DIASTASE ENZYME ACTIVITY AS A QUALITY AND CLINICAL SAFETY PARAMETER OF PURE HONEY IN PHARMACIES IN THE PASAR KEMIS

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ABSTRACT

Diastase enzyme is a natural constituent of honey highly sensitive to heat and storage, making its activity a primary indicator for bio-integrity and authenticity. This study aims to evaluate diastase activity in pure honey at pharmacies to ensure compliance with quality standards and consumer safety. The population included all packaged pure honey in pharmacies, using purposive sampling for five brands (samples A, B, C, D, and E). Instruments used included a spectrophotometer at 660 nanometers, with simple linear regression to determine the Diastase Number. Analysis revealed significant differences in enzymatic activity between samples. Sample A demonstrated optimal quality (7.49 DN), while samples B (0.12 DN), C (0.15 DN), and D (2.53 DN) were far below the minimum threshold of 3 units as per Indonesian National Standard 8664:2024. This decrease reflects molecular protein denaturation that is visually undetectable but implies therapeutic loss and toxic degradation risk. There is a significant anomaly between marketing authorization legality and biological quality in retail, necessitating systemic strengthening of post-market surveillance by regulatory authorities.

Keywords: *Pure Honey; Diastase Enzyme; Consumer Safety*

ABSTRAK

Enzim diastase merupakan komponen alami madu yang sangat sensitif terhadap panas dan penyimpanan, sehingga aktivitasnya menjadi indikator utama integritas biologis dan keasliannya. Studi ini bertujuan untuk mengevaluasi aktivitas diastase pada madu murni di apotek untuk memastikan kepatuhan terhadap standar mutu dan keamanan konsumen. Populasi yang diteliti meliputi semua madu murni kemasan di apotek, menggunakan pengambilan sampel bertujuan untuk lima merek (sampel A, B, C, D, dan E). Instrumen yang digunakan meliputi spektrofotometer pada 660 nanometer, dengan regresi linier sederhana untuk menentukan Angka Diastase. Analisis menunjukkan perbedaan signifikan dalam aktivitas enzimatik antar sampel. Sampel A menunjukkan kualitas optimal (7,49 DN), sedangkan sampel B (0,12 DN), C (0,15 DN), dan D (2,53 DN) jauh di bawah ambang batas minimum 3 unit sesuai Standar Nasional Indonesia 8664:2024. Penurunan ini mencerminkan denaturasi protein molekuler yang secara visual tidak terdeteksi tetapi menyiratkan kehilangan terapeutik dan risiko degradasi toksik. Terdapat anomali signifikan antara legalitas izin pemasaran dan kualitas biologis di pasar ritel, yang mengharuskan penguatan sistematis pengawasan pasca-pemasaran oleh otoritas pengatur.

Kata Kunci: Madu Murni; Enzim Diastase; Keamanan Konsumen

INTRODUCTION

Pure honey is a natural product with high biochemical complexity, in which diastase enzyme activity serves as a critical parameter for assessing its biological integrity and quality. As a temperature-sensitive amylolytic enzyme, diastase ensures that the product is processed and stored under appropriate conditions to preserve the stability of other bioactive compounds. Within the health product distribution chain, this molecular quality assurance is essential because a decrease in enzymatic activity below a certain threshold can compromise the nutritional profile and natural characteristics of the honey.[1] Therefore, standardizing diastase enzyme activity is a key tool for maintaining the consistent quality of honey products distributed to consumers.

In Indonesia's regulatory framework, packaged pure honey products must comply with the Indonesian National Standard (SNI) 8664:2024, which includes a minimum diastase activity requirement of 3 Diastase Numbers (DN). Badan Pengawas Obat dan Makanan (BPOM), or the Food and Drug Monitoring Agency, plays a central role in certification and post-market surveillance to ensure the quality of products available to consumers. However, the technical challenges of maintaining the stability of biological products throughout their shelf life and distribution at the retail level necessitate regular monitoring. The presence of a distribution permit number on a product is intended to signify ongoing compliance with quality standards in accordance with established regulations.[2]

The dynamics of product quality at the retail level, particularly in the Pasar Kemis area, represent a critical aspect that requires scientific evaluation to provide a comprehensive understanding of the actual condition of biological products within the community. Monitoring enzymatic activity in honey with distribution permits is essential to obtain objective data on product stability throughout the downstream distribution chain. Given the importance of natural product quality for consumers, this study was conducted to evaluate the diastase activity in pure honey samples from local markets. The results are expected to serve as a scientific reference to support the enhancement of quality management and sustainable consumer protection efforts.

METHODS

This study employed a descriptive analytical design with a laboratory experimental approach to evaluate the enzymatic activity of pure honey. The research was conducted in October 2025, with samples collected from three pharmacies in the Pasar Kemis, Tangerang Regency. The study population comprised all packaged pure honey products available at pharmacies in the Pasar Kemis, Tangerang Regency. Samples were selected using purposive sampling, with inclusion criteria requiring pure honey to have an official distribution permit from Badan Pengawas Obat dan Makanan (BPOM) and to be within its valid shelf life. A total of six samples were analyzed, representing five different brands; one brand was sampled from two different pharmacies to assess quality consistency throughout the distribution chain. All tests were conducted in triplicate to ensure data precision. The independent variables in this study were the brand of pure honey and the location of the pharmaceutical service facility, while the dependent variable was diastase enzyme activity, expressed as the Diastase Number value.[3]

The primary instruments used include a spectrophotometer equipped with a 1 cm cuvette, set to a wavelength of 660 nm, a water bath maintained at 40 ± 0.2°C, and a stopwatch. The testing procedure begins with solution preparation and standardization, which involves preparing an iodine stock solution, a pH 5.3 acetate buffer solution, and a 0.5 M NaCl solution. Before sample testing, the starch solution is standardized to determine the appropriate volume of distilled water (aquadest) required to achieve a precise absorbance value of 0.760 ± 0.02. During sample preparation, 5 g of the sample is dissolved in 15 ml of distilled water and 2.5 ml of acetate buffer, then transferred to a 25 ml volumetric flask containing 1.5 ml of NaCl solution, and the volume is adjusted to the calibration mark.[2]

Enzyme activity determination began with separate incubations of 10 ml of sample solution and 5 ml of starch solution at a constant temperature of 40°C for 15 minutes in a water

bath. Once the target temperature was reached, the two solutions were mixed to initiate the enzymatic hydrolysis reaction, and the time was immediately recorded using a stopwatch. At 5-minute intervals, 1 ml of the mixture was periodically pipetted into 10 ml of iodine solution, then diluted with distilled water using a specific volume (x ml) determined from previous standardization results. Absorbance values were measured using a spectrophotometer at a wavelength of 660 nm until reaching a target value below 0.235, the reaction endpoint.[4]

Data analysis was conducted by evaluating the kinetics of starch degradation by the diastase enzyme using a simple linear regression approach. This method involves plotting absorbance (Y) against incubation time in minutes (x) to generate a linear equation of the form Y = a + bx. Here, represents the intercept or initial absorbance, while is the regression coefficient (slope) indicating the rate of enzymatic hydrolysis. To ensure accuracy, the time (t) required to reach a specific absorbance target of 0.235 is determined by rearranging the regression equation as t = (0.235 - a) / b. This time value is then converted to the Diastase Number (DN) using the formula: DN = 300/t. Throughout the analysis, the correlation coefficient (r) is monitored to confirm the linearity of the relationship. The required standard for the Diastase Number (DN) is a minimum value of 3 DN.[2]

RESULTS

The population in this study comprised packaged pure honey products that had obtained official distribution permit numbers from regulatory authorities. The sample included five brands of pure honey (A, B, C, D, and E) sourced from three different pharmacies in the Pasar Kemis area (X, Y, and Z). The purpose of selecting samples from these pharmaceutical outlets was to investigate the correlation between regulatory approval and the products' actual biological quality upon reaching consumers. Testing was performed in triplicate to ensure data accuracy. The results of the diastase enzyme activity test for all pure honey samples are presented in Table 1.

Table 1. Diastase Enzym Test Results

Pharmacies	Honey	Diastase Enzym (DN)			Average ±SD
		1	2	3	
X	A	7,25	7,14	7,38	7,2566 ±0,12
	B	0,16	0,09	0,12	0,1225 ±0,03
Y	C	0,19	0,11	0,15	0,15 ±0,03
	A	7,49	7,32	7,67	7,4933 ±0,14
Z	D	2,53	2,29	2,79	2,536667 ±0,2
	E	6,59	6,72	6,4	6,57 ±0,13

DISCUSSION

Diastase enzyme activity in honey directly reflects the bees' physiological processes and the quality of post-harvest handling. Chemically, diastase is an amylolytic enzyme that catalyzes the hydrolysis of starch into maltose and dextrin. From a phytochemical standpoint, diastase serves as a highly reliable indicator of biological authenticity.

Based on the latest SNI 8664:2024 standard, the diastase number (DN) is established as a mandatory primary quality indicator with a minimum threshold of 3 DN. This value represents a biologically critical point, indicating that the honey retains residual enzymatic activity and is valid as a natural product.[5]

The findings of this study reveal a significant anomaly in the biological product quality assurance system. Although all tested

samples had received official distribution permits from Badan Pengawas Obat dan Makanan (BPOM), laboratory results indicated severe enzymatic degradation in samples B (0.12 DN), C (0.15 DN), and D (2.53 DN). In contrast, honey sample A exhibited an optimal profile with an average diastase number above 7.49 DN. The presence of products with diastase activity well below the minimum standard in authorized facilities such as pharmacies has sparked critical discussion regarding the effectiveness of post-market surveillance. This phenomenon suggests that quality validation conducted during the pre-market (registration) stage does not necessarily guarantee consistent molecular quality in the field.[3]

Honey with low diastase activity often does not exhibit any noticeable physical changes; its color, aroma, and viscosity remain normal to consumers. This visual characteristic presents a significant challenge because biological damage caused by enzyme protein denaturation occurs at the invisible molecular level. Therefore, spectrophotometric analysis of these enzymes is necessary to detect clinically significant losses in catalytic function in honey.[6,7]

The fundamental principle of diastase activity testing involves monitoring the degradation kinetics of the starch-iodine complex using a spectrophotometer at a wavelength of 660 nm.[4] The spectrophotometer measures the intensity of light absorption from the bluish-violet color formed when iodine molecules are trapped within the helical structure of the amylose fraction in starch.[8] High absorbance values at the beginning of the test indicate an abundance of intact starch helical structures. As the reaction proceeds, the diastase enzyme in honey, which is a mixture of α- and β-amylases, hydrolyzes the starch by breaking the α-1,4 glycosidic bonds in the starch polymer chain, producing simpler molecules such as maltose, maltotriose, and dextrin.[9] This fragmentation of the starch chain disrupts the amylose helical structure, causing iodine molecules to lose their binding sites. Consequently, the color intensity in the solution gradually fades: the initial bluish-violet color changes to reddish-purple, then to red-brown, and finally to yellow, which is the original color of the dilute iodine solution when no longer bound to starch.[10] The decrease in absorbance to a specific target value of 0.235 quantitatively indicates that most of the starch substrate has been degraded into dextrin and simple sugars that no longer form a color complex with iodine.[4] Therefore, the time required for the sample to reach this absorbance endpoint is directly proportional to the rate of enzymatic activity: the shorter the time, the higher the bio-integrity of the diastase enzyme in the honey.[10] Conversely, if the absorbance does not significantly decrease, this indicates that the enzyme protein has undergone molecular denaturation, resulting in the loss of catalytic function and biological vitality of the honey preparation.[11]

The activity of the diastase enzyme in pure honey is fundamentally governed by the principles of enzyme kinetics and the stability of macromolecular proteins. As a polymer of amino acids, diastase possesses a highly specific three-dimensional (tertiary) structure essential for maintaining its catalytic function in hydrolyzing starch. Heating or storage at extreme temperatures increases the system's kinetic energy beyond the activation energy of the hydrogen bonds that stabilize the protein's folding, leading to irreversible denaturation.[11] The low Diastase Number (DN) values observed in most samples indicate that the enzyme's structural integrity has been permanently compromised at the molecular level, resulting in the loss of the active site's ability to bind to the starch substrate.[12]

From a food thermodynamics perspective, the decrease in enzymatic activity often precedes or occurs simultaneously with the Maillard reaction. In tropical climates with high humidity, reducing sugars such as fructose and glucose tend to react with the amino groups of free amino acids present in honey. This process not only diminishes enzyme effectiveness but also gradually alters the chemical profile of honey through the formation of dark polymeric compounds called melanoidins, which are not yet visually detectable to the naked eye. This dynamic confirms that pure honey is an unstable biological system; failure to maintain cold chain management during distribution accelerates internal chemical reactions, thereby shortening the product's biological shelf life before its administrative expiration date.[8]

Pharmacognostically and phytochemically, diastase serves as a bio-indicator for the presence of other labile micronutrient compounds. Standard pharmacognosy textbooks emphasize that if the enzymatic index falls below a minimum threshold, it indicates that sensitive secondary metabolites, such as polyphenols and flavonoids, have undergone oxidative degradation.[13] This condition fundamentally alters the pharmacological status of honey, changing it from a functional food with therapeutic activity to merely a simple carbohydrate preparation that has lost its medicinal value.[14] Therefore, consuming honey with a low diastase number (DN) will not provide the immune modulation effects or clinical recovery expected in natural medicine practices.[15]

The health implications of low diastase levels in market-approved honey are significant. Diastase inactivation strongly indicates overall phytochemical degradation and the potential formation of the sugar degradation compound hydroxymethylfurfural (HMF).[16] Long-term exposure to HMF can induce the formation of free radicals in body cells, potentially causing oxidative stress and DNA damage. The use of substandard honey for therapeutic purposes becomes ineffective due to the typically reduced activity of The use of substandard honey for therapeutic purposes becomes ineffective because the activity of glucose oxidase (an enzyme that naturally produces antibacterial hydrogen peroxide/H_2O_2) is typically reduced. For consumers, especially those with liver disorders or diabetes, consuming thermally damaged honey increases the burden on liver detoxification due to exposure to chemical residues resulting from degradation. [17,18] Therefore, honey with a poor enzymatic profile can no longer be considered a health-supporting product; rather, it is regarded as a preparation that has lost its biological safety standards.

Pharmacologically, clinically safe honey must preserve its complete ecosystem of active substances, including polyphenols and flavonoids. When diastase activity declines significantly due to errors in distribution, processing, or storage, the integrity of these antioxidant compounds is typically compromised through oxidation.[19] This degradation causes honey to lose its ability to modulate the immune system and maintain blood glucose balance. Therefore, adhering to the diastase activity threshold is not merely an administrative requirement but a guarantee that patients purchasing honey from pharmacies receive a safe, high-quality product capable of delivering therapeutic effects consistent with medical expectations.

From a biochemical perspective, the correlation between diastase activity and honey's therapeutic properties is rooted in the intrinsic instability of honey's complex matrix. As explained in food chemistry, enzymatic activity, particularly diastase (α-amylase), serves as a sensitive indicator of a product's thermal history and freshness because enzymes are labile proteins that are easily denatured by heat or improper storage.[20] The degradation of this enzymatic fraction reflects broader chemical changes within the food system; the same high temperatures or prolonged storage conditions that disrupt diastase structure also accelerate oxidative reactions that degrade phenolic acids and flavonoids.[20] These polyphenolic compounds are the primary contributors to honey's antioxidant capacity, a property fundamentally dependent on its chemical integrity. According to biochemical principles, the antioxidant function of these molecules is exerted through their ability to donate hydrogen atoms or electrons to neutralize free radicals, a mechanism that is nullified if their structure has been oxidized.[21] Thus, decreased diastase levels are not an isolated event but rather a reliable marker for the more pharmacologically critical depletion of non-enzymatic antioxidants responsible for modulating the human body's metabolic and immune pathways.

The presence of legally recognized products that do not meet SNI standards for molecular quality highlights the need for more stringent periodic audits of distribution permit holders. This situation emphasizes the necessity for reform in regulatory oversight management. As an authority equipped with comprehensive laboratory infrastructure, BPOM's role should extend beyond administrative tasks to include continuous spot testing, ensuring that each batch in circulation maintains its biological integrity. The failure to detect quality degradation within the retail chain represents a

public safety risk that demands immediate corrective action.[22]

Strengthening BPOM's post-market surveillance through regular laboratory testing at the retail level is essential to ensure consistent molecular quality of products. Standardizing diastase activity should be established as an absolute criterion for functional food safety, accompanied by a robust recall mechanism for products identified as substandard. Pharmacy practitioners are encouraged to be more selective when choosing suppliers and to enhance consumer education on proper honey storage to prevent further enzymatic degradation.

CONCLUSIONS

This study concluded that there was a significant anomaly between the legal status of distribution permits and the actual biological quality of pure honey in the Pasar Kemis area. The majority of samples (B, C, and D) were found to be non-compliant (TMS) according to SNI 8664:2024 with values <3 DN, despite being marketed in authorized pharmaceutical facilities. This molecular damage could not be visually identifiable, creating a risk of information asymmetry for consumers. This low enzymatic activity not only negates the therapeutic benefits of honey but also indicates a failure of the quality surveillance system in maintaining product integrity until it reaches the end consumer.

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