maturing honey is high but, at the time of pumping, the sucrose content may remain at 10 to 25%. During further storage, the sucrose content is set at the level of 0 to 1.0%. The same processes of sucrose hydrolysis occur in sugar honey (Polishchuk et al., 2017).

According to the indicators shown in Table 2, it follows that the water mass fraction in linden honey after aerosol treatment of bees with a neomycin solution was significantly lower by 0.77% ($P < 0.001$) than in the control. At the same time, when bees were fed syrup with neomycin, the water mass fraction in linden honey was significantly higher by 7.03 ($P < 0.001$) and 7.80% ($P < 0.001$) compared with aerosol treatment and the control, respectively.

The level of diastase number in linden honey both after the aerosol treatment of bees and feeding them syrup with neomycin was significantly lower by 9.9 ($P < 0.05$) and 35.3% ($P < 0.001$), respectively, compared to that in the control. When bees were fed syrup with neomycin, the diastase number was significantly lower by 28.2% ($P < 0.001$) than after the aerosol treatment of bees.

The hydroxymethylfurfural content in linden honey tended to increase when feeding bees syrup with neomycin compared with the control, as well as aerosol treatment.

The acidity of linden honey, both after aerosol treatment of bees and feeding them syrup with neomycin was significantly lower by 30.9 ($P < 0.01$) and by 38.2% ($P < 0.001$), respectively, compared with the control. When feeding syrup with neomycin, the acidity of

2. Physicochemical parameters of linden honey on 30th day of storage after treatment of honey bee colonies with neomycin (M ± m, n = 3)

Parameter	1st group (control)	Experimental groups	
		2nd (aerosol treatment)	3rd (syrup feeding)
Water mass fraction, %	17.10 ± 0.06	16.33 ± 0.06●	24.13 ± 0.06**▲
Diastase number, Gothe units	12.06 ± 0.13	10.86 ± 0.29●●●	7.80 ± 0.14**▲
Hydroxymethylfurfural content, mg/kg	0.53 ± 0.07	0.53 ± 0.17	0.80 ± 0.23
Acidity, mEq NaOH per 1 kg	9.16 ± 0.33	6.33 ±0.16●●	5.66 ± 0.16**▲▲▲
Mass fraction of reducing sugars, %	87.96 ± 0.46	87.26 ± 0.40	89.33 ± 0.57▲▲▲
Sucrose mass fraction, %	3.63 ± 0.12	4.77 ± 0.24●●●	6.08 ± 0.11**▲▲

Note: similar to Table 1.

honey was significantly lower by 10.6% ($P < 0.05$) compared with the aerosol treatment of bees.

The mass fraction of reducing sugars in linden honey when feeding syrup with neomycin was significantly higher by 2.07% ($P < 0.05$) compared to the aerosol treatment of bees with this antibiotic.

The sucrose mass fraction in linden honey was significantly higher by 2.45 ($P < 0.001$) and 1.14% ($P < 0.05$) compared with the control both after feeding syrup to bees and aerosol treatment of bees with a neomycin solution. At the same time, the sucrose mass fraction in honey was significantly higher by 1.31% ($P < 0.01$) after feeding syrup with neomycin compared with the value after aerosol treatment of bees with this solution.

After aerosol treatment of bees with neomycin (Table 3), the water mass fraction in linden honey was significantly lower by 0.67% ($P < 0.01$) compared with the control.

However, when bees were fed syrup with this antibiotic, the water mass fraction was significantly higher by 6.87 ($P < 0.001$) and 7.54% ($P < 0.001$) compared with the control and aerosol treatment, respectively.

The diastase number of honey when feeding syrup with neomycin was significantly lower by 36.4% ($P < 0.01$) compared with the control, and by 30.4% ($P < 0.01$), compared with the aerosol treatment of bees with this antibiotic. Also, there was only a tendency to reduce the diastase number of linden honey after aerosol treatment of bees with neomycin.

In terms of hydroxymethylfurfural content in linden honey, there is a tendency to increase under different methods for treatment of bees with neomycin and to decrease after feeding syrup with this antibiotic compared to aerosol treatment of bees.

The acidity of linden honey after aerosol treatment of bees with neomycin and feeding syrup was significantly lower by 25.0 ($P < 0.01$) and 12.5% ($P < 0.01$) compared with the control, respectively. However, when feeding syrup with neomycin, the acidity of linden honey was significantly higher by 16.5% ($P < 0.05$) compared with the aerosol treatment of bees. According to the mass fraction of reducing sugars, there is a tendency to decrease in their content in linden honey after aerosol

3. Physicochemical parameters of linden honey on the 120th day of storage after treatment of honey bee colonies with neomycin (M ± m, n = 3)

Parameter	1st group (control)	Experimental groups	
		2nd (aerosol treatment)	3rd (syrup feeding)
Water mass fraction, %	17.13 ± 0.07	16.46 ± 0.06●●	24.00 ± 0.11**▲
Diastase number, Gothe units	12.43 ± 0.55	11.36 ± 0.26	7.90 ± 0.45*▲▲
Hydroxymethylfurfural content, mg/kg	0.53 ± 0.13	1.06 ± 0.29	0.60 ± 0.11
Acidity, mEq NaOH per 1 kg	9.33 ± 0.16	7.00 ± 0.28●●	8.16 ± 0.16*▲▲▲
Mass fraction of reducing sugars, %	86.80 ± 0.72	88.33 ± 0.8	89.23 ± 0.49
Sucrose mass fraction, %	3.73 ± 0.09	4.05 ± 0.16	5.93 ± 0.07**▲

Note: similar to Table 1.

treatment of bees with neomycin, and increase after feeding syrup with this antibiotic compared to both the control and aerosol treatment of bees with a neomycin solution.

The sucrose mass fraction in linden honey when feeding bees syrup with this antibiotic was significantly higher by 2.20 ($P < 0.001$) and by 1.88% ($P < 0.001$) compared with the control and aerosol treatment of bees with a solution of neomycin, respectively.

The water mass fraction after feeding bees syrup with neomycin was higher than the requirements of the current DSTU 4497:2005. According to this indicator, such honey is prohibited for sale and is not amenable to long-term storage.

There is no sufficiently substantiated information in the scientific literature on the effect of antibiotic residues on the quality of honey. It is known that the water content below 20% is characteristic of good, mature honey, which can be stored for a long time. DSTU 4497:2005 allows the water content in commercial honey up to a maximum of 21%. This limit is applied to honey, which is intended for immediate consumption, for honey, which is sent for storage, such water content is unacceptable because there is a tendency to delamination and fermentation.

At the same time, it is known that there is a clear difference between the botanical varieties of honey by the average values of diastase number. Thus, according to some authors, the highest diastase number have buckwheat (48.12) and pine (33.15) honey, and the lowest – acacia (9.82) and sunflower (16.6). The linden, flower, and herb honey have diastase number at the level of 19–25 Gothe units (Adamchuk & Bilotserkivets, 2015). Other scientists prove that diastase activity is very low in some types of natural honey – white acacia, clover, linden, sunflower, fireweed (Samarghandian et al., 2017). However, the indicators of diastase number in our experiment met the requirements of DSTU 4497:2005.

It is known that hydroxymethylfurfural content in freshly pumped honey is minimal and constitute 1–5 mg/kg (Kovalskyi & Kyryliv, 2001). At the same time, linden honey is characterized by a higher value of active acidity. If all light-colored honey has a pH value from 3.5 to 4.1 units (for example, in sunflower honey, this figure does not exceed 4.15; heather – 4.14; acacia – 4.11; melilot – 3.95; sainfoin – 3.85; raspberry – 3.8; phacelia – 3.77), then aqueous solutions of linden honey have a pH from 4.5 to 7 units (Samarghandian et al., 2017).

Conclusions and future perspectives

The dynamics of physicochemical parameters in linden honey on the 10th day of the experiment and after 30 and 120 days of storage depended on the method for treatment of honey bee colonies with 1% neomycin solution.

At the same time, most of the physical and chemical parameters met the requirements of DSTU 4497:2005. "Natural honey. Technical conditions", except for the mass fractions of water and sucrose when feeding bees with syrup containing 0.1% neomycin solution.

After feeding syrup with 0.1% neomycin solution, the water mass fraction did not meet the requirements of the current national standard and constituted $24.16 \pm 0.08\%$ on the 10th day, $4.13 \pm 0.06\%$ on the 30th day, and $24.00 \pm 0.11\%$ on the 120th day.

The sucrose mass fraction in linden honey did not meet the current national standard when feeding syrup with the studied antibiotics. Thus, on the 10th day

after feeding the syrup with 0.1% neomycin solution, the mass fraction of sucrose in linden honey decreased compared to the control group (9.17 ± 0.13%) and amounted to 8.23 ± 0.09%; on the 30th day – was higher compared to the control group (3.63 ± 0.12%) and amounted to 6.08 ± 0.11%; on the 120th day of storage of linden honey – was higher compared to the control group (3.73 ± 0.09%) and amounted to 5.93 ± 0.07%.

On the 10th day after aerosol treatment of bees with 0.1% solution of neomycin, the mass fraction of sucrose in linden honey did not meet the requirements of the current national standard and was 8.00 ± 0.15%, although it was lower compared to the control group (9.17 ± 0.13%).

In the future, further research is needed to determine the residual amounts of antibiotics in honey by an improved method of enzyme-linked immunosorbent assay.

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Анотація. *Наша держава займає перше місце в Європі та третє у світі за показниками виробництва меду. Проте, з експортом меду в Україні виникають проблеми, які пов'язані з виявленням антибіотиків. Окрім відповідності показникам безпечності відповідно до стандартів України та Європейського Союзу, контроль якості меду проводять за фізико-хімічними показниками.*

Метою дослідження було визначити якісні показники меду з липи після обробки бджолиних сімей 1% розчином неоміцину, антибіотику, що часто використовується пасічниками в лікуванні та профілактиці інфекційних хвороб бджіл.

В експериментальному дослідженні використали 25 груп бджолиних сімей, які знаходилися за природних умов на пасіці лабораторії технологічних і спеціальних заходів профілактики хвороб бджіл ННЦ «Інститут бджільництва ім. П. І. Прокоповича».

У результаті проведеного дослідження встановили, що більшість фізико-хімічних показників відповідала вимогам ДСТУ 4497:2005 «Мед натуральний. Технічні умови», окрім масової частки води та масової частки сахарози за згодовування бджолам сиропу з 0,1% розчином неоміцину.

Показники масової частки води та сахарози за згодовування сиропу з 0,1% розчином неоміцину не відповідали вимогам чинного національного стандарту і становили: води – на 10 добу – 24,16 ± 0,08%; на 30 добу – 24,13 ± 0,06%; на 120 добу – 24,00 ± 0,11%; сахарози – на 10 добу – 8,23 ± 0,09%; на 30 добу – 6,08 ± 0,11%; на 120 добу – 5,93 ± 0,07%.

Таким чином, динаміка фізико-хімічних показників у меді з липи на 10 добу експерименту та після 30 і 120 доби зберігання залежала від обробки бджолиних сімей 1% розчином неоміцину.

Ключові слова: *мед із липи, неоміцин, показники якості меду, способи обробки*