POTENTIAL ADDITION OF *Lactobacillus casei* AND FLOUR OF AMBON BANANA TO INHIBIT PATHOGENS AT YOGURT

(POTENSI YOGURT DENGAN PENAMBAHAN *Lactobacillus casei* DAN TEPUNG PISANG AMBON DALAM MENGHAMBAT PATOGEN)

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ABSTRACT

This study aims to determine the effect of *Lactobacillus casei* and Ambon banana flour on the viability of lactic acid bacteria and inhibition of pathogens in yogurt. The studies used the Randomized Block Design (RBD) *2 factor*, starter type and ambon banana flour concentration. The starters type consisting of *Streptococcus thermophillus* and *Lactobacillus bulgaricus*; *S. thermophilus*, *L. bulgaricus* and *L. casei*. The banana flour concentration consisting of 0; 2.5; 5.0; 7.5 and 10%. Analyzed used one-way ANOVA and continued with Duncan's Multiple Range Test. Variables observed including the viability of lactic acid bacteria, inhibition of pathogen (*Escherichia coli* and *Staphylococcus aureus*), and yogurt preference. The results showed that the addition of *L. casei* to yogurt cultures decreased the viability of lactic acid bacteria and inhibition of *E. coli* but increased inhibition of *S. aureus*. Increasing the concentration of banana flour causes increased viability of lactic acid bacteria, inhibiting against *E. coli* and *S. aureus*. The highest lactic acid bacteria viability has resulted in 10% banana flour concentration. Yogurt without the addition of banana flour cannot inhibit *E. coli* and *S. aureus*. Increasing the concentration of banana flour added to yogurt causes a decrease in preference.

Key words: Ambon banana flour, *E. coli*, inhibition, *L.casei*, *S. aureus*

ABSTRAK

Penelitian bertujuan untuk mengetahui pengaruh penambahan *Lactobacillus casei* dan tepung pisang ambon terhadap viabilitas bakteri asam laktat dan kemampuannya dalam menghambat patogen pada yogurt. Penelitian menggunakan Rancangan Acak Kelompok (RAK) dengan 2 faktor pengujian yaitu jenis starter dan konsentrasi tepung pisang ambon. Data hasil penelitian dianalisis menggunakan ANOVA dan dilanjutkan uji Jarak Berganda Duncan. Jenis starter terdiri dari *Streptococcus thermophillus* dan *Lactobacillus bulgaricus*; *S. thermophilus*, *L. bulgaricus* dan *L. casei*. Konsentrasi tepung pisang terdiri dari 0; 2.5; 5,0; 7,5 dan 10%. Variabel yang diamati meliputi viabilitas bakteri asam laktat, penghambatan terhadap pathogen (*Escherichia coli* dan *Staphylococcus aureus*), dan tingkat kesukaan terhadap yogurt. Hasil penelitian menunjukkan penambahan *L. casei* pada kultur yoghurt menurunkan viabilitas bakteri asam laktat dan penghambatan terhadap *E. coli* tetapi meningkatkan penghambatan *S. aureus*. Peningkatan konsentrasi tepung pisang menyebabkan peningkatan viabilitas bakteri asam laktat, penghambatan terhadap *E. coli* dan *S. aureus*. Viabilitas bakteri asam laktat tertinggi dihasilkan pada konsentrasi tepung pisang sebesar 10%. Yogurt tanpa penambahan tepung pisang tidak dapat menghambat *E. coli* dan *S. aureus*. Peningkatan konsentrasi tepung pisang yang ditambahkan pada yogurt menyebabkan penurunan kesukaan.

Kata kunci: Tepung pisang ambon, *E. coli*, penghambatan, *L.casei*, *S. aureus*

INTRODUCTION

Probiotics, prebiotics, and synbiotics are functional foods currently being developed, especially in the food industry. Functional food can provide health benefits beyond the benefits provided by its nutrients. The concept of functional food emphasizes that food is vital for life and plays a role in preventing and reducing the factors that cause illness and improving critical physiological functions (Pandey et al., 2015).

Probiotics and prebiotics are functional food ingredients that have become the focus of great interest to the general public, the food industry, and scientists. The concept of food as medicine is being intensified, which is a way to optimize functional food to treat disease. Diarrhea is a digestive tract disease that is still a severe problem, especially in developing countries like Indonesia. Diarrhea is also a significant disease in infants and a leading cause of death in infants and children under five years apart from pneumonia (Radlović et al., 2015). Bacteria that cause diarrhea include *Escherichia coli*. Besides *E. coli*, *S. aureus* could cause foodborne diseases. The type of illness caused by *S. aureus* is intoxication. Lactic acid bacteria, especially probiotics, have been known to play a role in inhibiting pathogens (Annuk et al., 2003, Mohammaddoost Chakoosari et al., 2015, Rahayu et al., 2011).

Prebiotics can increase lactic acid bacteria growth. Prebiotics that digestive enzymes can not hydrolyze in the human digestive tract will be utilized by good bacteria in the large intestine and increase this good bacteria, which improves their host's health (Davani-Davari et al., 2019). Inulin and fructooligosaccharides were known as prebiotics, found in leeks, onions, wheat, asparagus (*Asparagus officianalis*, garlic, Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichoriumintybus*) root (Abed et al., 2016). Inulin can selectively stimulate the intestinal bacteria's growth and activity associated with health and well-being. Synbiotics are food products that contain probiotics and prebiotics.

Synbiotic products are believed to provide a better effect because they deliver probiotics beneficial to their host and ingredients needed for probiotic growth. Tari & Handayani (2015) stated that probiotic yogurt and purple sweet potato reduced enteropathogenic *E. coli* by 4 log cycles for two weeks of storage at 4ºC in vitro. Tari et al. (2016) stated yogurt with the addition of purple sweet potato can reduce diarrhea in mice that have been infected with enteropathogenic *E. coli*.

Banana (*Musa paradisiaca*) contains lots of inulin and fructooligosaccharides. Inulin is considered an indefinitely usable prebiotic model. Bananas contain about 1% inulin (Hardisari & Amaliawati, 2016), while Moshfegh et al. (1999) state bananas contain about 0.3-0.7%. Furthermore, Hardisari & Amaliawati (2016) conducted a study on using kepok banana as a prebiotic agent to increase the viability of *Lactobacillus casei* in de Mann Roguse and Sharpe (MRSB) media. The results showed increased kepok banana flour concentration, which was added to MRSB media from 2% to 10%, increasing *L. casei* cells biomass.

This study evaluates the effect of the addition of *L. casei* and ambon banana flour in yogurt manufacturing to lactic acid bacteria viability and inhibiting pathogens. The enteric pathogens used consisted of *E. coli* and *S. aureus*. The novelty of ambon banana flour research as a prebiotic agent increases lactic acid bacteria's growth and inhibits enteric pathogens.

RESEARCH METHODS

Equipment

Tools used in this research include autoclave (Hi Clave, Hirayama), oven sterilizer (Heraeus, Type UT 5042 EK), UV-Vis spectrophotometer (Shimadzu-UV 1650 PC), centrifuge (Heraeus, Labofuge 200), pH meter (Thermo scientific), incubator (Memmert), vortex (Thermoline, type 37600), transferring pipette (Ependove), laminar airflow (Gelman, Sciences type BH 20), digital scale, deep freezer, refrigerator and glassware such as beaker glass, erlenmeyer, test tube, and petri dish.

Bacteria and chemicals

S. thermophillus FNCC 0040, *L. bulgaricus* FNCC 0041, *L casei* FNCC 0090, *E. coli* FNCC 0091, *S. aureus* FNCC 0047 were obtained from Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta. The medium and chemicals used include MRS (Merck), glucose (Merck), $CaCO_3$ (Merck), NaCl (Merck), Rochelle

Salt (Merck), $NaHCO_3$ (Merck), Na_2SO_4 (Merck), $CuSO_4$ $5H_2O$, H_2SO_4 (Merck). All chemicals used were pro analyze grade.

Analysis of variables

Statistical analyses performed using one-way analysis of variance (ANOVA) followed by the Duncan Multiple Range Test (DMRT) to compare several means. Values of $p<0.05$ were considered significant.

Banana flour manufacturing

The manufacturing of banana flour refers to Hardisari & Amaliawati (2016) with modifications. The modification was done by adding a blanching treatment (steam blanching). Blanching is done on unpeeled bananas. Ambon bananas are chosen which have the optimum maturity, namely yellow banana peels, but the structure is still a bit hard. After blanching, the banana is peeled and thinly sliced, dried using a cabinet dryer at 80°C until dry, and then the siege was carried out. The flour is sieved using an 80 mesh siever.

Yogurt manufacturing

First, probiotics yogurt manufacturing is done by making a starter culture of lactic acid bacteria followed by making yogurt. Preparation bacterial starter cultures refer to Jimenez-Diaz et al. (1993) and Tari & Handayani (2015). Making yogurt refers to Tari et al., (2016), while banana flour refers to Hardisari & Amaliawati (2016). Fresh milk (200 mL) was added with 2% (w/v) of skimmed milk, 10% sugar and banana flour (with concentrations of 0; 2.5; 5; 7.5 and 10% w/v). The mixture of ingredients sterilized at 115°C for 10 minutes, then cooled to 40-45°C. Working culture of *S. thermophillus*, *L. bulgaricus* with and without *L. casei* as much as 5% (v/v) inoculated to the mixture. Comparing *S. thermophillus* and *L. bulgaricus* was 1:1, while comparing *S. thermophillus*, *L. bulgaricus*, and *L. casei* was 1:1:1. The mixture was then shaken until homogeneous and incubated in the incubator at 40°C for 18 hours to produce yogurt. The resulting yogurt was then analyzed, including the number of lactic acid bacteria and the ability to inhibit *E. coli* and *S. aureus*.

The number of lactic acid bacteria cells was carried out using the pour plate count method by adding 1 ml of the sample containing lactic acid bacteria into 9 ml of sterile 0.85% NaCl. Then the solution was taken as much as 1 ml and then carried out a series of dilutions. From each dilution series, planting to MRS agar supplemented with $CaCO_3$ then incubation at 37°C for 48 hours. Lactic acid bacteria that grow will form a clear zone around the colony. The colonies formed were then counted using a standard plate count.

In vitro inhibition testing of pathogens was carried out using the diffusion method to refer to Tari et al., (2016). A starter of pathogenic bacteria was poured on NA media in petridish and stored for 1 hour at 4°C in the refrigerator to hardens. Then a well was made for each pathogenic bacterial culture. A total of 50 µL of synbiotic banana-yogurt starter was added to the well and incubated at 37°C for two days. A clear zone around the well indicates the inhibitory ability of pathogens by the activity of lactic acid bacteria in yogurt.

Preference Test

The preference test for yogurt was carried out using the scoring method. The panelists used were semi-trained panelists of 20 people

RESULTS AND DISCUSSION

Lactic Acid Bacteria

Bananas contain inulin and FOS, known as prebiotics (Thammarutwasik et al., 2009). Prebiotics are substrates used by lactic acid bacteria to stimulate their growth but cannot be digested in the human digestive tract. Banana flour also contains simple sugars used as a carbon source to grow lactic acid bacteria.

The results showed that a starter consisting of *S. thermophillus* and *L. bulgaricus* produced higher viability of lactic acid bacteria than a starter composed of *S. thermophillus*, *L. bulgaricus*, and *L. casei*. The viability of lactic acid bacteria in various starter types is shown in Figure 1.

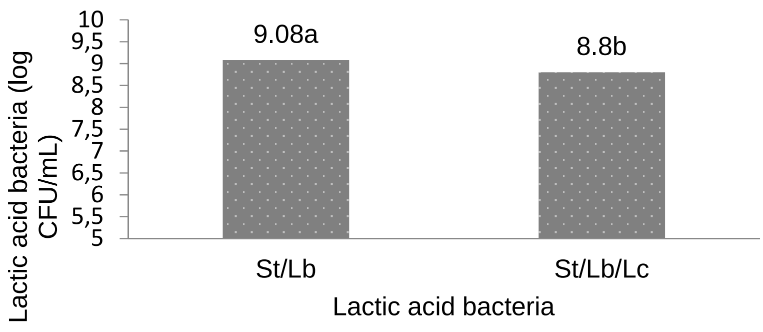


Figure 1. A total number of lactic acid bacteria in various starter types.

Figure 1 shows that starter types affected the viability of lactic acid bacteria. The more diverse of starters suspected provide higher competition usage of nutrients. Setiarto et al., (2017) state that using a different starter in making synbiotic yogurt with taro flour produces different amounts of yogurt lactic acid bacteria.

An increase in ambon banana flour concentration caused enhancement of lactic acid bacteria in yogurt. The number of lactic acid bacteria in the variation of ambon banana flour concentration is shown in Figure 2.

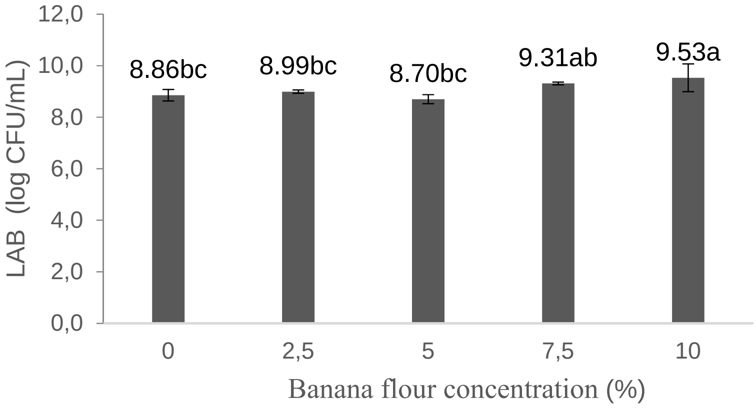


Figure 2. The number of lactic acid bacteria at various banana flour concentrations.

Increasing banana flour concentration from 7.5% to 10% enlarges lactic acid bacteria viability. The number of lactic acid bacteria at the beginning of fermentation was 7.35 log CFU/mL. During 24 hours of fermentation at 37^{o}C, the cells increased from 1.51 to 2 log cycles. The 10% of banana flour concentration produced the highest level of lactic acid bacteria (9.53 log CFU/mL). Growing of cells is influenced by various factors, such as nutrients. The higher banana flour concentration enhances simple sugars and prebiotics content needed for lactic acid bacteria growing. Hapsari & Lestari (2016) reported that bananas contain carbohydrates as much as 16,72 -35.24 g /100g. Some of these carbohydrates are sucrose, fructose, and glucose, which needed probiotic bacteria's growth. The results also align with Kumalasari et al., (2013). They state that the increasing of longan extract in yogurt's manufacturing increases the number of lactic acid bacteria caused by the rise in simple sugars in longan extract. Hardisari & Amaliawati (2016) reported that adding kepok banana flour from 0 to 10% into MRS media increased the *L. casei* cell. According to Hardisari & Amaliawati (2016), an increase in kepok banana flour concentration increases prebiotics (inulin and FOS) as a nutrition source, resulting in higher *L. casei* cells. Pandey et al. 2015) stated that lactic acid bacteria's growth could improve by prebiotics.

The inhibition against *E. coli and S. aureus*

Digestive tract infection is one of the diseases that is still a problem in Indonesia (Tari et al., 2016). *E. coli* is known as the bacteria that cause diarrhea. Lactic acid bacteria is one of the bacteria that can inhibit *E. coli*. This research showed that the addition of *L casei* to yogurt culture starter resulted in different inhibitions of *E. coli* and *S. aureus*. The inhibition of *E. coli* and *S aureus* in the various starters type is shown in Figure 3.

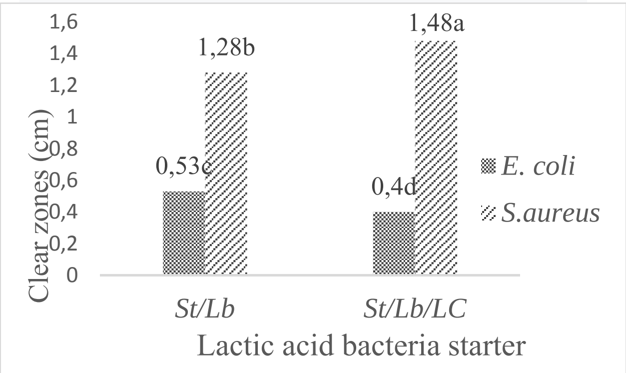


Figure 3. Inhibition of *E. coli* and *S. aureus* in various starter types.

Figure 3 showed that the inhibition zone of *S. aureus* is more extensive than *E. coli*. *S. aureus* is a Gram-positive bacteria with a simpler cell wall structure than Gram-negative bacteria (*E. coli*). The cell wall of Gram-positive bacteria consists of peptidoglycan and teichoic acid (Fardiaz, 1989). Gram-negative bacterial cells have a multi-layered structure and relatively higher fat content (11-12%), resulted in more resistance to environmental changes (Pelezar and Chan, 1986). An outer membrane of Gram-negative bacteria composed of 30% lipoproteins, 40-45% phospholipids, and 40-45% protein better survive against the external environment antibacterial compounds (Tari et al., 2015).

Lactic acid bacteria can produce antimicrobial compounds that are known to inhibit pathogens. The antimicrobial compounds produced by lactic acid bacteria include organic acids, hydrogen peroxide, carbon dioxide, and bacteriocins (Pınar & Yalçın, 2015). Bacteriocins produced by lactic acid bacteria can inhibit Gram-positive and negative bacteria (Jiménez-Diaz et al., 1993; Aslam et al., 2011). The inhibition of *E. coli* and *S aureus* increased with the enhancement of banana flour concentration. The inhibition of *E. coli* and *S aureus* in the variation of banana flour concentration is shown in Figure 4.

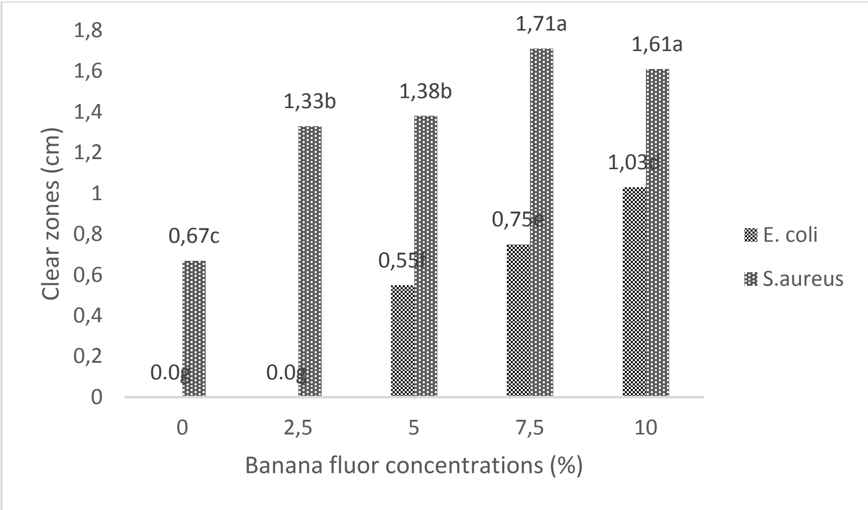


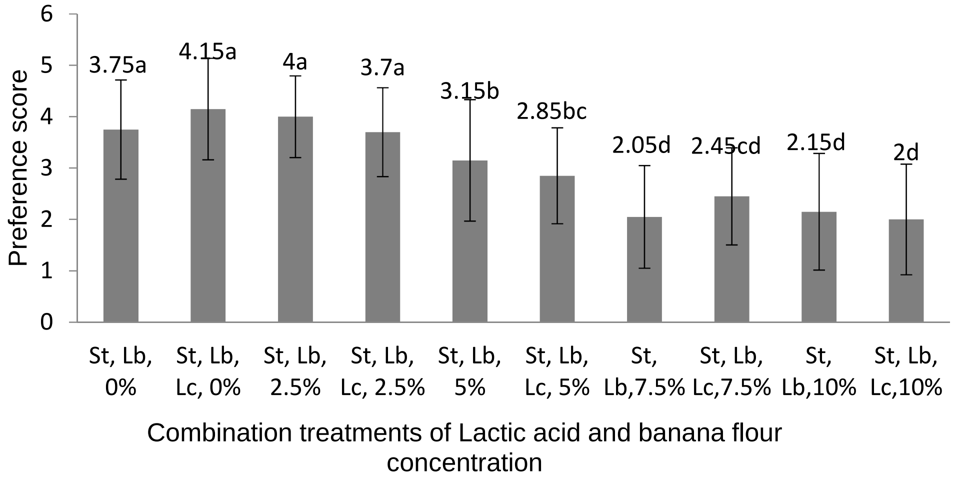
Figure 4. Inhibition of *E. coli* on variations banana flour concentration.

Enhancement of banana flour concentration increased the pathogen's inhibition, indicated by the larger of clear zones. Inhibition of *E.coli* was produced by 5 to 10% banana flour concentration, while 2.5 to 10% of *S. aureus*. Yogurt with 2.5% addition of banana flour concentration and without the addition of banana flour could not inhibit *E.coli*, while the addition of 2.5% banana flour could inhibit *S. aureus*. This result indicated that the metabolic products of lactic acid bacteria in yogurt and banana flour more inhibit *S aureus* than *E. coli*.

According to Tari et al. (2015), Davis & Stout (1971), antibacterial activity is determined based on the bacterial inhibition zone's diameter against indicator bacteria. Generally pathogenic bacteria with a range of> 20 mm = very strong, 10-20 mm = strong, 5-10 mm = moderate and <5 mm = weak. Based on these criteria, yogurt with the addition of 5-10% banana flour has moderate to intense inhibitory activity. Lactic acid bacteria can produce antibacterials, including nisin, bacteriocin, and lactic acid.

Yogurt preference

The result showed that the type of starter and banana flour concentration significantly affected yogurt's preference. The effect of starter type treatment and banana flour concentration on yogurt preference is shown in Figure 5.



St: *Streptococcus thermophillus*
Lb: *Lactobacillus bulgaricus*
Lc: *Lactobacillus casei*

Preference score:
1: extremely don't like
2: don't like
3: rather like
4: like
5: extremely like

Figure 5. The preference of yogurt on the treatment of variations of starter types and banana flour concentration.

The difference in impression or level of liking for yogurt is related to yogurt taste. The acidity products strongly influence the overall acceptance level of fermented products with LAB starter. The increasing level of acidity decreases the panelist acceptance level. The highest yogurt preference is 4.15, produced from *S. thermophillus*, *L. bulgaricus*, and *L. casei* without banana flour, while the lowest is 2.0 (yogurt with *S. thermophillus* and *L. bulgaricus*, 7.5% banana flour). These results were not significantly different from yogurt in the addition of 2.5% banana flour with and without *L. casei*, so it functions as a prebiotic yogurt. An increase in banana flour concentration caused decreased preference due to banana flour producing a higher sour taste and a rougher texture. The increasing level of acidity in yogurt decrease the panelist acceptance level.

CONCLUSION

The addition of *L. casei* to yogurt cultures (*S. thermophillus* and *L. bulgaricus*) decreased the viability of lactic acid bacteria and inhibited *E. coli* but increased the inhibition against *S. aureus*. An increase in banana flour concentration caused decreased preference due to banana flour producing a higher sour taste and a rougher texture. The addition of *L.*

casei and 2.5% ambon banana flour made synbiotic yogurt which the panelists favored

Conflict of interest

The authors declare that there are no conflicts of interest.

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