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Changes in the Chemical Composition and Antimicrobial Activity of Linden, Buckwheat and Sunflower Honey Stored at Low Temperatures

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Abstract

Natural bee honey can change its physicochemical and biological properties during storage. Literature data on long-term storage of honey at low temperatures (from 0 to -20 °C) indicate that this ensures the stability of some physicochemical parameters. Despite the potential benefits of these temperature regimes for practical use, it is important to consider their potential negative effects on honey quality. The aim of this study was to investigate the influence of various temperature conditions on the physicochemical and biochemical parameters of linden, buckwheat, and sunflower honeys during storage for 12 months. For the first time, a wide range of physicochemical parameters of honey samples was analyzed before and during storage for 12 months at temperatures of 18, 10, 5, 0, -5, -10 and -18 (±2) °C. The evaluation of the physicochemical parameters before storage demonstrated that the samples fully complied with the Interstate Standards. Throughout the storage period, the HMF level remained stable at -18 °C, whereas it significantly increased at higher temperatures; for example, after 12 months at 18 °C, the increase ranged from 472.5% to 488.1%, depending on the botanical origin of the honey. However, maximum permissible concentration – MPC (25 mg/

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kg) was not exceeded. A decrease in the activity of diastase, D-glucose-1-oxidase and catalase was observed at all temperature conditions already during the first month of storage. Minimal changes were observed at 0 and 5 °C. Hydrogen peroxide (H_2O_2) remained stable in this temperature range. Moisture content, total mass fraction of reducing sugars, and acidity did not change significantly in all samples. An antimicrobial study using test cultures found that honey stored at 5 and 0 °C had the greatest inhibitory effect. The data obtained demonstrates that the optimal temperature range for 12-month storage of honey is between 5 and 0 °C. These findings can be used as supplementary guidance when making amendments to regulatory documents governing storage requirements for this product.

Introduction

For many centuries, natural honey has been considered not only as a valuable food but also as a medicinal product with significant antimicrobial activity. This activity is primarily attributed to the presence of monosaccharides, enzymes, organic acids, and phenolic compounds in the product.[1–5] According to some literature sources, the antimicrobial activity of honey depends on the content of hydrogen peroxide (H_2O_2) in it.[6–8]

The beneficial properties of honey are preserved for a long time, but only if certain storage conditions are followed. According to the requirements of the Interstate Standard "Natural honey. Technical conditions", this product should be stored in places inaccessible to direct sunlight, at a temperature not exceeding 20 °C in tightly sealed containers for 12 months from the date of examination, in hermetically sealed containers for 24 months from the date of packaging and 12 months after opening hermetically sealed packaging.[9]

There is data on the storage of honey at low temperatures.[10–13] Most often, this approach is used to suppress fermentation that can occur in honey with a moisture content of more than 20%, as well as to prevent crystallization.[10,14–16] It is known that exposure to low temperatures (from 0 to -20 °C) for a long time leads to a slowdown in chemical processes and, as a result, stabilization of physical and chemical indicators of honey such as electrical conductivity, humidity, acidity and content of phenolic compounds.[10] It is also important to note that storing honey at these temperatures slows down the formation and accumulation of hydroxymethylfurfural (HMF), a product of monosaccharide dehydration that has neurotoxic, cytotoxic, genotoxic, and mutagenic effects.[17–19]

Thus, the storage of honey at low temperatures might appear promising for practical application. However, it is essential to bear in mind that, while attempting to prevent the formation of HMF, we must also take into account the potential adverse effects of low temperatures on other physicochemical parameters of honey, particularly those that determine its antimicrobial activity.

In Russia, Commonwealth of Independent States (CIS) countries and Europe some of the most common and important honey plants are buckwheat (buckwheat, *Fagopyrum esculentum Moench*.), linden (lime linden, *Tilia cordata Mill*.) and sunflower (annual sunflower, *Helianthus annuus L*.). For example, in Russia (mainly in the forest-steppe zone of the European part of the country, Altai Territory, Transbaikalia and the Primorye-Amur region), as well as in Kazakhstan, buckwheat provides over 50% of marketable honey.[20] The honey productivity of buckwheat can reach 60–362 kg/ha.[21] Another valuable honey plant, small-leaved linden, occupies a large area in the broad-leaved forest zone of the European part of Russia, spreading to the Urals, and is also found in the Crimea and the Caucasus. Its honey productivity averages 700–1000 kg/ha.[22] Annual sunflower is a honey-bearing and pollen-bearing crop that provides honey production in Russia, Kazakhstan, Ukraine, as well as in the countries of southern and south-eastern Europe. Honey productivity is at the level of 40–50 kg/ha.[23]

In connection with the above, the purpose of this work was to study the influence of different

temperature storage conditions of honey of different botanical origins on its physicochemical and biological parameters during storage for 12 months.

Material and Methods

Samples Collection

The samples of sunflower *Helianthus annuus L*. (n = 36), linden Tilia cordata Mill. (n = 31) and buckwheat honey *Fagopyrum esculentum Moench*. (n = 34) admitted to the laboratory of veterinary sanitation and environmental safety in beekeeping of Russian Research Institute of Experimental Veterinary Medicine from the following regions of Russia: Rostov, Volgograd, Kursk, Voronezh and Saratov regions, as well as from the Krasnodar Territory in period 2021-2022. The samples were stored in climatic test chambers M-60/100-500 KTVH (JSC LOIP, Russia).

Palynological Analysis

The palynological composition of honey was studied in accordance with Interstate Standard 31769-2012.[24] Trinocular microscope AmScope T390C (AmScope, China) with digital camera Levenhuk M1000 PLUS (Levenhuk, USA) was used to perform optical light microscopy.

Pollen grains in the samples were calculated using the following formula:

$$X_p\ (\%) = G \cdot 100n^{-1} \quad ...(1)$$

where $G = \Sigma G_i$ is the number of pollen grains of linden, buckwheat or sunflower in all examined fields; $n = \Sigma n_i$ – total number of pollen grains; 100 – conversion factor to percentage.

Physicochemical studies

Physicochemical studies of honey were carried out in accordance with current Interstate Standards, the requirements of which are no less stringent than those of the Codex Alimentarius and Council Directive 2001/110/EU.[25,26] Also, it should be noted that the methods of some analyzes in the above standards are for the most part similar to the methods of Harmonized methods of the international honey commission.[27]

Determination of Moisture

The moisture (mass fraction of water) of the studied samples was determined by the refractometric method according to Interstate Standard 31774-2012[28] using an IRF-454 B2M refractometer (JSC Kazan Optical-Mechanical Plant (KOMZ), Russia).

Determination of HMF Concentration

HMF was determined by reverse phase HPLC in accordance with the Interstate Standard.[29] The study used Shimadzu LC-20 Prominence chromatograph with DAD (simultaneously detectable wavelength range 210-400 nm, working wavelength 283 nm), as well as Eclipse XDB-C18 column (150 × 4.6 mm, 5 µm). The mode used was gradient, the flow rate – 1.0 mL/min, and the peak retention time – 3.83 min. Standard solutions were prepared from the analytical standard HMF (purity no less than 99%, Sigma-Aldrich, USA). The calibration curve was constructed using the following concentrations of HMF: 150.0; 100.0; 50.0; 30.0; 25.0; 20.0; 15.0; 10.0; 5.0; 1.0 µg/mL using the formula:

$$M_{5\text{-}HMF} = C_{HMF} \cdot V_{sample} / m_{honey} \quad ...(2)$$

where C_{HMF} – the HMF concentration, determined from the calibration graph (µg/mL); V_{sample} – volume of analyzed sample (mL); m_{honey} – mass of the sample (g).

Carrez solutions I and II for precipitation of proteins and stabilization of HMF in aqueous solution were also prepared according to the specified Interstate Standard.

Determination of Total Mass Fraction of Reducing Sugars

Determination of the glucose and fructose (in %) was carried out according to Interstate Standard 32167-2013 using the colorimetric method.[30] The essence of this method is to determine the optical density of a solution of potassium iron sulfide – $K_3[Fe(CN)_6]$ (≥99.5%, LenReaktiv, Russia) after its interaction with reducing sugars of honey. The analysis was carried out on KFK-2 photocolorimeter (JSC Zagorsk Optical-Mechanical Plant, Russia) at a wavelength λ = 440 nm.

Determination of Free Acidity

To determine free acidity, aqueous solutions of honey samples were titrated with a 0.1 M solution of sodium hydroxide – NaOH (99.2%, LenReaktiv, Russia) to pH 8.30. The pH value was monitored using a potentiometric analyzer – Hanna edge with

pH electrode HI11310 (Hanna Instruments, USA). The results were expressed in milliequivalents of HCl in 1 kg of honey – meq/kg.[31]

Free acidity (K) in milliequivalents of HCl in 1 kg of honey was calculated using the formula:

$$K = V \cdot 10 \quad ...(3)$$

where V – the volume of sodium hydroxide solution of concentration c(NaOH) = 0.1 mol/dm^3, consumed for titration, cm^3; 10 – conversion factor for the mass of honey 1 kg.

Determination of the Diastase Activity

Diastase activity (Gothe units) was determined by the colorimetric method according to the Interstate Standard. Diastasis matched to the amount of starch digested down by honey enzymes.[32]

The following formula was used for calculation:

$$X = 100 \cdot 80(D_C - D_{test}) \cdot D_C^{-1} \cdot (100 - W)^{-1} \quad ...(4)$$

where 80 – the conversion factor; D_C – optical density of the control solution; D_{test} – optical density of the test solution; W – mass fraction of water in honey (%).

Determination of Catalase Activity

Catalase activity was determined in accordance with the method developed by Aganin A.V.,[33] according to which the activity was expressed in mm^3 of oxygen released when catalase contained in 1 g of honey is exposed to 10 mL of 1% (wt.) H_2O_2 solution for 24 h.

Determination of D-Glucose-1-Oxidase activity

D-glucose-1-oxidase activity was determined according to the method developed by Flanjak I. *et al*.[34] Under the influence of peroxidase, H_2O_2 is reduced to water. The 3,3'-dimethoxybenzidine used produces a colored product that can be detected colorimetrically. The results were expressed in μg H_2O_2/h g honey.

Determination of Hydrogen Peroxide Content

H_2O_2 in honey was detected using iodometry and spectrophotometry. The potassium iodide used in the reaction reacts with the H_2O_2 contained in the honey.[35] The molecular iodine released as a result of the reaction can be detected using spectrophotometer (PE5400UF, Ekroskhim, Russia). Analytical solutions of honey samples were obtained by weighing 1.0±0.01 g of each sample, after which 2.0 mL of HPLC water (Khimiya XXI Vek, Russia) was added to them and mixed for 3-5 min on Vortex mixer VM-300S (Joan Lab Equipment, China) or ultrasonic homogenizer Bandelin Sonopuls HD 2200 (Bandelin, Germany) until completely dissolved. From the resulting analytical solutions, 1.0 mL was taken and 1.0 mL of 0.2 M sulfuric acid (H_2SO_4, ≥93.6%, LenReaktiv, Russia) was added, after which carbon dioxide was passed through. At the last stage of sample preparation, a 5% solution of potassium iodide (KI, 99.5%, Rushim, Russia) was added to the analytical solutions, through which carbon dioxide was previously passed. The prepared analytical solutions of honey samples were incubated at room temperature for 24 h in a dark place. The absorption maximum was observed at λ = 351 nm.

The H_2O_2 concentration was calculated using the formula:

$$C = R \cdot (D - D_0) / \varepsilon, \quad ...(5)$$

where C – H_2O_2 concentration in the analytical solution (μmol/L); D – optical density of the analytical solution; D_0 – optical density of the control solution; R – dilution coefficient; ε –extinction coefficient (ε_{351}= 26400 L · mol^{-1} · cm^{-1}).

Physicochemical studies of honey samples were carried out at room temperature (22±2 °C).

Determination of Antimicrobial Activity

Microorganism Strains

In this work the following strains of microorganisms were used: *Staphylococcus aureus* 209-P, Escherichia coli 1257 and Bacillus cereus 96 (Table 1).

Microbial Test

To determine the antimicrobial activity of honey samples before and at the 12th month of storage at the indicated temperatures, the method of inoculating microorganisms (*S. aureus, E. coli, B. cereus*) on MPA was used. Samples in amount of 100 μg were added to a well made in the center of a Petri dish with MPA seeded with a microorganism. The antimicrobial activity of honey was manifested in the size of the growth inhibition zone.

Table 1: Some characteristics of microorganism strains

Micro-organism	Isolation	Temperature optimum, °C	Enzymatic activity		Antibiotic sensitivity	Antibiotic resistance
			+	—		
S. aureus	Salt meat-peptone agar (MPA) and salt meat-peptone broth (MPB), Egg-Yolk Salt Agar, Baird Parker Agar	30–37	acid phosphatase alcohol dehydrogenase alkaline phosphatase beta-galactosidase	alpha-chymotrypsin alpha-fucosidase alpha-galactosidase alpha-mannosidase	Aztreonam Colistin Nystatin	Penicillin G Ampicillin Imipenem Tetracycline Fosfomycin Linezolid Kanamycin Nitrofurantoin Pipemidic acid
E. coli	MPA, MPB, Endo Agar, Levin agar, MacConkey agar (MAC)	37–38	acid phosphatase alkaline phosphatase beta-galactosidase leucine arylamidase trypsin	alcohol dehydrogenase alpha-chymotrypsin alpha-fucosidase alpha-galactosidase alpha-glucosidase	Penicillin G Oxacillin Lincomycin Bacitracin Clindamycin Nystatin Linezolid	Cefotaxime Imipenem Norfloxacin Moxifloxacin Ampicillin Ticarcillin Pipemidic acid
B. cereus	MPA, MPB	30–37	alcohol dehydrogenase amylase caseinase lecithinase oxidase	beta-galactosidase gamma-glutamyl transferase lysine decarboxylase urease	Oxacillin Aztreonam Colistin Nystatin	Chloram-phenicol Erythromycin Clindamycin Linezolid Teicoplanin Ceftriaxone Amikacin

Statistical Analysis

Statistical processing of the results was carried out using MS Excel 2010 software. All measurements were performed in triplicate. The significance of differences in mean values was established using Student's t-test at a significance level of $p < 0.05$. Results are presented as means (M) and standard errors of the means (±SEM).

Results and discussion

Results of Palynological Analysis

According to Interstate Standard "Monofloral honeys. Technical conditions"[36] natural linden and buckwheat honey must contain at least 30% of dominant pollen grains, sunflower honey – at least 45%. Moreover, the total number of pollen grains during palynological analysis cannot be less than 500.

The studied samples fully complied with the specified requirements: amount of pollen grains of linden honey exceeded 600, of which 42±2% were identified as pollen grains of linden (*Tilia*). Palynological analysis of buckwheat and sunflower honey also showed their compliance with the Standard: amount of pollen grains was 620 and 680, respectively. Of these, the dominant grains were 45±3% (*Fagopyrum*) and 63±2% (*Helianthus*). Figure 1 shows photographs of pollen grains of these honey plants (×400).

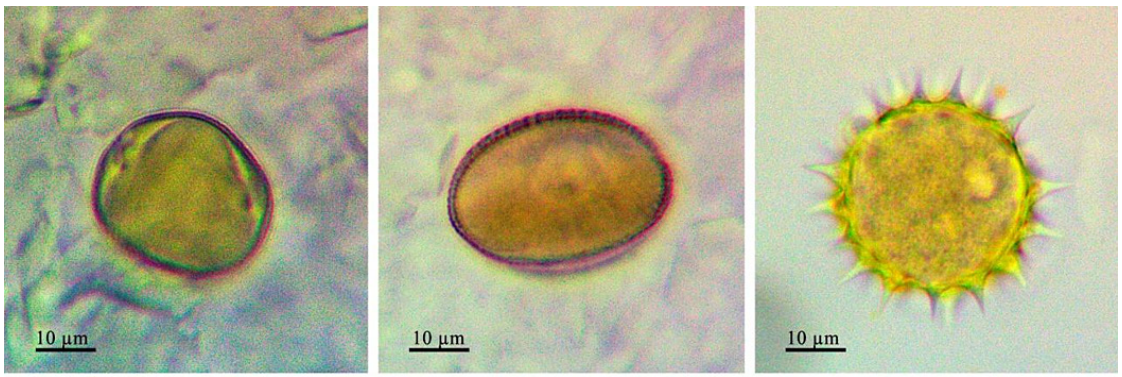

Fig. 1: Photographic images of pollen grains (×400): a – linden (*Tilia cordata Mill.*), b – buckwheat (*Fagopyrum esculentum Moench.*), c – sunflower (*Helianthus annuus L.*)

As you can see, the grain of small-leaved linden is three-furrowed, spherical-flattened, color is light yellow. Buckwheat has an ellipsoidal shape, a reticulate structure, and the grain color is dark yellow. The grain of the annual sunflower is golden, spherical in shape with a characteristic prickly sculpture, the arrangement of the thorns is uniform. This description completely coincides with the characteristics of the pollen of these honey plants given in literary sources.[37,38]

Results of Physicochemical Analysis

Before storing samples of freshly pumped linden, buckwheat and sunflower honey, their physicochemical parameters were assessed (Table 2).

As can be seen from the table, all parameters regulated by Interstate Standards met their requirements. Currently, the activity of catalase and D-glucose-1-oxidase, as well as the H_2O_2 content, are not regulated. Therefore, we were guided by data from literature sources, which provide positive results of using these indicators to determine the quality of honey.[33, 34, 39]

In order to study changes in physicochemical and biological properties of honey during prolonged storage under different temperature conditions, initial values obtained were used as a control.

The equal weight of honey samples (100±0.1 g) were placed in plastic containers with hermetically screwed lids and stored in climatic chambers at temperatures of 18, 10, 5, 0, -5, -10 and -18 (±2) °C, humidity 55 (±3)% for a period of 12 months. The choice of temperatures was due to the fact that 18±2 °C is the maximum temperature limit for

honey storage according to Interstate Standard requirements (no more than 20 °C), storage at 10, 5, 0, -5, -10 and -18 (±2) °C – modeling the effect of low temperatures at which changes in physicochemical parameters were observed. The resulting dynamics are presented in Figure 2.

Table 2: Analysis of the chemical composition of linden, buckwheat and sunflower honey

Analyzed para -meter, units	Regulated norm by Interstate Standards	Samples		
		Linden honey	Buckwheat honey	Sunflower honey
Interstate standard 31766-2022				
Moisture content, %	not more than 20.0* not more than 19.0** not more than 18.0***	18.2±0,5	17.9±0,7	16.8±0,8
Reducing sugars content, %	at least 66.0* at least 68.0** at least 71.0***	86.6±3.9	86.8±3.6	89.9±1.6
Free acidity, meq/kg	10.0–25.0* 10.0–40.0** 10.0–30.0***	24.6±0.8	26.3±1.1	25.1±0.9
Diastase, Gothe units	at least 8.0* at least 18.0** at least 15.0***	17.5±0.8	40.2±0.8	16.5±0.8
Interstate standard 19792-2017				
HMF content, mg/kg	not more than 25.0	2.36±0.11	3.25±0.15	2.44±0.1
Non-regulated parameters				
Catalase, mm^3 O_2	–	330±14	485±22	850±22
D-glucose-1-oxidase, μg H_2O_2/h g	–	432.1±10.5	385.9±12.3	187.8±9.1
H_2O_2 concentration, $\times 10^{-4}$ M	–	1.71±0.05	1.48±0.07	0.79±0.03

The mass fraction of HMF in all analyzed samples increased gradually at all temperatures, expect for -18 °C. This process began to occur most intensively after the 3rd month of storage. The dependence of the increase in HMF on storage temperature is monitored in the figure 2. Samples stored at 18 °C exhibited the highest HMF content. At the 12th month, its increase relative to the initial value was found to be 472.5% in samples of linden honey and by 484.0 and 488.1% for buckwheat and sunflower honey. However, these values did not exceed the maximum permissible concentration (MPC) of 25 mg/kg. Throughout the entire study period, the HMF content was stable at -18 °C. In samples of linden and sunflower honey during storage, a slight decrease was noted: in the 12th month – by 5.9 and 2.5%, respectively. The data obtained are consistent with the results of research by I.N. Pasias *et al*.[11] and M. Kędzierska-Matysek *et al*.,[10] which demonstrated the stability of HMF in honey samples of various botanical origins stored at sub-zero temperatures, and an increase in the level of this indicator in samples stored at room temperature.

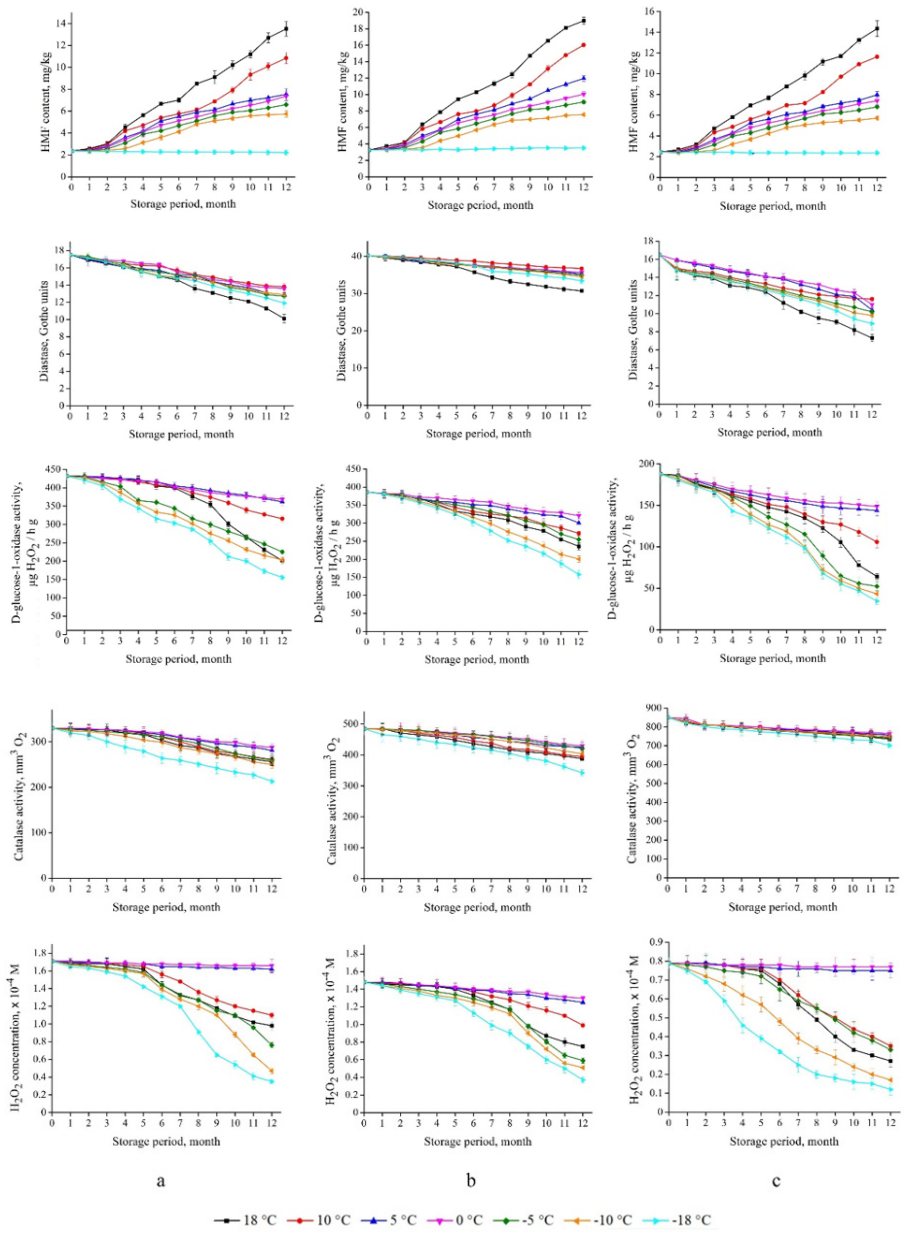

Fig. 2: Changes in the physicochemical parameters of linden (a), buckwheat (b) and sunflower (c) honey samples during storage for 12 months at temperatures: 18 °C (1), 10 °C (2), 5 ° C (3), 0 °C (4), -5 °C (5), -10 °C (6), -18 °C (7). 0 month – initial data (control) obtained before storing samples. Note: $p < 0.05$

A decrease in the diastase activity of honey samples occurred already in the first month of storage under all temperature conditions (Fig. 2). However, for buckwheat honey samples, stored under all specified temperatures for the entire study period, this parameter remained within the limits set by the Interstate Standard. A similar trend was observed for linden honey, except for samples stored for 12 months at 18 °C (average deviation from the standard – 0.9 Gothe units). The diastase

of sunflower honey was below the permitted norm in all experimental options, except for storage at 0 °C and 5 °C for 3 months.

It should be noted that as a result of storage at 18 °C, the diastase activity of all samples decreased significantly by the end of the study. Thus, in the 12th month of the experiment, in samples of linden honey it decreased by 42.3%, in samples of buckwheat honey – by 66.4%, in sunflower honey – by 55.8%. Temperature -18 °C also had an unfavorable effect: at the 12th month in linden honey, the diastase number was 11.9±0.96 Gothe units, which is 32.0% less than before storage; in buckwheat – 33.4±0.7 Gothe units (difference from the initial value of 16.9%) and in sunflower – 8.9±0.7 Gothe units (the difference was 46.1%). We noted that minimal changes in this parameter occurred at 0, 5 and 10 °C. I.N. Pasias *et al*.[11] when studying samples of pine, eucalyptus, cotton and thyme honey (Lamia, Greece) showed a decrease in diastase by 33.0-44.4% as a result of storage at room temperature for 12 months and by 5.6-9.1% at 0 °C, as well as no significant changes in this indicator at -18 °C. Due to the fact that the researchers recorded the results once every three months, it is quite difficult to track at what stage the decrease in diastase began to occur. M. Kędzierska-Matysek *et al*.[10] reported a decrease in diastase activity of rapeseed honey (*Brassica napus L*.), stored for a year at -20 °C, by more than 7% relative to the control. Thus, there is no data on this parameter that completely coincides with each other. This may be due to differences in the resistance of the enzyme to low temperatures, depending on the botanical origin of the honey.

The D-glucose-1-oxidase activity began to fall in the first month of storage at all temperature conditions just like the diastase number (Fig. 2). The intensity of the decrease in enzyme activity depended on the storage temperature: the maximum was observed in the 12th month at -18 °C: by 64.0, 59.1 and 81.6% (compared to the control) for samples of linden, buckwheat and sunflower honey, respectively. Also, significant changes (by 34.0-76.9% depending on the botanical origin of honey) occurred as a result of storage at -10, -5 and 18 °C. The most suitable storage condition was found to be at temperatures between 5 and 0°C, as the enzyme activity in the 12-month study period decreased by only 14.6%-23.5%.

Analysis of catalase enzyme content in stored honey samples demonstrated its greater resistance to both low and room temperatures compared to the enzymes described above. Despite the fact that, as in the case of diastase and D-glucose-1-oxidase, a gradual decrease in its activity was noted throughout the entire period of research, this process did not occur so intensely at all temperature conditions (Fig. 2). It should be noted that in samples of sunflower honey, characterized by an initially high content of catalase (850±22 mm^3 O_2), minimal changes in this indicator were noted: in the 12th month, catalase decreased by 10.0-17.4%, depending on the storage temperature. At the same time, for samples of linden and buckwheat honey in which the initial level of catalase activity was significantly lower (330±14 and 485±19 mm^3 O_2, respectively), the activity decreased by 12.7-35.5 % and 11.3-29.5 %, respectively, by the end of the experiment. Overall, it can be noted that regardless of botanical origin, minimal changes in enzyme activity were observed at temperatures of 5 and 0 °C.

The content of H_2O_2 in honey samples was quite stable at temperatures of 5 and 0 °C: in the 12th month, its concentration in linden honey samples decreased by 5.3 and 2.9%, respectively, in buckwheat samples – by 15.5 and 12.2%, respectively, sunflower – by 5.1 and 2.5%, respectively. In samples stored at 18 °C, a significant decrease in H_2O_2 began at the 6th month, which coincides with our previously obtained data. The same trend was observed for samples stored at 10 and -5 °C. At the same time, storage at -10 and -18 °C led to a decrease in this indicator already in the first month (Fig. 2).

It was found that after 12 months of storage at the specified temperatures, the moisture content, total mass fraction of reducing sugars, and free acidity of honey samples did not change significantly (the difference from the control was less than 5%), and they remained within the limits regulated by Interstate Standards. In this regard, Table 3 presents the data obtained at the end of the experiment (after 12 months) as well as the initial values of these parameters (control).

Table 3: Content of moisture, reducing sugars and free acidity in linden, buckwheat and sunflower honey samples stored at different temperatures for 12 months

Analyzed parameter	Control	Storage temperature, °C						
		18	10	5	0	-5	-10	-18
Linden honey								
Moisture content, %	18.2±0.5	17.3±0.2	17.4±0.2	17.4±0.2	17.4±0.3	17.5±0.3	17.6±0.3	17.9±0.6
Reducing sugars content, %	86.6±3.9	83.8±3.7	84.5±2.9	84.6±1.7	84.8±3.5	85.1±3.8	85.2±3.5	85.7±2.3
Free acidity, meq/kg	24.6±0.8	25.7±1.2	25.6±0.3	25.5±1.2	25.5±0.9	25.4±0.7	24.8±0.6	24.7±0.2
Buckwheat honey								
Moisture content, %	17.9±0.7	17.1±0.5	17.2±0.6	17.3±0.5	17.4±0.4	17.5±0.7	17.8±0.3	17.8±0.5
Reducing sugars content, %	86.8±3.6	83.9±1.3	84.3±2.6	84.9±2.4	85.3±2.8	85.6±2.8	86.2±1.9	86.7±3.1
Free acidity, meq/kg	26.3±1.1	27.3±0.7	27.1±0.9	27.0±0.5	26.9±1.3	26.8±1.1	26.8±1.2	26.6±0.6
Sunflower honey								
Moisture content, %	16.8±0.8	16.0±0.2	16.1±0.3	16.2±0.1	16.2±0.1	16.2±0.2	16.3±0.3	16.4±0.2
Reducing sugars content, %	89.9±1.6	88.4±1.4	89.4±2.7	89.5±2.9	89.6±3.1	89.7±2.8	89.8±2.5	89.8±3.8
Free acidity, meq/kg	25.1±0.9	25.8±1.3	25.6±1.2	25.6±0.8	25.5±1.2	25.4±0.7	25.3±0.9	25.2±1.1

Note: $p < 0.05$

The maximum decrease in the moisture content in linden honey samples was 4.9% at a temperature of 18 °C (relative to the control). Buckwheat and sunflower honey had a decrease of 4.5% and 4.8%, respectively, at the same temperature. Minimal deviations from the initial values were observed at 0 °C and negative temperatures. The results obtained are consistent with the data obtained by Kędzierska-Matysek M. *et al*.[10] The stability of the moisture of samples stored at low temperatures can be explained by the faster transition of water molecules into a bound form.[40]

The same trend was observed in relation to reducing sugars: the smallest amount of them was noted in the analyzed samples stored at 18 °C. Thus, for samples of linden and buckwheat honey the deviation was 3.3%, and for sunflower honey – 1.7%. According to G.P. Ribeiro *et al*.[12] the content of reducing sugars in honey samples stored at -18 °C for 180 days changed by 4.9%. The results presented in the work of S.N. Esenkina,[41] demonstrated a deviation of this indicator by 4.2% after 3 months of storage at a similar temperature.

Free acidity for 12 months at all temperatures, on the contrary, it increased slightly. The following pattern was identified: the higher the storage temperature, the higher the acidity value. At 18 °C this parameter increased by 4.5% (for linden honey), 3.8% for buckwheat honey, and 2.7% for sunflower honey. F. Braghini *et al*. noted the same trend when studying honey stored at 22 °C.[13]

The changes that occurred in the content of reducing sugars and free acidity are due to the speed of the dehydration process, as a result of which simpler compounds are formed from monosaccharides, including organic acids, as well as HMF (which explains its stabilization at low temperatures). This is consistent with the findings of Kędzierska-Matysek,[10] who studied rapeseed honey. It showed that when the storage temperature of this product is lowered, chemical reactions proceed much more slowly in it.

Results of Antimicrobial Analysis

As previously mentioned, according to the Interstate Standard,[9] honey should be stored at a temperature not exceeding 20 °C. This requirement applies

to all types of honey, whether it is monofloral or polyfloral, regardless of botanical origin. There is literature on the antimicrobial activity of honey stored at room temperature and 4 °C, as well as on heat-treated honey. Researchers gave noted that various components contribute to honey's inhibitory effect of against gram-positive and gram-negative microorganisms, such as sugar content, polyphenolic compounds, acids, 1,2-dicarbonyl compounds, bee defensin-1, and H_2O_2. These elements are present in varying concentrations in honey depending on the source of the nectar and type of bees, so the antimicrobial activity of different types of honey is not uniform.[4, 42–47] There is evidence of high activity of Manuka honey from the nectar of *Leptospermum scoparium* flowers. Manuka honey is a source of phenolics and unique chemical compounds, such as methylglyoxal, dihydroxyacetone, leptosperin glyoxal, methylsyringate and leptosin.[48, 49] Among Russian monofloral honeys, linden honey is considered one of the most highly active, which may be due to its rather high H_2O_2 content.[50]

However, it is not yet clear how the antimicrobial effect of honey is affected by storing it at different temperature conditions for a long time. To investigate this, we conducted the experiment on *Escherichia coli* (strain 1257), *Staphylococcus aureus* (strain 209-P) and *Bacillus cereus* (strain 96, vegetative form). These microorganisms are commonly used as test cultures in microbiological studies and for investigating various biochemical mechanisms. Additionally, *E. coli* and *S. aureus* are known as contaminants of the mucous and skin epithelium of humans and animals, therefore, under certain conditions, they can cause inflammatory processes and food poisoning. *B. cereus*, although a representative of soil bacterium, can also be a cause of toxic infections.[51–54]

The studied samples of freshly pumped honey from various botanical origins had a clear inhibitory effect on the growth of all test cultures (Fig. 3).

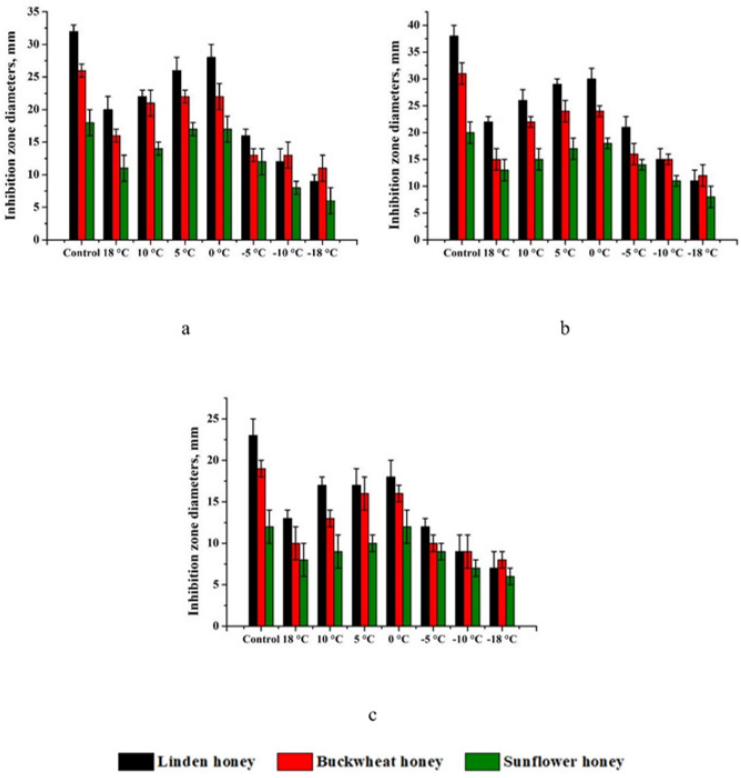

Fig. 3: Inhibition of the growth of *E. coli* (a), *S. aureus* (b) and *B. cereus* (c) by samples of linden, buckwheat and sunflower honey: before storage (control), as well as on the 12th month of storage at temperatures: 18 °C, 10 °C, 5 °C, 0 °C, -5 °C, -10 °C and -18 °C

The antimicrobial activity of linden honey against *S. aureus* (with an average inhibition zone diameter is 38 mm) was superior to the effect on *E. coli* (32 mm) and on *B. cereus* (23 mm). Buckwheat honey also had a greater inhibitory effect on *S. aureus* (31 mm) than on *E. coli* (26 mm) and *B. cereus* (19 mm). Sunflower honey similarly suppressed the growth of *Staphylococcus aureus* (20 mm) to a greater extent than *E. coli* (18 mm) and *B. cereus* (12 mm).

Staphylococcus aureus exhibited the least resistance to honey, regardless of its botanical origin. This is explained by the varying resistance of microorganisms to external factors.

In all honey samples, a decrease in antimicrobial activity was observed after 12 months of storage at different temperatures. The level of this decrease was greatest at -18 °C, followed by -10 °C, -5 °C, 18 °C, 10 °C and 5 °C. This trend was observed for all honey types examined.

Thus, the average degree of decrease in the antimicrobial activity of linden honey (compared to the control) against *S. aureus* at -18 and 0 °C was 71% and 21%, respectively; for *E. coli* – 72% and 12.5%; *B. cereus* – 69.5% and 21.7%. The inhibitory effect of buckwheat honey against *S. aureus* decreased by 61.3% and 22.6% at -18 and 0 °C, respectively; for *E. coli* – by 57.7% and 15.4%; *B. cereus* – by 57.9% and 15.8%. The same trend was noted for sunflower honey: 60.0% at -18 °C and 10.0% at 0 °C for *S. aureus*; 66.7% and 5.5% for *E. coli*; 50.0% and 0.0% (not including deviation from the mean) for *B. cereus*.

To a large extent, these changes were associated with the level of H_2O_2 in honey samples, which is known to be a factor in oxidative stress.[55, 56] However, the dependence was not direct, which is explained by the influence of other factors on it as well.

Therefore, storage of all types of honey at temperatures outside the range of 0–5 °C resulted in a decrease in H_2O_2 levels, enzymes activity, an increase in HMF, and a reduction in the inhibitory effect on test cultures.

H_2O_2 is synthesized in honey in a reaction with β-D-glucose, catalyzed by the enzyme D-glucose-1-oxidase. This reaction is a two-stage oxidation-reduction process. In the first stage, D-glucose-1-oxidase catalyzes the oxidation of β-D-glucose by transferring two electrons and a proton from the hydroxyl group of β-D-glucose to flavin adenine dinucleotide (FAD). In this case, glucose is oxidized to glucono-1,5-lactone. Then, the resulting glucono-1,5-lactone is hydrolyzed to gluconic acid. In the second stage, the reduced $FADH_2$ is oxidized by molecular oxygen. Oxygen accepts two electrons from $FADH_2$, turning into H_2O_2. Excess H_2O_2 is decomposed into water and oxygen by the iron containing enzyme catalase in two stages.[7, 57] In the first stage,H_2O_2 oxidizes Fe^{III} to oxyferryl $Fe^{IV}O$. In this process, the O–O bond in the H_2O_2 molecule is broken, which ultimately leads to the formation of water and oxygen. In the second stage, the intermediate oxyferryl $Fe^{IV}O$ is reduced by a second H_2O_2 molecule to the original catalase. Thus, the relationship between the H_2O_2content and the activity of D-glucose-1-oxidase and catalase is obvious. The decrease in the activity of D-glucose-1-oxidase can be explained by its high temperature sensitivity.[58] At the same time, catalase is a thermostable enzyme.[59]

HMF is a low-melting cyclic aldehyde. The mechanism of its formation in honey is that as a result of exposure to high temperatures, as well as during long-term storage, one or two water molecules are split off from sugars (dehydration). In this case, anhydrides are formed, which can combine either with each other or with an unchanged sugar molecule and form so-called condensation (reversion) products. Then the third water molecule is split off and HMF is formed. Subsequently, it decomposes with the destruction of the carbohydrate skeleton and the formation of formic and levulinic acids. The most intensive processes described occur when honey is heated.[60, 61] In our studies, during storage of honey samples at low temperatures, we observed minor changes in sugar content and acidity, as well as no excess of the permissible concentration of HMF.

It should be noted that physicochemical parameters can be used as indicators of honey quality, since their significant change signals inadequate storage conditions for this product. However, honey is a complex biological system, therefore, to obtain more accurate information about possible negative factors, a comprehensive analysis should be

carried out taking into account the main criteria. For example, the detection of a high HMF content in combination with a low H_2O_2 concentration and reduced enzymatic activity often indicates heat treatment or adulteration of honey.[62–64]

As already mentioned, the inhibitory effect of honey is due to the various compounds present in it. Considering the absence of significant changes in the content of sugars and acidity in the studied honey samples, it can be assumed that H_2O_2 is the leading factor in their antimicrobial activity against *E. coli*, *S. aureus* and *B. cereus*. The effectiveness of H_2O_2 is explained by its strong oxidizing property. H_2O_2 affects several biomolecules within the cell, causing peroxidation and destruction of membrane layers, oxidation of thiol groups, inhibition of enzymes, oxidation of nucleosides, disruption of energy production, disruption of protein synthesis, and ultimately leading to cell death.[65–67]

Conclusions

As a result, it can be concluded that the physicochemical and biological analysis of samples of linden, buckwheat, and sunflower honey, which was carried out for 12 months and stored at different temperatures, made it possible to identify certain changes in the physicochemical parameters and biological activity of the studied samples.

A positive result of using negative temperatures (-5, -10 and, in particular, -18 °C) for long-term storage of honey was a slowdown in the rate of chemical processes, which ultimately led to minimal changes in the content of the toxic compound – HMF. However, such temperature storage conditions had a negative impact on the enzymatic activity and H_2O_2 concentration, which, along with the content of sugars and acids, are factors in the antimicrobial activity of honey. Similar changes occurred with samples stored at 18 and 10 °C, in which, in addition, a higher content of HMF was noted. Chemical processes occurred less intensely at 5 and 0 °C. The HMF content in samples stored at these temperatures for 12 months ranged from 7.37 to 11.97 mg/kg, depending on the botanical origin of honey, which is significantly below the MPC.

At the 12th month of storage, all honey samples met the requirements of Interstate Standards for all indicators. The exception was diastase activity in samples of sunflower honey. Nevertheless, a test conducted to study the antimicrobial activity of honey against *E. coli*, *S. aureus* and *B. cereus* demonstrated that samples stored at 5 and 0 °C had the greatest inhibitory effect. Therefore, these temperatures seem to be optimal for storing honey for a long time. The results of this study can be used as additional recommendations when organizing honey storage.

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The authors contributed equally to the research and the manuscript.

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References

1. Seraglio S. K. T., Schulz M., Brugnerotto P., Silva B., Gonzaga L. V., Fett R., Costa A. C. O. Quality, composition and health-protective properties of citrus honey: A review. *Food Res Int.* 2021;143:110268. DOI: https://doi.org/10.1016/J.FOODRES.2021.110268
2. Proaño A., Coello D., Villacrés-Granda I., Ballesteros I., Debut A., Vizuete K., Brenciani A., Álvarez-Suarez J. M. The Osmotic Action of Sugar Combined with Hydrogen Peroxide

and Bee-Derived Antibacterial Peptide Defensin-1 is Crucial for the Antibiofilm Activity of Eucalyptus Honey. *LWT - Food Sci Technol.* 2021;136(2):110379. DOI: https://doi.org/10.1016/j.lwt.2020.110379

3. Bucekova M., Jardekova L., Juricova V., Bugarova V., Di Marco G., Gismondi A., Leonardi D., Farkasovska J., Godocikova J., Laho M., Klaudiny J., Majtan V., Canini A., Majtan J. Antibacterial activity of different blossom honeys: New findings. *Molecules.* 2019;24(8):1573. DOI: https://doi.org/10.3390/molecules24081573
4. Almasaudi S. The Antibacterial Activities of Honey. *Saudi Journal of Biological Sciences.* 2021;28(4):2188–2196. DOI: https://doi.org/10.1016/j.sjbs.2020.10.017
5. Ayoub W. S., Ritu, Zahoor I., Dar A. H., Farooq S., Mir T. A., Ganaie T. A., Srivastava Sh., Pandey V. K., Altaf A. Exploiting the polyphenolic potential of honey in the prevention of chronic diseases. *Food Chem Adv.* 2023;3:100373. DOI: https://doi.org/10.1016/j.focha.2023.100373
6. Alygizou A., Grigorakis S., Gotsiou P., Loupassaki S., Calokerinos A.C.. Quantification of Hydrogen Peroxide in Cretan Honey and Correlation with Physicochemical Parameters. *J Anal Methods Chem.* 2021;2021:5554305. DOI: https://doi.org/10.1155/2021/5554305
7. Brudzynski K. A current perspective on hydrogen peroxide production in honey. A review. *Food Chem.* 2020;332:127229. DOI: https://doi.org/10.1016/j.foodchem.2020.127229
8. Mohammed M. E. A., Alarjany W., Suleiman M. H. A., Al-Gramah H. A. Hydrogen Peroxide and Dicarbonyl Compounds Concentration in Honey Samples from Different Botanical Origins and Altitudes in the South of Saudi Arabia. *Curr. Res. Nutr Food Sci.* 2019;7(1):150–160. DOI: https://doi.org/10.12944/CRNFSJ.7.1.15
9. Interstate Standard 19792-2017. Natural honey. Specifications. Moscow: Standartinform; 2017.
10. Kędzierska-Matysek M., Florek M., Wolanciuk A., Skałecki P. Effect of freezing and room temperatures storage for 18 months on quality of raw rapeseed honey (Brassica napus). *J Food Sci Technol.* 2016;53(8):3349–3355. DOI: https://doi.org/10.1007/s13197-016-2313-x
11. Pasias I. N., Raptopoulou K. G., Makrigennis G., Ntakoulas D. D., Lembessis D., Dimakis V., Katsinas R., Proestos C. Finding the optimum treatment procedure to delay honey crystallization without reducing its quality. *Food Chem.* 2022;381:132301. DOI: https://doi.org/10.1016/j.foodchem.2022.132301
12. Ribeiro G. P., Villas-Bôas J.K., Spinosa W. A., Prudencio S. H. Influence of freezing, pasteurization and maturation on Tiúba honey quality. *LWT - Food Sci Technol.* 2018;90:607–612. DOI: https://doi.org/10.1016/j.lwt.2017.12.072
13. Braghini F., Biluca F. C., Ottequir F., Gonzaga L. V., da Silva M., Vitali L., Micke G. A., Costa A. C. O., Fett R. Effect of different storage conditions on physicochemical and bioactive characteristics of thermally processed stingless bee honeys. *LWT - Food Sci Technol*. 2020;131:109724. DOI: https://doi.org/10.1016/j.lwt.2020.109724
14. Zaikina V. I. Examination of honey and methods for detecting its falsification: Textbook (in Russ.). Moscow: Izdatel'sko-torgovaya korporatsiya “Dashkov i K”; 2012.
15. Ji P., Liu X., Yang Ch., Wu F., Sun J., Cao W., Zhao H. Natural crystallization properties of honey and seed crystals-induced crystallization process for honey performance enhancing. *Food Chem.* 2023;405:134972. DOI: https://doi.org/10.1016/j.foodchem.2022.134972
16. Villacrés-Granda I., Proaño A., Coello D., Debut A., Vizuete K., Ballesteros I., Granda-Albuja G., Rosero-Mayanquer H., Battino M., Giampieri F., Alvarez-Suárez J.M. Effect of thermal liquefaction on quality, chemical composition and antibiofilm activity against multiresistant human pathogens of crystallized eucalyptus honey. *Food Chem.* 2021;365(15):130519. DOI: https://doi.org/10.1016/j.foodchem.2021.130519
17. Besir A., Yazici F., Mortas M., Gul O. A novel spectrophotometric method based on Seliwanoff test to determine 5-(Hydroxymethyl) furfural (HMF) in honey: Development, in house validation and application. *LWT - Food Sci Technol.* 2021;139:110602. DOI: https://

doi.org/10.1016/j.lwt.2020.110602
18. Salhi I., Samet Y., Trabelsi M. Direct electrochemical determination of very low levels of 5-hydroxymethyl furfural in natural honey by cyclic and square wave voltammetric techniques. *J Electroanal Chem.* 2020;837:114326. DOI: https://doi.org/10.1016/j.jelechem.2020.114326
19. Laolue P., Lerdsri J. Development of square wave voltammetry method using working electrodes modified with nickel oxide and carbon black for determination of 5-hydroxymethylfurfural in honey. *J Food Compos Anal.* 2023;124:105699. DOI: https://doi.org/10.1016/j.jfca.2023.105699
20. Esenkina S. N. Useful properties of buckwheat honey (in Russ.). Pchelovodstvo. 2022;2:50–51.
21. Naumkin V. P. Bees on buckwheat (in Russ.). Pchelovodstvo. 2002;5:20–21.
22. Yakimov M. V., Absalyamov R. R., Yakimov D. V. Influence of weather conditions on honey yield during flowering period of small-leaved linden in Udmurt republic (in Russ.). *Forestry Bulletin.* 2022;26(1):41–49. DOI: https://doi.org/10.18698/2542-1468-2022-1-41-49
23. Mazalov V. I., Naumkin V. P. Crop yield of new sunflower varieties and hybrids (in Russ.). *Pchelovodstvo.* 2021;6:24–25.
24. Interstate Standard 31769-2012. Honey. Determination of the relative frequency of pollen. Moscow: Standartinform; 2019.
25. Codex Alimentarius. 1981. Standards for honey. CODEX STAN 12-1981 revised 2001.
26. Council Directive 2001/110/EC. On honey. *Official Journal of the European Communities.* 2002;L10:47-52.
27. International Honey Commission. Harmonised Methods of the International Honey Commission. 2009.
28. Interstate Standard 31774-2012. Honey. Refractometric method for determination of water. Moscow: Standartinform; 2018.
29. Interstate Standard 31768-2012. Natural honey. Methods for determination of hydroxymethylfurfural. Moscow: Standartinform; 2019.
30. Interstate Standard 32167-2013. Honey. Method for determination of sugars. Moscow: Standartinform; 2018.
31. Interstate Standard 32169-2013. Honey. Method determination of pH and free acidity. Moscow: Standartinform; 2019.
32. Interstate Standard 34232-2017. Honey. Methods for determination of sucrose activity, diastase activity, insoluble matters. Moscow: Standartinform; 2017.
33. Aganin A. V. Honey and its research (in Russ.). Saratov: Izdatel'stvo Saratovskogo universiteta; 1985.
34. Flanjak I., Strelec I., Kenjerić D., Primorac L. Croatian produced unifloral honeys characterized according to the protein and proline content and enzyme activities. *J Apicult Sci.* 2015;60(1):39–48. DOI: https://doi.org/10.1515/jas-2016-0005
35. Lobanov A. V., Rubtsova N. A., Vedeneeva Yu. A., Komissarov G. G. Photocatalytic activity of chlorophyll in hydrogen peroxide generation in water. Doklady Chemistry. 2008;421(2):190–193. DOI: https://doi.org/10.1134/S0012500808080065
36. Interstate Standard 31766-2022. Monofloric honeys. Specifications. Moscow: Standartinform; 2022.
37. Burmistrov A. N., Nikitina V. A. Honey plants and their pollen: Directory (in Russ.). Moscow: Rosagropromizdat; 1990.
38. Karpovich I., Drebezgina E., Elovikova E., Legotkina G., Zubova E., Kuzyaev R., Khismatullin R. Pollen Atlas (in Russ.). Ekaterinburg: Uralsky Rabochiy; 2015.
39. Gruznova O. A., Lobanov A. V., Sokhlikov A. B., Gruznov D. V. Determination of the correlation between the content of 5-hydroxymethylfurfural and hydrogen peroxide in honey (in Russ.). *Chem Safety Sci.* 2022;6(2):215–226. DOI: https://doi.org/10.25514/CHS.2022.2.23014
40. Bakier S. Characteristics of water state in some chosen types of honey found in Poland. *Acta Agrophysica.* 2006;7:7–15.
41. Esenkina S. N. The impact of negative temperatures on honey quality (in Russ.). Pchelovodstvo. 2022;3:49–50.
42. Brudzynski K., Abubaker K., St-Martin L., Castle A. Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey. *Front. Microbiol.* 2011;2:213. DOI: https://doi.org/10.3389/fmicb.2011.00213
43. Hussain M. B., Hannan A., Akhtar N., Fayyaz

G. Q., Imran M., Saleem S., Qureshi I. A. Evaluation of the antibacterial activity of selected Pakistani honeys against multi-drug resistant Salmonella typhi. *BMC Complementary and Alternative Medicine.* 2015;15(1):549. DOI: https://doi.org/10.1186/s12906-015-0549-z

44. Bucekova M., Buriova M., Pekarik L., Majtan V., Majtan J. Phytochemicals-mediated production of hydrogen peroxide is crucial for high antibacterial activity of honeydew honey. *Scientific Reports*. 2018;8:9061. DOI: https://doi.org/10.1038/s41598-018-27449-3
45. Chen C., Campbell L. T., Blair Sh. E., Carter D. A. The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. *Front. Microbiol.* 2012;3:265. DOI: https://doi.org/10.3389/fmicb.2012.00265
46. Sowa P., Grabek-Lejko D., Wesołowska M., Swacha S., Dzugan M. Hydrogen peroxide-dependent antibacterial action of Melilotus albus honey. *Letters in Applied Microbiology.* 2017;65:82–89. DOI: https://doi.org/ 10.1111/lam.12749
47. Poli J.-P., Guinoiseau E., Luciani A., Yang Y., Battesti M.-J., Paolini J., Costa J., Quilichini Y., Berti L., Lorenzi V. Key role of hydrogen peroxide in antimicrobial activity of spring, Honeydew maquis and chestnut grove Corsican honeys on Pseudomonas aeruginosa DNA. *Letters in Applied Microbiology.* 2018;66(5):427–433. DOI: https://doi.org/10.1111/lam.12868
48. Schmidt C., Eichelberger K., Rohm H. New Zealand mānuka honey - A review on specific properties and possibilities to distinguish mānuka from kānuka honey. *LWT - Food Sci Technol*. 2021;136(1):110311. DOI: https://doi.org/10.1016/j.lwt.2020.110311
49. Wang S., Qiu Yi, Zhu F. An updated review of functional ingredients of Manuka honey and their value-added innovations. *Food Chem*. 2024;440:138060. DOI: https://doi.org/10.1016/j.foodchem.2023.138060
50. Gruznova O. A., Lobanov A. V., Sokhlikov A. B., Gruznov D. V. Comparison of organoleptic and physicochemical parameters of natural and falcified honey (in Russ.). *Rus. J. "Problems of Veterinary Sanitation, Hygiene and Ecology*". 2023;3(47):314–320. DOI: https://doi.org/10.36871/vet.san.hyg.ecol.202303009
51. Krupyanskii Yu. F., Generalova A. A., Kovalenko V. V., Loiko N. G., Tereshkin E. V., Moiseenko A. V., Tereshkina K. B., Sokolova O. S., Popov A. N. DNA Condensation in Bacteria. *Russian Journal of Physical Chemistry B*. 2023;17(3):517–533. DOI: https://doi.org/10.1134/S1990793123030211
52. Chhawchharia A., Haines R. R., Green K. J., Barnett T. C., Bowen A. C., Hammer K. A. In vitro antibacterial activity of Western Australian honeys, and manuka honey, against bacteria implicated in impetigo. *Complementary Therapies in Clinical Practice.* 2022;49:101640. DOI: https://doi.org/10.1016/j.ctcp.2022.101640
53. Bucekova M., Godocikova J., Kohutova L., Danchenko M., Barath P., Majtan J. Antibacterial activity and bee-derived protein content of honey as important and suitable complementary tools for the assessment of honey. *J Food Compos Anal.* 2023;123:105610. DOI: https://doi.org/10.1016/j.jfca.2023.105610
54. Rahnama H., Azari R., Yousefi M. H., Berizi E., Mazloomi S. M., Hosseinzadeh S., Derakhshan Z., Ferrante M., Conti G. O. A systematic review and meta-analysis of the prevalence of Bacillus cereus in foods. *Food Control.* 2023;143:109250. DOI: https://doi.org/10.1016/j.foodcont.2022.109250
55. Karbyshev M. S., Abdullayev Sh. P. Biochemistry of oxidative stress (in Russ.). Moscow: Izdatel'stvo FGBOU VO RNIMU imeni N.I. Pirogova Minzdrava Rossii. 2018.
56. Faúndez X., Báez M.E., Martínez J., Zúñiga-López M. C., Espinoza J., Fuentes E. Evaluation of the generation of reactive oxygen species and antibacterial activity of honey as a function of its phenolic and mineral composition. *Food Chem.* 2023;426:136561. DOI: https://doi.org/10.1016/j.foodchem.2023.136561
57. Gruznov D. V., Gruznova O. A., Lobanov A. V., Sokhlikov A. B., Shcherbakova G. Sh., Stepanova S. P., Popov N. I. Influence of Different Heat Treatment Regimes on the Change of the Chemical Composition and Antibacterial Activity of Bee Honey. *Rus. J. Phys. Chem. B.* 2024;18(1):258–265. DOI:

https://doi.org/10.1134/S1990793124010275

58. Ning X., Yanli Zh., Tiantian Yu., Qingbin Li, Jian T., Weishi G., Bo L., Wei Zh., Xinxin X., Yuhong Zh. Enhanced Thermostability of Glucose Oxidase through Computer-Aided Molecular Design. *Int J Mol Sci.* 2018;19(2):425. DOI: https://doi.org/10.3390/ijms19020425
59. Scott J. W., Stee J. J. Extremophile enzyme activity lab: using catalase from Pyrobaculum calidifontis to highlight temperature sensitivity and thermostable enzyme activity. *J. Microbiol. & Biol. Education.* 2024;25(1):1–5. DOI: https://doi.org/10.1128/jmbe.00065-23
60. Khalil, M.I., S.A. Sulaiman and S.H. Gan. High 5-hydroxymethylfurfural concentrations are found in Malaysian honeysamples stored for more than one year. *Food and Chem Toxicol*. 2010;48:2388–2392. DOI: https://doi.org/10.1016/j.fct.2010.05.076
61. Mansoor S. S., Roufegari-nejad L. A Study on the Formation and Safety of HMF in Honey. *Advances in Environmental Biology.* 2014;8(11):1228–1231.
62. Gruznov D. V., Gruznova O. A., Lobanov A. V., Sokhlikov A. B. Detection of honey adulterations based on physicochemical analysis. *Chemical Safety Science*. 2023;7(2):153–165. DOI: https://doi.org/10.25514/CHS.2023.2.25009
63. Villacr´es-Granda I., Proaño A., Coello D., Debut A., Vizuete K., Ballesteros I., Granda-Albuja G., Rosero-Mayanquer H., Battino M., Giampieri F., Alvarez-Suarez J. M. Effect of thermal liquefaction on quality, chemical composition and antibiofilm activity against multiresistant human pathogens of crystallized eucalyptus honey. *Food Chem.* 2021;365:130519. DOI: https://doi.org/10.1016/j.foodchem.2021.130519
64. Amiry S., Esmaiili M., Alizadeh M. Classification of adulterated honeys by multivariate analysis. *Food Chem.* 2017;224:390–397. DOI: http://dx.doi.org/10.1016/j.foodchem.2016.12.025
65. Imlay J. A. The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nature Reviews Microbiology.* 2013;11:443–454. DOI: DOI: https://doi.org/10.1038/nrmicro3032
66. Zeng, F., Cao, S., Jin, W., Zhou, X., Ding, W., Tu, R., Han, S. F., Wang, C., Jiang, Q., Huang, H., Ding, F. Inactivation of chlorine-resistant bacterial spores in drinking water using UV irradiation, UV/Hydrogen peroxide and UV/peroxymonosulfate: efficiency and mechanism. *Journal of Cleaner Production.* 2020;243:118666. DOI: https://doi.org/10.1016/j.jclepro.2019.118666
67. Abdelshafy A. M., Neetoo H., Al-Asmari F. Antimicrobial Activity of Hydrogen Peroxide for Application in Food Safety and COVID-19 Mitigation: An Updated Review. *Journal of Food Protection*. 2024;87(7):100306. DOI: https://doi.org/10.1016/j.jfp.2024.100306.