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Determination of the shelf life of fermented glutinous rice using the Arrhenius equation

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Abstract
Fermented glutinous rice, popularly known as tape ketan, is a traditional Indonesian food produced through a fermentation process using a yeast starter. Predicting the shelf life of this product is essential for ensuring quality and safety during distribution. This study aimed to determine the shelf life of fermented glutinous rice using the Accelerated Shelf Life Test (ASLT) based on the Arrhenius model. The samples were stored at 3 different temperatures (2°C, 22°C, and 29°C) for a duration of 8 days. Quality degradation was monitored through pH parameters as a primary indicator of acidification during storage. The results indicated that the quality reduction of fermented glutinous rice follows a zero-order reaction kinetics, yielding a linear regression equation of y = -0.015x + 4.96. Based on the Arrhenius calculation using the pH parameter, the estimated shelf life of fermented glutinous rice stored at a refrigerated temperature of 2°C is 3 days. At higher storage temperatures of 22°C and 29°C, the shelf life significantly decreased to approximately 2 days. This research confirms that storage temperature significantly impacts the reaction rate of chemical degradation and the overall durability of the product.

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INTRODUCTION

Fermented glutinous rice, locally known as tape ketan, is a popular traditional Indonesian snack made from steamed white or black glutinous rice fermented with a yeast starter (e.g. Saccharomyces cerevisiae). During the process, complex carbohydrates in glutinous rice are enzymatically hydrolyzed into simpler sugars by amylolytic enzymes, followed by their conversion into ethanol, organic acids, and other metabolites. This biochemical transformation not only alters the chemical composition but also softens the texture and increases the moisture content, resulting in a semi-solid product with a characteristic aroma (Zhao et al., 2021). This fermentation process produces a unique sensory profile characterized by a combination of sweet, slightly sour, and alcoholic flavors. Beyond its sensory appeal, fermented glutinous rice is widely consumed for its functional health benefits (X. Xu et al., 2019). The addition of an appropriate amount of sugar during fermentation can produce a balanced sweet and sour flavor, thereby enhancing the palatability and overall appeal of the fermented product (Y. Xu et al., 2021).

Despite its popularity, fermented glutinous rice is highly perishable due to the continuous nature of the fermentation process during storage. Environmental factors, particularly temperature, play a critical role in maintaining its quality. Inappropriate storage temperatures can lead to rapid quality degradation through ongoing microbial activity, esterification, and oxidation. Consequently, cold storage is often employed to slow down these biochemical reactions and maintain the freshness of the product until it reaches the consumer.

To ensure quality assurance and food safety, it is essential to provide a reliable estimation of the product's shelf life. A widely recognized approach for this purpose is the Accelerated Shelf-Life Testing (ASLT) method using the Arrhenius equation. The ASLT method estimates shelf life by accelerating the quality reduction reactions under storage conditions above normal levels (Labuza, 2007). In this model, temperature is the primary parameter, as higher temperatures generally correlate with faster spoilage rates. The Arrhenius equation allows researchers to mathematically determine the relationship between temperature and the rate of degradation, providing a predictable timeframe for the product's stability.

The purpose of this research is to determine the shelf life and analyze the quality reduction of fermented glutinous rice stored at various temperatures. By applying the ASLT method with the Arrhenius equation, this study aims to provide an accurate prediction of the product's durability, which is crucial for both producers and consumers in the traditional food industry

METODE

Time and Place of Research

This research was conducted from July 10, 2024 to July 18, 2024. The sample preparation and quality monitoring were performed at the Laboratory of Industrial Microbiology, Chemical Engineering Department, UPN “Veteran” Jawa Timur.

Materials and Equipment

The primary material used was white glutinous rice purchased from a local market in Surabaya. The fermentation was initiated using a commercial yeast starter (*Saccharomyces sp.*). The equipment used in this research included a traditional stainless steel steamer, a digital thermometer for temperature monitoring, a refrigerator (for 2 °C storage), and a laboratory incubator (for 29 °C storage). The pH measurements were conducted using Universal pH indicator strips (pH 0-14, Merck, Germany).

Research Design

This research employed an experimental design utilizing the Accelerated Shelf-Life Testing (ASLT) method with the Arrhenius model approach. The independent variable was the storage temperature, tested at three levels: 2 °C (refrigerated), 22 °C (room temperature), and 29 °C (warm temperature). The dependent variable was the pH value of the fermented glutinous rice, which served as the critical indicator of quality degradation over time.

Sample Preparation

The fermented glutinous rice samples were prepared in the laboratory following these procedures:

1. Preparation: White glutinous rice was washed thoroughly to remove impurities and subsequently soaked in water for approximately 4 hours.
2. Steaming: Following the soaking process, the rice was transferred to a stainless-steel steamer and heated until it was completely cooked and gelatinized.
3. Cooling: The cooked rice was evenly distributed on a clean tray and left to cool naturally until it reached room temperature (approximately 28 °C).
4. Inoculation: After cooling, powdered yeast starter was added at a concentration of 1% (w/w). The rice was then mixed thoroughly to promote uniform distribution of the starter throughout the substrate.
5. Primary Fermentation: The inoculated rice was transferred into airtight containers and fermented at room temperature for 48 hours. Upon completion of the primary fermentation, the fermented samples were placed under three storage conditions, namely 2 °C, 22 °C, and 29 °C, and monitored for a period of 8 days.

Data Analysis and Arrhenius Model

The degradation rate of the product quality was evaluated according to zero-order and first-order kinetic models. The reaction model that best described the deterioration process was selected by comparing the correlation coefficients (R^2) obtained from the linear regression between pH values and storage duration. The reaction rate constant (k) calculated at each storage temperature was subsequently used to establish the Arrhenius relationship by plotting ln k against the reciprocal of absolute temperature (1/T). This analysis was performed to obtain the activation energy (E_a) associated with the quality degradation process. Finally, the product shelf life was estimated by calculating the reaction rate constant (k) at the specified storage temperature through extrapolation using the Arrhenius equation :

$$k = A_0[e]^{-E_a/RT} \quad (1)$$

where:
k = Arrhenius equation
A_0 = rate constant as T approach infinity
E_a = activation energy

Within the ASLT approach, storage temperature is considered a critical factor influencing the rate of food deterioration. An increase in temperature generally accelerates the reactions responsible for quality loss and, consequently, shortens the product's storage stability. The effect of temperature on the rate of quality degradation can be quantitatively described using the Arrhenius model (Kaymak-Ertekin & Gedik, 2005).

The Accelerated Shelf-Life Testing (ASLT) approach based on the Arrhenius model provides an efficient alternative for estimating product shelf life without requiring prolonged storage periods or substantial costs. In this approach, food products are exposed to elevated storage temperatures to accelerate quality deterioration, after which the shelf life is estimated through extrapolation to the intended storage temperature. Temperature-dependent oxidation, which may lead to rancidity, can be employed as an indicator of product deterioration. The extent of rancidity can be evaluated using the thiobarbituric acid (TBA) value (Riyandi et al., 2022). The ASLT procedure using the Arrhenius model generally involves storage at three different temperature conditions. Shelf-life estimation is subsequently performed by determining the reaction kinetics and applying the Arrhenius relationship to describe the effect of temperature on the rate of quality degradation. The Arrhenius equation is expressed as follows:

Zero order:

$$r = \frac{dA}{dt} = k \quad (2)$$

$$A = A_0 + kt \quad (3)$$

First order:

$$r = -\frac{dA}{dt} = kA \quad (4)$$

$$\ln(\frac{A}{A_0}) = kt \quad (5)$$

Second order:

$$r = -\frac{dA}{dt} = kA^2 \quad (6)$$

$$\frac{1}{A} - \frac{1}{A_0} = kt \quad (7)$$

(Levenspiel, 1999)

The deterioration of food quality can be described using either zero-order or first-order reaction kinetics. The reaction rate constant (k) at each storage temperature can be estimated through an Arrhenius plot by establishing the relationship between Ln k and the reciprocal of absolute temperature (1/T). The resulting linear relationship can then be extrapolated to estimate the reaction rate at the desired storage temperature. This approach is particularly applicable to fermented glutinous rice products, whose quality characteristics are susceptible to temperature variations. Through the Accelerated Shelf-Life Testing (ASLT) approach, changes in storage temperature are intentionally applied to accelerate product deterioration, allowing shelf-life estimation within a shorter experimental period (Nurhayati et al., 2017). In the Arrhenius-based ASLT approach, shelf life is estimated by exposing the product to controlled temperature conditions and subsequently determining the temperature dependence of the deterioration rate. The procedure generally consists of several stages:

a. Determining the changes in the selected quality parameter at different temperatures using both zero-order and first-order kinetic models;
b. Constructing regression plots for each reaction order to obtain the reaction rate constant (k_0), intercept, and coefficient of determination (R^2);
c. Establishing the Arrhenius relationship by plotting ln k against 1/T for the respective storage temperatures.

The Arrhenius plot provides the corresponding reaction rate constant, intercept, and coefficient of determination. The value of K represents the slope obtained from the linear regression analysis of the quality degradation data collected under the three storage temperature conditions.

RESULTS AND DISCUSSION

Organoleptic Test

The organoleptic test evaluated the flavor, color, aroma, and consistency of the fermented glutinous rice samples. Initially, the product exhibited a bright white appearance and a

characteristic sweet-sour profile. However, during the storage period, the color transitioned to a yellowish pale, accompanied by a more pronounced alcoholic aroma. Furthermore, the samples showed increased syneresis (watery texture), which is attributed to the ongoing enzymatic breakdown of starch structures during fermentation.

Table 1. Organoleptic quality change of fermented glutinous rice

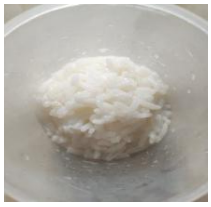
Day	Temperature		
	2 °C	22 °C	29 °C
0			
2			
4			
6			
8			

Table 2. Organoleptic test of fermented glutinous rice at 2°C

Temperature	Day	Taste	Colour	Aroma
2 °C	0	Neutral	White	Odorless
	2	Sweet, slightly astringent	White to pale	Alcoholic
	4	Sweet, slightly astringent	Pale white	Alcoholic
	6	Sweet and sour	Bright yellow	Alcoholic
	8	Sweet and sour	Yellowish pale	Strong alcoholic

Table 3. Organoleptic test of fermented glutinous rice at 22 °C

Temperature	Day	Taste	Colour	Aroma
22 °C	0	Neutral	White	Odorless
	2	Sweet, slightly astringent	White to pale	Alcoholic
	4	Sweet, slightly astringent	Pale white	Alcoholic
	6	Sweet and sour	Bright yellow	Alcoholic
	8	Sweet and sour	Yellowish pale	Strong alcoholic

Table 4. Organoleptic test of fermented glutinous rice at 29 °C

Temperature	Day	Taste	Colour	Smell
29 °C	0	Neutral	White	Odorless
	2	Sweet, slightly astringent	Bright white to pale	Alcoholic
	4	Sweet, slightly astringent	Pale white	Alcoholic
	6	Sweet and sour	Bright yellow	Strong alcoholic
	8	Sweet and sour	Yellowish pale	Strong alcoholic with undesirable odor

Notes : From table 1, 2, 3 and 4 inform that the organoleptic conditions of the glutinous rice have been observed. The decline in this condition can be influenced by many factors. This decrease in organoleptic quality serves as a direct indicator of the storage duration and the extent of internal biochemical reactions (Wang et al., 2018). The yellowish discoloration is likely caused by the oxidation of phenolic compounds or non-enzymatic browning that occurs as fermentation progresses. Meanwhile, the increase in wateriness (syneresis) is attributed to the continued activity of amylolytic enzymes that break down starch structures into simpler sugars and water. This result is consistent with research by (Y. Xu et al., 2021). which demonstrated that prolonged fermentation in glutinous rice significantly alters the physical matrix and volatile aroma profile due to advanced microbial metabolism.

pH and Temperature Test

The pH value of the fermented glutinous rice samples showed a consistent decline during storage, ranging from 5 to 4. This acidification process is a key indicator of quality degradation and is directly influenced by both storage temperature and duration.

The reduction in pH during fermentation can be associated with the metabolic processes carried out by lactic acid bacteria and yeast contained in the fermentation starter. These microorganisms utilize the remaining sugars, particularly glucose, as substrates and produce several organic acids, including lactic acid, acetic acid, and gluconic acid (Law et al., 2011). The observed decrease in pH is also related to the biochemical progression of fermentation, in which sugars are initially metabolized into alcohol and subsequently undergo oxidation to form organic acids. The accumulation of these acidic compounds increases the concentration of hydrogen ions (H^+), thereby resulting in a more acidic fermentation environment

While some studies on pigmented rice suggest that anthocyanin degradation can influence chemical shifts (Irwanda et al., 2022), in the context of white glutinous rice, the pH drop is predominantly driven by this acid production. Furthermore, higher storage temperatures (22 °C

and 29 °C) provide the thermal energy required to accelerate these enzymatic and microbial reactions, as described by the Arrhenius principle. According to (Tiwari et al., 2008), increased temperatures enhance the molecular collision frequency between reactants, thereby increasing the reaction rate constant (k) and leading to a more rapid decline in pH compared to refrigerated conditions (2°C).

The changes of pH value the product for storage ranged from 5 – 4. Changes in pH values along with decreasing temperature and storage time are thought to be due to the anthocyanins in the product experience degradation, causing the pH value to decrease changed. Heating causes conformation of the anthocyanin structure where anthocyanin turns into unstable anthocyanidins (Irwanda et al., 2022).

Table 5. Observation data of the pH values during various temperature storage

Time (day)	pH		
	2 °C	22 °C	29 °C
0	5	5	5
2	4,8	4,9	4,7
4	5	5	4,6
6	4,9	4,8	4,1
8	4,8	4,7	4

Table 6. Linear Regression Equation for pH Value parameters against temperature

Temperature (°C)	Regression Equation		R^2 value	
	Zero Order	First Order	Zero Order	First Order
2	y=-0,015x+4,96	y=-0,0031x+1,6013	0,225	0,2235
22	y=-0,035x+5,02	y=-0,0072x+1,6137	0,7206	0,7231
29	y=-0,13x+5	y=-0,0291x+1,6126	0,9548	0,9518

Table 6 shows the kinetics of changes in the pH fermented glutinous rice storage at temperatures of 2 °C, 22 °C, and 29 °C following a zero-order reaction. This shows that the reaction speed of the pH value is constant. The results of the linear regression of pH values obtained values coefficient close to one ($R^2 \approx 1$) which means the storage time has an effect on the pH value of the product. The longer the storage, the pH value of the product decreases (Mancini et al., 2005).

In the context of reaction kinetics, the reduction in food quality can be seen through first order and zero order (Tiwari et al., 2008). The zero order can be detected by making a graph between the pH value as the Y axis and the storage time as the X axis. Meanwhile, the first order can be detected by making a graph between the ln pH value as the Y axis and the storage time as the X axis . The reaction order used can be determined by comparing the R^2 value, where the reaction order with the higher R^2 value is considered dominant.

Linear regression equation from the plot of ln k and 1/T on changes in pH of fermented glutinous rice results are y= -5873,6x + 17,042 with R^2=0,8385.

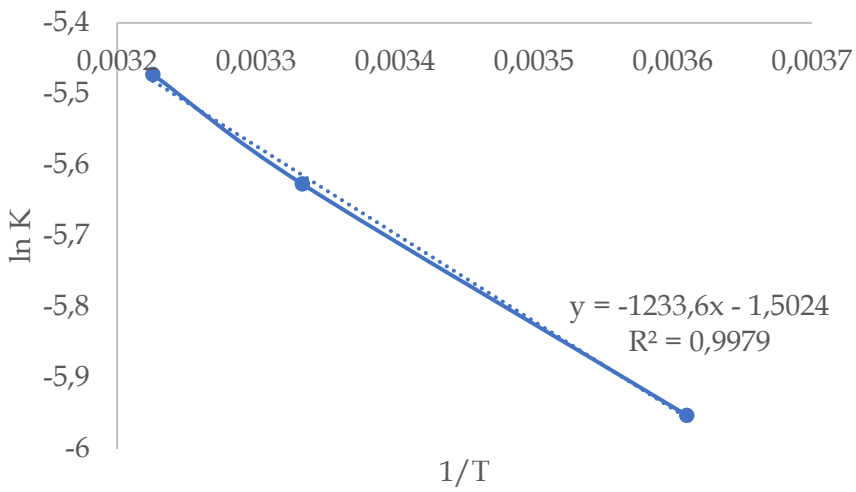


Figure 1. Arrhenius Plot of Changes in pH values during stored

The shelf life of fermented glutinous rice was calculated using a linear regression equation from parameters that have activation energy values (Vyazovkin, 2016). From this equation, the k value can be obtained which can be used for determining the shelf life of the product. The k value is obtained from the formula $\ln_k$=$\ln k_0$-Ea/R(1/T), where $\ln k_0$=intercept, Ea/R=slope. The k value obtained is then entered into the equation. Reaction kinetics based on reaction order. The pH parameters follow zero order reaction kinetics, so the shelf-life equation is:

$$k_0 = A_0[e]^{-E_a/RT_0} \quad (8)$$

Arrhenius equation can be substituted:

$$\ln k = k_0 - E_a/RT \quad (9)$$

where:

T_0 = reference temperature at k = k_0
Slope (E_a/R) = 5873,6
Intercept (k_0) = 17,042

Critical Comparison of Predicted Shelf Life

The Arrhenius-based prediction estimated a shelf life of 3 days at 2 °C. When compared to the study by Nurhayati et al. (2017) on the shelf life of traditional fermented products, the shelf life of this laboratory-produced tape ketan is relatively short. This difference can be critically analyzed through several factors:

- Moisture Content: High moisture levels in tape ketan facilitate faster microbial growth compared to drier fermented products.
- Initial Microbial Load: The use of traditional yeast starters without standardized pasteurization means that endogenous enzymes remain highly active even at low temperatures.
- Packaging: The sensitivity of the product to oxygen (leading to ethanol oxidation into acetic acid) might further shorten the shelf life if the containers are not perfectly hermetic.

Table 7. Shelf-Life Estimation of Fermented Glutinous Rice using Arrhenius Model

Temperature (°C)	k	Shelf life (day)
2	0.015	2.721466301
22	0.035	1.767868678
29	0.13	1.716488856

Notes: The data indicates a correlation between storage temperature and the reaction rate constant (k), where higher temperatures accelerate the acidification process of the fermented glutinous rice. Consequently, the predicted shelf life, determined using a critical pH threshold of 4.0, significantly decreases as the storage temperature rises, with the product exhibiting its maximum stability of approximately 2.72 days at a refrigerated temperature of 2°C.

These findings align with previous studies by Nurhayati et al. (2017), which demonstrate that low-temperature storage effectively prevents over-fermentation by suppressing microbial activity. This suppression prevents the product from becoming excessively acidic and watery, thereby maintaining the organoleptic properties of the fermented rice. The results of this study suggest that cold storage at 2 °C alone is insufficient for long-term preservation. Consequently, additional processing steps, such as heat treatment or specialized packaging, are necessary to further extend the shelf life.

CONCLUSION

This study concludes that storage temperature significantly dictates the shelf life of fermented glutinous rice. Based on the Arrhenius model, the estimated shelf life is 2.7 ≈ 3 days at 2 °C which decreases to 1.76 ≈ 2 days at 22 °C and 1.71 ≈ 2 days at 29 °C. The results show that as temperature increases, the product becomes unstable more quickly. Higher storage temperature increases the reaction rate (k), which causes the quality of the fermented rice to drop rapidly. This degradation is characterized by significant acidification (pH drop), yellowish discoloration, and increased syneresis (wateriness) due to accelerated starch hydrolysis.

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