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ASSESSMENT OF MICROBIOLOGICAL QUALITY OF BELORUSSIAN NECTAR HONEYS

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ABSTRACT

The main objective of the research was to evaluate the microbiological quality of 10 honeys from Belorus collected from beekeepers in the area of Grodno. The study was conducted in 2013 at the Apiculture Division of Warsaw University of Life Sciences-SGGW. The aim of the research was the count of total number of microorganisms, count of the genus *Bacillus spp.*, and also yeasts and molds. An assessment of microbial count in honey was made with the use of three methods: submerged culture, surface culture and spiral plate to select the best of them. Furthermore were tested selected physicochemical properties such as pH, water content and water activity. The presence of *Bacillus spp.*, yeasts and molds, and the total number of microorganisms was found in all honeys. It was also ascertained that the appropriate method for testing the microbiological quality of honey is the classic submerged method. Tested honeys proved to be a good value. Most of the studied parameters was in the standards or were on the borders. All honeys were characterized by low pH (range 3.08-3.42), while the water activity (0.622 - 0.538 aw) and a water content (16.7-21.7%) was adequate to hinder the microbial growth.

Keywords: Belorussian honey, microbiological quality.

INTRODUCTION

Honey is a sweet substance produced by bees. It is collected and matured in the honeycomb, gaining there the right consistency and composition [Bornus, 1986]. Since ancient times people were engaged in extraction of honey. Its consumption was a culturally and historically shaped. For centuries, it is present in gastronomy and is an important component of the diet. Honey has also its application in medicine and health-oriented prevention. As far back as ancient times people had knowledge of its nutritional and medicinal properties [Mika et al., 2013]. Honey is a valuable nutritional product, and has a cleansing and anti-inflammatory properties [Broadhurts, 2012]. Today, thanks to modern research methods, we are able to deepen knowledge on the composition, properties and quality of honey. It is known the basic composition of this product, most of the physicochemical and biological properties. One of the main components of honey is water.

The water in the honey determines its properties biological, chemical as well as physical. It is a good environment for chemical reactions and affect the microflora. The content and water activity in honey are the important parameters affecting the condition of the product. The proportion of water in the processes occurring in honey and its availability can be determined on the basis of the water activity (aw) [Rybak-Chmielewska, Szczęsna, 1996]. Its activity is defined as the ratio of the water vapor pressure above the solution to the water vapor pressure above pure water at constant temperature and pressure. This is very important factor as it affects the qualities of honey, such as smell, texture, taste, long shelf life, appearance [Janowicz et al., 2007]. The water activity in honey is 0.6 aw and the clean water 1 aw [Kędzia, Hołderna-Kędzia, 2008]. The water content in honey is usually 17-20%. It depends on many

factors, and comes from honeydew and nectar [Cymanow 2009]. Weather factors (humidity, temperature), conditions in which the honey matures and the geographical area of origin of honey have an impact on the percentage of water in honey [Kędzia, Hołderna-Kędzia, 2008].

Another parameter determining the condition of the honey is its acidity. For the determination of the acidity is used pH-negative logarithm of the hydrogen ion concentration as a scale. Average pH for honey is 4.2 and is too low for the development of various bacteria. It is recognized that the acidity is one of the mechanisms of defense against bacteria [Buzzini, 1994]. Another way of defense is proper storage of honey, but even in properly stored honey are present microorganisms [Różańska, 2011]. But among all microbes it should not be included any microorganisms which may be pathogenic. In the honey is present a large number of microorganisms which are typically originated from the nectar, honeydew and water. In addition, a source of contamination of honey is the mouthparts of bees (esophagus and crop) [Kędzia, Hołderna-Kędzia, 2008)\]. The microflora of honey varies with the maturation in honeycomb. In the open honey dominate aerobic bacteria but fungi are found in small amounts. In immature honey it is usually found bacteria of the genus Gluconobacter (gram-negative), and Lacto*bacillus* (gram-positive). With the maturation of honey and with the change of the composition (decreases amount of water and increases the concentration of sugar) changes its microflora. In the mature honey there are mainly fungi, which have a perfect environment for development. In the majority of floral honey standard plate count is 1000 in 1 g of honey [Snowdown et al., 1996]. In the honey may be found micro-organisms which are pathogenic to plants and bees (Table 1).

Microorganisms pathogenic to human are: *Escherichia coli, Klebsiella pneumoniae* and *Shigella spp.* (Dysentery bacilli), *Salmonella, Cirtobacter, Edwardsiella, Staphylococcus, Enterococcus*. Spores of aerobic and anaerobic bacteria, Especially *Bacillus spp.*, and *Clostridium spp.* are responsible for microbial contamination of honey with its vegetative forms and toxins. It was also ascertained the presence of spores of *Clostridium botulinum*, causing botulism infection in infants fed with honey [Kędzia, Hołderna-Kędzia, 2008]. Botulism is a set of symptoms caused by the action of bacteria *Clostridium botulinum* [Midura et al., 1979]. The incidence of this bacteria in honey depends on the geographical region from which the honey origin. In Poland, botulism caused by consumption of contaminated honey is very rare [Kędzia, Hołderna-Kędzia, 2008]. To eliminate the risk it is advised not to fed honey to children under one year old [Kędzia, 2009].

Table 1.

Microorganisms pathogenic to plants and bees occurring in honey [Kędzia, Hołderna-Kędzia, 2008]

Plants pathogens	Microorganisms pathogenic to bees
Ustilago (*Ustiliginales*) Pseudoperonospora (*Peronosporales*) Urediniomycetes (*Uredinales*)	*Peanibacillus laevae (Bacillus larvae)* *Bacillus alvei* *Enterobacter hafniae (Hafnia alvei)* *Pseudomonas aeruginosa* *Aspergillus flavus* *Nosema apis* *Malpighamoeba mellifica*

So, the safety of consumers of honey depends on honey quality [Arszułowicz, 2006]. Over the past decade outlines the growing awareness and importance of healthy eating. This forces the need to produce food of high quality, accepted by customers, and above all, food safe for health. Only products of the highest standard have a chance of survival and success in the market and only those can be accessed by the purchaser. Mediocre products or at low quality not gain the acceptance. The importance therefore have all research related to product quality included microbiological quality of honey.

The aim of the study was to evaluate the microbiological quality of flower honey from Belorussia for the total number of microorganisms, bacteria of the genus *Bacillus spp.*, and also yeasts and molds. An assessment of microbial count in honey was made using three methods: submerged culture, surface culture and spiral plate to determine which of them is the best. Furthermore was tested chosen physicochemical properties such as pH, water content and water activity.

MATERIAL AND METHODS

The study was conducted in 2013 at the Apiculture Division Warsaw University of Life Sciences - SGGW. Research material consisted of 10 samples of honey from Belorussia, Grodno region. All of the honeys were obtained from Belorussian bee-keepers in 2013. The honey have been purchased at apiaries to avoid possible secondary contamination of the studied material. Microbiological and physico-chemical analysis were carried out for all 10 samples of nectar honey Belorussian origin (2 buckwheat, 2 sunflower, 2

Dandelion, 2 Heather and 2 Multifloral).

Methods of microbiological analysis

Ten grams of each sample has been homogenized with 90 ml of buffered peptone water (BPW) using Somacher 400 instrument (IUL Instruments, Germany). To perform decimal dilutions BPW has been used. The microbiological quality of honeys has been tested using the reference plate 3 methods, performing analysis on two parallel plates. An assessment of microbial honey was made using three methods: submerged culture, surface culture and spiral plate. The analysis was carried out for the total number of microorganisms, *Bacillus spp.*, and also yeasts and molds. The number of microorganisms is expressed as colony forming units per gram of honey (cfu/g). Determination of total mesophilic aerobic bacteria (TMAB) were performed using the ISO standard [ISO 7218:2007/A1:2013] with Plate Coumt Agar (PCA Biokar Diagnostics, Austria). The plates were incubated at 30°C for 3 days. The number of mold and yeast were determined on Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar, Becton Dickinson and Co.), after incubation at 25° C for 5 days also according ISO standard [ISO 21527-2., 2008)]. Honeys were tested for the presence of mesophilic spores of *Bacillus spp.*, using Glucose Bromocresol Purple Agar (BCP Biokar Diagnostics), after incubation at 37°C for 5 days.

Methods of physico-chemical analysis

Chosen physicochemical parameters such as acidity, content and water activity of the honeys were tested. The pH of honey was determined using Lab 860 pHmeter (SI Analytics GmbH, Schott Instruments, Germany). Samples of honeys was prepared in the following dilution: 10 g of honey in 10 ml of distilled water. The water activity was measured at 25 °C (± 0.2°C) using Aqualab TE series 4 analyzer (Decagon Devices, Pullman, Washington, USA), with a temperature stable sampling environment, calibrated with saturated salt solutions in the aw range of 0.40-0.70. This device operates based on electronic dew-point measurements. AquaLab analyzer continues the analyses of water activity until the difference of three consecutive measurements is less than 0.0005 aw. Water content in undiluted honeys was established using PAL-22S refractometer (Conbest Sp. z.o.o, Poland) - three/four measurements has been performed.

RESULTS

Result of microbiology analysis. The results of cultures for total microorganisms count (TBC).

In all examined honeys it was revealed the presence of micro-organisms (Figure 1). In inoculations with the use of submerged culture method the highest count of micro-organisms have been observed in dandelion honeys no 1.2 (2.34 log cfu /g; 1.64 log cfu/g) and the lowest in multifloral honeys no 1.2 (0.77 log cfu/g; 0.56 log cfu/g). Inoculations performed using surface culture method the largest number of microorganisms presented in the heather honeys no. 1.2 (1.48 log cfu/g; 1.53 log cfu/g). The lowest count of TBC was observed in sunflower honey no. 2 (0.60 log cfu/g) and multifloral no. 2 (0.64 log cfu/g). In inoculations performed using spiral plate method in buckwheat honey 2, there was no microbial growth. The dandelion honeys no 1 and 2 occurred the highest count (2.26 log cfu/g; 1.55 log cfu/g), and the lowest count in sunflower honey no. 1 and 2 (0.3 log cfu/g 0.47 log cfu/g).

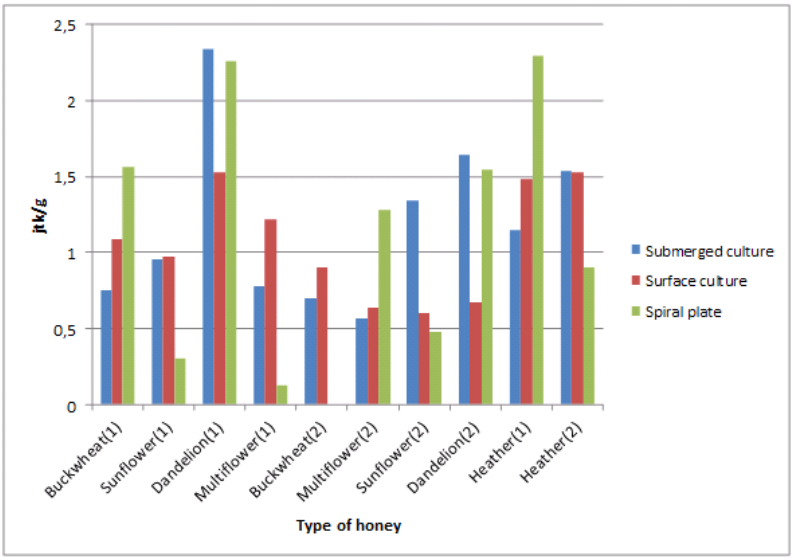

Figure 1. The results of microbiological analyses for total microorganisms count (TBC) in Belorussian honeys.

The most consistent results in the case of above studies were in the submerged culture method.

The results of cultures for Bacillus spp.

In inoculations performed using submerged culture method in nine honeys was observed the growth of bacteria of the *Bacillus spp.* genus. They only were not present in multifloral honey no. 2. The largest growth of the colonies occurred in the sunflower honeys no. 1.2 (1.13 log cfu/g; 1.09 log cfu/g). The largest number of colonies of bacteria of the genus *Bacillus spp* (surface method) was found in multifloral honey no. 1 (2.4 log cfu/g), while the smallest in dandelion honey no. 1 (1.9 log cfu/g). The results of this culture are very aligned. Inoculations performed by the using spiral plate method showed that in all honeys, there were numerous colonies of *Bacillus spp.* bacteria. The highest count of bacteria were in the buckwheat honey no. 1 (2.43 log cfu/g) and dandelion honey no 1 (2.42 log cfu/g), while the lowest in sunflower honey 2 (2.26 log cfu/g). In all honeys participating in the study were present bacteria *Bacillus spp.* It was found that the results from the surface plate method is most uniform (Figure 2).

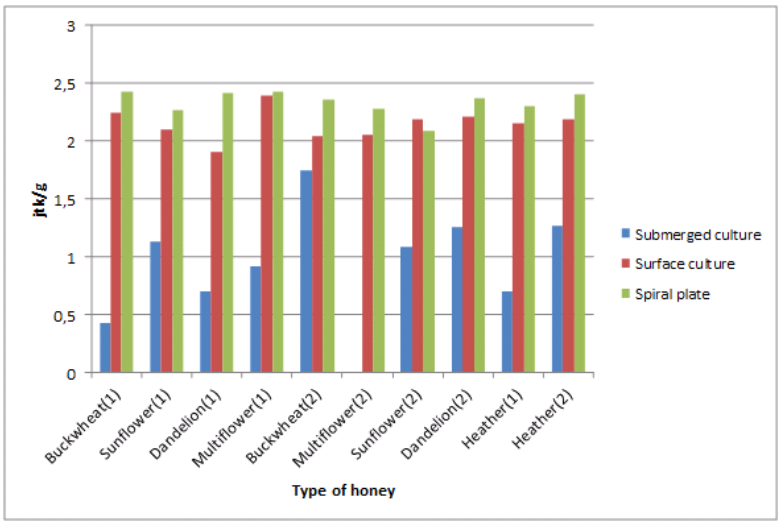

Figure 2. The results of microbiological analyses for *Bacillus spp* in Belorussian honeys.

The results of cultures for the yeast and molds.

In inoculations for yeasts and molds made using submerged culture method in all honeys were detected colonies. The highest count of them were in heather honey 2 and the lowest number of yeast and mold were in multifloral honeys no. 1 and no. 2 (2.7 log cfu/g, 2.2 log cfu/g). Inoculations performed by surface culture method showed that colonies was only in heather honey1 where were detected fungi (0.78 log cfu/g). In inoculations performed using a spiral plate method in three honeys was detected the presence of fungi. Most colonies were observed in the heather honey 1 (0.56 log cfu/g) and the least in sunflower honey 2 (0.11 log cfu/g).

In all honeys were observed the presence of yeasts and molds. It has been found that the submerged culture method is able to provide uniform results (Figure 3).

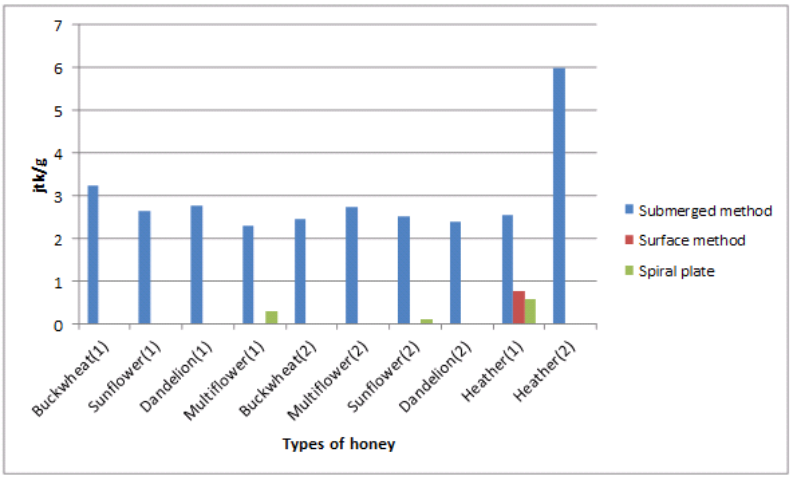

Figure 3. The results of microbiological analyses for yeast and molds in Belorussian honeys.

The results of physicochemical analysis. The total acidity of honeys.

All honeys were characterized by high acidity and were in the pH range of 3.08-3.42. The highest pH was observed in buckwheat honey 1 (pH 3.42), while the lowest in the heather honey 2 (pH 3.08) (Figure 4).

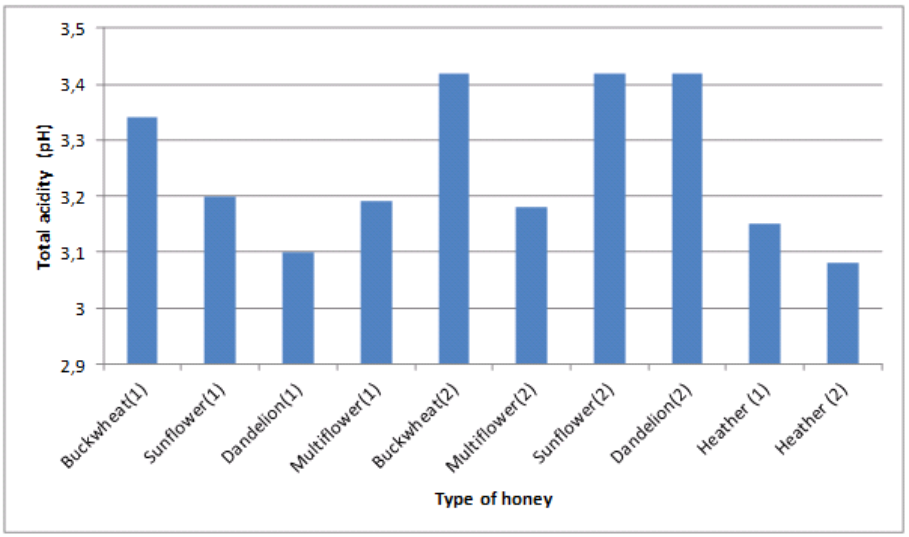

Figure 4. The results of physicochemical analyses for total acidity (pH) of tested honeys.

The water content in honey.

In most honeys water content was below 20%. The only honey that exceed this value and at the same time contain the most water was the multiflower honey no. 2 (21.8%). Honey which contained the least water was dandelion no.1 (16.7%) (Figure 5).

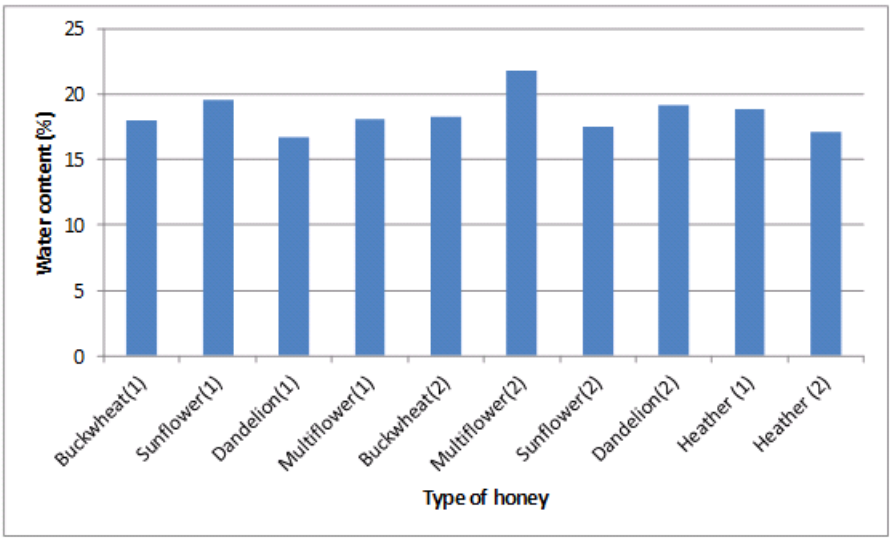

Figure 5. The results of physicochemical analyses for water content in tested honeys.

The water activity of honeys.

The highest water activity was found in buckwheat honey 1 (0.622 aw), while the lowest in the heather honey 2 (0.538 aw) (Figure 6).

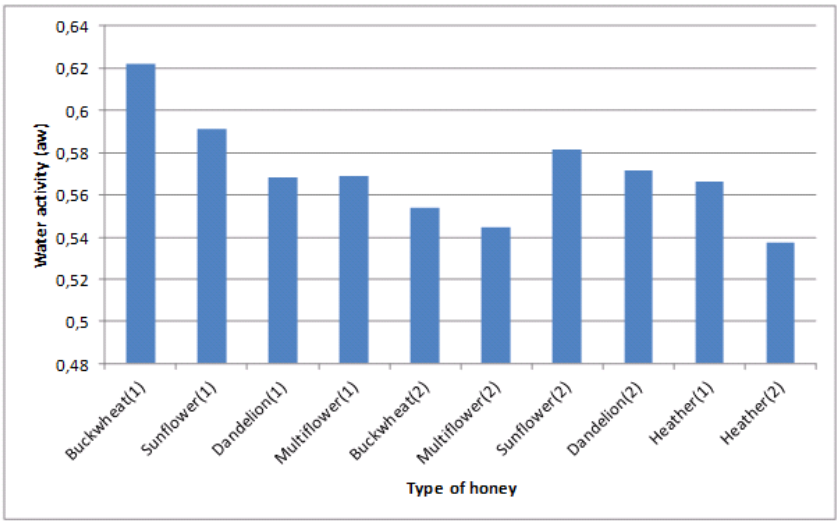

Figure 6. The results of physicochemical analyses for water activity of tested honeys.

DISCUSSION

The possibility of discussion on microbiological analysis of honey is limited because of the small popularity of the topic that is likely due to the high degree of difficulty while performing these analyzes. Honey is a very demanding material and requires particularly steril conditions to carry out time-consuming research. Besides this, so far, there are no standards in this area which makes it very difficult to interpret the results. Microbiological studies in our experience has shown TBC impurities in an amount not exceeded 2.34 log cfu/g. In all samples it was revealed the presence of aerobic microorganisms (Figure 1).

Similar results were obtained by other authors in Portugal, where 5 samples has been labeled of 200 cfu/g and contamination of aerobic microflora in the case of the 4 samples did not exceeded 10 cfu/g [Gomes, 2010]. Higher results were found by Turkish reseraches, who in twenty samples of honey from Turkey had found the content of all the mesophilic aerobic bacteria from 2.00 to 3.96 log cfu/g [Tornuk et al., 2013]. In contrast, a much lower results received authors [Nevas, 2005] and they reported that the contamination of 35 honey were at the level of 0-83 cfu/g. The presence of yeast or mold had been found in all tested samples. Heather honey 2 stood out in terms of the degree of contamination (Figure 3).

The water content and water activity of the honeys was normal therefore honey did not fermented. There is a close relationship between water content, the amount of yeast-like cells and condition of honey. If the water content exceeds 20%, the presence of single colony of yeast may be sufficient to initiate fermentation. Bacteria *Bacillus spp.*, occurred in nine samples of honey at the level not higher than 2.4 log cfu/g. In other studies [Iurlina et al.,2006] *Bacillus spp.* was found in the 38.5% of tested samples. Currently, there is no clear microbiological criteria directly involved in the honey in terms of assessing their quality. This also applies to European regulations [EU, 2001]. The pH value of the Examined honeys ranged from 3.08 to 3.42 (Figure 4).

The obtained pH values were similar to the values obtained by other authors [Azeredo et al., 2003; Semkiw et al., 2008; Szczęsna et al., 2011]. The water content was associated with external agents apperaing during the honey maturity and storage. The permissible level is 20 g per 100 g of honey. The water content in honey varied within the borders of the adopted standards; except the multiflower honey no. 2, wherein the water content was 21.8% (Figure 5).

The water content is an important parameter in the assessment of contamination of honey to fermentative microorganisms. Tested honeys characterized by the balanced water activity at the level of 0.538 to 0.622 (Figure 6). Similar observation has been reported by other authors [Chirife et al., 2006].

CONCLUSIONS

1. It was found that the tested honey proved to be a good value. Most of the studied parameters was within the standards or were on their borders.
2. All honeys were characterized by low pH (range 3.08-3.42), while the water activity (0.622 - 0.538 aw) and a water content (16.7-21.7%) was adequate hinder microbial growth.
3. The presence of the bacteria *Bacillus spp.*, yeasts and molds and the total number of microorganisms were found in all tested honeys.
4. It has been shown that the best method to implement the inoculation was submerged culture

method due to the high repeatability. The least useful method was spiral plate. The WASP camera distributed solutions unevenly, and specialized computer programme incorrectly interpreted the results. It has been found that the reason of that failure were hand poured plates with agar, so that they are not exactly equal. In the future, the test should be repeated on the factory filled plates.

5. During the tests conducted on the quality of honey it should ensured sterile conditions, because the product is very sensitive to pollution and can be easily infected. It should be noted how important is the honeys storage, because it can significantly affects its quality. The cause of the deterioration of the quality of the product may be high humidity in storage. These conditions prevent the evaporation of the water that collects on the top layer of honey, where in consequence grow colonies of yeasts and molds. These in turn initiate the fermentation process, which lowers the pH of honeys.

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ОЦЕНКА МИКРОБИОЛОГИЧЕСКОГО КАЧЕСТВА БЕЛОРУССКОГО МЕДА

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АННОТАЦИЯ

Основная цель исследования заключалась в оценке микробиологического качества 10 видов меда из Белоруссии, собранных пчеловодами в районе Гродно. Исследование было проведено в 2013 году на кафедре пчеловодства Варшавского университета естественных наук - SGGW. Целью исследования является подсчет общего числа микроорганизмов, рассчет количества представителей рода *Bacillus spp.*, а также дрожжевых и плесневых грибков. Оценка микробного числа в меде было сделано с использованием трех методов: погруженной культуры, поверхностной культуры и спиральных пластин, с целью выбора наиболее подходящего. Кроме того, анализировали физико-химические свойства меда, такие как pH, содержание воды и водной активности. Оценка на наличие *Bacillus spp.*, дрожжевых и плесневых грибков, а также общего количества микроорганизмов было проведено для всех образцов меда. Было также установлено, что наиболее подходящим методом для тестирования микробиологического качества меда является классический метод погружения. Проанализированные меда оказалась хорошего качества. Большинство изученных параметров находились в пределах стандартных значений. Все меда характеризовались низким pH (диапазон 3.08 - 3.42), в то время как водная активность (0.622 - 0.538 aw) и содержание воды (16.7 - 21.7%) была на уровне, чтобы препятствовать росту микроорганизмов.

Ключевые слова: белорусский мед, микробиологическое качество