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Determination of Optimum Temperature for Amount Lactic Acid Bacteria and Antioxidant Activity from Pickled Bamboo Betung Boobs (*Dendrocalamus asper*)

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Abstract: Pickled betung bamboo shoots (Dendrocalamus asper) are a traditionally fermented product and can potentially be a source of probiotics. Pickled bamboo shoots contain good microorganisms, namely LAB, which are bacteria that contribute to the food sector for natural fermentation. Fermentation of bamboo shoots produces antioxidant compounds at an optimum temperature range. The research aims to understand the optimum temperature for the amount of LAB and antioxidant activity in pickled betung bamboo shoots. This research is a laboratory experiment with 3 treatments, namely 100 grams of bamboo shoots fermented with pickled bamboo shoots at a temperature of 15℃ (K1), a temperature of 37℃ (K2), and a temperature of 40℃ (K3). The three treatments were incubated for 72 hours with 4 repetitions. The research stages included processing pickled bamboo shoots, isolating bacteria using the dilution series-pour plate method, and antioxidant testing using the DPPH method. The number of LAB is calculated using the Quebec Colony Counter. Data analysis used Anova and Duncan's test. The results obtained include that temperature influences the amount of BAL in pickled bamboo shoots with a value of p=0.016. The number of LAB at a temperature of 150C was 59.50, at a temperature of 37 129.74, and a temperature of 40 107.57. Temperature also affects antioxidant activity. The average values at temperatures of 15.37, 40℃ are 30.60%, 25.241%, and 16.8782% (% inhibition), 36.3655, 41.6775, 51.0757 (IC50). The optimum temperature for the number of BAL pickled bamboo shoots is 37°C, and the optimum temperature for the number of antioxidants is 15°C.

Keywords: Antioxidant; Bamboo Betung; Lactic Acid Bacteria; Pickled Bamboo Shoots; Temperature.

Introduction

Indonesia has various types of traditional food. Many well-known types of conventional food are fermented foods. Food made using the fermentation method is better than other culinary delights because it can increase digestibility and preserve most nutritional constituents with various tastes, aromas, and textures [1]. One of the fermented foods is pickles. Pickles are listed in the fermentation of acid, lactic acid, which in the event of fermentation is automatic fermentation. Asi, nan can be made from various fruits, foods, and vegetables. One of the types of *asinan* in vegetables is *asinan* from rebung bambu betung [2].

Bacteria lactate acid is a bacteria whose contribution is relatively high in the food sector. This bacteria is also used to preserve fermented foods naturally. Bacteria and bacteria are used as ingredients to protect food naturally by applying biopreservative techniques. This technique has been widely developed to apply these bacteria directly. It is used as a metabolite of anti-microbial agents [3]. LAB microbial activity is usually used in the fermentation of pickled bamboo shoots. Therefore, through this process, fermented bamboo shoots form the taste and texture of food, increasing the bioavailability of carbohydrates for humans [4].

During fermentation of LAB, bamboo shoots produce several antioxidant compounds such as 1,3,5-trimethyl benzene, limonene, toluene, naphthalene, palmitic acid, vitexin, orientin, vitamin E, curcumin polyphenol and flavonoids [5]. Flavonoids can provide an antioxidant effect by preventing the production of hydroxyl radicals, hydroxyl frees, single oxygens, hydrogen peroxylates, alkoxyl radicals, peroxyl radicals, and superoxide anions [6]. One class of flavonoids is ant, cyanin. Anthocyanins are natural pigments in red, blue, purple, and black vegetables, fruits, and grains [7]. As an antioxidant, anthocyanins help prevent diabetes, colon cancer, and cardiovascular disease. Fermented products made from plants can undoubtedly contain anthocyanins. However, because anthocyanins are unstable, their antioxidant properties may be reduced. The stability of anthocyanins occurs due to various variables, namely enzymes, pH, light, and temperature. The stability of anthocyanins increased below 40°C. Anthocyanin fermentation in the laboratory is one method to maintain the stability of anthocyanins [8].

Temperature determines the fermentation of lactic acid bacteria to proceed well. Lower temperatures cause lactic acid bacteria to grow more slowly, which results in reduced production and damage to fermented products, so they rot. Slight growth of lactic acid bacteria will reduce antioxidant activity [9]. According to [10], the higher the temperature, the faster LAB produces lactic acid through metabolic activity, and the lower the temperature, the slower LAB produces lactic acid through metabolism.

Fermentation production Growth rate and lactic acid bacteria were linearly correlated. Because it is proven that the growth of lactic acid bacteria is closely related to the

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elements of the growth media and environmental conditions, they can be classified as probiotics [11]. One ecological element that can impact the production and growth of fermented lactic acid bacteria is temperature [12]. Lactic acid bacteria can grow at 5-45°C [13]. Each lactic acid bacteria has a different optimum temperature range. Microorganisms are known to grow best in three different temperature ranges: thermophilic (45°C-30°C), mesophilic (30°C-40°C), and psychrophilic (7°C-30°C) [14]. Based on the background information provided, the author wants to research "Determining the Optimum Temperature of the Number of Lactic Acid Bacteria and Antioxidant Activity of Pickled Betung Bamboo Shoots (Dendrocalamus asper).

This research aimed to determine the optimum temperature for the amount of LAB in pickled bamboo shoots and their antioxidant activity. It was conducted to provide information about the optimum LAB temperature for pickled bamboo shoots in producing antioxidant activity and to advance science, especially in food microbiology, regarding the best temperature for storing pickled bamboo shoots. It can inform the public of the importance of consuming processed fermented foods to make humans healthy.

Research Methods

This research is a laboratory experiment with a single-factor method of fermentation of bamboo shoots, divided into three treatments, namely (P1) 100 grams of bamboo shoots fermented with pickled bamboo shoots at a temperature of 15℃, (P2) 100 grams of bamboo shoots fermented at a temperature of 37℃, and (P3) 100 grams of bamboo shoots fermentation was carried out at a temperature of 40℃. The three treatments were incubated for 72 hours with four repetitions. During the fermentation of bamboo shoots, 2.5% salt was added to each preparation, after which the bamboo shoots were tested for antioxidant activity.

Processing of Pickled Bamboo Shoots

First, make 100g of cleaned bamboo shoots, red chillies, and garlic. Put them in a jar filled with boiled water, stir while sprinkling with 2.5% salt, cut the bamboo shoots into pieces, and add them one by one to the jar, then close tightly. The fermentation process lasts 3 days at various temperatures (15oC, 37oC, 40oC).

Isolation of Bacteria

Certain isolation media, often known as selective media, are used in LAB isolation. Because of its uniqueness, this selective media grows and maintains certain microorganisms, especially LAB. Only certain bacteria can thrive in this media. MRS agar or de Man Rogosa Sharpe Agar is a medium used in BAL isolation.

The pour dilution technique used de Man Rogosa and Sharpe (MRS) media to isolate Lactic Acid Bacteria (LAB) from pickled bamboo shoots. The pickled bamboo shoots were taken aseptically using a spatula under the LAF and then mixed in a test tube containing 9 mL of MRSB medium. The inoculum was homogenized in a vortex until the vibration reached the bottom of the tube so that it was mixed completely. The inoculum was then incubated for 48 hours at 37°C.

Antioxidant Test

Using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) technique, the antioxidant chemicals present in the sample and the vitamin C reference were reacted to determine antioxidant activity. IC50 and % Inhibition values were used to calculate antioxidant activity.

Data analysis

The collected data was analysed statistically using a one-way analysis of variance test (one-way ANOVA); if the test provides significant results, Duncan's test determines the results.

Results and Discussion

This research is a laboratory experiment with a single-factor method of fermentation of bamboo shoots, divided into three treatments, namely (P1) 100 grams of bamboo shoots fermented with pickled bamboo shoots at a temperature of 15℃, (P2) 100 grams of bamboo shoots fermented at a temperature of 37℃, and (P3) 100 grams of bamboo shoots fermentation was carried out at a temperature of 40℃. The three treatments were concentrated for 72 hours with four repetitions. During the fermentation of bamboo shoots, 2.5% salt was added to each preparation, after which the bamboo shoots were tested for antioxidant activity.

One type of bacteria found in fermented products is lactic acid bacteria (LAB). LAB converts fruit and vegetables containing carbohydrates into lactic acid, characterised by decreased pH due to fermentation. Decreased pH levels Occur when β-galactosidase breaks down lactose into lactic acid, which increases the acid level as the duration of fermentation increases. Lactic acid will be produced from glucose during the fermentation process. An increase in H+ concentration, resulting in decreased pH, will occur when lactic acid levels increase. This is because lactic acid bacteria ferment food to produce lactic acid [2].

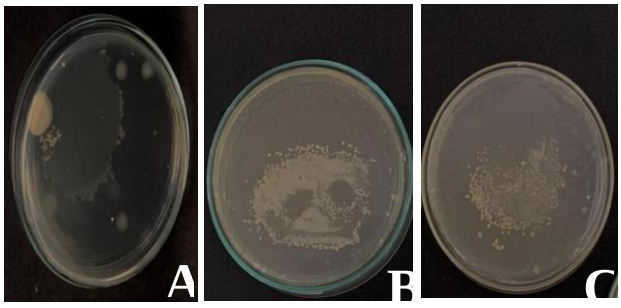

Figure 1. Number of LAB Colony Growth in Each Temperature Variation (Source:
Personal Documentation (A. Colony sample dilution 10-4 at 150C
B. Colony sample of 10-4 dilution at 370C
C. Colony sample of 10-4 dilution at 400C

From the results of further research on the relationship between concentration and the antioxidant inhibition value of pickled bamboo shoots for free radicals,

you can use the IC50 value because it is a potent antioxidant. The lower the IC50 value, the stronger the antioxidant power [14]. From this research, the results obtained at a temperature of 15°C were the lowest IC50 value among the three temperature factors at 36.3655. Meanwhile, the highest IC50 value is at 40°C, 51.0757 (see table 4.4). This explains that a temperature of 15°C is optimal for increasing antioxidant activity in pickled betung bamboo shoots. It can be concluded that temperature can affect the ability of pickled bamboo shoots as an antioxidant in warding off free radicals [15]. One of the roles of temperatures of 15°C and 37°C is that it can inhibit the work of pathogenic microorganisms such as Erwinia cartovora, Pseudomonas Flourescens, and Clostridium bacteria whose activity is influenced by temperature and prevent the growth of fungi. Apart from that, 15°C and 37°C temperatures can inactivate enzymes. - the enzyme in the polyphenol oxidase enzyme material, which plays a role in forming quinones with phenolic compounds as the substrate [16].

Based on the table, the antioxidant activity of pickled bamboo shoots at temperatures of 15°C and 37°C and also vitamin C at each temperature shows a very strong category because (IC<50) [17]. Meanwhile, pickled bamboo shoots at a temperature of 40°C experienced a significant decrease because the IC value of more than 50 was said to be a weak antioxidant. It could be caused by high temperatures destroying the phenol chemicals found in bamboo shoots [18]. Because phenolic compounds are thermosensitive, high temperatures can cause these compounds to hydrolyze, and their content decreases [19]. In this study, vitamin C was used as a comparison because it works as a secondary antioxidant by preventing reactions and has very high antioxidant properties. The activity of pickled bamboo shoots at each temperature has a higher IC50 value than vitamin C. These results show that pickled bamboo shoots do not have strong antioxidant properties like vitamin C. This is because vitamin C is a pure molecule, while pickled bamboo shoots consist of several combinations of ingredients—chemistry [20].

Based on research conducted by [21], the responses tested include pH value, water content, ash content, fat content, protein content, carbohydrate content, crude fibre content, and a number of microbes. Organoleptic responses were tested for color, aroma, taste, and texture. The results showed that salt concentration influenced water content, total microbes, and the smell of kimchi produced after fermentation. The length of fermentation influences the kimchi's ash, fat, protein, color, and texture. Salt concentration and fermentation time influence bamboo shoot kimchi's pH, crude fiber, and taste.

Shelf-life testing is carried out by placing grape seeds in capsules; Capsules containing 50 mg of grape seeds were packaged using glass bottles and metalised plastic and stored at 40°C for 28 days. Antibacterial activity test using the Kirby-Bauer method against Escherichia coli and Staphylococcus aureus. MIC determination was carried out by diluting the extract into several concentrations, namely, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml, 1.56 mg/ml and 0.78 mg/ml, dissolved with media and bacterial culture [22].

At the 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12-hour intervals, total bacterial analysis was carried out using the Total Plate Count (TPC) method, lactic acid content analysis using 0.01 N NaOH titrimetry method and pH using a pH-meter and 0,3,6,9,12 hours analysis of antioxidant activity using the DPPH method. The analysis results at the 12th hour showed that the highest number of probiotic bacteria in corn tempeh yogurt was 6.7x107 cfu/ml, and the highest antioxidant activity in soy tempeh was 80.22%. Yogurt fermentation time is better until the 7th hour of fermentation to obtain the highest total probiotic bacteria and lactic acid levels as well as pH according to SNI with high antioxidant activity [23].

The addition of extracts significantly affected acceptability ($\rho = 0.0001$), taste, color, and texture, but not aroma ($\rho = 0.266$). The optimal product in this study was synbiotic yoghurt with the addition of 0.1% red ginger extract. Conclusion: The addition of red ginger extract significantly affects antioxidant activity, taste, color, and texture, but it does not affect lactic acid bacteria or aroma [24].

The first factor was the concentration of guava concentrate (K), consisting of 5 levels (0%, 5%, 10%, 15%, and 20%), and the second factor was the addition of glucose (G) that consisted of 3 levels (2%, 3%, and 4%). Homogeneity and additivity data were analyzed using Barlett and Tuckey tests. The data were analyzed using Analysis of Variance, then further analyzed using orthogonal polynomial at a significant level of 5% or 1%. The results showed that the addition of 15% guava concentrate and 2% glucose produced the best characteristics of green grass jelly synbiotic beverage with a concentration of lactic acid bacteria of 11,209 log colonies/ml, taste of 3.48 (somewhat like), flavor of 3.35 (rather like), the appearance of 3.18 (somewhat like) and 3.57 overall acceptance (like), and antioxidant activity of 67.14% [25].

The significant differences between treatments were determined using the Duncan Test New Multiple Range Test (DMRT) at a 95% confidence level. Thus, this study aims to assess the viability of lactic acid bacteria and investigate the antioxidant activities in the bitter melon fermented drink during storage conditions. The viability of lactic acid bacteria in fermented bitter melon juice throughout the 30 days of refrigerated storage condition (4oC) showed that the bitter melon juice fermented with L. pentosus LLA8 and L. fermentum LLB3 has good viability until the 12th day. For instance, lactic acid bacteria have good viability until the 15th day. The amount of living cells is still in the minimum range of probiotic drinks, which is 106–107 CFU of probiotic bacteria per milliliter. This result also showed that different LAB strains show various amounts of antioxidant activity. After 30 days of refrigerated storage (4oC), bitter melon juice with L. pentosus LLA8 has the highest antioxidant activity until 89.42±0.99 (%) on day 0. Still, on the 30th day, bitter melon juice with L. fermentum LLB3 showed the highest antioxidant activity compared to another strain of 70.94±1.20 (%). The pH and total soluble solid (sugar concentration) of fermented bitter melon juice significantly changed during storage conditions, but the range wasn't comprehensive and relatively stable [26].

Conclusion

At a temperature of 15°C, the number of LAB colonies was 59.50; at 37°C, there were 129.74; and at 40°C, there were 107.57 colonies of lactic acid bacteria with a

p=value of 0.016. The optimum temperature for observing the number of lactic acid bacteria in pickled betung bamboo shoots is 37°C. The optimum temperature for observing antioxidant activity is 150C, with an average %inhibition value of 30.60%, and the IC50 value is 36.3655.

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