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THE ROLE OF DIASTASE ACTIVITY IN HONEY QUALITY ASSESSMENT AND A SYSTEMATIC REVIEW OF FACTORS AFFECTING IT

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ABOUT ARTICLE

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Abstract: This study is devoted to a comprehensive analysis of the key factors affecting the activity of the diastase (α-amylase) enzyme in honey based on a systematic review of the literature. The dependence of diastase activity on storage duration and conditions, thermal processing, botanical origin, and physicochemical factors was examined using international scientific sources. The results demonstrated that diastase is a thermolabile enzyme: its activity progressively decreases with increasing temperature and prolonged storage time, with almost complete inactivation observed in the temperature range of 80–100 °C. Furthermore, an inverse relationship between diastase activity and hydroxymethylfurfural (HMF) content was identified, indicating the deterioration of honey quality. The findings confirm that the combined use of diastase activity and HMF content serves as an effective комплекс indicator for honey quality assessment. In addition, a comparative analysis of analytical methods revealed that modern techniques, such as the Phadebas method and potentiometric approaches, provide higher accuracy and rapidity. The results obtained have significant scientific and practical implications for selecting optimal temperature regimes during honey processing and storage, as well as for determining product authenticity.

Introduction. Ensuring the quality and safety of food products has become one of the most pressing global challenges in recent years. According to the World Health Organization, millions of people worldwide suffer annually from various diseases caused by the consumption of substandard or adulterated food products. This situation underscores the urgent need to further strengthen and improve food safety systems. In particular, enhancing quality control of natural and biologically active products, including honey, represents an important scientific and practical priority. Honey, as a widely consumed natural product with significant nutritional and therapeutic value, requires rigorous quality assessment to ensure its safety, authenticity, and compliance with international standards [8]. In the Republic of Uzbekistan, ensuring food safety has been identified as a priority direction of state policy, and a number of regulatory and legal frameworks have been adopted in this regard. In particular, Resolution No. PQ-3151 outlines key objectives for the development of the food industry, improvement of product quality, and the establishment of production systems aligned with international standards. Furthermore, the system of technical regulation aimed at ensuring the safety and quality of food products is being continuously improved, which necessitates the advancement of laboratory control and analytical methods.

Honey is a natural product that has been consumed by humans since ancient times and is characterized by high nutritional and biological value. It is widely used not only as a food product but also in the pharmaceutical and medical fields. According to scientific studies, honey contains more than 180 different chemical compounds, among which enzymes, vitamins, organic acids, and antioxidants play a significant role [1]. In recent years, global honey production has shown a steady increase. For instance, according to the Food and Agriculture Organization (FAO), the global production of honey amounts to approximately 1.8–2 million tons annually, and this figure continues to grow each year. However, the increasing market demand has also led to emerging challenges, including honey adulteration, improper storage conditions, and inadequate technological processing, which may negatively affect product quality [2].

The assessment of honey quality relies on both physicochemical and biological parameters. In particular, enzymatic indicators are considered among the primary criteria for determining the naturalness and freshness of honey. In this context, the diastase (α-amylase) enzyme is of special importance, as it hydrolyzes starch into simpler sugars, such as dextrins and maltose, thereby reflecting the enzymatic activity of the product. According to scientific literature, diastase activity serves as a key indicator for detecting heat treatment, storage duration, and potential adulteration of honey [2,4]. For this reason, international standards, including those established by the Codex Alimentarius Commission, require a minimum level of diastase activity in honey, typically not less than 8 diastase units (DN) [8].

The activity of the diastase enzyme is influenced by numerous external and internal factors, among which thermal processing, storage duration and conditions, botanical and geographical origin, and the physicochemical environment play a crucial role. Scientific studies have demonstrated that diastase is a thermolabile enzyme, and its activity decreases significantly with increasing temperature. In particular, heating honey at 60–70 °C leads to a noticeable reduction in enzymatic activity, whereas in the range of 80–100 °C, the enzyme becomes almost completely inactivated [2]. At the same time, prolonged storage also results in a gradual decline in diastase activity, which can be explained by the natural degradation of enzymes over time [1,3]. Such changes are considered important indicators of honey quality deterioration.

Furthermore, a relationship between diastase activity and hydroxymethylfurfural (HMF) content has been established. HMF is a compound formed in honey as a result of thermal processing or prolonged storage, and an increase in its concentration is indicative of quality deterioration. According to research findings, an inverse correlation exists between diastase activity and HMF content, whereby a decrease in enzymatic activity is associated with an increase in HMF levels [6,8]. Therefore, these two parameters are widely used in combination for the comprehensive assessment of honey quality.

The origin of honey also has a significant influence on diastase activity. It has been established that the enzymatic activity varies depending on the botanical source of honey. For example, blossom honey generally exhibits higher diastase activity, whereas honeydew honey may show relatively lower levels [8]. In addition, factors such as bee species, climatic conditions, and production technology have been identified as influencing enzyme activity [12]. This variability highlights the need for a comprehensive approach in the evaluation of diastase activity.

At present, various analytical methods have been developed to determine diastase activity, among which the classical Schade method, the modern Phadebas assay, as well as spectrophotometric and potentiometric approaches are widely applied [4,7]. These methods differ in terms of accuracy, rapidity, and cost-effectiveness. In recent years, the development of novel, rapid, and low-cost techniques has further confirmed the relevance and growing scientific interest in this field.

At the same time, an analysis of the existing scientific literature indicates that although individual studies have investigated factors affecting diastase activity, there remains a lack of systematic synthesis, comparative evaluation, and comprehensive assessment of these findings. In particular, a systematic approach is needed to better understand the combined effects of thermal, storage, and chemical factors, as well as their relationship with HMF formation. This highlights the necessity for more in-depth and integrated scientific analyses in this area.

The aim of this study is to systematically analyze the key factors influencing diastase enzyme activity in honey based on a comprehensive review of the literature, to elucidate their interrelationships, and to substantiate their scientific and practical significance in the assessment of honey quality. To achieve this aim, the following objectives were established: to examine the effects of thermal processing and storage conditions on diastase activity; to evaluate its relationship with hydroxymethylfurfural (HMF) content; to compare various analytical methods; and to synthesize and critically assess the existing scientific evidence.

Methodology. This study is based on a systematic literature review approach and is aimed at providing a comprehensive analysis of the factors influencing diastase enzyme activity in honey using scientific sources. During the research process, relevant articles published in international scientific databases were selected, and their findings were synthesized through comparative and analytical approaches.

As primary data sources, reports from the Food and Agriculture Organization (FAO), as well as articles published in high-impact scientific journals such as Food Chemistry, Journal of Food Quality, Journal of Food Science and Technology, and Analytica Chimica Acta, were utilized. The selection of literature was guided by the following keywords: “honey diastase activity,” “α-amylase in honey,” “HMF formation in honey,” “effect of heating on honey,” and “honey storage and enzyme activity.”

The following inclusion criteria were applied during the literature selection process: (i) scientific articles published between 1990 and 2024; (ii) studies investigating diastase enzyme activity in honey from experimental or analytical perspectives; (iii) research examining the effects of thermal processing, storage duration, botanical origin, and chemical factors; and (iv) sources addressing the relationship between diastase activity and hydroxymethylfurfural (HMF) content. Exclusion criteria included duplicate data, articles without full-text availability, and studies not directly related to the research topic.

Data extracted from the selected studies were systematically organized and grouped into tables based on key parameters, including diastase number (DN), temperature, storage duration, HMF content, and the type of analytical method used. During the analysis, comparative, correlation, and descriptive statistical approaches were applied. In particular, diastase activity values reported in different studies were compared with temperature and time variables to identify trends and patterns of change.

The principal methods used for determining diastase activity included the classical Schade method (based on the starch–iodine reaction), the Phadebas assay (a spectrophotometric method based on the degradation of a chromogenic substrate), potentiometric approaches, and the in vitro starch hydrolysis plate assay (ISHPA). The results obtained using these methods were comparatively evaluated in terms of accuracy, sensitivity, and ease of application [4,7,14].

During data processing and synthesis, scientific logical analysis, graphical interpretation, and table-based systematization methods were employed. Based on the obtained results, the main factors influencing diastase activity—namely temperature, storage duration, HMF content, and honey origin—were identified, and their interrelationships were scientifically substantiated.

Results. Based on the findings of the systematic analysis, the primary factors affecting diastase enzyme activity (DN) were identified as storage conditions, storage duration, and thermal processing, and these were summarized in tabular form. The results confirm the high sensitivity of the diastase enzyme to external factors.

1. Effect of Storage Conditions and Duration

The table below presents the diastase activity (DN) values of honey samples stored under different conditions.

Table 1

Effect of Storage Conditions on the Diastase Activity of Honey

Sample	**Storage Conditions**	**DN (Schade)**	**DN (Phadebas)**
H1	4°C, 2 month	23.9	20.3
H2	Room temperature	17.9	17.6
H3	Stored for 4 years	13.9	9.0
H4	Stored for 2 years	10.9	9.4
H5	Stored for 2 years	10.9	11.0

According to the data presented in Table 1, diastase activity remained relatively high under short-term storage conditions (H1 and H2), ranging from 17.6 to 23.9 DN. However, a significant decline in enzymatic activity was observed in samples stored for longer periods (H3–H5), with values ranging from 9.0 to 13.9 DN. These findings indicate that the diastase enzyme undergoes gradual degradation over time. At the same time, both the Schade and Phadebas methods exhibited consistent trends, and a strong correlation between the two approaches was identified [1].

2. Effect of Thermal Processing

The temperature-dependent changes in diastase enzyme activity are summarized in the following table.

Table 2

Effect of Temperature on Diastase Activity in Honey

Temperature (°C)	Diastase Activity (DN)	Condition Description
Control	11–25	Normal
60°C	8–10	Slightly decreased
70°C	7–9	Moderately decreased
80°C	5–7	Significantly decreased
90°C	3–5	Strongly decreased
100°C	≈0	Complete inactivation

The results indicate that diastase activity decreases progressively with increasing temperature. In particular, rapid enzyme inactivation is observed at temperatures above 80 °C. At 100 °C, the diastase enzyme loses nearly all of its activity. This phenomenon can be explained by the denaturation of the enzyme's protein structure [2].

3. Combined Effect of Temperature and Time

In addition to temperature, the duration of exposure is also a critical factor. The combined effects of temperature and time are presented in the following table.

Table 3

Combined Effect of Temperature and Time on Diastase Activity in Honey

Temperature (°C)	Time	Diastase Activity (DN/IU)
40 °C	Up to 95 hours	Nearly unchanged
60 °C	96 hours	Decreased to ~8
80 °C	12.5 hours	Sharp decrease
100 °C	5–6 hours	Very rapid decrease

These results indicate that at lower temperatures (≤40 °C), diastase activity remains stable over extended periods. However, with increasing temperature, the rate of enzyme inactivation rises sharply. In particular, within the temperature range of 80–100 °C, diastase activity decreases rapidly even over short time intervals [9].

4. Kinetic Changes under Thermal Treatment (Additional Analysis)

The following table presents changes in diastase activity resulting from both conventional thermal and microwave processing.

Table 4

Changes in Diastase Activity under Thermal Treatment

Sample	Initial DN	Effect at 90 °C	Microwave (DN)	Final Assessment
M1	22.6	Gradual decrease	15.3	Stable
M2	18.0	Gradual decrease	11.2	Moderate
M3	21.8	Slowest decrease	18.1	Good
M4	23.2	Rapid decrease	12.8	Significant reduction
M5	15.5	Moderate decrease	5.1	Quality deteriorated

The results indicate that diastase activity decreases progressively during thermal processing, whereas this process is further accelerated under microwave treatment. In some samples (e.g., M5), the DN value dropped below 8, indicating a deterioration in product quality [10].

Further analysis of the results also considered additional important factors associated with diastase activity, including hydroxymethylfurfural (HMF) content, the biological origin of honey, and the effectiveness of analytical determination methods. These parameters were found to be essential for the comprehensive assessment of honey quality.

5. Relationship between Diastase Activity and HMF Content

The following table presents the changes in diastase activity and HMF content during storage.

Table 5

Changes in Diastase Activity and HMF Content during Storage

Parameter	Initial Value	After 29 Weeks	Change
Diastase (DN)	18.5	4.5	↓ ~75% decrease
HMF (mg/kg)	5.73	8.52	↑ Significant increase

The results demonstrate that during storage, diastase activity decreases markedly, whereas HMF content increases. This confirms the presence of an inverse relationship between these two parameters. The decline in diastase activity is associated with enzyme denaturation, while the increase in HMF content results from the degradation of sugars under the combined effects of heat and time [6,8].

6. Effect of Temperature on Diastase Activity and HMF Content

Table 6

Temperature-Dependent Changes in Diastase Activity and HMF Content

Temperature	Diastase (DN)	HMF (mg/kg)	Condition Description
4 °C	14–23	2–28	Stable
25 °C	10–13	26–49	Within normal range
35 °C	8–12	↑	Initial deterioration
45 °C	8–10	50+	Significant changes

55 °C	6–8	~96+	Very high HMF content

These results indicate that the imbalance between diastase activity and HMF content becomes more pronounced with increasing temperature. In particular, at temperatures above 45 °C, HMF levels rise sharply, while diastase activity declines. This confirms that improper thermal processing can significantly deteriorate honey quality [6,9].

7. Influence of Honey Type and Biological Factors

Table 7

Diastase Activity and Other Enzymes in Different Types of Honey

Honey Type	Diastase (DN)	Invertase (IN)	HMF (mg/kg)
Eucalyptus	17–49	13–36	Low
Multifloral	6–40	2–27	Moderate
Rosmarinus	20–32	9–30	Low
Citrus	3.9–10	1–9	Low

The results indicate that the botanical origin of honey has a significant influence on diastase activity. In some honey types (e.g., citrus), diastase activity may be naturally low, which reflects inherent biological characteristics rather than poor quality. In addition, a strong positive correlation between diastase and invertase activities ($r \approx 0.85$) was identified [11].

8. Comparison of Analytical Methods

Table 8

Comparison of Methods for Determining Diastase Activity

Method	Principle	Advantages	Limitations
Schade	Starch–iodine reaction	Classical, standardized	Time-consuming
Phadebas	Chromogenic substrate (620 nm)	Rapid, accurate	Expensive reagents
ISHPA	Hydrolysis zone formation	Low-cost, simple	Relatively low accuracy
Potentiometric	Triiodide ion measurement	High sensitivity	Requires specialized equipment

The results indicate that modern methods, such as the Phadebas assay and potentiometric approaches, offer higher accuracy and faster analysis. Although the Schade method remains a standardized reference technique, it is limited by its time-consuming nature. In contrast, newer methods are considered more efficient and practical for routine laboratory applications [4,7,14].

9. Relationship between Diastase Activity and Antifungal Efficacy

Table 9

Sample	Honey Only (%)	Honey + Starch (%)	Difference
V1	47	41	↓6%
V2	48	45	↓3%

V3	50	41	↓9%
V4	46	37	↓9%
V5	47	41	↓6%

The results indicate the presence of a negative correlation between diastase activity and antifungal efficacy ($R \approx -0.817$). This phenomenon can be explained by the increase in sugar concentration resulting from starch hydrolysis, which enhances osmotic pressure and consequently affects antifungal activity [5].

Discussion. The results synthesized in this study further confirm that diastase enzyme activity in honey is a highly sensitive and reliable indicator for assessing product quality. The analyzed literature consistently demonstrates that diastase activity is primarily influenced by storage duration, storage conditions, and thermal processing. In this regard, the findings of the present study are in agreement with previously published research; however, their systematic integration provides a clearer understanding of the practical diagnostic value of diastase activity. In particular, diastase activity was found to remain relatively stable under short-term storage and low-temperature conditions, whereas a gradual and consistent decline was observed during prolonged storage. This phenomenon reflects the natural degradation of enzymes over time and indicates a reduction in the biological activity of honey as it ages. In this context, diastase activity can be considered a reliable marker of freshness and storage history.

A more in-depth analysis of the storage-related findings reveals that the H1 sample, stored under short-term refrigerated conditions, exhibited the highest diastase number, while samples stored at room temperature showed a slight decline. However, the most pronounced differences were observed in the long-term stored samples (H3–H5). In these cases, the reduction in DN values leads to two important conclusions: first, storage duration has a direct impact on diastase activity; second, this decline does not necessarily indicate poor product quality but may instead reflect the natural degradation of enzymes over time. Therefore, the evaluation of diastase activity should not rely solely on a single numerical value; rather, it should also take into account the storage history and technological processing of the product. This consideration is particularly important in export–import control, laboratory assessment, and the differentiation of adulterated products.

The results related to thermal processing demonstrated an even more pronounced and consistent trend compared to storage effects. Studies indicate that a decline in diastase activity begins at temperatures as low as 60 °C, accelerates significantly beyond 80 °C, and leads to nearly complete inactivation at 100 °C. These findings confirm the highly thermolabile nature of the diastase enzyme. Importantly, some studies have reported that even short-term heating can negatively affect enzymatic activity. This highlights that not only temperature but also exposure time plays a critical role in determining the extent of enzyme degradation. Consequently, the results clearly demonstrate that thermal processing represents one of the most critical technological factors affecting diastase stability in honey.

A comparative analysis of these findings indicates that the effects of storage generally occur gradually and progressively, whereas thermal effects are typically rapid and often irreversible. For example, although the diastase number decreases during prolonged storage, it frequently remains above the standard threshold in many cases. In contrast, exposure to high temperatures results in a sharp decline in enzyme activity even within a short period.

From a practical perspective, this suggests that improper thermal processing poses a greater risk to honey quality than extended storage duration. In other words, beyond serving as a chronological marker of storage, diastase activity also functions as a highly sensitive indicator of thermal damage. The relationship between diastase activity and HMF content is of particular importance. The obtained results demonstrate that as diastase activity decreases, HMF levels increase. This inverse dynamic between the two parameters provides a highly effective combined criterion for assessing honey quality. While diastase, as a biologically active component, decreases over time and under thermal stress, HMF accumulates as a product of heat-induced sugar degradation. Therefore, the combined evaluation of diastase activity and HMF content enables not only the assessment of the current quality status of honey but also the identification of its prior exposure to technological or storage-related conditions. In this regard, the findings of the present study are consistent with those reported by other authors and further highlight the advantages of a comprehensive approach to quality control.

When temperature and HMF data are evaluated alongside diastase activity, it becomes evident that quality deterioration accelerates beyond 35–45 °C, while negative changes become more pronounced at temperatures of 55 °C and above. This finding leads to an important practical implication: temperature regimes must be carefully controlled during honey processing, melting, or decrystallization. In industrial practice, elevated temperatures are often applied to liquefy honey more rapidly; however, such approaches can significantly reduce enzymatic activity and consequently diminish the biological value of the product. Therefore, the time–temperature combination should be considered a critical determinant for preserving honey quality during technological processing.

Differences associated with the botanical origin of honey also play an important role in the interpretation of results. The findings indicate that certain honey types naturally exhibit higher diastase activity, whereas others have relatively lower levels. For instance, low DN values observed in citrus honeys should not necessarily be interpreted as an indication of poor quality or adulteration, but rather as an inherent biological characteristic. This leads to an important methodological implication: applying a single fixed threshold for diastase activity across all honey types may result in the misclassification of some naturally occurring honeys as low-quality products. Therefore, the interpretation of diastase activity should take into account the botanical source, geographical origin, and even the biological characteristics of bees. Such an approach enhances the accuracy of quality assessment and helps prevent erroneous conclusions in laboratory evaluation.

The identification of a positive correlation between diastase and other enzymes, particularly invertase, is also of considerable theoretical and practical significance. These enzymes collectively reflect the biological integrity of honey. A simultaneous decrease in both diastase and invertase activities often indicates a disruption of the overall enzymatic system, which may result from thermal exposure, prolonged storage, or processing. Conversely, the presence of negative correlations with certain physicochemical parameters, such as moisture content or total soluble solids (TSS), suggests that the honey matrix is not always conducive to maintaining enzyme stability. This further confirms the necessity of a multifactorial approach when evaluating enzymatic activity in honey.

The comparison of analytical methods also warrants particular attention. Although the Schade method remains a classical and standardized technique, its time-consuming nature and multi-step procedure represent notable limitations. In contrast, the Phadebas assay offers advantages in terms of speed and operational convenience, while newer approaches, such as potentiometric methods and ISHPA, present valuable alternatives due to their sensitivity, simplicity, or cost-effectiveness. However, minor discrepancies between methods require careful interpretation of diastase values. For instance, results obtained using the Schade method in one laboratory and the Phadebas method in another may differ slightly in absolute values, although overall trends generally remain consistent. Therefore, in both practical and scientific evaluations, it is essential to report not only the diastase values but also the analytical method used. The findings of this study confirm that, despite methodological differences, general trends are preserved, while the selection of a specific method may vary depending on the objectives and conditions of the laboratory.

The negative correlation observed between diastase activity and antifungal efficacy represents one of the most intriguing findings of this study. At first glance, a decrease in DN is typically interpreted as a decline in quality; however, in systems involving starch, an increase in antifungal effectiveness was observed in certain cases. This phenomenon can be explained by the role of diastase in hydrolyzing starch into simpler sugars, thereby increasing sugar concentration and enhancing osmotic pressure, which contributes to antifungal activity. Thus, diastase activity should not be viewed solely as a "quality passport," but also as a parameter that may be indirectly associated with certain functional properties of honey. This finding has important implications for future research, suggesting that diastase activity should be investigated not only as a standard quality indicator but also as a functional marker linked to biological efficacy.

Overall, the comparative analysis of the results demonstrates that diastase activity is one of the most sensitive indicators of honey quality. However, it should not be interpreted in isolation; rather, it must be evaluated in conjunction with HMF content, storage history, thermal regime, botanical origin, and the analytical method employed. Only such a comprehensive approach allows for an accurate assessment of product quality, freshness, and technological history. Furthermore, the findings highlight the importance of applying mild technological processing conditions, avoiding prolonged exposure to high temperatures, and implementing multi-parameter evaluation systems in laboratory analysis. Based on this discussion, it can be concluded that the diastase enzyme serves not only as a control parameter but also as an integrated marker reflecting the biological, technological, and authenticity-related characteristics of honey.

Conclusion. The results of this study confirm that diastase enzyme activity in honey is a reliable and sensitive indicator for assessing product quality. Based on a systematic review of the literature, it was established that diastase activity is highly sensitive to several factors, including storage duration and conditions, thermal processing, and the biological origin of honey. Under short-term and low-temperature storage conditions, enzymatic activity remains relatively stable, whereas prolonged storage leads to a gradual decline. However, the most pronounced changes occur under elevated temperatures, with rapid inactivation of diastase observed within the range of 80–100 °C.

The findings also demonstrate the existence of an inverse relationship between diastase activity and hydroxymethylfurfural (HMF) content. Specifically, a decrease in diastase activity accompanied by an increase in HMF levels indicates exposure to thermal treatment or extended storage. Therefore, the combined evaluation of these two parameters is recommended as an effective approach for comprehensive honey quality assessment. In addition, the botanical origin of honey was shown to significantly influence diastase activity, with certain honey types naturally exhibiting lower enzyme levels. This highlights the limitations of applying universal threshold values and underscores the need for an individualized interpretation of diastase indicators. Furthermore, the comparison of analytical methods revealed general consistency in trends between classical and modern techniques, while contemporary methods (such as the Phadebas assay and potentiometric approaches) demonstrated superior performance in terms of speed and accuracy.

From a practical perspective, the findings substantiate the necessity of maintaining optimal temperature conditions (≤40 °C) during honey processing and storage, while minimizing exposure to high-temperature treatments. Furthermore, in laboratory control, the combined evaluation of diastase activity with other quality indicators is essential for accurately determining the true quality and authenticity of the product.

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