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THE IMPACT OF STORAGE AND HEAT TREATMENT ON THE DIASTASE NUMBER OF HONEY

Honey is known as a valuable product, useful for adults and children. Regular consumption of this delicious product helps the body adapt to the negative effects of the external environment. Special attention should be paid to the antimicrobial and bactericidal properties of honey, which strengthen the body's resistance, especially during growth, to various infectious and cold diseases. It is worth noting that different varieties of honey have different levels of antimicrobial activity. For example, researchers[1] note that dark honey has the most pronounced antimicrobial properties. In addition to its characteristic taste, honey has a high nutritional value. Natural honey contains simple monosaccharides (glucose, fructose), which are easily absorbed and quickly enter the bloodstream, replenishing the body's energy reserves. Honey also contains amino acids, essential oils, hormones, enzymes, organic acids, minerals, vitamins, antidiabetic and other useful substances that contribute to

1 Megherbi, M., Herbreteau, B., Faure, R., & Salvador, A. (2009). Polysaccharides as a marker for detection of corn sugar syrup addition in honey. *Journal of Agricultural and Food Chemistry*, *57*(6), 2105-2111. URL: https://doi.org/10.1021/jf803384q

the health of the body. In total, honey contains about 300 different substances that are a product of beekeeping.

Honey is known to be rich in both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard products, amino acids and proteins[2].

Unfortunately, due to the high demand for honey, some fraudsters are trying to get rich by adding cheap sweeteners to the product. These sweeteners may include high fructose corn syrup, corn sugar syrup, invert sugar syrup, cane sugar syrup, palm sugar, high fructose syrup, inulin, and others[3].

Scientists [4] say that falsification of honey is a serious problem in the food and processing industry, and the most common methods of falsification are the addition of sugar, syrups, water and other substances to natural honey. This deteriorates the quality of the product and violates the trust of consumers in honey producers. Falsification of honey also leads to unfair competition and losses for legitimate producers. But the most serious consequence is the potential harm to the health of consumers, since honey, which contains additional ingredients, artificial additives and preservatives, can be harmful to the body[5]

1. Using cheap sugars and industrial syrups to fake honey.

This chapter deals with the important topic of using cheap sugars and industrial syrups to falsification honey. The scientists' work[6]. provides important data on the various components used in honey adulteration, such as sugar cane and sugar beet syrups, including corn syrup, high fructose corn syrup, glucose syrup, sucrose syrup, invert syrup and high fructose inulin syrup. . It is known that the use of such counterfeit components can affect the chemical and biochemical characteristics of honey, including enzymatic activity, electrical conductivity and the content of specific compounds[7].

2 Svyatnenko, R., Marynin, A., Litvynchuk, S., & Pasichnyi, V. (2023). Study on the quality of honey from different botanical sources and one re-gional origin. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Food Technologies, 25(99), 56–60. URL: https://doi.org/10.32718/nvlvet-f9910

3 Мельник, О. П., Шевченко, О. Ю., Маринін, А. І., & Літвинчук, С. І. (2022). ФАЛЬСИФІКАЦІЯ МЕДУ І МЕТОДИ ЇЇ ВИЯВЛЕННЯ. Наукові праці НУХТ. Том 28, № 5. 54-64. URL: https: 10.24263/2225-2924-2022-28-5-8.

4 Megherbi, M., Herbreteau, B., Faure, R., & Salvador, A. (2009). Polysaccharides as a marker for detection of corn sugar syrup addition in honey. *Journal of Agricultural and Food Chemistry*, *57*(6), 2105–2111. URL: https://doi.org/10.1021/jf803384q

5 Мельник, О. П., Маринін, А. І., Шевченко, О. Ю., Літвинчук, С. І., & Святненко, Р. С. (2023). ВИКОРИСТАННЯ МЕТОДУ ЯМР-СПЕКТРОСКОПІЇ ДЛЯ ДОСЛІДЖЕННЯ ІДЕНТИФІКАЦІЇ ТА ФАЛЬСИФІКАЦІЇ МЕДУ. *Вісник ЛТЕУ. Технічні науки*, (34), 21–31. URL: https://doi.org/10.32782/2522-1221-2023-34-03

6 Ismail, M.M.; Ismail, W.I.W. Development of stingless beekeeping projects in Malaysia. In Proceedings of the CSSPO International Conference 2018: Towards Inclusive & Sustainable Agriculture-Harmonizing Environmental, Social and Economic Dimensions: Is it Possible? Sarawak, Malaysia, 9–11 July 2018; p. 5

7 Soares, S., Amaral, J. S., Oliveira, M. B. P., & Mafra, I. (2017). A comprehensive review on the main honey authentication issues: Production and origin. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1072–1100. URL: https://doi.org/10.1111/1541-4337.12278

The selection of ingredients for falsification honey is based on several factors, such as the region of origin, economic benefits, and the presence of sugar or sweeteners. An important example is the use of rice and wheat syrup extracts as additional components for the adulteration of honey in Turkey and France. Plant syrups can be obtained by heating plant juices or partial enzymatic hydrolysis [8].

Recent studies [9] show that high-fructose inulin syrup is often used in European countries to adulterate honey.

Among the other components used for falsification of honey is cane sugar, which consists of two molecules of monosaccharides - glucose and fructose. Although glucose and fructose are monosaccharides with the same chemical formula ($C_6H_{12}O_6$),), their chemical conformations are different. These monosaccharides are linked by a weak glycosidic bond, creating a molecule of sucrose ($C_{12}H_{22}O_{11}$), which is a disaccharide [10].

Additionally, the study[11] points to the use of cane sugar as an ingredient for direct and indirect honey adulteration. In the direct adulteration study, 10, 20 and 40% syrup was added to the honey sample, and in the indirect method, bees were fed syrup.

Another important component is invert sugar, which is formed by the decomposition of sucrose into its monosaccharides: fructose and dextrose. The inversion process is often carried out by heating sucrose syrup in the presence of acids, bases or invertase [12]. This sugar is obtained from beet and cane plants, and its composition can mimic the sugar profile of natural honey. The wide application of invert sugar in the food and semi-industrial industries allows the creation of a variety of products, including non-crystallized creams, jams, artificial honey and liquid sugar[13]. Invert sugar is a safe substance and is recommended taking into account the individual characteristics of patients who have diabetes or specific genetic abnormalities.

8 Tosun, M. (2013). Detection of adulteration in honey samples added various sugar syrups with 13C/12C isotope ratio analysis method. *Food chemistry*, *138*(2-3), 1629-1632. URL: https://doi.org/10.1016/j.foodchem.2012.11.068.

9 Corradini, C., Bianchi, F., Matteuzzi, D., Amoretti, A., Rossi, M., & Zanoni, S. (2004). High-performance anion-exchange chromatography coupled with pulsed amperometric detection and capillary zone electrophoresis with indirect ultra violet detection as powerful tools to evaluate prebiotic properties of fructooligosaccharides and inulin. *Journal of Chromatography a*, *1054*(1–2), 165–173. URL: https://doi.org/10.1016/j.chroma.2004.07.109

10 Low, H., & Hammond, D. A. (1996). Detection of high fructose syrup from inulin in apple juice by capillary gas chromatography. *Fruit Processing*, *6*, 135–139.

11 Li, S., Zhang, X., Shan, Y., Su, D., Ma, Q., Wen, R., & Li, J. (2017). Qualitative and quantitative detection of honey adulterated with high-fructose corn syrup and maltose syrup by using near-infrared spectroscopy. *Food chemistry*, *218*, 231–236. URL: https://doi.org/10.1016/j.foodchem.2016.08.105

12 European Chemical Agency. (2010). Evaluation of New Scientific Evidence Concerning the Restrictions Contained in Annex XVII to Regulation (EC) No. 1907/2006 (REACH): Review of New Available Information for Di-'isononyl'Phthalate (DINP).

13 Se, K. W., Ghoshal, S. K., Wahab, R. A., Ibrahim, R. K. R., & Lani, M. N. (2018). A simple approach for rapid detection and quantification of adulterants in stingless bees (Heterotrigona itama) honey. *Food Research International*, *105*, 453–460. URL: https://doi.org/10.1016/j.foodres.2017.11.012.

In addition, rice syrup, which is formed during the hydrolysis of rice polysaccharide, is used as an additional component in honey in China. It contains three types of sugars: maltotriose, maltose and glucose, rice syrup acts as 100% glucose in the body.

Inulin, a natural polysaccharide that belongs to the class of fructans, is also an important component found in a variety of sources, from wheat to sunflower [14].

Particular attention should be paid to palm sugar, which is made from palm flower buds. It is a natural sweetener with a low glycemic index and is used to falsification honey in India[14].

2. Falsification of honey and methods of detection.

This section discusses various types of honey falsification and methods of their detection. Falsification of honey is a serious problem because it can lead to a loss of product quality and also has negative consequences for consumers. Detection of falsification is an important task for ensuring the quality and safety of food products, in particular honey.

Direct falsification

Direct falsification of honey involves adding different types of syrups to the product to change its taste, aroma and consistency. This practice is often used to enhance the appearance of inferior types of honey and mask their poor quality. The addition of various syrups, such as cane sugar, beet sugar and glucose syrup, can change the properties of honey, which can be difficult to detect with traditional methods. [15].

Indirect falsification

Indirect falsification of honey consists in the fact that bees are fed sugar syrups, which affects the quality and composition of honey. This approach leads to an increase in the volume of honey produced by the hives, but at the same time the quality of the product decreases. The use of low-quality ingredients can affect the taste and aroma of honey, as well as its beneficial properties[16].

Blending falsification

Blending falsification involves mixing high-quality honey with lower-quality or completely different origin. This approach can be used to reduce costs or

14 Yamada, T., Iida, T., Takamine, S., Hayashi, N., & Okuma, K. (2015). Safety evaluation of rare sugar syrup: Single-dose oral toxicity in rats, reverse mutation assay, chromosome aberration assay, and acute non-effect level for diarrhea of a single dose in humans. *Shokuhin Eiseigaku zasshi. Journal of the Food Hygienic Society of Japan*, *56*(5), 211–216. URL: doi:10.3358/shokueishi.56.211

15 Zábrodská, B., & Vorlová, L. (2015). Adulteration of honey and available methods for detection–a review. *Acta Veterinaria Brno*, *83*(10), 85–102.

16 Al-Awadhi, M. A., & Deshmukh, R. R. (2021, December). A review on modern analytical methods for detecting and quantifying adulteration in honey. In *2021 international conference of modern trends in information and communication technology industry (MTICTI)* (pp. 1–6). IEEE.

improve the appearance of a product. The blend can also be used to sell old honey, adding flavorings, additives and colorings to improve its appeal to consumers. [17].

Methods of detecting falsification of honey

Detecting falsification of honey is a difficult task, as modern methods of adulteration can be very inventive and difficult to detect using traditional means. However, scientists and specialists have developed various methods and techniques to detect falsification of honey:

Infrared (IR) spectroscopy: This method allows the identification of various impurities in honey, particularly from different plants. The use of IR spectroscopy allows quick and accurate identification of honey components.

Raman spectroscopy: This method complements the information obtained from IR spectral data and allows the detection of various sugar impurities in honey. It allows you to get additional information about the structure of molecules[18]. Nuclear Magnetic Resonance (NMR): Used to detect honey falsificated with sugar syrups. NMR-spectroscopy allows to study the chemical composition of the product in detail[19]. Chromatographic methods: liquid chromatography (HPLC), gas chromatography-mass spectrometry, micellar electrokinetic chromatography (HPAEC) are used to evaluate the composition of hydrocarbons, including various types of sugars and other substances[20].

Physico-chemical indicators: Thermal properties of honey, moisture content, enzymatic activity of diastase can help detect falsification. Analysis of thermal characteristics allows to detect the presence of impurities or changes in the composition of honey.

3. The effect of temperature and heating conditions of honey on the diastase number in Goethe units.

Among the adulteration of honey, heating of honey is widely used. Heating honey is a common practice, but improper heating can result in the loss of valuable physical and chemical properties of the product. As a result of heating, honey can lose its natural consistency, aroma, taste and nutritional value. In addition, there is a risk of the formation of harmful compounds

17 Мельник, О. П., Шевченко, О. Ю., Маринін, А. І., & Літвинчук, С. І. (2022). Фальсифікація меду і методи її виявлення. *Наукові праці НУХТ*, *28*(5), 55–62.

18 Li, S., Shan, Y., Zhu, X., Zhang, X., Ling, G. (2012). Detection of honey adulteration by high fructose corn syrup and maltose syrup using Raman spectroscopy. Journal of Food Composition and Analysis. 28 (1): 69–74. URL: https://doi.org/10.1016/j. jfca.2012.07.006.

19 Bertelli, D, Lolli, M, Papotti, G, Bortolotti, L, Serra, G., Plessi, M. (2010). Detection of honey adulteration by sugar syrups using one-dimensional and two-dimensional high-resolution nuclear magnetic resonance. J Agric Food Chem, 58: 8495–8501. URL: https://doi.org/10.1021/jf101460t

20 Arroyo-Manzanares, N., Garcia-Nicolas, M., Castell, A., Campillo, N., Vinas, P., Lopez-Garcia, I., Hernandez-Cordoba, M. (2019). Untargeted headspace gas chromatography–Ion mobility spectrometry analysis for detection of adulterated honey. Talanta. 205:120123. URL:https://doi.org/10.1016/ j.talanta.2019.120123.

such as hydroxymethylfurfural (HMF), which can be a consequence of overheating the sugars in honey.

An important catalyst in honey production are Goethe numbers, which are of great importance. These numbers help to assess the quality and naturalness of the product. One of the key roles of this enzyme is to break down starch and other polysaccharides into disaccharides, and they are generally present in all types of honey. An important characteristic of diastase is its interaction with other enzymes, which makes it an indicator of the overall activity of enzymes in honey. Even more, this enzyme is characterized by high stability, which allows it to be used to assess the presence of other enzymes in honey. The presence or absence of diastase in honey can be an indicator of product quality and affect consumer perception[21].

Scientists studied the effect of various heating processes on the activity of diastase (enzyme) in honey. The study was conducted on different types of heating, and included heating the honey in a water bath, in an oven, and in a microwave oven. The results of the study showed that different heating processes have different effects on the activity of diastase in honey.

The purpose of our research is to study the influence of different temperatures and methods of heating and duration of storage of buckwheat honey on diastase number in Goethe units.

The research involves conducting experiments with heating buckwheat honey, which was purchased from private beekeepers of Boryspil district of Kyiv region, in a water bath and a thermostat at different temperatures, and further monitoring changes in Goethe units in honey during one month of storage of heated samples. The diastase number of honey was determined according to the standardized method specified in DSTU 4497:2005 «Natural honey. Technical requirements»[22].

In order to study the influence of temperature and methods of heating, honey was heated to 60 °C and 80 °C for 14 seconds in a PolyScience water bath and in a TS-80M-2 dry air thermostat. Buckwheat honey was stored at room temperature for a month to study the change in diastase number depending on temperatures of 60 °C and 80 °C and methods of heating.

The results of the study of buckwheat honey with different heating methods and temperatures are presented in Fig. 1.

21 Святненко Р.С., Маринін А.І., Шевченко О.Ю., Позняк О.М., Літвинчук С.І. (2023). ФІЗИКО-ХІМІЧНІ ТА ОРГАНОЛЕПТИЧНІ ПОКАЗНИКИ МЕДУ РІЗНОГО БОТАНІЧНОГО ТА ОДНОГО РЕГІОНАЛЬНОГО ПОХОДЖЕННЯ Науковий журнал «Вчені записки ТНУ імені В.І. Вернадського. Серія: Технічні науки» Том 34 (73) № 3, 110–114.

22 ДСТУ 4497:2005 «Мед натуральний. Технічні вимоги»

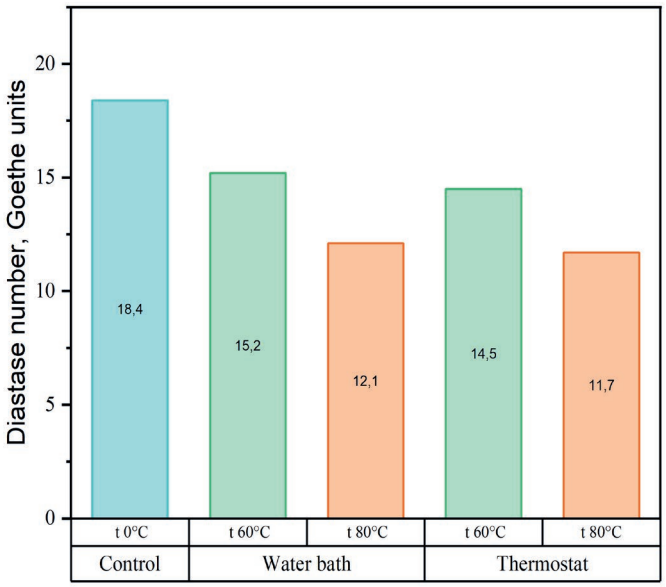

Fig. 1. Effect of temperature and heating method on the diastase number

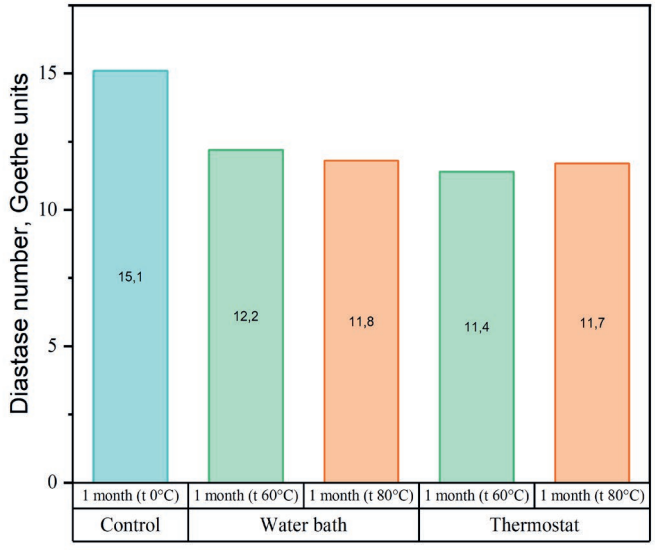

Fig. 2. Change in diastase number after storage for one month due to precipitation from the heating method.

It was found that when honey was heated in a water bath at a temperature of 60°C, the content of diastase number in honey was 15.2, which is significantly lower compared to the control sample, where the content was 18.4. A further increase in temperature to 80°C led to an even greater decrease in the diastase number, which was 12.1. A similar effect was found when honey was heated in a

thermostat. At a temperature of 60°C, the content of diastase number was 14.5, which is also less than in the control sample. Increasing the temperature to 80°C led to a decrease in the diastase number, which was 11.7.

After conducting a study of the influence of different temperatures and methods of heating honey on the content of diastase number, we investigated changes in the diastase number of honey during one month of storage. From the obtained results (Fig. 2) it was established that the storage of honey after heating in a water bath and a thermostat at temperatures of 60°C and 80°C for one month also led to a further decrease in the diastase number.

In this work, we considered possible methods of falsification of honey and its physical and chemical characteristics at different temperatures and methods of heating.

The first chapter examines the problem of using cheap sugars and industrial syrups to falsification honey. The conducted literature analysis provides important data on various components used in the adulteration of honey, such as syrups from sugar cane, sugar beet and others. The importance of choosing components for falsification of honey depending on the region, economic benefits and availability of sugar or sweeteners was also considered.

The second chapter is devoted to the concept and types of honey falsification. Various methods and approaches for detecting falsification were considered, including stable carbon isotope analysis, IR and Raman spectroscopy, chromatography, and other techniques. It turns out to be important to use the diastase number to assess the quality and naturalness of honey.

In chapter three, the influence of temperature and methods of heating, as well as its storage, on the diastase activity of honey was investigated, and it was established that temperature treatment and storage after it can significantly affect the reduction of the diastase number.

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