KINETIC STUDY OF QUALITY CHANGES AND SHELF-LIFE PREDICTION OF *Gracilaria* sp. SEAWEED-BASED ANALOG RICE USING THE ARRHENIUS MODEL

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Submitted: 23 January 2025/Accepted: 25 April 2025
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How to cite (APA Style 7th): Salsabila, L., Purwaningsih, S., & Ramadhan, W. (2025). Kinetic study of quality changes and shelf-life prediction of *Gracilaria* sp. seaweed-based analog rice using the Arrhenius model. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 28(4), 392-405. http://dx.doi.org/10.17844/jphpi.v28i4.62203

Abstract

Seaweed-based analog rice has emerged as a novel functional food due to its high fiber content and the presence of bioactive compounds. However, information regarding its shelf life remains limited, particularly in relation to its chemical and physical stability during storage. This study aimed to determine the shelf life of *Gracilaria* sp.-based analog rice using the Arrhenius accelerated shelf-life model, and evaluate the physical and chemical quality changes during storage. Accelerated shelf-life testing (ASLT) was conducted at three different storage temperatures namely at 35, 45, and 55°C, applying the Arrhenius kinetic model. Temperature effects were analyzed through kinetic parameters, including changes in thiobarbituric acid (TBA) levels, color, and sensory attributes (rancid odor and color intensity). Changes in physical and chemical quality were observed on day 0 and day 30 based on crystallinity index, proximate, and fiber content analysis. The results identified TBA levels as the most critical quality parameter, described by the equation $y = -731.83x - 2.68$, with a coefficient of determination (R^2) of 0.9873. Based on the Arrhenius model, the estimated shelf life of the seaweed-based analog rice was approximately 210 days at room temperature. Storage periode day 0 and 30 has significant effect to physical and chemical changes such as in dietary fiber content from 7.35% to 5.84%, fat content from 4.17% to 3.27%, and the crystallinity index from 40,74% to 53,30%. This study provides valuable insights into how storage conditions affect the quality of analog rice, with particular emphasis on fiber stability and lipid oxidation, and provides a reference for distributors and consumers in determining shelf life.

Keywords: accelerated shelf-life testing, degradation, fiber, stability, TBA

Studi Kinetika Perubahan Kualitas dan Prediksi Umur Simpan Beras Analog Berbasis Rumput Laut *Gracilaria* sp. menggunakan Model Arrhenius

Abstract

Beras analog berbahan dasar rumput laut telah diusulkan sebagai pangan fungsional baru karena kandungan serat yang tinggi dan senyawa bioaktif. Informasi mengenai umur simpan beras analog masih terbatas, terutama yang dapat memengaruhi stabilitas kimia dan fisik selama penyimpanan. Penelitian ini bertujuan untuk menentukan umur simpan beras analog *Gracilaria* sp. menggunakan model Arrhenius dan mengevaluasi perubahan kualitas fisik dan kimia selama penyimpanan. Penelitian ini menggunakan *metode accelerated shelf-life testing* (ASLT) dengan model kinetika Arrhenius pada tiga suhu penyimpanan, yaitu 35, 45, and 55°C. Pengaruh suhu terhadap umur simpan dianalisis melalui parameter kadar asam tiobarbiturat (TBA), warna, dan mutu sensoris selama 30 hari penyimpanan. Perubahan kualitas fisik dan kimia diamati pada hari ke-0 dan hari ke-30 melalui parameter indeks kristalinitas, proksimat, dan serat. Kadar TBA merupakan parameter mutu yang paling kritis, dengan persamaan y = -731.83x – 2.68 dan nilai R^2 sebesar 0.9873. Berdasarkan persamaan Arrhenius, masa simpan beras analog berbasis rumput laut diperkirakan mencapai 210 hari pada suhu ruang. Waktu penyimpanan hari ke-0 dan ke-30 berpengaruh signifikan terhadap perubahan fisik dan kimia beras analog terutama kadar serat dari 7,35% menjadi 5,84%, lemak dari 4,17% menjadi 3,27%, dan indeks kristalinitas dari 40,74% menjadi 53,30%. Penelitian ini memberikan pemahaman lebih lanjut tentang dampak penyimpanan terhadap kualitas beras analog, khususnya mengenai stabilitas serat dan degradasi lemak selama penyimpanan, serta memberikan acuan bagi distributor dan konsumen dalam menentukan masa simpan.

Kata kunci: accelerated shelf-life testing, degradasi, serat, stabilitas, TBA

INTRODUCTION

Diabetes is a chronic disease with one of the highest global prevalence rates and ranks among the top ten leading causes of death worldwide. The International Diabetes Federation (IDF) estimates that the number of people with diabetes in Indonesia may reach 28.57 million by 2045 (Magliano & Boyko, 2021). Diabetes mellitus represents a growing global health challenge, largely attributed to unhealthy dietary patterns. One effective strategy for individuals with diabetes mellitus to maintain stable blood glucose levels is the consumption of low-glycemic index foods (Purbowati & Kumalasari, 2023). Analog rice has emerged as a promising functional food for individuals with diabetes mellitus due to its low glycemic index. It is typically formulated from ingredients such as mocaf (modified cassava flour), corn, mung beans, and seaweed. Studies have demonstrated that analog rice has a lower glycemic index compared to conventional rice, which is considered to have a medium glycemic index (Firdausia *et al*., 2021), suggesting its suitability for individuals with type 2 diabetes. Analog rice mimics the shape and texture of regular rice and is commonly produced using extrusion technology (Liu *et al.,* 2022; Mishra *et al*., 2012)and these broken kernels are not generally accepted by consumers. These broken kernels can be mixed with some desired additives to improve their quality and extruded for the preparation of reconstituted rice kernels or rice analogues. Various studies have been conducted for the preparation of the rice analogues in the past few decades, and recently attempts have been made to fortify these analogues with protein, certain vitamins and minerals. The main features such as colour, shape, size, texture, and cooking characteristics and cooking time of these rice-like grains can be tailored to the requirements of specific applications by modification of the extrusion parameters. Various organisations, such as Wuxi NutriRice Co. (DSM/Buhler. Its popularity stems from its ability to address specific nutritional needs and functional health benefits.

Traditionally, analog rice has been fortified with land-based ingredients such as corn, cassava, and konjac. However, seaweed-fortified analog rice—commonly referred to as seaweed-based analog rice—is gaining increasing attention. Research by Damat *et al.* (2021)with red seaweed (*Gracilaria* spp. demonstrated that incorporating seaweed into analog rice significantly increases its resistant starch content, thereby enhancing its dietary fiber value. Among various seaweed types, *Gracilaria* sp. exhibits higher dietary fiber content compared to *Kappaphycus, Eucheuma,* and *Sargassum*, which generally contain fiber with lower digestibility. Purwaningsih (2022) reported that *Gracilaria* sp. contains 64.74% dietary fiber, —substantially higher than other seaweed species. A recent study by Purwaningsih *et al.* (2024)a red seaweed, serves as the primary ingredient in the agar industry and is subsequently utilized in food, biotechnological fields, nutraceuticals, and pharmaceutical applications due to its rich nutritional and bioactive compounds beneficial for human health. In fact, this seaweed has been cultivated in many regions and countries, especially in Indonesia. Several areas, particularly Java and Lombok Island, are known as the primary producers of *Gracilaria* seaweed and its derivatives in Indonesia. However, the current state of research lacks comprehensive exploration regarding the relationship or correlation between cultivation areas and the resultant quality of derived seaweed products. It is especially valuable to investigate the dataset concerning its nutrition and bioactive profile. Thus, this study aims to investigate and provide the chemical composition and bioactive compound of Estuarine Seaweed *Gracilaria* from four different cultivation areas in Java and Lombok Island in Indonesia. There are three areas in Java, specifically Karawang, Situbondo, and Pasuruan, and one area in Lombok, as the main location of sampling. These seaweed samples were then evaluated for their proximate composition, dietary fiber, selenium, iodine, carotene, antioxidant, and bioactive compound profiles. *Gracilaria* seaweed from Lombok Island, Situbondo, Pasuruan, and Karawang displayed moisture content in the range of 9-11%, ash content of 5-6%, fat content ranging from 0.26-0.62%, protein content between 9-17%, and carbohydrate content varying from 64-73%. The content of seaweed from Karawang, Pasuruan, Situbondo, and Lombok were recorded as 66.35%, 59.94%, 57.41%, and 72.56%, respectively. The analysis revealed that *Gracilaria* from the Lombok area had a selenium content of 18.82 mcg/100 g, whereas Karawang Seaweed showed 31.04 mcg/100 g of selenium. The Situbondo area exhibited iodine content (19676.96 mcg/100 g further revealed that the highest dietary fiber content was found in *Gracilaria sp.* samples from Lombok (72.56%), followed by those from Karawang (66.35%). Recent studies have also demonstrated that *Gracilaria* sp. can enhance the characteristics of fermented food products such as yogurt, through its contributions of protein, fiber, and bioactive compounds (Fauziah *et al.*, 2023). This high fiber content plays a crucial role in slowing glucose absorption in the body, making *Gracilaria* sp. a more effective dietary option for blood glucose regulation than other lower-fiber seaweeds. In addition to its fiber content, *Gracilaria* sp. also contains phlorotannin, a bioactive compound with antioxidant properties and potential health benefit applications (Purwaningsih *et al.*, 2024)a red seaweed, serves as the primary ingredient in the agar industry and is subsequently utilized in food, biotechnological fields, nutraceuticals, and pharmaceutical applications due to its rich nutritional and bioactive compounds beneficial for human health. In fact, this seaweed has been cultivated in many regions and countries, especially in Indonesia. Several areas, particularly Java and Lombok Island, are known as the primary producers of *Gracilaria* seaweed and its derivatives in Indonesia. However, the current state of research lacks comprehensive exploration regarding the relationship or correlation between cultivation areas and the resultant quality of derived seaweed products. It is especially valuable to investigate the dataset concerning its nutrition and bioactive profile. 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However, as with most food products intended for long-term storage, maintaining quality throughout storage period is a critical factor. Understanding and accurately determining a product's shelf life is essential to ensure its quality and safety during distribution and storage. While conventional rice can have a shelf life of up to 8 months (Hadipernata & Hidayah, 2020), analog rice generally exhibits a shorter shelf life. For instance, analog rice fortified with bran and broken rice kernels has a shelf life of only 23 days (Hermanianto *et al.*, 2000), while corn-based analog rice can last for three months and 17 days (Kusnandar *et al.*, 2017). Kinetic modeling is a widely accepted approach for predicting quality changes in food products over time (Remini *et al.*, 2015; Zhang, W *et al.*, 2021). One such method is Accelerated Shelf-Life Testing (ASLT), which utilizes the Arrhenius model to simulate and accelerate chemical reactions through increased temperature (Brilian *et al.*, 2023). Kusnandar *et al.* (2017) noted that the Arrhenius approach in ASLT is particularly suitable for analog rice products that degrade due to chemical reactions, such as lipid oxidation, resulting in rancid odors and color changes. Thiobarbituric acid (TBA) analysis serves as a key parameter in assessing analog rice degradation, as it effectively measures lipid oxidation—an indicator of rancidity and overall quality deterioration during storage.

Although some studies have investigated the shelf life of analog rice, to the best of our knowledge, no research has been conducted on shelf-life prediction for aquatic-based analog rice, particularly those made from *Gracilaria* sp. Therefore, this study aims to determine the shelf life of *Gracilaria* sp.-based analog rice using the Arrhenius accelerated shelf-life model, and evaluate the physical and chemical quality changes during storage. The findings of this study are expected to provide valuable insights into the shelf life of food products made from aquatic raw materials and to highlight the role of fiber structure in preserving quality during storage.

MATERIALS AND METHODS

Analog Rice Production

The material used in this study was commercially produced *Gracilaria* sp. analog rice, fabricated according to the method described by Ramadhan *et al.* (2024). The production process consisted of several stages, including ingredient weighing, mixing, extrusion, and drying. All ingredients were blended using a 15 kg-capacity mixer to ensure homogeneity, then shaped into rice-like grains using a twin-screw extruder (Jinan RQ(Y) KX-5-5, Jinan, China). The extrusion process was performed at controlled temperatures: 90°C at the inlet, 150°C during processing, and 70°C at the outlet. The extruded product was subsequently dried in a conveyor oven at 110°C for 30 minutes to reduce moisture content and enhance shelf stability.

Degradation Experiments

The degradation process refers to Kusnandar *et al.* (2017) that observations were carried out by storing the product in an incubator set at temperatures of 35°C, 45°C, and 55°C. Two types of analyses were performed: (1) assessment of analog rice quality changes—including crystallinity, proximate composition, and dietary fiber—on days 0 and 30; and (2) estimation of shelf life through color analysis, thiobarbituric acid (TBA) value, and organoleptic assessment over a 30-day period. Samples were collected every seven days for storage at 35°C and 45°C, and every four days at 55°C. All samples were maintained under a controlled relative humidity (RH) of 75–80%.

Deteorization Kinetic Analysis

The deterioration kinetics were evaluated using the Accelerated Shelf-Life Testing (ASLT) method based on the Arrhenius equation, following the approach described by Diniyah *et al.* (2015). In this method, several quality parameters were monitored, including thiobarbituric acid (TBA) values, color attributes (L^*, a^*, b^*), and the intensity of rancid aroma in both raw and cooked rice stored at different temperatures. Data collected over time were used to generate linear equations (y = a + bx), where the slope (b) represents the deterioration rate constant (*k*). The reaction order for each parameter was determined by identifying the model that produced the highest coefficient of determination (R^2). The Arrhenius equation was then applied by plotting the natural logarithm of the rate constant (ln k) against the inverse of absolute temperature (1/T), where the slope corresponds to the activation energy (Ea) when multiplied by the gas constant (R = 1.986 cal/mol·K). Shelf-life prediction was subsequently carried out using both zero-order and first-order kinetic models, applying the respective formulas:

$$\text{Zero-order: } t = (A_0 - A_t) / k$$

$$\text{First-order: } t = \ln(A_0 / A_t) / k$$

Note:
A_0 is the initial value of the quality parameter
A_t is the final value
k is the rate constant
t is the estimated shelf life in days

Proximate Analysis

The proximate composition of *Gracilaria* sp. analog rice—including moisture (BSN, 2020), protein (BSN, 2006), fat (BSN, 2017), ash (BSN, 1992), and carbohydrate content (AOAC, 2019))—was determined using standard methods.

Total Dietary Fiber

The total dietary fiber content was analyzed using the AOAC 985.29-1986 method (2003), which involves an enzymatic-gravimetric approach using α-amylase and amyloglucosidase.

Analysis of Thiobarbituric Acid (TBA)

TBA analysis method was refers to Apriyantono *et al.* (1989). Ten grams of powdered analog rice were homogenized with 50 mL of distilled water for 2 minutes. The mixture was transferred to a distillation flask, rinsed with an additional 47.5 mL of distilled water, and combined with 2.5 mL of 4 M HCl to adjust the pH to 1.5. The flask was connected to a distillation apparatus and heated for 10 minutes to obtain 50 mL of distillate. After stirring, 5 mL of distillate was mixed with 5 mL of TBA reagent and heated in boiling water for 35 minutes. Absorbance was measured at 528 nm using a blank as a reference. The TBA value was calculated using the formula: TBA = 7.8 × A528.

Color Analysis

Color analysis method was refers to Elnemr *et al.* (2022). Color was evaluated using a colorimeter with RGB scale values. Samples were placed in transparent containers under the device's measuring head and analyzed three times. The RGB values were converted into *L** (lightness), *a** (redness), and *b** (yellowness) using a color conversion tool (colormine.org).

Sensory Evaluation

Sensory evaluation method was refers to Kusnandar *et al.* (2017). Sensory evaluation of aroma and color was conducted with 15 trained panelists. Aroma was assessed using a 9-point intensity rating scale, ranging from 1 ("Extremely weak") to 9 ("Extremely strong"). Color was evaluated using the triangle test, in which the panelist were presented with three samples simultaneously (two identical, one different). Both raw and cooked samples (5 g each) were coded with randomized three-digit numbers and arranged sequentially from the control to the most deteriorated sample.

X-ray Diffraction (XRD) Analysis

X-ray Diffraction method was refers to Fatimah *et al.* (2022). The crystalline structure of analog rice was analyzed using an X-ray diffractometer (D8, Bruker, Karlsruhe, Germany) operating at Cu-Kα radiation (40 kV, 40 mA). Powdered samples were sieved through a 0.25 mm mesh, and 0.5 g was placed in a scanning pan and stored at 4 °C for 24 hours. X-ray diffraction was recorded at a wavelength of 0.154 nm with 0.02° intervals over a 2θ range of 0°–40°.

Data Analysis

Quality changes in analog rice were analyzed using an Independent Samples T-Test with IBM SPSS Statistics version 25. This test was used to determine statistically significant differences in mean values between pre- and post-storage samples. The experiment was conducted with three independent replications to ensure reliable estimation of variability. Prior to analysis, the data were assessed for normality using the Shapiro-Wilk test and for homogeneity using Levene's Test. Since the data met the assumptions of normal distribution and homogeneity of variance, the Independent Samples T-Test was performed. A significance level of $p < 0.05$ was used to determine statistical significance.

Shelf life was estimated using both linear and non-linear Arrhenius models, with all experiments conducted in triplicated. The reaction rate constant (k) for key quality attributes was plotted against the inverse of absolute temperature (1/T) to estimate the activation energy (Ea).

RESULTS AND DISCUSSION

Kinetics of Quality Changes in Products During Storage

The results demonstrated that all measured quality parameters followed zero-order reaction kinetics (Table 1). This conclusion is supported by the higher correlation coefficients (R^2) observed for zero-order reactions compared to first-order reactions. Among the parameters analyzed, the most significant quality indicators—identified based on their highest R^2 values—were the Thiobarbituric Acid (TBA) value, rancid aroma in both raw and cooked rice, and rice color.

The TBA value showed strong linearity across different storage temperatures, with R^2 values of 0.9468, 0.9585, and 0.9345 at 35°C, 45°C, and 55°C, respectively (Table 1). These results indicate that the TBA value is a reliable indicator for monitoring lipid oxidation, a key factor in determining the product's shelf life. Compared to other parameters such as color attributes (L^*, a^*, and b^*), which demonstrated only moderate correlation values, the TBA value emerged as the most sensitive and accurate indicator for predicting quality degradation. These findings contrast with those for corn-based analog rice, where the TBA value fluctuated during storage and quality changes were more strongly associated with color values, particularly the b^* value (Kusnandar *et al.*, 2017). This highlights that the critical indicator for predicting product stability may vary depending on the raw materials used in the production of analog rice.

The increasing trend in TBA value during storage, from 0.02 to 0.19 mg MDA/kg, further supports the occurrence of lipid oxidation (Table 3). Lipid oxidation involves the degradation of unsaturated fatty acids, leading to the formation of reactive compounds such as malondialdehyde (MDA), which is measured via the TBA test (Ayala *et al.*, 2014). In the formulation of *Gracilaria*-based analog rice, fats are primarily derived mainly from palm oil and *Gracilaria* sp. seaweed. Although the polyunsaturated fatty acid (PUFA) content of *Gracilaria* is relatively low (approximately 0.36%), it remains highly susceptible to oxidation due to its chemical reactivity (Illijas *et al.*, 2012) Supelco, PA, USA. Palm oil contributes unsaturated fatty acids such as oleic and linoleic acids, which are prone to oxidation during storage (Boateng *et al.*, 2016). Additionally, the observed decline in fat content from 4.17% to 3.27%, further indicating the conversion of lipids into secondary oxidation products, including MDA.

Determination of Critical Parameters

The relationship between ln k and 1/T for selected quality parameters—namely the TBA value, aroma of raw and cooked rice, and rice color—is presented in Figure 1. Among these, the TBA value exhibited the strongest correlation, with the highest R^2 value of 0.9873. This parameter also demonstrated the lowest activation energy (E_a), making it the most sensitive to quality changes during storage (Table 2).

Furthermore, aroma parameters also demonstrated significant correlations, particularly at 45°C, with R^2 values of 0.9401

Table 1 Linear regression equations and R^2 for each quality parameter
Tabel 1 Persamaan regresi linear dan R^2 masing-masing parameter mutu

Parameters	Temperature (°C)	Zero Order		First Order	
		Equation	R^2	Equation	R^2
TBA value	35	y = 0.0064x – 0.0026	0.9468	$y = 0.0175e^{0.0893x}$	0.9349
	45	y = 0.0068x + 0.0036	0.9585	$y = 0.0203e^{0.0889x}$	0.8904
	55	y = 0.0074x	0.9345	$y = 0.0234e^{0.0857x}$	0.9309
L* color	35	y = -0.1601x + 66.805	0.5188	$y = 66.767e^{-0.002x}$	0.5269
	45	y = -0.2562x + 66.528	0.6567	$y = 66.497e^{-0.004x}$	0.6626
	55	y = -0.516x + 69.548	0.7915	$y = 69.968e^{-0.009x}$	0.7754
a* color	35	y = -0.0428x + 2.3462	0.5007	$y = 2.4162e^{-0.028x}$	0.4406
	45	y = -0.0133x + 1.773	0.0800	$y = 1.7034e^{-0.008x}$	0.0797
	55	y = -0.0184x + 2.1483	0.1201	$y = 2.1152e^{-0.011x}$	0.1138
b* color	35	y = 0.0524x + 23.26	0.5182	$y = 23.25e^{0.0022x}$	0.5214
	45	y = 0.0432x + 23.362	0.3017	$y = 23.346e^{0.0018x}$	0.3070
	55	y = 0.0868x + 23.542	0.6060	$y = 23.543e^{0.0035x}$	0.5998
Aroma of raw rice	35	y = 0.0581x + 1.2667	0.8146	$y = 1.2568e^{0.0318x}$	0.7551
	45	y = 0.0924x + 0.8933	0.9401	$y = 1.0684e^{0.0443x}$	0.9754
	55	y = 0.067x + 1.4222	0.7163	$y = 1.4282e^{0.0318x}$	0.6598
Aroma of cooked rice	35	y = 0.0581x + 1.2667	0.8146	$y = 1.2568e^{0.0318x}$	0.7551
	45	y = 0.1152x + 1.1333	0.9453	$y = 1.2521e^{0.0481x}$	0.8882
	55	y = 0.0788x + 1.5056	0.8506	$y = 1.5002e^{0.0352x}$	0.7293
Rice color	35	y = 0.2857x + 5.8	0.8929	$y = 5.8768e^{0.0326x}$	0.7006
	45	y = 0.3286x + 5.4	0.9121	$y = 5.5467e^{0.0371x}$	0.7624
	55	y = 0.3065x + 6.0833	0.9037	$y = 6.3204e^{0.0321x}$	0.6283
Cooked rice color	35	y = 0.4x + 3.2	0.8245	$y = 3.6653e^{0.0529x}$	0.8899
	45	y = 0.3286x + 5.2	0.7919	$y = 4.5816e^{0.046x}$	0.6901
	55	y = 0.4554x + 4.5	0.8661	$y = 4.5074e^{0.054x}$	0.7973

Table 2 Arrhenius equations and activation energy for selected parameters
Tabel 2 Persamaan Arrhenius dan energi aktivasi parameter terpilih

Parameters	Arrhenius model	R^2	E_a (kJ/mol)
TBA number	ln k = -731.83(1/T) – 2.68	0.9873	6.08
Rice aroma	ln k = - 596.74(1/T) + 0.8872	0.2336	4.96
Cooked rice aroma	ln k = -6851.9(1/T) + 20.118	0.8265	56.99
Rice color	ln k = -821.44(1/T) + 1.2525	0.3548	6.83

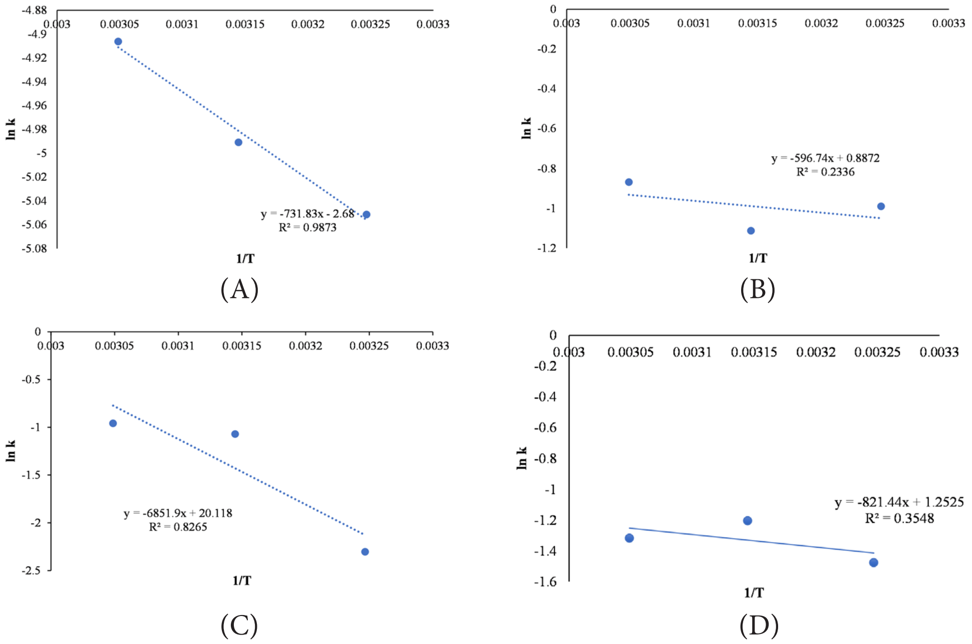

Figure 1 Relationship between ln k and 1/T for TBA number parameter (A); rancid rice aroma parameter (B); rancid cooked rice aroma parameter (C); rice color parameter (D)
Gambar 1 Hubungan antara ln k dan 1/T parameter bilangan TBA (A); parameter aroma tengik beras (B); parameter aroma tengik nasi (C); parameter warna beras (D)

and 0.9453, respectively. These results indicate that aroma changes are closely associated with lipid oxidation and degradation processes during storage. The oxidation of free fatty acids produces volatile compounds, such as hexanal, which contribute to rancid odors and serves as a marker of lipid oxidation (Indrasari & Mardiah, 2012). Color parameters, particularly *L** value (lightness), showed noticeable changes during storage. However, the correlation with reaction kinetics was weaker, with R^2 values ranging from 0.5188 to 0.7915 depending on the temperature. This suggests that while color changes is visually perceptible, it may not serve as a primary indicator of quality degradation in seaweed-based analog rice. In this product, lipid stability, as represented by the TBA value, is a more reliable parameter than color or aroma. Consequently, quality control strategies should prioritize lipid oxidation during storage and distribution. Previous studies have emphasized that peroxide value and free fatty acid content are also critical in determining the shelf life of analog rice (Diniyah *et al.*, 2015).

Arrhenius Equation and Activation Energy of Selected Parameters

The linear regression results derived from the Arrhenius model for various quality parameters of analog rice are presented in Table 2. Among the evaluated parameters, the Thiobarbituric Acid (TBA) value exhibited the strongest correlation (R^2 = 0.9873) and the lowest activation energy (Ea = 6.08 kJ/mol). This finding suggests that the lipid oxidation process, as indicated by the TBA value, requires the least energy to occur, making it the most temperature-sensitive deterioration reaction in analog rice. Shelf-life prediction at room temperature (28°C) based on the TBA kinetics estimated a storage duration of 210 days. This confirms the potential of analog rice to maintain acceptable quality for extended periods under ambient conditions.

The findings confirm that the TBA value serves as a critical parameter for predicting the shelf life of seaweed-based analog rice. Due to its low activation energy and strong linear correlation with temperature, the TBA value enables more consistent and accurate predictions of product degradation compared to other quality attributes such as aroma or color. This aligns with prior studies suggesting that quality parameters with low activation energy (*Ea*) are more responsive to temperature changes, making them more practical for shelf-life modeling (Haouet *et al.*, 2018). However, these results contrast with those of Kusnandar *et al.* (2017) on corn-based analog rice, in which TBA values exhibited greater variability and color parameters, particularly *b**, were more indicative of quality deterioration. These differences highlight the influence of raw materials on dominant degradation pathways and reinforce the need for tailored shelf-life models for each analog rice formulation. The estimated shelf life of 210 days for analog rice stored at 28°C underscores its potential as a stable food source for medium-term storage. Nevertheless, future studies should examine additional variables, such as relative humidity, and investigate interactions between quality parameters to further enhance the robustness of shelf-life predictions.

Physicochemical Changes in Analog Rice During Storage

Table 3 presents the physicochemical changes in *Gracilaria* sp. seaweed-based analog rice during storage. Several quality attributes displayed statistically significant changes over the 30-day period.

During the storage of *Gracilaria* sp. analog rice, a slight decrease in moisture content was observed, from 9.61% to 9.38% after 30 days (Table 3). Although this reduction appears minor, it can still influence the texture and organoleptic properties of the product, particularly in terms of softness and chewiness. Interestingly, the water activity (aw) increased from 0.52 to 0.55, despite the decline in total moisture. This rise in aw is likely attributed to the redistribution of water within the rice matrix during storage. Such changes are consistent with previous studies, which suggest that storage-induced degradation or molecular alterations—such as starch hydrolysis or lipid oxidation—can reduce the material's water-binding capacity, leading to increased availability of free water (Wiebach *et al.*, 2020). Oxidative and hydrolytic reactions also contribute to elevated aw by increasing the amount of unbound water, thereby raising the risk of microbial growth even as the total moisture decreases (Bhunia *et al.*, 2023). This highlights the critical role of aw in shelf-life assessment: higher aw levels can accelerate spoilage through microbial proliferation. Research has consistently demonstrated a direct relationship between increased aw and the acceleration of microbial and chemical deterioration (Bradford *et al.*, 2016) measuring seed or grain moisture content in the field can be difficult, particularly in rural locations in developing countries. Because seed/commodity moisture content is uniquely

Table 3 Quality changes in *Gracilaria* sp. seaweed-based analog rice during storage
Tabel 3 Perubahan kualitas beras analog rumput laut *Gracilaria* sp. selama penyimpanan

Parameters	Day 0	Day 30
Moisture (%wb)	9.61±0.01	9.38±0.22*
Protein (%wb)	2.55±0.01	2.51±0.05*
Ash (%wb)	0.17±0.01	0.48±0.01
Fat (%wb)	4.17±0.02	3.27±0.09*
Carbohydrates (%wb)	83.75 0.06	84.36±0.08
Fiber (%wb)	7.35±0.09	5.84±0.17*
aw	0.52±0.00	0.55±0.00
L* Color	67.83±0.79	52.86±3.42*
a* Color	-1.36±0.48	-1.60±0.23*
b* Color	21.67±0.65	26.25±0.71*
TBA Value (malonaldehyde/kg sample)	0.02±0.0	0.19±0.17*

related to equilibrium relative humidity at a given temperature, moisture content can be estimated by measuring the relative humidity of a sample enclosed in a sealed container. Relative humidity can be measured using electronic meters, or even more inexpensively using indicator paper that changes color in response to relative humidity. We describe this method and provide a spreadsheet to convert relative humidity to moisture content for many common seeds and commodities. This simple method can be used in the field for quickly estimating seed or commodity moisture content to determine whether further drying is needed or is suitable for storage, milling or other uses (Bradford *et al*., 2016).

The significant reduction in fat content—from 4.17% to 3.27%—during the storage period may be attributed to lipid oxidation. Coconut oil, which serves as the primary lipid source in the analog rice formulation, contains approximately 90% saturated fatty acids and around 9% unsaturated fatty acids, such as oleic and linoleic acids (Boateng *et al*., 2016). Prior studies have shown that coconut oil can exhibit increased peroxide values during extended storage, indicating the initiation of lipid oxidation (Moigradean *et al.,* 2012). Lipid oxidation is a key mechanism of fat degradation in food products, especially those containing unsaturated fats or seaweed-derived ingredients (Fereidoon & Ying, 2010). This process generates free radicals and reactive oxygen species, forming peroxides and volatile compounds that reduce total fat content. In seaweed-based products, this oxidative degradation often leads to the formation of aldehydes, particularly malondialdehyde (MDA), which is quantified as Thiobarbituric Acid Reactive Substances (TBARS). In this study, the TBA value increased significantly from 0.02 to 0.19 malonaldehyde/kg sample, indicating ongoing lipid oxidation and the potential onset of rancidity. As such, TBA serves as a reliable indicator of fat degradation and a critical metric for evaluating the shelf-life stability of seaweed-based analog rice (Harrysson *et al*., 2021) fatty acids, ascorbic acid and colour of Porphyra and Ulva after oven-drying at 40 °C, and during subsequent storage for ≥370 days under light, semi-light and dark conditions. Part of the seaweed was pre-soaked in freshwater or pre-coated with a whey protein mixture. Controls consisted of freeze-dried seaweeds. Throughout storage there was a moderate development of the lipid oxidation-derived aldehydes, malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-trans-2-nonenal, while there was a great loss of unsaturated fatty acids and ascorbic acid. Light storage and freeze-drying stimulated the fatty acid loss as well as pigment bleaching, seen as increased a*-values. For Ulva, the coating reduced malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-trans-2-nonenal formation during drying and slightly prevented loss of polyunsaturated fatty acids during light storage. Pre-soaking in freshwater had no effect on the seaweed stability, although it reduced the ash content and thereby increased the relative content of ascorbic acid and fatty acids of the biomasses. (Harrysson et al., 2021.

In addition to lipid degradation, fiber content in *Gracilaria* sp. analog rice decreased significantly, from 7.35% to 5.84% (Table 3). This reduction may result from the breakdown of fiber structure, thereby reducing the product's functional benefits as a dietary fiber source. Fiber degradation is often associated with increased crystallinity, as evidenced by the rise in crystallinity index from 40.74% at day 0 to 53.30% at day 30, suggesting starch retrogradation. Similar findings were reported by Harrysson *et al.* (2021) fatty acids, ascorbic acid and colour of *Porphyra* and *Ulva* after oven-drying at 40 °C, and during subsequent storage for ≥370 days under light, semi-light and dark conditions. Part of the seaweed was pre-soaked in freshwater or pre-coated with a whey protein mixture. Controls consisted of freeze-dried seaweeds. Throughout storage there was a moderate development of the lipid oxidation-derived aldehydes, malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-trans-2-nonenal, while there was a great loss of unsaturated fatty acids and ascorbic acid. Light storage and freeze-drying stimulated the fatty acid loss as well as pigment bleaching, seen as increased a*-values. For *Ulva*, the coating reduced malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-

http://dx.doi.org/10.17844/jphpi.v28i4.62203

trans-2-nonenal formation during drying and slightly prevented loss of polyunsaturated fatty acids during light storage. Pre-soaking in freshwater had no effect on the seaweed stability, although it reduced the ash content and thereby increased the relative content of ascorbic acid and fatty acids of the biomasses. (Harrysson *et al.*, 2021), who observed increased crystallinity and structural changes in seaweed fibers during storage. Interestingly, X-ray diffraction patterns of *Gracilaria* sp. analog rice remained stable before and after storage, displaying characteristic type A starch crystal peaks at 2θ angles of 15°, 17°, 18°, 20°, and 23° (Figure 2). This suggests that although storage increased crystallinity, it did not alter the crystal type—consistent with findings from other algal-based products (Harrysson *et al.*, 2021)fatty acids, ascorbic acid and colour of *Porphyra* and *Ulva* after oven-drying at 40 °C, and during subsequent storage for ≥370 days under light, semi-light and dark conditions. Part of the seaweed was pre-soaked in freshwater or pre-coated with a whey protein mixture. Controls consisted of freeze-dried seaweeds. Throughout storage there was a moderate development of the lipid oxidation-derived aldehydes, malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-trans-2-nonenal, while there was a great loss of unsaturated fatty acids and ascorbic acid. Light storage and freeze-drying stimulated the fatty acid loss as well as pigment bleaching, seen as increased a*-values. For Ulva, the coating reduced malondialdehyde, 4-hydroxy-trans-2-hexenal and 4-hydroxy-trans-2-nonenal formation during drying and slightly prevented loss of polyunsaturated fatty acids during light storage. Pre-soaking in freshwater had no effect on the seaweed stability, although it reduced the ash content and thereby increased the relative content of ascorbic acid and fatty acids of the biomasses. (Harrysson *et al.*, 2021).

Color parameters also changed significantly during storage (Table 3). The *L** value (lightness) decreased from 67.83 to 52.86, indicating diminished brightness. The *a** value (green-red axis) shifted slightly from -1.36 to -1.60, while the *b** value (blue-yellow axis) increased from 21.67 to 26.25, signifying enhanced yellow intensity. These shifts could affect consumer perception and product appeal.

The interplay between kinetic deterioration (e.g., TBA increase) and physicochemical changes (e.g., fat loss, a_w increase, and color shifts) offers a more comprehensive understanding of quality degradation in seaweed-based analog rice

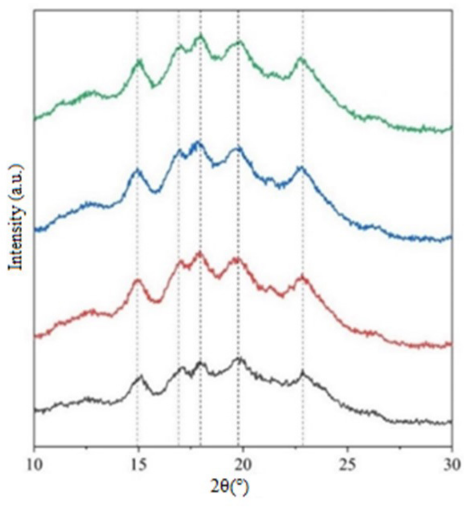

Figure 2 X-ray diffractogram of *Gracilaria* sp. seaweed-based starch analog rice before storage (▬), and after storage in an incubator at 35°C (▬), 45°C (▬), 55°C (▬)

Gambar 2 Difraktogram sinar-X analog pati beras dari rumput laut *Gracilaria* sp. sebelum penyimpanan (▬), dan setelah penyimpanan dalam inkubator 35°C (▬), 45°C (▬), 55°C (▬)

during storage. For instance, the rise in TBA from 0.02 to 0.19 mg MDA/kg aligns with a fat reduction from 4.17% to 3.27%, confirming lipid oxidation. Concurrently, the increase in aw from 0.52 to 0.55 supports the hypothesis that oxidative byproducts contribute to water redistribution, increasing the risk of spoilage. This integration of kinetic and physicochemical data reinforces the value of TBA as a key marker for evaluating product stability and provides a holistic framework for assessing storage-related quality changes in aquatic food products.

CONCLUSION

The results indicated that TBA levels are the most critical quality parameter. The product is estimated to have a shelf life of approximately 210 days at room temperature (28°C). This study demonstrates that during storage, *Gracilaria* sp. seaweed-based analog rice undergoes significant physicochemical changes, particularly in the reduction of fiber and fat content and an increase in TBA values.

ACKNOWLEDGEMENT

The author would like to thank the ‘Promoting Research and Innovation Through Modern and Efficient Science and Technology Parks Project’ (PRIME STeP) - Asian Development Bank Program for the grant received (number 13468/IT3. L1/HK.07.00.P/B/2023) under the coordination of Science and Techno Park IPB University, led by Prof. Dr. Ir. Sri Purwaningsih, M.Si. We also thank PT Fits Mandiri Indonesia for the invaluable support and facilities in the production process of seaweed-based analog rice using its extruder machine.

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