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THE USE OF PURPLE EGGPLANT FRUIT CALYX STEM (*Solanum melongena* L.) AS AN ALTERNATIVE SOURCE OF LACTASE-PRODUCING LACTIC ACID BACTERIA

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ABSTRACT

Lactose intolerance is a condition where the body cannot digest lactose due to a deficiency of the lactase enzyme in the digestive system, leading to various health issues and indirectly increasing the risk of stunting. To prevent lactose intolerance, supplements containing the lactase enzyme or foods and drinks low in lactose, such as yoghurt, are recommended. Yoghurt production requires microorganisms capable of synthesizing the lactase enzyme, such as lactic acid bacteria (LAB). This research is an experimental study exploring the potential of purple eggplant stalks and petals (Solanum melongena L.) as a new source of LAB capable of synthesizing the lactase enzyme. The experiment began with making yoghurt from milk with added purple eggplant calyx stems as initial proof of LAB presence. Milk was chosen as the initial growth medium because it contains lactose, which acts as a substrate for the lactase enzyme found in LAB. The obtained yoghurt was then inoculated into selective media, resulting in the growth of gram-positive bacilli colonies. The lactase enzyme was isolated from LAB using the sonication method. Optimum enzyme activity was achieved at 70°C and pH 7, measuring 0.0061 U/Mg. This research concludes that purple eggplant stalks and petals contain LAB capable of synthesizing the lactase enzyme. Hopefully, this research will progress to purifying the lactase enzyme and developing lactase enzyme supplements, which can be tested as a therapy for lactose intolerance.

Keywords: *lactase enzyme, lactose intolerance, lactic acid bacteria, Solanum melongena L., stunting*

INTRODUCTION

One of the risk factors for stunting in children under five years old is inadequate nutrition. Ensuring proper nutrition is crucial for preventing stunting, and milk plays a vital role due to its lactose content, which aids in the absorption of calcium and magnesium [1]. Another contributing factor to stunting is lactose intolerance, a condition where the body cannot digest lactose due to a deficiency in the lactase enzyme, leading to digestive issues such as stomach pain, bloating, and diarrhea [2, 3]. The root cause of this deficiency is a mutation in the LCT gene, which is responsible for coding the lactase enzyme [4]. In Indonesia, lactose intolerance is prevalent, affecting around 75% of individuals, spanning from children to adults [2, 5, 6]. To address lactose intolerance, providing food that contains the lactase enzyme can alleviate digestive disorders and optimize lactose utilization, particularly for calcium absorption [7, 8].

Yoghurt is a safe dairy product for individuals with lactose intolerance because it contains the lactase enzyme produced by lactic acid bacteria (LAB) during fermentation. This enzyme aids in lactose digestion, reducing digestive issues for lactose-intolerant individuals when consuming dairy products or lactose-containing foods [9]. Despite yoghurt's benefits, the market prices for yoghurt and lactase enzyme supplements remain relatively high. To tackle this issue, lactase-rich products must be developed using readily available and cost-effective ingredients.

Indonesia boasts rich floral diversity, including the purple eggplant (*Solanum melongena* L). Eggplants are widely available throughout the country and are not seasonally dependent, making them cost-effective in the market. Currently, the utilization of eggplants is limited to the fruit, with no research exploring other parts of the purple eggplant, such as the stalk. Previous studies have demonstrated lactase enzyme activity in chilli stalks, which belong to the same plant order as the purple eggplant [10]. Additionally, research indicates that purple eggplants can enhance yoghurt stability, highlighting their probiotic activity [11]. Purple eggplant stalks, considered household waste, remain underutilized. Building on prior evidence, there is a need to further explore the potential isolation of the lactase enzyme from purple eggplant stalks.

To confirm the presence of the lactase enzyme in eggplant stalks, an experiment involving milk fermentation as an initial medium for lactic acid bacteria growth is essential. Subsequently, isolating the lactase enzyme from the fermentation results and determining its specific activity, total protein concentration, and basic characteristics is crucial for understanding the obtained enzyme concentration and the molecular weight of the lactase enzyme isolated from the flower stalk.

The results of this research aim to repurpose purple eggplant stalks, a household waste material, as an alternative source of the lactase enzyme. These findings serve as a foundation for further research, specifically the development of lactase enzyme supplement products using easily accessible and affordable ingredients, providing an alternative therapy for lactose intolerance at an affordable price for the public.

METHODS

Material. Milk containing lactose (Greenfield), MRS Broth (SRL), MRS Agar (SRL), o-nitrophenyl-β-D-galactopyranoside (OnpGal) Substrate (SRL), Na_2CO_3 (MERCK), Bradford reagent, Phosphate (MERCK) Buffer pH, 4,7,10 (MERCK), Yoghurt (Greenfield).

Plant identification tests. Plant identification tests were conducted up to the species level, with testing taking place at the Herbarium Depokensis (UIDEP), Biota Room, Universitas Indonesia, on October 9, 2023.

Milk fermentation using eggplant stalks. Heat 250 ml of fresh milk to a temperature of 40-45°C, which is the optimal temperature for inoculating starter bacteria. After reaching the desired temperature, pour the milk into a sterile container. Next, add three stalks of eggplant to the milk. Close the container tightly and incubate the milk for 24 hours at 37°C [12]. The results at this stage produce fermented milk called yoghurt.

Control. In this case, milk is the initial medium for LAB growth because it contains lactose. LAB will break down the lactose in milk into glucose and galactose, change the consistency of the milk to become thicker and lower the pH of the milk. The controls in this study were commercial yoghurt, which was used as a yoghurt starter and mixed with milk (sample) called control a, and milk without any mixture which was incubated at 37°C, called control b.

Isolation of lactic acid bacteria (LAB). Inoculate 1 ml of yoghurt sample into 5 ml of MRS Broth, then incubate at 37°C for 24 hours. As much as 0.1 ml of the results obtained will be continued to the isolation stage in a specific medium, namely MRS agar, to which 1% $CaCO_3$ has been added and incubated for 24 hours at 37°C. The expected result is to obtain LAB colonies that appear as colonies surrounded by a clear zone [13, 14].

Isolation of the lactase enzyme. Lactic acid bacteria that grew on MRS agar were dissolved in phosphate buffer pH 7 at a speed of 4500 rpm for 60 minutes. The biomass obtained was sonicated at 40 Hz for 30 minutes, then continued with centrifugation again at the same speed to separate cell debris with the lactase enzyme [15]. In this research, the sonication parameters such as temperature, time, and ultrasound intensity were fixed and amounted respectively at 20°C.

Testing total protein levels. Testing for total protein levels was carried out by measuring absorption using the Bradford method by reading absorption at a wavelength of 595 nm. The protein standard that will be used to determine protein levels is Bovine serum albumin (BSA), with a concentration range of 0.1-0.5 mg/mL [16].

Quantitative test of lactase enzyme activity. The quantitative test of lactase enzyme activity aims to determine the concentration of the lactase enzyme produced from the isolation of LAB from eggplant stalks. A total of 1000 µl of 0.1M phosphate buffer pH 7 and 100 µl of test samples were pipetted into a reaction tube then incubated for 15 minutes at 37°C, then 200 µl of o-nitrophenyl-β-D-galactopyranoside (OnpGal) 4 mg/ml was added as substrate and continued incubation for 15 minutes at 37°C, then added a solution that acts as a stop solution, namely 1000 µl Na_2CO_3 1M. Next, the absorbance was read using a UV-Vis spectrophotometer at 420 nm. Enzyme activity (U/mL) is the product formed in the form of the amount of o-nitrophenol (oNP) formed per minute per millilitre of the enzyme at a temperature of 37°C in µmol units [17].

Enzyme activity is calculated by the equation:

$$\text{Enzyme Activity (U/ml) [18]} = \frac{\text{(micromoles oNP)}}{\text{(vxt)}} \quad \text{................ (1)}$$

Calculation of the specific activity of the lactase enzyme is carried out using the following equation:

$$\text{The specific activity of the lactase enzyme (U/mg protein) [18]} = \frac{\text{enzyme activity}}{\text{protein concentration}} \quad \text{............ (2)}$$

Characterization of the lactase enzyme. Characterization of the lactase enzyme in this study was limited to testing enzyme activity against variations in pH and incubation temperature. These parameters were selected based on factors that influenced the enzyme's action. To determine the optimal pH of the lactase enzyme, enzyme activity testing was carried out using a phosphate buffer solution with a pH range of 4, 7, and 10. To determine the optimal temperature for the lactase enzyme, enzyme activity testing was carried out at various incubation temperatures, namely 25, 37, 40, 50, 60, 70, 80, 90, 100°C. It is expected that the results of the characterization of the lactase enzyme will provide information on the general properties of the lactase enzyme isolated from purple eggplant stalks.

RESULTS AND DISCUSSION

This research marks the beginning of a series of studies aimed at producing a pure lactase enzyme product from a new source. The use of purple eggplant stalks represents an effort to utilize household waste as an alternative source of lactase enzyme. Lactase enzymes are known to thrive in the digestive system, particularly in the lumen of the mammalian small intestine, and in certain microorganisms such as yeast cells and lactic acid bacteria (LAB). LABs known for their lactase activity include species from the Lactobacillus and Streptococcus genera [19].

This study builds upon previous research evidence indicating that chilli pepper stalks contain LAB capable of producing lactase, making them suitable for yoghurt production [10]. To confirm the presence of lactase enzymes in purple eggplant stalks, milk was fermented using these stalks, resulting in acidic milk (yoghurt). This step was essential to identify the presence of LAB, particularly Lactobacillus species, in the purple eggplant stalks.

The purple eggplant calyx stems used in the study were sourced from eggplants cultivated by the research team. Identification tests were conducted up to the species level to ensure accuracy in scientific nomenclature. The plant, identified through tests at UIDEP, was confirmed to be Solanum melongena L (Figure 1).

Figure 1. Purple eggplant with calyx stem. a. Eggplant whole fruit; b. Eggplant calyx stem only

For fermentation, three purple eggplant calyx stems were used to ferment 250 ml of Ultra High Temperature (UHT)-packaged cow's milk. Positive and negative controls were included and subjected to the same process. The fermentation was carried out over 24 hours, resulting in yoghurt with a pH of 4 (Figure 2).

The yoghurt resulting from the fermentation of milk using eggplant stalks produces results as shown in Figure 2a. The change in the consistency of the milk becomes thicker and separates into two layers. Similarly, the results of milk fermentation using commercial yoghurt are shown in Figure 2b, where there is also a change in consistency, becoming thicker and forming two layers. In contrast, Figure 2c shows milk left for 24 hours at 37°C with no visible change in consistency.

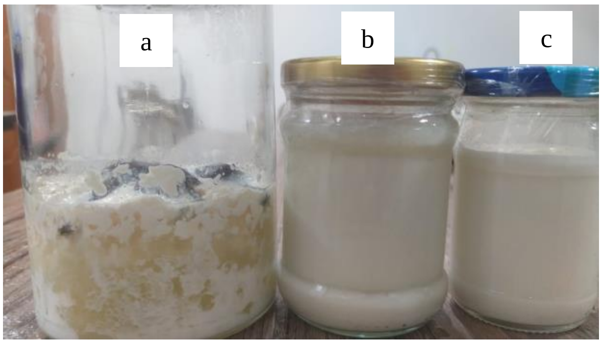

Figure 2. Yoghurt produced from the fermentation of milk with purple eggplant calyx stalks. a. Yoghurt from fermented milk with purple eggplant stalks; b. Fermentation of milk with commercial yoghurt (control a); c. Results of milk incubation alone (control b)

The next stage is involved inoculating the samples into LAB selective media, specifically MRS (de Man, Rogosa, and Sharpe) broth. The growth of lactic acid bacteria in MRS broth, supplemented with 1% lactose, was carried out at 37°C for 24 hours. The resulting turbidity or cloudiness of the media indicated the growth of lactic acid bacteria on the MRS broth media (Figure 3).

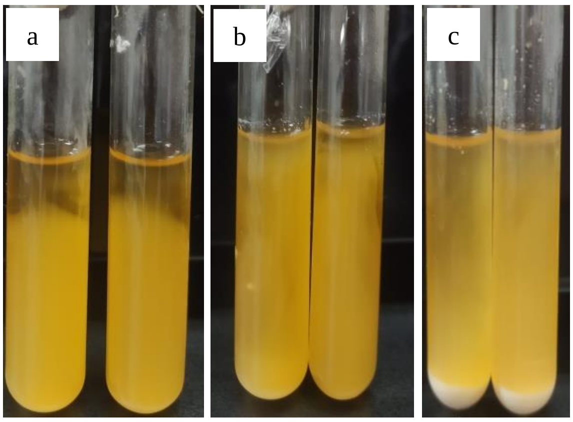

Figure 3. LAB culture on MRS Broth media (duplo). a. LAB growth from samples (Yoghurt from purple eggplant stalks); b. LAB growth control a (yoghurt mixed with milk); c. LAB growth control b (milk incubated at 37°C)

MRS Broth is a selective medium for the growth of *Lactobacillus*. However, other lactic acid bacteria (LAB) like *Streptococcus*, *Pediococcus*, and *Leuconostoc* can also thrive in this medium [19]. To determine the types of LAB growing on MRS Broth, the next step involves inoculation on MRS Agar medium for 24 hours. The result obtained shows colonies growing on this medium with similar characteristics: they are bulbous, smooth, milk-white in color, and slightly convex (Figure 4).

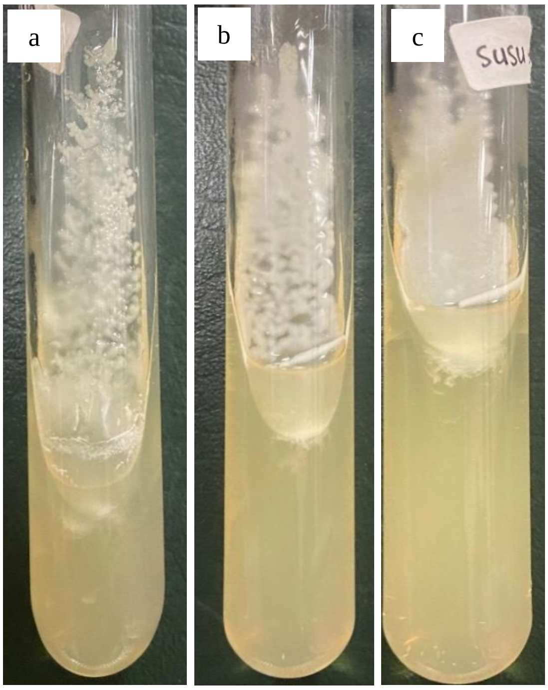

Figure 4. LAB growth on MRS Agar. a. Sample; b. Control a; c. Control b

After inoculation on MRS Agar, a single type of colony was obtained, as evidenced by the uniform appearance of colonies on the medium. To confirm that these colonies represent the same type of bacteria, morphological identification was conducted using Gram staining and observed using a microscope with a magnification of 10 x 100. The results obtained indicated Gram-positive bacilli (Figure 5).

To determine the enzyme activity in the lactic acid bacteria (LAB) grown on MRS Agar, it is necessary to isolate the enzyme using sonication, a technology that utilizes sound waves with vibrational energy exceeding the limits of human hearing (20-100 kHz). Sonication employs sound waves to agitate particles in a solvent by converting electrical signals into physical vibrations, which can cause cell wall lysis, releasing intracellular substances from LAB, including lactase enzymes [20].

The sonicated isolates were then subjected to lactase enzyme activity tests to verify whether the LAB cultured from the purple eggplant stalks produced lactase enzymes. The enzyme activity and total protein content of each colony were tested, and the results are presented in Table 1. Enzyme activity was tested using o-nitrophenyl-β-D-galactopyranoside (ONPGal), a synthetic substrate resembling lactose. Lactase enzymes produced by LAB break down the ONPGal substrate into galactose and o-nitrophenol, resulting in a yellow color. The intensity of the yellow color can be measured at 420 nm, indicating the presence of lactase enzymes [21]. Table 1 elucidates the activity of the lactase enzyme isolated from different LAB sources when examining the enzyme activity using a pH of 7 and an incubation temperature of 37°C. A simple characterization test was conducted to observe the distinctive characteristics of each colony by examining enzyme activity at various temperatures and pH levels. Enzyme activity tests at different pH levels were incubated at 37°C, and specific enzyme activity was calculated using the same total protein concentration in Table 1, and the result is explained in Table 2.

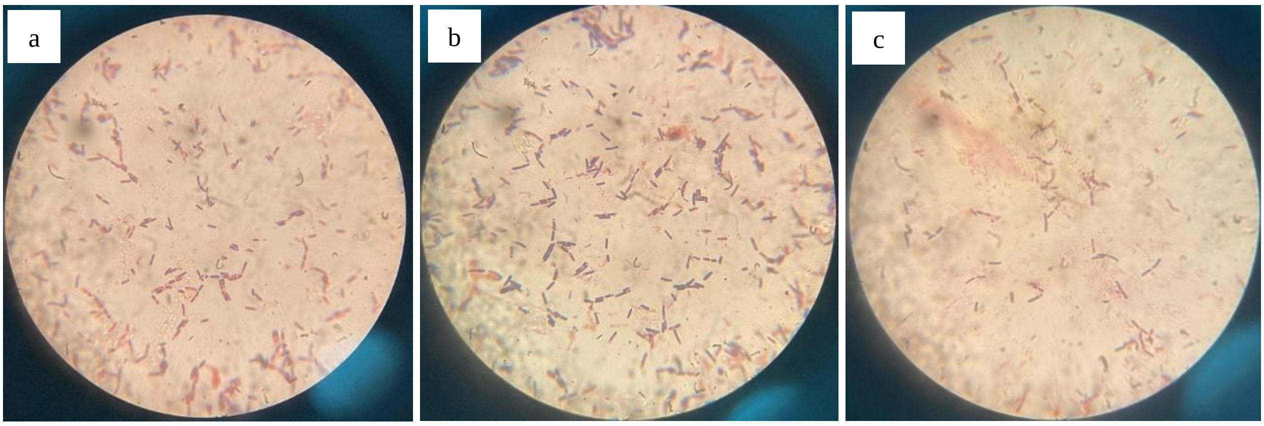

Figure 5. LAB microscopic characterization from MRS Agar. a. Sample; b. Control a; c. Control b

Table 1. Total protein and specific enzyme activity of each colony

	Concentration		
	Sample	**Control a**	**Control b**
Protein Total	18.56 mg/ml	18.85 mg/ml	16.71
Enzyme Activity Test	1.765 U/ml	8.156 U/mL	0.441
Enzyme-Specific Activity Test	0.095 U/mg	0.432 U/mg	0.026

Table 2. Specific enzyme activity at various pH variations

pH Variations	Enzyme Activity (U/ml)	Enzyme Specific-Activity (U/mg)	Enzyme Activity (U/ml)	Enzyme Specific-Activity (U/mg)	Enzyme Activity (U/ml)	Enzyme Specific-Activity (U/mg)
	Sample		Control a		Control b	
pH 4	0.041	0.002	0.031	0.001	0.012	0.0007
pH 7	1.714	0.092	8.236	0.436	0.472	0.028
pH 10	0.081	0.004	0.043	0.002	0.056	0.003

Table 3. Enzyme activity at various temperature variations

Temperature variations (°C)	Enzyme activity (U/ml)	Specific enzyme activity (U/mg)	Enzyme activity (U/ml)	Specific enzyme activity (U/mg)	Enzyme activity (U/ml)	Specific enzyme activity (U/mg)
	Sample		Control a		Control b	
2-8	0.034	0.001	0.012	0.00063	0.010	0.0006
25	0.865	0.046	0.882	0.046	0.041	0.002
37	1.765	0.095	1.237	0.065	**0.487**	0.029
50	1.891	0.101	1.769	0.093	0.432	0.025
70	**3.452**	0.185	**2.176**	0.115	0.332	0.019
90	0.753	0.040	0.652	0.034	0.278	0.017

Tables 1 and 2 demonstrate that lactase enzymes isolated from different sources share similar characteristics, with their optimal pH being pH 7. To determine another characteristic, namely the optimal temperature, the activity of the lactase enzyme was tested at various incubation temperatures while maintaining the optimal pH at 7 (Table 3).

Table 3 explains the optimum temperature of the lactase enzyme isolated from different sources. There is a notable difference in the optimum temperature between the two lactase enzymes. The lactase enzyme isolated from the petal stems of LAB birds exhibits an optimum temperature of 70°C, while the lactase enzyme isolated from commercial yoghurt and milk shows an optimum temperature of only 37°C. This discrepancy in optimum temperature could be attributed to differences in the LAB species isolated. LAB from commercial yoghurt typically consists of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, known for thriving at higher temperatures during yoghurt fermentation. Conversely, the specific type of LAB isolated from eggplant calyx stem is unknown, but its lactase enzyme displays thermostable characteristics due to its optimal temperature of 70°C.

The thermostability of the lactase enzyme from eggplant petal stems presents advantages for its application in the food industry. It can withstand high temperatures, making it suitable for cooking without degradation. This contrasts with the lactase enzyme from commercial yoghurt, which is optimal at lower temperatures. Despite the convenience of using *Lactobacillus species* in dairy product production due to their ease of isolation and the heating process involved before adding starter bacteria, the thermostable lactase enzyme from eggplant petal stems offers potential benefits for various food processing applications.

Enzyme activity is influenced by various factors such as temperature, pH, substrate concentration, and enzyme concentration. These factors can affect enzyme structure, particularly the conformation of the active site involved in substrate binding. Temperature, in particular, plays a crucial role in enzyme activity by influencing the reaction rate through increasing the energy of substrate molecules. Each enzyme has its unique characteristics, including its optimum temperature, which allows for the optimal conformation of the enzyme's active site to bind to a specific substrate. However, it's important to note that enzymes, being proteins, can experience decreased activity at high temperatures due to the denaturation of their three-dimensional structure [17].

Certain enzymes, particularly those isolated from LAB, exhibit thermostable characteristics, enabling them to withstand high temperatures. This thermostability is advantageous, as seen in the yoghurt production industry where LAB-derived lactase enzymes are utilized, capable of functioning at temperatures above 50°C. In this study, the results showed that both the sample and control A (commercial yoghurt) had the same optimum temperature of 70°C, indicating that the lactase enzyme isolated from LAB of purple eggplant stalks possesses thermostable characteristics. Conversely, control B (solely milk) showed an optimum temperature of 37°C, suggesting the presence of different LAB species.

Lactase enzymes can be synthesized not only by LAB but also by fungi. Further research is needed to determine the specific species of LAB present in the samples. Previous studies have shown that lactase enzymes isolated from *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have an optimum temperature of 70°C [22, 23], which aligns with the findings in control A and the sample. This indicates similarities in the characteristics of the lactase enzyme between control A and the sample, but additional research is required to confirm the LAB species in the sample.

Other research also states that the lactase enzyme can survive in the temperature range of 20-70°C, and each lactase enzyme has different characteristics, especially different optimum temperatures, and this can be caused by the structural conformation of the lactase enzyme. Apart from temperature, enzyme activity is also influenced by pH. Changes in pH can affect the ionization of amino acids in the active site of the enzyme, thereby affecting the conformation of the enzyme and the binding between the enzyme and the substrate. In this study, no difference was found in the optimum pH of the lactase enzyme from the two sources. A pH variation characterization test has been carried out with a pH range of 4-10, and the optimum pH obtained from samples control a and control b is pH 7 (Table 2). According to previous research reports, lactase can be active in the low pH range and tend to be alkaline, but almost all of the studies report that the optimum pH for LAB is 7 [24, 25, 26].

The presence of lactic acid bacteria (LAB) in milk fermentation can occur naturally or through inoculation from a starter culture. Milk serves as a natural habitat for LAB, and the spontaneous growth of LAB during the fermentation process has been observed over centuries of human practices in milk fermentation. Various types of LAB are commonly found in milk, including *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Enterococcus*. The specific microbiota present in milk can significantly influence the characteristics of the fermented product. During fermentation, microorganisms produce lactate, which impacts the texture, taste, and aroma of the final product. Additionally, the microbial composition of milk can affect its quality and storage properties and may also have implications for human health [27].

Lactose intolerance affects approximately 70% of the world's adult population and is characterized by limited expression of the lactase enzyme responsible for digesting lactose. Individuals with lactose intolerance often experience symptoms such as gastrointestinal discomfort after consuming lactose-containing foods. Treatment typically involves reducing or eliminating lactose from the diet until symptoms subside. However, cow's milk is a significant source of calcium and various essential vitamins and minerals. Therefore, individuals with lactose intolerance may find it challenging to obtain these nutrients from dairy products. Yoghurt presents a suitable option for individuals with lactose intolerance as it is lower in lactose compared to fresh milk. The fermentation process involved in yoghurt production partially breaks down lactose, making it easier to digest for individuals with lactose intolerance. Thus, yoghurt provides a way for individuals with lactose intolerance to still benefit from the nutritional components of milk while minimizing digestive discomfort [28].

CONCLUSION

This research has successfully demonstrated that purple eggplant stalks, a common household waste material, can serve as a viable source of lactic acid bacteria (LAB), which in turn can be utilized to produce new lactase enzymes. The deliberate choice of using milk as the initial medium for LAB growth on purple eggplant stalks stems from the ultimate objective of this research: to develop yoghurt suitable for consumption by individuals with lactose intolerance. By harnessing LAB from purple eggplant stalks, the aim is to create yoghurt products that are both natural and cost-effective, offering a practical solution for individuals with lactose intolerance. The optimal activity of the lactase enzyme isolated in this research occurs at pH 7 and an incubation temperature of 70°C. This characterization aligns with the lactase enzyme activity observed in the control group, specifically the result obtained from milk fermentation using commercial yoghurt. The similarity in lactase enzyme characteristics between the research sample and the control group underscores the potential of purple eggplant stalks as a valuable source of LAB and lactase enzymes for yoghurt production.

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