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THE EFFECT OF LONG STORAGE ON MANGROVE (*Sonneratia caseolarisi*) FRUIT SLICE JAM ON TOTAL PLATE COUNT (TPC) VALUE AND ANTIOXIDANT ACTIVITY

Pengaruh Lama Penyimpanan Terhadap Nilai *Total Plate Count* (Tpc) Dan Aktivitas Antioksidan Pada Selai Lembaran Buah Mangrove (*Sonneratia Caseolarisi*)

Rossa Maharani[1], Chandra Trisna Pangestu[2], Eka Saputra[3], Sapto Andriyono[3*]

[1]Fishery Product Technology Study Program Airlangga University, [2]Masters Program in Fisheries and Marine Biotechnology Airlangga University, [3]Department of Marine Science Airlangga University

Surabaya, East Java 60115, Indonesia

[*]Correspongin Author: sapto.andriyono@fpk.unair.ac.id

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ABSTRACT

The mangrove apple (*Sonneratia caseolarisi*) has potential as an alternative food source due to its high nutritional and antioxidant content. This study aims to analyze the effect of storage duration on mangrove apple-based fruit slices' microbiological and chemical quality by adding rosella extract, focusing on Total Plate Count (TPC) value, antioxidant activity, and proximate analysis. The slices were made by adding 50% rosella extract based on the weight of the fruit pulp and were tested during storage at 0, 7, 14, 21, and 28 days. The research methods included TPC testing to count microorganisms, antioxidant activity testing using the DPPH method, and proximate analysis to determine moisture, fat, protein, ash, and carbohydrate content. The results showed that TPC values increased significantly with longer storage time, with the highest increase in the fourth week. Antioxidant activity also decreased over time, indicating the degradation of antioxidant compounds due to oxidation. Proximate testing revealed an increase in moisture and fat content, while protein, ash, and carbohydrate levels decreased as storage duration increased

Keywords: Mangrove apple, rosella extract, TPC, antioxidant, proximate.

ABSTRAK

Buah pedada (*Sonneratia caseolarisi*) memiliki potensi sebagai sumber pangan alternatif dengan kandungan nutrisi dan antioksidan yang tinggi. Penelitian ini bertujuan untuk menganalisis pengaruh lama penyimpanan terhadap kualitas mikrobiologis dan kimiawi selai lembaran berbasis buah pedada dengan penambahan ekstrak rosella yang fokus pada nilai *Total Plate Count* (TPC), aktivitas antioksidan, dan analisis proksimat. Selai dibuat dengan penambahan ekstrak rosella sebesar 50% dari berat bubur buah dan diuji selama penyimpanan

0, 7, 14, 21, dan 28 hari. Metode penelitian mencakup uji TPC untuk menghitung jumlah mikroorganisme, uji aktivitas antioksidan menggunakan metode DPPH, dan uji proksimat untuk menentukan kadar air, lemak, protein, abu, dan karbohidrat. Hasil menunjukkan bahwa nilai TPC meningkat secara signifikan seiring dengan lamanya penyimpanan, dengan peningkatan tertinggi pada minggu ke-4. Aktivitas antioksidan juga menurun seiring waktu penyimpanan yang menunjukkan degradasi senyawa antioksidan akibat oksidasi. Uji proksimat menunjukkan peningkatan kadar air dan lemak, sementara kadar protein, abu, dan karbohidrat menurun seiring lamanya penyimpanan.

Kata Kunci: Pedada, ekstrak rosella, TPC, antioksidan, proksimat.

INTRODUCTION

Mangroves are coastal ecosystems that have significant ecological and economic value. One of the potential uses of this ecosystem is as an alternative food source. Pedada fruit (*Sonneratia caseolarisi*) has the potential to be developed in the context of food diversification, which aims to utilize natural ingredients with high nutritional and antioxidant content. This food diversification is important to expand food sources and support national food security. Indonesia, with its abundant mangrove resources, has a great opportunity to optimize the use of mangrove fruit in increasing food security and the economic value of processed mangrove products. This approach is expected to not only contribute to meeting the nutritional needs of the community, but also encourage local economic development through the processing and marketing of mangrove-based products (Salsabila, 2022) Pedada fruit (*Sonneratia caseolarisi*) has distinctive characteristics such as green color, fragrant aroma, and unique sour taste. In addition, this fruit is non-toxic and can be consumed directly (Manalu, 2013). In several coastal areas, pedada fruit (Sonneratia caseolarisi) has been processed into various food products such as syrup (Rajis, 2017), ice cream (Jariyah, 2019), flour (Verdiantika, 2022), and jelly candy (Ramadani, 2020). Previous studies have shown that mangrove apple fruit contains high levels of antioxidants and fiber, thus providing health benefits (Lubis, 2020).

Sheet jam from pedada fruit is one of the innovations in diversification of processed products that has great market potential. This product has an elastic, dense, and non-sticky texture, which is favored by consumers. This innovation not only expands the choice of mangrove-based products but can also increase economic value and competitiveness in a wider market (Ramadhan, 2017).

Sheet jam based on pedada fruit (*Sonneratia caseolarisi*) is a semi-solid jam modified in the form of sheets with unique elastic texture characteristics. The process of making this sheet jam involves several important variables that affect the quality and texture of the final product.

These variables include the level of fruit ripeness, ingredient composition, sugar content, thickener concentration (such as pectin and carrageenan), and cooking temperature and duration. Variations in these factors affect the physical and chemical characteristics of sheet jam, including hardness, color brightness, and non-stickiness (Ariani, 2023)

The quality of sheet jam can be evaluated based on several aspects, including organoleptic, physical, and microbiological characteristics. Organoleptic quality includes color brightness, distinctive fruit aroma, and natural flavor without the addition of artificial sweeteners such as saccharin or cyclamate, which are avoided to maintain food safety. In terms of microbiology, the quality standard for sheet jam requires the number of coliform bacteria to be less than 3 MPN/gram and a Total Plate Count (TPC) of no more than $1x10^3$ CFU/g (Abdillah, 2021). In addition, a minimum soluble solids content of 65% based on mass fraction is also important to maintain product consistency (Wati, 2021). Therefore, proper processing and storage techniques are very important in maintaining quality and shelf life. (Antika *et al.*, 2024).

Shelf life is an important parameter for processed fruit products such as jam sheets, because it reflects the duration for which the product can be stored under certain conditions without experiencing significant quality degradation. Good shelf life helps maintain product quality during the recommended storage period (Susanti, 2024). Storage conditions, such as temperature, oxygen content in the packaging, humidity, and light exposure greatly affect the shelf life of the product. In addition, factors such as sugar and thickener content, water activity (Aw), and proper pasteurization process also play a role in inhibiting the growth of microbes that can damage the product and shorten its shelf life (Abdillah, 2021).

Jam sheets based on pedada fruit (*Sonneratia caseolarisi*) require a specific shelf life assessment to determine how long the product remains edible with maintained quality. Shelf life assessment is important considering the natural sensitivity of pedada fruit to storage conditions. This study will analyze critical parameters such as Total Plate Count (TPC) and antioxidant activity in jam sheet products. TPC is used to measure the total number of microbes in the product which functions as an indicator of cleanliness and safety during storage. In addition, antioxidant activity will be assessed to evaluate the product's ability to prevent oxidation that can affect aroma, color, and nutritional content during the storage period.

This research is expected to contribute to the diversification of mangrove fruit-based foods, while also answering the challenges of food security in Indonesia. With increasing consumer awareness of functional foods that are not only nutritious but also beneficial for health, pedada fruit-based sheet jam has the potential to become a popular product and has high demand. The results of this study will provide insight into the right storage conditions to maintain the quality and shelf life of sheet jam, thereby increasing the economic value of pedada fruit as a food source. In addition, this study is expected to encourage the utilization of high-value local resources, which ultimately contribute to the welfare of coastal communities and national food security.

METHODS

Place and Time

This research was conducted from December to February 2024 in the Chemistry Laboratory, Analysis Laboratory and Food Laboratory, and Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Airlangga University, Surabaya.

Tools and Materials

This study used various tools and materials that support the process of making and testing the storage time of mangrove fruit sheet jam. The main ingredients used include pedada fruit from Wonorejo, Surabaya, and dried rosella petals, while supporting materials include PCA media, physiological NaCl, carrageenan flour, granulated sugar, water, methanol, distilled water, aluminum foil, and DPPH solution. The tools used in this study include N4S UV-Vis Spectrophotometer (Shanghai, China), glassware, vortex, centrifuge, 100-1000µl micropipette, analytical balance, autoclave, incubator, petri dish, water bath, and various equipment for proximate analysis such as Kjeldahl apparatus complete with destruction and distillation, desiccator, Soxtec apparatus, oven, porcelain cup, and ashing furnace.

Research methods

This study used a completely randomized design (CRD) with five treatments and four replications, resulting in 20 experiments. The treatments in the jam shelf life test followed the reference of Nanda *et al*., (2019), where the treatments were P0 (storage period 0 days), P1 (storage 7 days), P2 (storage 14 days), P3 (storage 21 days), and P4 (storage 28 days). The variables used in this study consisted of independent variables, namely storage period (X), dependent variables including the Total Plate Count (TPC) test value, antioxidant activity, and proximate test results (Y), and controlled variables (Z) including the type of pedada fruit, use

of carrageenan, storage media and temperature, jam production process, and tools and materials used in the study.

Research Procedures

A. Rosella Extraction

Rosella extraction was carried out using a modified maceration method from the modified Puspita (2019) method. The purpose of this extraction is to obtain anthocyanin and phenolic compounds from rosella flower petals. This process involves grinding dried rosella flowers into powder, which is then mixed with warm water in a ratio of 1:10 (w/w). The mixture is stirred until the color pigment comes out optimally, then filtered to obtain rosella extract 100%.

B. Making Pedada Fruit Porridge

Making pedada fruit pulp was carried out using a modified Simamora (2017) method. This process begins by weighing 500 grams of pedada fruit which has been peeled and washed clean. The pedada flesh is then mixed with boiled water in a 1:1 ratio and stirred until porridge forms. This pulp is strained to separate the seeds and ensure a smooth texture.

C. Making Pedada Fruit Jam

The formulation and process of making pedada fruit sheet jam refers to the method in the research of Puspita (2019) and Simamora (2017) and which was modified. The process of making pedada fruit sheet jam is done by mixing pedada fruit pulp with rosella extract (50% of the weight of the fruit pulp), sugar, margarine, and carrageenan according to the specified formulation (Table 1). The mixture is stirred until homogeneous and cooked at a temperature of 50-60ºC for 3 minutes until it thickens. The cooked jam is poured into a baking pan as a mold and allowed to harden for 2 minutes before being put into a tightly closed plastic container for storage.

Table 1. Formulation of Pedada Fruit Jam

No	Ingredients	Formulation with rosella extract	
		Gram	%
1	Pedada Fruit Porridge	100	50
2	Rosella Flower Extract	50	25
3	Carrageenan	2,6	1,25
4	Margarine	3	1,5
5	Sugar	44	22
	Total	199,6	100

D. Testing of Pedada Fruit Jam

Testing of pedada fruit sheet jam was carried out to assess its quality through several tests that were adjusted to SNI. These tests include the Total Plate Count (TPC) test, antioxidant activity test, and proximate test. A detailed explanation of each of these tests is as follows:

1. Total Plate Count (TPC) Test

The Total Plate Count (TPC) test aims to calculate the total number of microbes in a sample of pedada fruit sheet jam. This method uses a dilution of the sample planted on solid media so that the microbial colonies can grow and be counted visually. The calculation results are expressed in Colony Forming Units (CFU) per gram of sample, with an average obtained from three repetitions. (Wenas, 2020).

2. Antioxidant Activity Test

Antioxidant activity test was conducted based on the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The stages of this test include sample preparation through centrifugation to obtain supernatant, making DPPH solution, and measuring absorbance using a spectrophotometer at a wavelength of 517 nm. Antioxidant activity is calculated based on the

percentage of DPPH free radical scavenging by antioxidant compounds in the sample. (Maesaroh, 2018).

3. Proximate Test

The proximate test on pedada fruit sheet jam aims to determine the main composition of the product, which includes water, protein, fat, ash, and carbohydrate content. Water content is measured by drying the sample at a temperature of 105°C until it reaches a constant weight, which provides information about the stability of the product and its shelf life (Rosaini, 2015). Protein content is determined using the Kjeldahl method, which measures the total nitrogen in the sample and then converts it to protein content. This process goes through the stages of destruction, distillation, and titration to obtain accurate results (Ispitasari, 2022). Fat content is measured using the Soxhlet method, where fat is extracted using an organic solvent (Mardhika, 2020), and ash content is calculated by burning the sample at a high temperature to determine the amount of minerals contained in the product (Rani, 2022). Carbohydrate content is calculated using the by difference method, which reduces the percentage of water, protein, fat, and ash content from 100% (Fitriyah, 2020).

Data Analysis

The research data were analyzed using homogeneity and normality tests, then statistical analysis was carried out using 1-factor Analysis of Variance (ANOVA). Treatments that showed significant results were continued with further Duncan Multiple Range Test (DMRT) tests using 95% accuracy. Data presentation was done by tabulating data in Microsoft Excel to create graphs and tables.

RESULT

Total Plate Count (TPC) testing

Total Plate Count (TPC) testing is used to measure the number of microorganisms in pedada fruit sheet jam products with various storage times. The complete TPC test results are presented as follows:

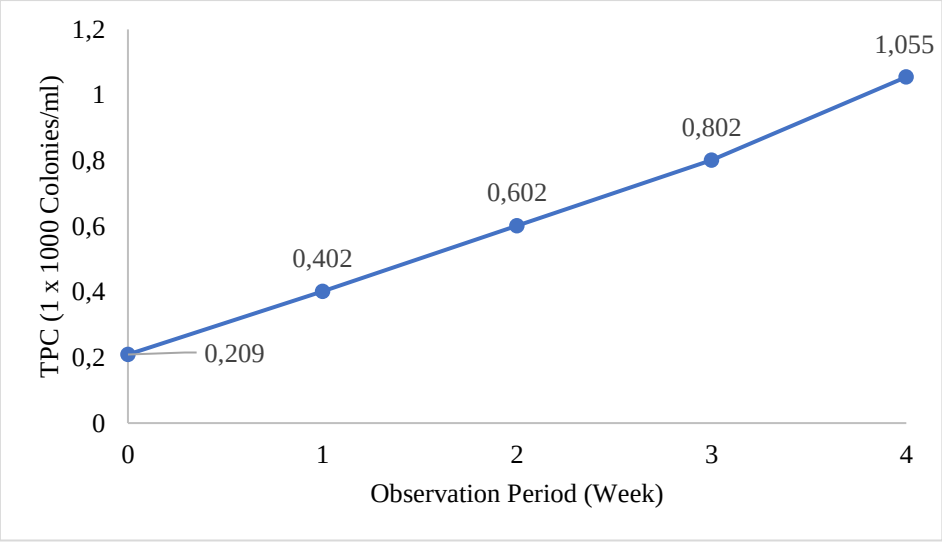

Figure 1. Total Plate Count (TPC) Test Results

Microbiological tests using the Total Plate Count (TPC) method aim to determine the number of microorganisms growing in food products by growing microorganisms on agar media and then calculating their number directly. The results of microbiological tests using the TPC method on pedada fruit sheet jam with variations in storage time (week 0, week 1, week

2, week 3, and week 4) showed significant differences in the TPC calculation results (Figure 1). In the 0th week of storage, the lowest TPC count was found. From week 1 to week 4, the TPC calculation results showed an increase. The highest TPC count was found in the 4th week of storage, so the storage time for pedada fruit sheet jam can affect the decline in product quality.

Antioxidant Activity Testing

Testing of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method aims to determine antioxidant activity quantitatively using a UV-Vis spectrophotometer with a wavelength of 517 nm. The value of antioxidant activity can be expressed by the inhibitory concentration value (IC_{50})

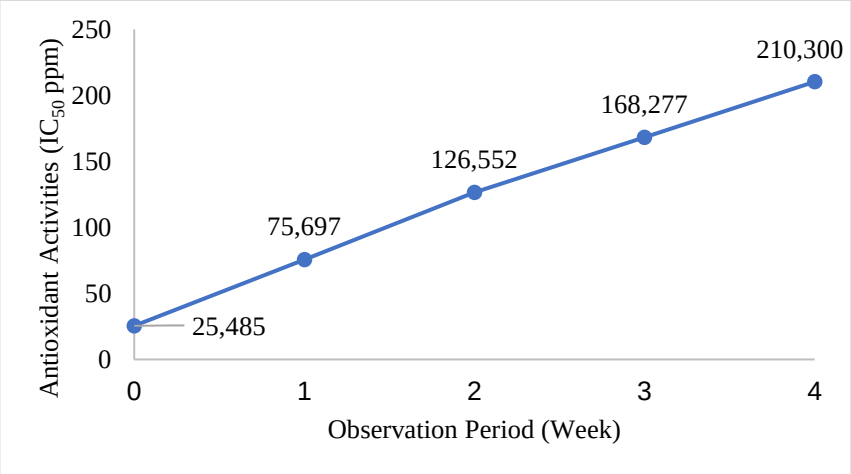

Figure 2. Results of Antioxidant Activity Testing

Based on the results of antioxidant activity testing (Figure 2), different storage times for pedada fruit sheet jam have significant differences in the inhibitory concentration (IC_{50}) value. In the 0th week of storage, the pedada fruit sheet jam was found to have the highest antioxidant activity value. The long storage time for pedada fruit jam is one of the factors causing the decrease in antioxidant activity values. During the 1st to 4th week of storage, a decrease in the antioxidant activity value of pedada fruit sheet jam was found. The lowest antioxidant activity value was found in the 4th week, so that pedada fruit jam with the addition of rosella flower extract with different storage times could have an effect on reducing the antioxidants contained in pedada fruit jam.

Proximate Testing

Proximate testing aims to determine the nutrient content of pedada fruit sheet jam. In this study, proximate tests were used in the form of water content, ash content, fat content, protein content, and carbohydrate content.

Table 2. Proximate Test Results of Pedada Fruit Sheet Jam

No	Observation	Proximate Percentage Average (%)				
		Carbohydrate	Protein	Fat	Ash	Water
1.	P0	46,710^{e}	1,587^{e}	1,822^{a}	1,810^{e}	48,070^{a}
2.	P1	44,395^{d}	1,372^{d}	2,122^{b}	1,727^{d}	50,382^{b}
3.	P2	42,332^{c}	1,140^{c}	3,152^{c}	1,662	51,712^{c}

4.	P3	40,000[b]	0,957[b]	4,282[d]	1,507[c]	53,252[d]
5.	P4	36,880[a]	0,770[a]	5,837[e]	1,452[b]	55,060[e]
					[a]	

Based on the results of proximate testing (Table 2), different storage periods of pedada fruit sheet jam showed significant differences. In the water content and fat content tests, the lowest values were found in the 0th week of storage. This is different from the ash content, protein content, and fat content tests which obtained the highest values in the 0th week of storage. Along with the length of storage time, the water content and fat content values increased and the ash content, protein content, and carbohydrate content values decreased. The length of storage time of pedada fruit sheet jam affects the nutrient content in the product.

DISCUSSION

Microbial growth at room temperature is faster than at cold temperatures. Room temperature is the main factor affecting microbial growth, this is because temperature can affect chemical reactions and enzymatic reactions (Khaira, 2016). In this study, the results of the TPC value from week 0 to week 3 in the pedada fruit sheet jam stored at room temperature were still safe for consumption according to the SNI reference for jam which has a maximum TPC value of 1 × 103 colonies/mL. In the 4th week, the pedada fruit sheet jam had a TPC value that exceeded the minimum limit based on the SNI reference, so the jam was not safe for consumption (SNI 3746, 2008).

The results showed that week 0 of storage produced the lowest TPC value of all treatments. This is because the storage time in week 0 is the initial part of microbial adaptation to adapt to the environment. In this adaptation process, microbial growth is slow and tends to be stable. This adaptation process takes place quickly and depends on the type of microorganisms that can grow on the food and the nutrients contained in the product (Astutiningsih, 2022). The increasing number of microbes in products is related to the nutritional content contained in the food ingredients used. In the storage treatment from week 1 to week 4, microbial growth occurred rapidly with the length of storage time. In this process, microbes enter a growth period characterized by the development of microbial cells. Microbes that grow on products use the nutrients contained in food as their energy source (Rima, 2017).

The results of this study indicate that the antioxidant activity of pedada fruit jam decreases with the length of storage time. This decrease was caused by oxidation of phenolic compounds, including polyphenols and vitamin C, which occurred due to exposure to oxygen even though the product was stored in tightly closed packaging (Mahardani, 2021). Antioxidant compounds such as ascorbic acid are very susceptible to oxidation, turning into dehydroascorbate which has reduced antioxidant function (Habibi *et al*., 2019). During storage, although oxidative enzymes are inactivated by heating, the heating process itself can damage and accelerate the release and degradation of antioxidant components (Ariadianti, 2015). In addition, water-soluble vitamin C is easily damaged during storage and heating which contributes to a decrease in the total antioxidant activity in the product (Maitimu, 2021). These results emphasize the importance of controlling storage conditions and post-production treatments to maintain antioxidant quality in pedada fruit-based food products. In addition, the addition of rosella extract contributed to increasing antioxidants from the resulting jam.

During storage, the water content in the sheet jam increases, caused by air humidity in the storage area and oxygen exposure due to non-vacuum packaging. In contrast, the ash and fat content tend to decrease. The decrease in ash content is related to the reduction of minerals

such as phosphorus, iron, and calcium in rosella extract (Prasetyani, 2022), while the decrease in fat content is caused by fat oxidation due to contact with oxygen in a humid environment. Carbohydrate and protein levels do not change significantly during storage, but the carbohydrate, sucrose, and sugar content can facilitate microbial growth, which contributes to the degradation of carbohydrates into acids and a decrease in product quality (Masyin, 2024). The increase in water content and the decrease in fat and ash indicate that storage conditions greatly affect the physical and chemical stability of the product, where non-airtight packaging accelerates the degradation process (Abdillah, 2021). By conducting nutritional content testing, ideal storage conditions need to be considered including storage space, storage methods and storage materials.

CONCLUSION

Room temperature in the storage process of mangrove fruit sheet jam products significantly affects the growth of bacteria in jam products. Storing products at room temperature for three weeks has a Total Plate Count (TPC) value that is still considered safe for consumption, but in the 4th week. This indicates that the jam product has rotted and is not suitable for consumption. The length of storage time significantly affects the quality of jam products. The antioxidant activity and nutritional value of sheet jam decreased in carbohydrate, protein, and ash parameters. While other parameters experienced an increase in water content and fat content parameters.

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