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Evaluating Honey Quality with Statistical and Analytical Methods

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ABSTRACT

Evaluating the quality of the honey requires a combination of statistical and analytical methods. These methods include laboratory analyses to determine the physical-chemical properties of honey and statistical analyses to interpret the data and confirm their significance. Bee honey, a dense, liquid, or crystallized food product, is produced by bees of the genus Apis. Various indicators from different samples of bee honey have been studied to establish its authenticity, verify its enzymatic activity, and assess its quality. The studied samples originate from the Blagoevgrad region, Bulgaria.
Keywords: analytical methods, enzymatic activity, honey quality, physico-chemical properties, statistical analyses.

INTRODUCTION

Current investigation assesses the quality and the naturality of the origin of the bee honey, with a primary focus on its diastase activity according to the Gothe scale (DA by Gothe) [1, 2]. Prolonged exposure of honey to high temperatures significantly reduces diastase activity and thus diminishes its beneficial properties. Other impurities in honey, such as various substances, also affect this indicator, often reducing its value substantially. Another key indicator related to enzymatic activity is hydroxy-methyl-furfural (HMF), which indicates the heating of honey. Honey is melted using a specific technology, slowly and at low temperatures, to preserve its antibacterial properties and enzymatic activity. Heating honey above 45°C results in loss of its beneficial properties. Stressful heating at 70 - 80°C keeps it in liquid form longer but unfortunately reduces its usefulness. A true indicator of honey is its tendency to crystallize, which demonstrates the absence of other substances and is a natural process due to the presence of fructose and glucose in its composition. The percentage of reducing sugars (RS) is another criterion for honey quality. Determining total sugars (TS) in % is necessary for calculating sucrose, which can be hydrolysed not only by acid but also by the action of the enzyme invertase. Mixing

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equal parts glucose and fructose results in the hydrolysis of ordinary sugar, known as invert sugar. Invert sugar crystallizes less readily and is sometimes used as a substitute for bee honey because it contains natural invert sugar.

Every honey consists of fructose and glucose in varying ratios depending on its composition, derived from the plants from which bees collect nectar for honey production.

EXPERIMENTAL

Analytical methods

Determination of diastase activity (DA, Gothe units)

Weigh 10 g of honey into a beaker and transfer quantitatively with a small amount of distilled water into a 100 cm^3 volumetric flask, then fill it up. In a dry Erlenmeyer flask (100 - 300 cm^3), pipette the following using a volumetric pipette: 8 mL of 0.1 % starch solution, 5 mL of phosphate citrate buffer with pH = 5, and 1 mL of 0.1N NaCl. Homogenize the mixture and leave it uncovered on a water bath at 40°C for 20 min. Add 1 cm^3 of the honey solution, shake gently, and return to the water bath for another 15 min. After the specified time, add 2 mL of 0.03N iodine solution. Prepare 9 mL of distilled water separately, to which add 1 mL of the resulting solution. Measure the optical density of the solution at 660 nm wavelength against a blank of distilled water.

Determination of reducing sugars (RS %)

Weigh approximately 1 g of honey sample into a beaker using an analytical balance. Transfer the sample with distilled water into a 100 cm^3 volumetric flask, fill up to the mark, and obtain the RS solution. Pipette the RS solution into an iodine flask.

These methods are employed to assess various aspects of honey quality, including enzymatic activity and sugar composition, critical for evaluating its naturalness and potential adulteration [3].

Blank Sample:

- 10 mL of Fehling's solution 1;
- 10 mL of Fehling's solution 2;
- 30 mL of distilled water.

Procedure for testing the sample:

- 10 mL of Fehling's solution 1;
- 10 mL of Fehling's solution 2;
- 10 mL of reducing sugars (RS %) solution;
- 20 mL of distilled water.

These procedures outline how to prepare and use the blank sample and the method for testing the sample, specifically focusing on determining the presence of reducing sugars using Fehling's solutions and distilled water as part of the analytical process.

The prepared solution is heated on a burner, and the following steps are followed:

- It should start boiling after 3 min;
- It should boil for 2 min;
- Quickly cool it under running water;
- Then add 2.5 g of KI and 10 cm^3 of HCl.

The flask is shaken well, closed with a stopper, and left in the dark for 5 min. Afterwards, titrate with 0.1N $Na_2S_2O_3$ using starch as an indicator until the solution turns beige. Continue titration until it lightens, then add 1 % starch 1 cm^3 and continue titrating until the colour changes.

Determining total sugars (TS %)

Using a volumetric pipette, take 50 cm^3 from the RS solution and transfer it to a 100 cm^3 volumetric flask. Heat the flask on a water bath preheated to 67 - 70°C for 10 min without adding water. Add 5 cm^3 of concentrated HCl and heat for an additional 5 min. Then, quickly cool the solution and neutralize it with phenolphthalein to a pale pink color. Top up the neutralized solution to the mark with distilled water. Determine invert sugar from the resulting solution. Solution TS.

The sample testing method continues as described in: Determination of reducing sugars (RS %).

To determine sucrose, it must first be hydrolyzed with concentrated hydrochloric acid, and then RS is determined. Optimal environmental conditions must be maintained during the test. Temperature: 18 - 25°C ± 2°C.

Determining the content of hydroxy-methyl-furfural (HMF) (mg kg^{-1})

Weigh 10 g of honey sample in a beaker. Quantitatively transfer it to a 50 cm^3 volumetric flask and fill it up to the mark. Prepare two 100 cm^3 Erlenmeyer flasks: in each, place 2 cm^3 of honey solution and 5 cm^3 of *p*-toluidine solution. To one flask, add 1cm^3 of distilled water (blank sample), and to the other flask, add 1 cm^3 of barbituric acid solution. Measure the optical density of the solution against the blank sample at 550 nm wavelength [4 - 6].

Statistical methods

The Control Chart is a tool that enables tracking changes in the quality indicator of a product or process over time, as well as studying the reasons behind these changes. At any given moment, the process may be running normally, but due to changes in its conditions, trends indicating deterioration over time may appear. The control chart allows such trends to be detected timely and addressed. The limits of the natural tolerance interval (m ± 3σ) for normally distributed variables are adopted as control limits. If the quality indicator follows the normal distribution and the process is under control, almost all observations should fall between the control limits [7, 8].

Histograms are one of the primary graphical tools for studying the distribution of a random variable based on the number of elements in a set. It is an estimate of the probability distribution of a continuous variable (quantitative variable) and it is presented by a bar plot where the x-axis represents the range of values, divided into intervals or bins, and the y-axis represents the frequency or count of observations that fall within each bin. They are constructed using data from a sample of the general population.

Each product or production process is characterized by one or several quality indicators. For a product to be of high quality, each of these indicators must have a specified value, often referred to as a target value. However, for various reasons, not every product achieves the exact target value for its quality indicator. Reasons for this include inaccuracies in component dosages, heterogeneous composition of raw materials used, deviations from the manufacturing process, and many others. As a result, deviations from the target value of the quality indicator occur. If these deviations fall within specified limits, known as tolerance limits, the product is considered acceptable; otherwise, it is defective [8].

RESULTS AND DISCISSION

An analysis was conducted on 19 samples of bee honey from the Blagoevgrad region, focusing on the following analytical parameters: Hydroxy-methyl-furfural (HMF), Diastase activity by Gothe scale (DA), reducing sugars (RS), and sucrose. Control charts for individual values were constructed to study the compliance with quality indicators of the honey.

Fig. 1 depicts a control chart of individual values for honey samples analysed for HMF. It can be observed that the process is out of control due to the presence of series of more than 8 points at one side of the central line, as well as one point exceeding the upper control limit (UCL = 81.3 mg kg^{-1}). The figure also shows the specification limit USL = 40 mg kg^{-1}, defined by the requirements of Ordinance No 2/27.03.2024. The analysis of sample No. 14 revealed a hydroxymethyl-furfural (HMF) content of 191.02 mg kg^{-1}, which significantly exceeds the legally permissible upper limit of 40 mg kg^{-1} specified for honey according to the quality requirements.

The high HMF content indicates that the honey was subjected to strong heating and/or improper storage, which led to the decomposition of the sugars and the formation of increased amounts

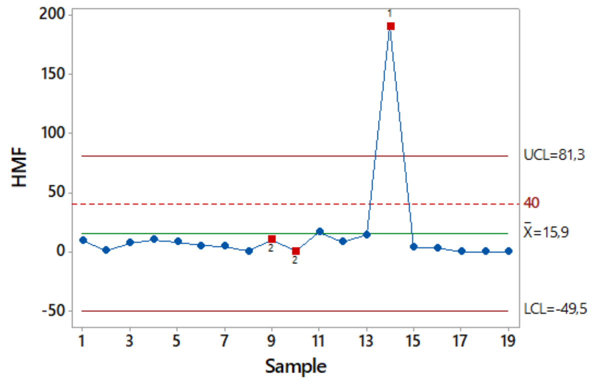

Fig. 1. Control chart of individual values for samples of honey analysed for HMF mg kg^{-1}.

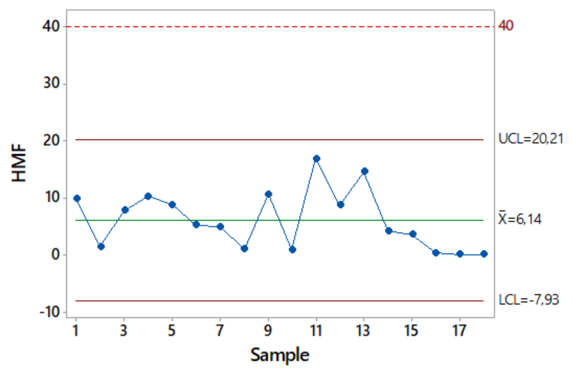

Fig. 2. Control chart of individual values for samples of honey analysed for HMF mg kg^{-1} (without sample No14).

of HMF. Freshly obtained honey practically does not contain HMF, with the increased values being due solely to thermal and oxidative processes. Regarding these facts, the result of point No. 14 does not reflect the actual technological state of the production, but is influenced by external factors, unusual for the normal production process. For this reason, point No. 14 is considered an anomalous value and is excluded from the control chart in order not to distort the statistical assessment of the process (Fig. 2).

The corresponding normal probability plots for HMF are built and presented in Fig. 3 and Fig. 4 for the initial dataset and the reduced with sample No14 dataset. In Fig. 3 it can be seen, that the considered observation No14 differs very much from the main data group - it is a bias. According to the Anderson-Darling test for normality, the distribution of HMF is not normal (probability $p < 0.05$). In Fig. 4 the presented normal probability plot, after reduction of the dataset, based on the Anderson-Darling test for normality shows that the distribution is normal (probability $p = 0.206$, $p > 0.05$).

Fig. 5 shows a control chart of individual values for honey samples analysed for DA. The process is under control, as the lower control limit (LCL = -1.08 Gothe units) is observed. The figure also presents the specification limit USL = 8 Gothe units, defined by the requirements of standard BSS 3050:1980 part 2.8. However, despite the process being under statistical control, there is an observation that does not meet the standard requirements (again sample No14). A deeper analysis is needed to determine the specific reasons for such deviation, but it can be assumed that the honey was subjected to strong heating and/or improper storage.

Fig. 6 presents a control chart of individual values for honey samples analyzed for RS. It can be observed that the process is out of control because one-point falls below the lower control limit (LCL = 42.75 %) and a series from more than 8 points at the one side of the central line. The figure also shows the specification limit USL = 60 %, defined by the requirements of BSS 3050:1980 part 2.3. There are specific circumstances related to that sample (sample No12) that need further investigation. It also should be removed from the dataset, since it indicates special circumstances during the bee honey production (Fig. 7).

The normal probability plot for RS % for the reduced with sample No12 dataset is presented in Fig. 8. The Anderson-Darling test shows that the RS data have normal distribution, since the probability $p = 0.611$ ($p > 0.05$).

Fig. 9 shows a control chart of individual values for honey samples analysed for sucrose.

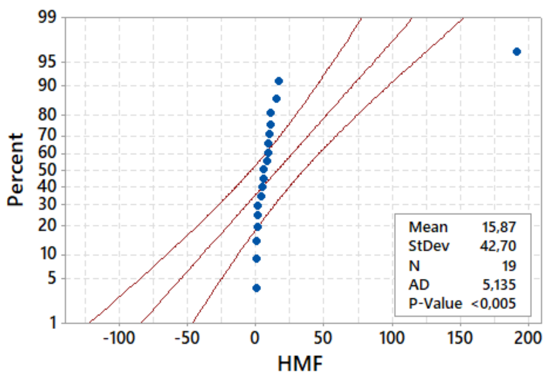

Fig. 3. Normal probability plot for HMF mg kg^{-1}.

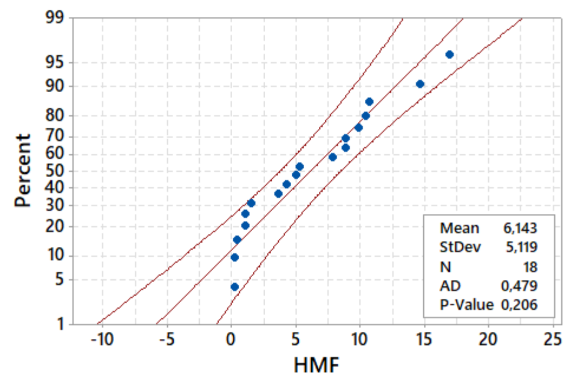

Fig. 4. Normal probability plot for HMF mg kg^{-1} (reduced dataset)

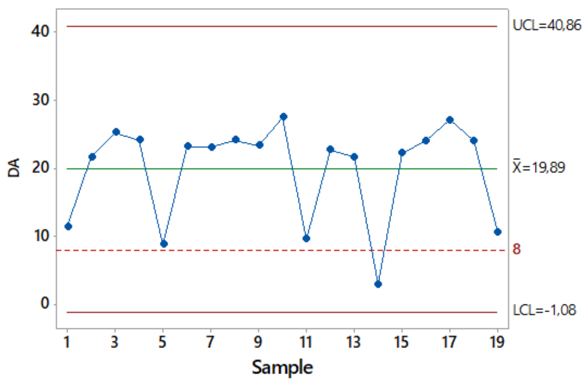

Fig. 5. Control chart of individual values for samples of honey analyzed for DA in Gothe units.

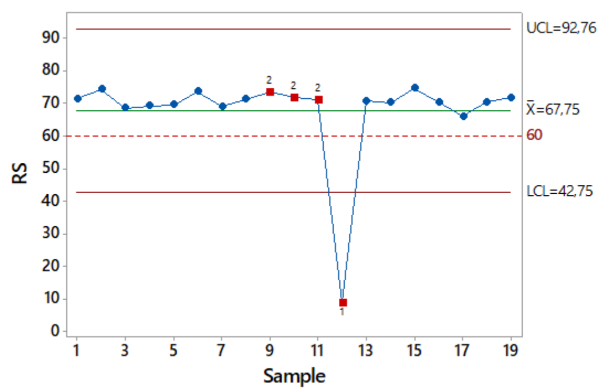

Fig. 6. Control chart of individual values for samples of honey analysed for RS %.

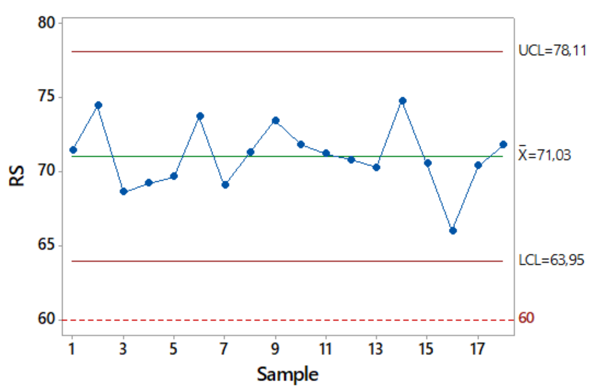

Fig. 7. Control chart of individual values for samples of honey analysed for RS % (reduced dataset).

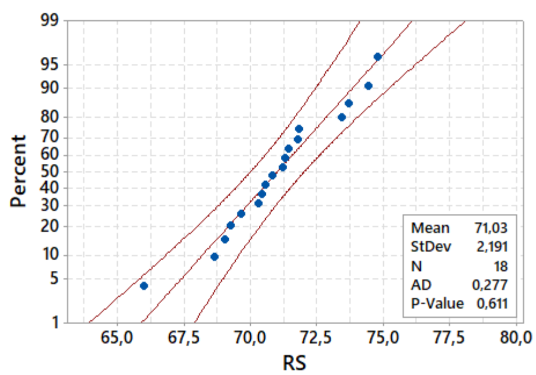

Fig. 8. Normal probability plot for RS % (reduced dataset).

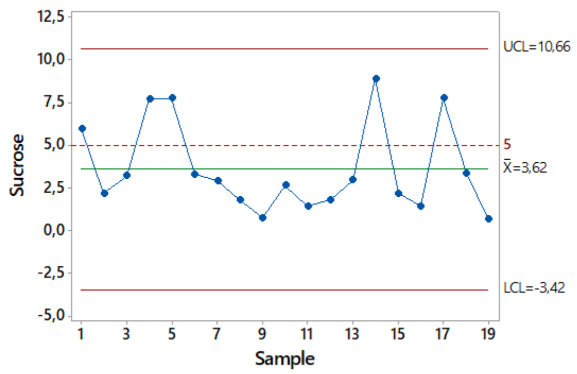

Fig. 9. Control chart of individual values for samples of honey analysed for sucrose

The process is under control, as there are no points observed outside the control limits (UCL = = 10.66 % and LCL = -3.42 %). The figure also presents the specification limit USL = 5 %, defined by the requirements of BSS 3050:1980 part 2.4, against which 5 points are observed above the specification limit.

The normality tests for the individual values (without and with reduction) for samples of honey analysed for DA and sucrose showed that their distributions are not normal. This fact is an indicator for the influence of other specific factors (except heating or improper storage – like sample No 14 and sample No12).

Among most samples, the HMF values shown in Fig. 10 are below 40 mg kg^{-1}, which is within the acceptable limits (assuming 40 mg kg^{-1} as the norm). The average value is 6.143 mg kg^{-1}, significantly lower than 40 mg kg^{-1}. The standard deviation is quite high (5.119 mg kg^{-1}), indicating substantial variation in HMF values across different samples. The exceptionally high value around 200 mg kg^{-1} may be due to error or a specific anomaly (such as exposure of honey to high temperatures or inadequate sealing of the honey container leading to oxidation) and requires further detailed investigation.

The average value of the diastase activity (DA) according to the Gothe method is 20.83, which is around the center of the distribution of samples, as shown in Fig. 11. The standard deviation of 6.097 indicates significant variation in the data. The red vertical line at a value of 8 represents the specification limit, below which the data are considered unusual or out of specification. A small number of samples are below this threshold, which may indicate potential issues or unusual cases deserving additional attention. This could be due to fresh honey that did not have the opportunity to mature and develop its beneficial qualities. The largest number of samples are

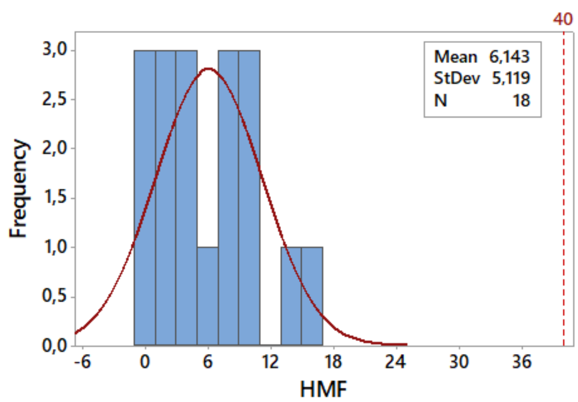

Fig. 10. Histogram of individual values of honey samples analysed for HMF mg kg^{-1} (reduced dataset).

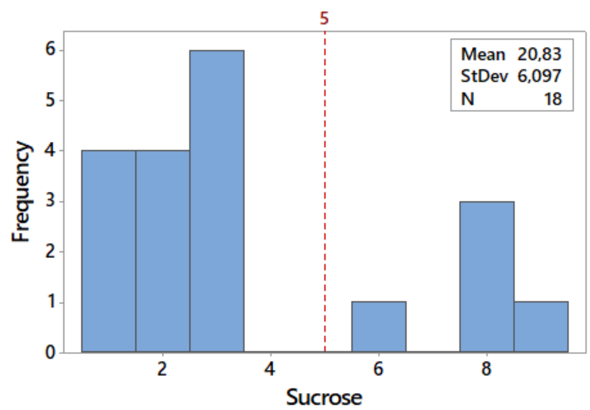

Fig. 11. Histogram of individual values of honey samples analysed for DA Gothe units (reduced dataset).

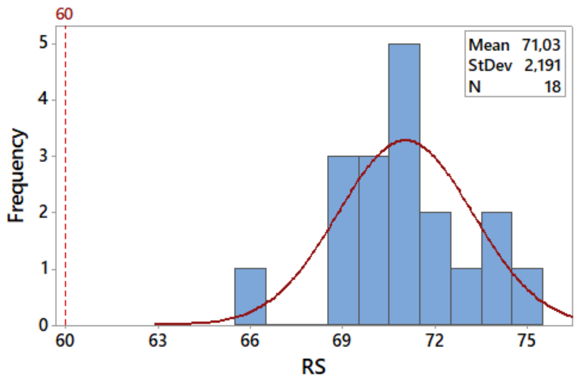

Fig. 12. Histogram of individual values of honey samples analysed for RS % (reduced dataset).

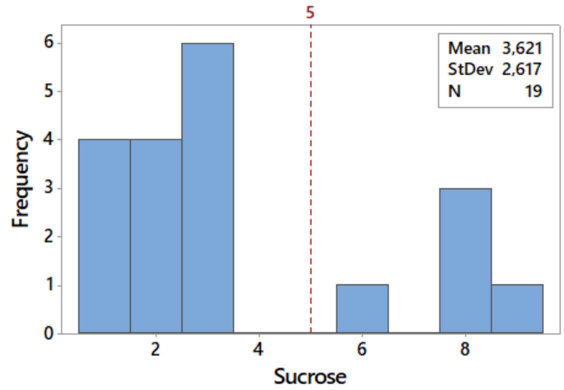

Fig. 13. Histogram of individual values of honey samples analysed for sucrose %.

found in the range of 20 - 25, indicating that in this range fall most frequently the observed samples (the distribution is not normal).

Fig. 12 shows that the average value of RS % is 71.03, with most samples concentrated in the range of 60 to 80 %. The standard deviation is 2.191. The red line at a value of 60 represents the minimum acceptable level or target value for reducing sugars. The fact that most samples are above this line is a positive indicator, but the presence of a small number of samples with very low values (around 20 %) indicates that there are some cases requiring further investigation and correction.

Fig. 13 the average value of sucrose percentage can be seen as 3.621, which is at the center of the distribution of samples. The standard deviation of 2.617 indicates significant variability in the data. The red line at a value of 5 represents the upper limit, although this may not apply uniformly to every type of honey. Several samples above this value indicate that some of the tested samples have a higher percentage of sucrose, which may be undesirable depending on the context of the study. The distribution is interrupted (not normal) and there is a skew towards lower values, suggesting that most samples are below the upper limit of 5%. The separation of the distribution relates to the bees' feeding and the resulting high percentage of the sucrose content.

CONCLUSIONS

The combination of laboratory and statistical methods provides a comprehensive and reliable approach to proving the quality of honey. Through detailed physical-chemical analysis and statistical interpretation of the results, we can assess the quality of honey according to established standards. This is essential for both producers and consumers to ensure that the honey meets quality and safety standards.

Heating honey above 45°C causes it to lose its beneficial properties. Heating it between 70 and 80°C allows honey to remain in liquid form for a longer period. Temperature fluctuations significantly impact its characteristics, including diastase activity.

Honey contains fructose and glucose, with their ratio varying depending on the plants from which bees gather nectar. The crystallization of honey serves as an indicator of its purity and sugar content.

The studies focus on diastase activity (DA) and hydroxy-methyl-furfural (HMF) as indicators of honey's quality and purity. DA is affected by high temperatures during honey processing, while HMF indicates the degree of honey heating.

In conclusion, the studies underscore the importance of controlling temperature regimes and the chemical composition of honey to maintain its beneficial properties and quality.

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