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Innovation in Bromelain Enzyme Production from Pineapple and Honey and Its Characteristics

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Abstract: Bromelain enzyme is one type of protease enzyme that has the ability to hydrolyze protein peptide bonds into amino acids. Bromelain enzyme can be found in pineapple (*Ananas comosus*) which consists of 95% cysteine protease mixture. Bromelain is often used by athletes to treat physical injuries or minor wounds. In obtaining the bromelain enzyme found in pineapple, an isolation process is required, namely by extracting it extracellularly. The method in this study does not go through the cell breakdown process so that it is easier to separate from other impurities and does not mix with other cell materials. This study aims to develop an innovative method in the production of bromelain enzyme using pineapple and honey as raw materials and to evaluate the organoleptic properties, pH, and the presence of lactic acid bacteria. This study is a descriptive study presented in the form of graphs and images. From the research that has been done, it can be concluded that the manufacture of bromelain enzyme using pineapple and honey has succeeded in producing a product that has an organoleptic value of liking (60%), pH 3 (acid) with the number of lactic acid bacteria 2.43 cells/ml.

Keywords: Bromelain enzyme; Elevation; Lactic acid bacteria; Pineapple (*Ananas comosus*)

Introduction

Bromelain enzyme is a type of protease enzyme that has the ability to hydrolyze protein peptide bonds into amino acids, making them easier for the body to digest (Tacias-Pascacio et al., 2024; Bahri et al., 2021). For years, Bromelain has been used in traditional medicine to treat various health problems (Nurnaningsih & Laela, 2022; Locci et al., 2024). Bromelain enzyme has been shown to be very helpful in curing upper respiratory tract infections such as bronchitis and sinusitis (Colletti et al., 2021; Desrosiers et al., 2011). Another use of bromelain enzyme is that it can stop Streptococcus mutans bacteria and plaque from growing in the teeth (Mosaddad et al., 2023; Janvier et al., 2024). Pineapple is one of the shrubby fruit plants with high levels of bromelain (Ujiani & Marhamah, 2019; Syahputra et al., 2024). The bromelain enzyme in pineapple is 95% a mixture of cysteine proteases that are heat resistant and can hydrolyze proteins (proteolysis) (Nineu et al., 2023; Santos et al., 2020).

In order to obtain the bromelain enzyme found in pineapple, an isolation process is required, namely by extracting it extracellularly. This method does not go through the cell breakdown process so that it is easier to separate from other impurities and does not mix with other cell materials. Cell residues are separated from this extracellular enzyme through a centrifugation process where this process is the initial stage of enzyme purification (Sari et al., 2022; Liu & Smith, 2021). According to Sebayang et al. (2017), honey is a thick brown liquid collected by Apis mellifera bees in their mothers.

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Diastase, invertase, glucose oxidase, catalase, glucosylceramidase, α-amylase, α-glucosidase, and protease are enzymes found in honey (Alaerjani et al., 2022; Alshareef et al., 2022). A study by Peixoto et al. (2016), Mahrus et al. (2023), and Al-Dhabi et al. (2020) found that products derived from pineapple and honey extracts contain proteolytic enzymes such as bromelain, trypsin, and chymotrypsin. From the data obtained, protease enzymes are found in pineapple and honey, therefore researchers conducted a study entitled "Innovation in Bromelain Enzyme Production from Pineapple and Honey and Knowing the Organoleptic, pH, and Lactic Acid Bacteria Tests".

Method

Research Location

This research was conducted from November 2023 to December 2023 at the Microbiology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Padang State University.

Tools and Materials

The tools and materials used in this study consisted of petri dishes, stereo microscopes, bromelain enzymes from pineapple and honey, object glass, MRSa medium, measuring cups, Erlenmeyer flasks, stirring rods, spreaders, laminar air flow, distilled water, Bunsen, alcohol, ose needles, crystal violet, hotplates, autoclaves, micropipettes, litmus paper, dropper pipettes.

Research Procedure

Making Bromelain Enzyme

Unripe pineapple is prepared then cut into small pieces including the stem. Then the pieces are put into a glass bottle filled with honey with an airtight lid. Make sure the pineapple pieces are evenly submerged in honey. Leave it for 40 days at room temperature. The enzyme that is ready to be harvested is filtered from the pulp. The enzyme is stored in a glass bottle and then put in the refrigerator.

Making MRSa Medium

Making MRSa medium is done by heating 13.64 grams with 200 ml of distilled water in an Erlenmeyer flask and then homogenizing it using a stirring rod on a hotplate. After that, it is left at room temperature and then given a stopper and aluminum foil. Then transferred to an autoclave for sterilization. After sterilization, transfer the MRSa medium into a petri dish until solid.

Sterilization

To prevent contamination during the study, tools made of glass and medium are sterilized using an autoclave at a temperature of 121 ^{o}C. While tools that cannot withstand high heat such as micropipettes are sterilized using 70% alcohol. Then the ose needle and spreader are sterilized using a bunsen.

pH Test

In the pH test, prepare the harvested bromelain enzyme and then test it using litmus paper. If the litmus paper is red it will produce an acidic pH and if the litmus paper turns blue it will produce an alkaline pH.

Organoleptic Test

Organoleptic test is conducted to determine the level of preference of respondents for the taste of the bromelain enzyme produced. The number of respondents needed for this test is 10 people with 4 parameters including, very much like, like, neutral and dislike.

Macroscopic Test of Lactic Acid Bacteria

Macroscopic test is conducted using a stereo microscope by looking at the color, elevation, margin and colony of lactic acid bacteria.

Microscopic Test of Lactic Acid Bacteria

In this test, the first stage carried out after the tools and materials are sterilized is to grow lactic acid bacteria from the bromelain enzyme into a petri dish using a micropipette. After being left for 48 hours, the lactic acid bacteria culture is taken using an ose needle to be spread on a glass object and then dripped with distilled water. Then fix it on a bunsen burner until it dries. Then continued with the coloring stage which was dripped by crystal violet for 60 seconds. Then flowed with running water and made microscopic observations.

Data Analysis

This research is a descriptive study, the data is presented in the form of graphs and images.

Result and Discussion

Pineapple plants (*Ananas Comosus* (L.) Merr) belong to the Bromeliaceae family and contain a protein known as bromelain or sulfhydryl proteolytic enzymes. Protease enzymes are one type of enzyme that plays a role in protein hydrolysis. One source of protease enzymes is pineapple (Dzulqaidah et al., 2021; Abril et al., 2023). Bromelain enzymes in pineapple plants can be found in the skin, crown, leaves, stems, tubers and flesh of pineapple tubers have a high bromelain content (Alfiyanti et al., 2020). This enzyme is one of the protease enzymes that can hydrolyze proteins (Mao et al., 2023; Irazoqui et al., 2024). Honey is a sweet liquid produced by bees where this honey is stored in honeycomb cells.

Honey contains minerals and salts such as iron, sulfur, magnesium, calcium, potassium, chlorine, sodium, phosphorus, and sodium, as well as antibiotics and digestive enzymes. Honey contains many minerals such as sodium, calcium, magnesium, aluminum, iron, phosphorus, and potassium. The vitamins found in honey are thiamine (B1), riboflavin (B2), ascorbic acid (C), pyridoxine (B6), niacin, pantothenic acid, biotin, folic acid, and vitamin K. Meanwhile, important enzymes in honey are diastase, invertase, glucose oxidase, peroxidase, and lipase (Osés et al., 2024). A study by Peixoto et al. (2016) found that products derived from pineapple and honey extracts contain proteolytic enzymes such as bromelain, trypsin, and chymotrypsin. From these data, researchers focused on pH tests, organoleptic tests, and lactic acid bacteria tests of bromelain enzymes derived from pineapple and honey.

pH Test

In the pH test, the bromelain enzyme produced had an acidic pH of 3. This is because the longer the fermentation process occurs, the more acid is produced. As the fermentation process progresses, lactic acid bacteria have more time to convert nutrients in the substrate and result in a decrease in pH value (Varilla et al., 2021).

Organoleptic Test

Organoleptic is a test of food ingredients based on preferences and desires for a product (Tuorila, 2015). Organoleptic testing utilizes the ability of the human senses to measure consumer acceptance of the goods being studied or felt (Juramang et al., 2023). Organoleptic testing is very important for quality implementation. The results of the organoleptic test can be seen in Figure 1.

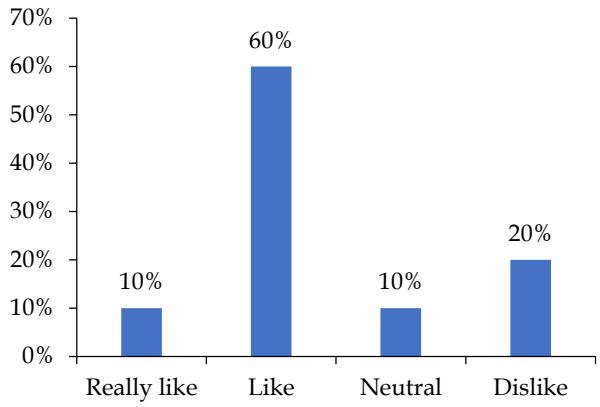


Figure 1. Organoleptic test results

Based on the graph above, there are 10 respondents who showed results of 10% really like, 60% like, 10% neutral, and 20% do not like the fermentation of bromelain enzyme produced from pineapple and honey. Based on organoleptic tests conducted on 10 respondents, it was found that in general respondents stated that the bromelain enzyme made had a sweet and sour taste and was slightly sour. One factor that can determine whether a product is accepted or not by consumers is taste. Taste is determined by our tongue (Lamusu, 2018). There are four main taste properties of the tongue, including sour, salty, sweet, and bitter (Spaggiari et al., 2020).

Lactic Acid Bacteria (LAB) Test

Macroscopic testing is a test that observes the shape and color observed visually (Cahya & Prabowo, 2019). The results of the macroscopic test of lactic acid bacteria can be seen in Table 1.

Table 1. Results of Macroscopic Observations of Lactic Acid Bacteria

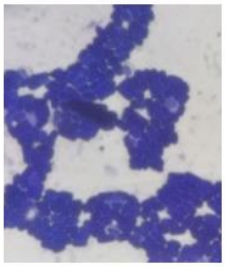
Picture	Elevation	Margin	Colony	Number of colonies	Color
	Convex	Smoot, Entire	Round	2.43 Cell/ml	White

Based on the table above, lactic acid bacteria have a convex elevation with a total of 2.4 × 103. Where according to Savitri et al. (2024), stated that the elevation in the colony that looks convex is called convex elevation. These lactic acid bacteria have smooth margins, are entire, have round colonies, and are white. Microscopic testing is a morphological test that requires a microscope in its observation. The results of the microscopic test of lactic acid bacteria can be seen in Table 2.

Based on the Table 2, lactic acid bacteria are classified as gram-positive bacteria that have a bacillus shape with a streptobacil arrangement. According to Widowati et al. (2015) stated that Lactobacillus is gram-

positive, catalase-negative, acid-tolerant, non-sporing, low G + C, and helps lactic acid from glucose substrates. The lactate fermentation process occurs when bacteria produce various food products, and is very important for safety and hygiene. Lactic Acid Bacteria (LAB) are a type of bacteria that produce lactic acid as the main metabolite (Ayivi et al., 2020).

Table 2. Results of Observations of Microscopic Tests of Lactic Acid Bacteria

Picture	Gram Type	Form	Composition
	Gram Positive Bacteria	Basil	Streptobacil

In addition to lactic acid, LAB also produces other active metabolites such as ethanol, hydroperoxides, bacteriocins (Ibrahim et al., 2017) and Exopolysaccharides (EPS) (Caggianiello et al., 2016) Lactic acid bacteria (LAB) involve varieties of bacteria that generally live at both mesophilic and thermophilic temperatures. LAB can grow at temperatures ranging from 10-45 °C, including types such as Lactobacillus, Enterococcus, Leuconostoc, and Pediococcus (Putri & Anita, 2017). Some examples of LAB strains include mesophilic groups with optimal temperatures of around ± 40 °C, such as Lactobacillus bulgaricus and Leuconostoc. There are also LAB strains that are optimal at temperatures around ± 50 °C, such as Enterococcus and Streptococcus thermophilus. In the pharmaceutical and food fields, bromelain is a very important and beneficial main component of pineapple.

Bromelain and fissin have the same protein-breaking function. The bromelain enzyme in pineapple is also considered very beneficial for individuals who have a cough. Bromelain has been shown to be beneficial in curing upper respiratory tract infections, such as bronchitis and sinusitis. In addition, the bromelain enzyme in pineapple can function as an anti-inflammatory agent, support digestion by lowering food in the stomach, inhibit the growth of cancer cells, inhibit platelet aggregation, and have fibrinolytic activity. Bromelain is often used by athletes to treat physical injuries or minor wounds. The bromelain enzyme found in pineapple is one of the natural ingredients that can prevent platelet aggregation (Fajarna et al., 2021). The bromelain enzyme is found in abundance in the stem (middle part) and stem of pineapple.

Conclusion

From the research that has been conducted, it can be concluded that the production of bromelain enzyme using pineapple and honey successfully produces a product that has an organoleptic value of liking (60%), pH 3 (acid) with a number of lactic acid bacteria of 2.43 cells/ml.

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Author Contributions
Conceptualization, W. Y.; methodology, I.; validation, Y. Y.; formal analysis, F. D. H.; investigation, D. K. N..; resources, N. P. A.; data curation, W. Y.: writing—original draft preparation, I; writing—review and editing, Y. Y.: visualization, F. D. H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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