Growth Ability of *Lactobacillus plantarum* and *Pediococcus acidilactici* at Various Concentrations of Banana Stem Flour

Ni Made Ayu Suardani Singapurwa[1*], *Ni Wayan* Nursini[2], *Purwaningtyas* Kusumaningsih[2], *I Putu* Candra[1], *A.A. Made* Semariyani[1], *Putu Diah* Wahyuni[1], *and Ni Wayan Yulia* Andriani[1]

[1]Food science and Technology, Faculty of Agriculture Warmadewa University
[2]Nutrition Science, Dhyana Pura University

Abstract. Banana stems contain bioactive components in the form of carbohydrates and dietary fiber prebiotics which have the potential as a fermentation medium for the growth of Lactic Acid Bacteria. The purpose of this study was to determine the ability to grow *Lactobacillus plantarum* and *Pediococcus acidilactici* in a banana stem flour fermentation medium. This study used a completely randomized design with the treatment of banana stem flour concentration Based on the results of the research, the characteristics of banana stem flour are as follows: moisture content of 6.59%, ash content of 16.44%, protein content of 0.12%, fat content of 3.20%, acidity degree of 7.29, total food fiber content of 61.66%, and carbohydrate content of73.29%. Based on the prebiotic test on the growth of *Pediococcus acidilactici* and *Lactobacillus plantarum* which increased in line with the increase in the concentration of carbon source (stone banana stem flour) given. The highest or most optimal total microbes result in thegrowth of *Pediococcus acidilactici* at a concentration of 0.6%, namely 4.6 x 10^{10} CFU/mL, and for *Lactobacillus plantarum* bacteria at a concentration of 0.2%, namely 1.3 x 10^9 CFU/mL. Therefore, stone banana stem flour (*Musa balbisiana* Colla) can be concluded to have the ability as a source of prebiotics.

1 Introduction

Prebiotics are a type of substance that cannot be digested by the human body but can be afood source for good bacteria in the digestive tract. Lactic acid bacteria have an important rolein maintaining the health of the human gut and digestive system [1]. Prebiotics play a key role in supporting the growth of these bacteria, thereby improving the health of the digestive tract and immune system [2]. The utilization of local natural

* Corresponding author: a.suardani@gmail.com

resources for the development of prebiotic ingredients is an interesting research focus to improve human gut health. One potential natural resource is the banana stem, which is often considered agricultural waste and underutilized. Banana stem flour contains dietary fiber, non-digestible compounds, and oligosaccharides, all of which have the potential as prebioticingredients [3].

Stone banana (*Musa balbisiana* Colla) is one of the wild banana species that has many seeds and is diploid. Parts of the banana plant that can be utilized are fruit, leaves, stump, stem, and heart [4]. Young banana stems have not been optimally utilized by the community. Banana stems have high fiber, and according to nutritionists they contain serotonin, norepinephrine, tannin, hydroxy tryptamine, vitamin B, dopamine, vitamin A, potassium, vitaminC, and sugar [5]. Banana stems contain cellulose, hemicellulose, and lignin. Raw banana stem fiber contains 21.1% hemicellulose, 48.0% cellulose, and 15.7% lignin, while the dignified residue contains 3.2% lignin, 7.6%hemicellulose, and 79.1% cellulose [6]. Another study mentioned that banana pseudo-stemfiber is mostly composed of cellulose, with approximately 50% cellulose, 30% hemicellulose,and 18% lignin [7]. Banana stems have a high nutritional composition withcrude protein of 3% including amino acids, nitrate amines, glycosides, N-containing, glycolipids, B vitamins, nucleic acids, extract material without nitrogen of 28.15%, ash of 25.12%, dry matter of 87.7%, the crude fiber of 29.40%, and crude fat of 14.23%. One of the cellulose contents in banana stems is alpha-cellulose, which has high fiber tensile strength and glossiness [8]. Flavonoids are known to be present in banana stems, and the majority of these chemicals have antioxidant properties [9].

The utilization of banana stem flour as a prebiotic ingredient has the potential to makea positive contribution to agricultural waste management while improving digestive health and overall human well-being. This study aims to determine the ability of banana stem flour as a prebiotic against lactic acid bacteria species *Lactobacillus Plantarum* and *Pediococcus acidilactici*.

2 Research methods

2.1 Place and time of research

This research was conducted at the Processing Laboratory and Food Analysis Laboratory of the Faculty of Agriculture, Warmadewa University, Denpasar City, Bali Province, and the Testing and Calibration Laboratory of the Agro Industry Centre, Bogor City,West Java. This research was conducted from July to September 2023.

2.2 Material and tools

2.2.1 *Material*

The materials used in this study were stone banana stems aged 6-8 months with a banana tree height of 165-175 cm obtained from Asahduren Village, Pekutatan District, Jembrana Regency, Bali Province. The sample used for testing is stem flour. Materials used for analysis were distilled water, NaOH, H_2SO_4, lowry D, laury E, petroleum benzine solvent, NaCl 0.9%, de Man, Rogosa, and Sharpe Agar (MRSA) media, and distilled water.

2.2.2 *Tools*

The tools used in the study were Erlenmeyer, analytical balance (Ohaus), test tubes, scales, beakers, measuring cups, oven (Memmert), test tube rack, Furnace (Ney m 525), Micropipette (Joanlab), laminar air flow, incubator (Memmert), gloves, mask, stirring rod, blue tip, aluminum foil, volumetric flask, porcelain cup, desiccator (Duran), spatula, dropper pipette, filter paper, electric stove (Maspion), pH meter (Hanna), and funnel.

2.3 Research design

This study used a completely randomized design (CRD) with the treatment of stem flour concentration, namely 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1% concentration. There were a total of 18 treatment units in this trial due to the fact that the treatment was repeated three times.

2.4 Procedures

Removing the banana stem from the tree is the first step in the research process to make banana stem flour. Water, salt, and banana stem are the components utilized in the preparation stage. The first step in the procedure is to weigh the banana stem, which is then divided into pieces that range in thickness from 2.74 to 4.57 mm. For ten minutes, the sliced stems were immersed in a solution containing 2.5% salt in a 1:3 ratio, meaning that one kilogram of stems was soaked in three liters of the solution. Soaking in saline solution is meant to make cleaning and sap removal easier. After being freed of sap, banana stems are rinsed under running water to remove any remaining sap and salt taste.

After washing, the stems of bananas are drained. Steaming was the procedure used to blanch the banana stems, with a temperature treatment of 75˚C for 10 minutes. After blanching, banana stems are submerged in water for a duration of two minutes. This is to prevent over-blanching due to the heat generated during the blanching process. The wet banana stems were subsequently drained and dried. An oven was used for the drying procedure, which lasted two days (48 hours) at 48±2 ˚C and 84±5 RH% and 22±12 RH% of humidity, respectively, on the first day. A blender was used to break the dried banana stems, and a 100-mesh sieve was used to strain them, signs that a banana is dry.

2.5 Test parameter

Stem flour is tested for chemical characteristics with test parameters including pH [10] Ash content test moisture content test, carbohydrate content test by different, fat content test,protein content test, and food fibre content test. The stem flour was then tested microbiologically by calculating the Lactic Acid Bacteria (*Lactobacillus plantarum* and *Pediococcus acidilactici*) according to the treatment [11]. Observation data were statistically analyzed using Analysis of Variance (ANOVA). Ifthe test results showed an effect, then *Duncan Multiple Range Test* (DMRT) was conducted.

3 Results and discussion

Based on the results of the study, data on the chemical characteristics of stone banana stem flour were obtained as shown in Table 1. The characteristics of banana stem flour with oven drying method and blanching temperature of 75°C are as follows: moisture content 6.59%, ash content 16.44%, protein content 0.12%, fat content 3.20%, total dietary fiber content 61.66%, and carbohydrate content 73.29%.

Table 1. Chemical Characteristics of Stone Banana Stem Flour

No.	Observation Variable	Characteristics
1.	Water content (%)	6,95 ± 0,069
2.	Ash content (%)	16,44 ± 0,164
3.	Protein (%)	0,12 ± 0,001
4.	Fat (%)	3,20 ± 0,032
5.	Degree of acidity (pH)	7,29 ± 0,073
6.	Carbohydrate content (by difference) (%)	73,29 ± 0,733
7.	Dietary fibre content	61,66 ± 0,616

Water content is an important component in food ingredients because water affects the flavor, appearance, and texture of food (Winarno, 2008). According to [12]. the moisture content of wheat flour is 7.80%, and according to SNI 01-3751-2006, the maximum moisture content in flour is 14.5%. The moisture content of stone banana stem flour is 6.59%,which means it meets the SNI for flour. Moisture content determines the quality of flour. The higher the moisture content, the lower the amount of dry solids in the flour. Flour specifications usually limit the moisture content of flour to 14% or less, and flour with a moisture content of more than 14% will be unstable at room temperature. Organisms naturally present in flour willstart to grow at high humidity resulting in odors and flavors [13].

A higher mineral content in the diet is typically indicated by ash content. Technical culture elements in the field during cultivation or planting, such as soil type, climate, and fertilizer composition and intensity, affect a material's ash content [14]. The ash content of stone banana stem flour in the study was 16.44%. The high ash content of stone banana stem flour is due to its mineral composition and the presence of non-endosperm parts. The mineral composition of banana stem flour, such as potassium and copper, contributes to the high ash content [15].

Protein content in this study was tested by the Lowry method, which is a method carried out using a visible spectrophotometer and is used to test soluble protein levels or proteins thatcan be absorbed by the body. The protein content of banana stem flour was 0.12%. Banana stems are low in protein content. The crude protein content of banana stems ranges from 2.40%. During processing into flour, protein content can be further reduced due to several factors, including temperature and drying time. The fat content of stone banana stem flour was 3.20%. Stone banana stem flour is alsohigh in carbohydrate content, which is 73.29%. stated that banana stem (*Musa acuminata* balbisiana) contains a fat content of 2.15% and carbohydrate content of 79.16%. Banana stems do contain good nutrition so they are good for food processing. [16]

The dietary fiber of stone banana stem flour was 61.66%. Banana pseudo-stem flour contains a lot of dietary fiber, with an insoluble dietary fiber component of 64.49%, soluble dietary fiber of 2.58%, and total dietary fiber of 67.07% [17]. Dietary fiber inbanana pseudo-stem flour consists of cellulose, hemicellulose, and lignin [18] High dietary fiber such as that found in banana pseudo-stem flour has the potential to be a source of prebiotics [19]. Prebiotics are compounds, mainlyoligosaccharides, that resist digestion in the human small intestine and reach the colon where they are fermented by the gut microflora [20].

Research has shown that various dietary fibers, including inulin and fructo-oligosaccharides, have prebiotic effects and selectively modulate the gut microbiota, thereby providing health benefits [21]. Prebiotic dietary fibers act as a carbon source for the growth of beneficial bacteria in the gut, providing specific or selective changes that confer metabolic-related health benefits.

The prebiotic activity of stone banana stem flour in this study can be seen from the growth of *Lactobacillus plantarum* and *Pediococcus acidilactici* that use the substrateof stone banana stem flour as a carbon source in bacterial growth media. The growth of *Lactobacillus plantarum* and *Pediococcus acidilactici* based on this prebiotic test wascarried out by measuring the growth of *Lactobacillus plantarum* and *Pediococcus acidilactici in the* form of an increase in total plate count (TPC) results. Based on the results showed that there was an increase in the growth of *Lactobacillus Plantarum* and *Pediococcus acidilactici* with visible morphology of bacterial isolates which are round and shiny white. The results of the calculation of the average total lactic acid bacteria using theTPC method are shown in Table 2 as follows.

Table 2. Colony count results of *Lactobacillus plantarum* and *Pediococcus acidilactici* at VariousConcentrations of stone banana stem flour using the TPC method

Concentration	***Pediococcus acidilactici* (CFU/mL)**	***Lactobacillus plantarum* (CFU/mL)**
0% (Control)	$5.2 \times 10^{8} \pm 1.4 \times 10^{5}$ c	$2.7 \times 10^{8} \pm 1.1 \times 10^{5}$ c
0,2 %	$1.4 \times 10^{10} \pm 2.2 \times 10^{5}$ ab	$1.3 \times 10^{9} \pm 2.7 \times 10^{5}$ a
0,4 %	$8.5 \times 10^{9} \pm 1.8 \times 10^{5}$ b	$4.3 \times 10^{8} \pm 1.8 \times 10^{5}$ b
0,6 %	$4.6 \times 10^{10} \pm 1.7 \times 10^{5}$ a	$1.1 \times 10^{9} \pm 1.2 \times 10^{5}$ ab
0,8 %	$2.6 \times 10^{10} \pm 2.3 \times 10^{5}$ ab	$8.2 \times 10^{8} \pm 2.8 \times 10^{5}$ b
1,0%	$3.4 \times 10^{10} \pm 3.1 \times 10^{5}$ ab	$4.8 \times 10^{8} \pm 3.2 \times 10^{5}$ b
Negative control	-	-
Sample control	-	-

Description: The average value followed by the same letter on the same column shows an intangible difference at the 5% Duncan level (n=3)

Based on the results of the calculation of bacterial colonies in Table 2, all sample requirements for TPC calculation. From the results of the calculation of bacterial colonies, *Pediococcus acidilactici* with a concentration of 0.6% has the highest average in stem flour, namely 4.6×10^{10} CFU/mL. While *Lactobacillus plantarum bacteria* with a concentration of 0.2% has the highest average value of 1.3×10^{9}. The high totalbacteria in prebiotic treatment is because the prebiotic sources given can stimulate the growthof beneficial potential bacteria by providing substrates in the form of prebiotics that can be digested by bacteria so that their population increases and can fight pathogenic bacteria [22].

Stone banana stem flour (*Musa balbisiana* Colla) can be a carbon source for the growth of *Pediococcus acidilactici* and *Lactobacillus plantarum*, this is related to the high total dietary fiber content in stone banana stems, namely 46.057% The food fiber is included in the prebiotic food requirements [23]. The soluble fiber in the gastrointestinal tract will be fermented in the caecum by anaerobic bacteria to produce *Short Chain Fatty Acid* (SCFA) which will quickly be absorbed by colony dwellers asa source of energy for reproduction [24].

The positive control media that was not given stem flour in this study obtained an average result of 5.24 x 10^8 CFU/mL for *Pediococcus acidilactici* while *Lactobacillus plantarum* obtained an average result of 2.7 x 10^8 CFU/mL. The results of the positive control showed lower results than the results of the treatment of 0.6% stem flour in *Pediococcus acidilactici* and 0.2% in *Lactobacillus plantarum* with a significant difference. This shows that stone banana stem flour can become a carbon source medium that can encourage the growth of probiotic bacteria and shows that stone banana stem flour has the ability as a prebiotic source that can be compared with commercial prebiotic sources.

The absence of bacteria or flour in the negative control media during the prebiotic test revealed that the media was free from contamination. This was demonstrated by the absence of bacterial growth on the negative control media. The same outcomes were observed in the control sample of stone banana stem flour, which did not receive any bacteria. This sample was found to be uncontaminated, as evidenced by the absence of fungi or other bacteria growing in it.

Previous research by Hidayat [25] on total lactic acid bacteria in yogurt drink media with the addition of mango fruit extract showed an increase in total lactic acid bacteria. This is because the addition of mango fruit extract can provide excess nutrients for lactic acid bacteria growth. Based on theresults of the sugar profile test, the glucose content in drink yogurt without the addition of mango fruit extract is 0.729%, while with the addition of mango fruit extract, it is 0.5410%, which means it shows that lactic acid bacteria in drink yogurt with the addition of mango fruit extract can use glucose in mango fruit to increase its growth [25].

Based on the results of the study, prebiotic activity shows the ability of the substratein the form of a carbon source of stone banana stem flour to increase the growth of *Pediococcus acidilactici* and *Lactobacillus plantarum*. The optimal growth of bacteria at the concentration shows that at that concentration the stone banana stem flour is optimal as a sourceof prebiotics, which in this study is shown at a concentration of 0.6% stone banana stem flour in *Pediococcus acidilactici* and 0.2% in *Lactobacillus plantarum* bacteria which is themost optimal compared to other treatments.

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