



The Effect of Different Temperatures on Addition of Powdered *Lactobacillus plantarum* Dad-13 on Peanut Chocolate Jam Towards Total Lactic Acid Bacteria (LAB), Sublethal Injury, pH, and Total Acids

Rizka Amalia Rahayu, Rafli Zulfa Kamil*, Anang Mohamad Legowo

Department of Food Technology, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia

*Corresponding author (rafli.zulfa@live.undip.ac.id)

Abstract

Lactobacillus plantarum Dad-13 is an indigenous probiotic that was isolated from Dadih. Peanut chocolate jam has the potential to be developed into a probiotic food product. However, peanut chocolate jam involves a heating process which can affect the number of probiotics and their metabolic processes. This research aims to determine the optimal temperature of *L. plantarum* Dad-13 probiotic powder in addition to peanut chocolate jam products. The different temperatures of probiotic powder *L. plantarum* Dad-13 addition at 30°C, 40°C, 50°C, and 60°C were used to measure total lactic acid bacteria (LAB), sublethal injury, pH value, and total acids. Total lactic acid bacteria and sublethal injury were measured by Total Plate Count (TPC), pH value was measured by pH meter and total acids were analyzed using the titration method. Results show significant differences ($p < 0.05$) on total lactic acid bacteria (LAB) and sublethal injury. Total lactic acid bacteria (LAB) resulting in a value of 8.04 ± 0.02 to 7.67 ± 0.05 and sublethal injury 0.04 ± 0.01 to 0.26 ± 0.03 . Meanwhile, no significant differences ($p > 0.05$) in pH value and total acids. The best temperature of *L. plantarum* Dad-13 probiotic powder addition on peanut chocolate jam was at 30–40°C because it showed the highest amount of total lactic acid bacteria (LAB) and the least sublethal injury.

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Introduction

The development of the globalization era affects the lifestyle of Indonesian, including daily food patterns that tend to be instantaneous. This habit can cause the emergence of various diseases, one of which is digestive disease. This encourages people to consume foods that are beneficial for digestive health, namely probiotic products. According to Setiarto et al. (2018) probiotics are microbial cells that are able to live through digestive tract conditions and provide beneficial effects by improving health for their host if consumed in sufficient quantities.

One of the local lactic acid bacteria that started to be developed is *L. plantarum* Dad-13 which was a new strain of indigenous probiotic that has been successfully isolated from Dadih, a spontaneously fermented buffalo milk (Kamil et al., 2021). These probiotic bacteria are categorized as mesophilic lactic acid bacteria which has optimum temperature for growth 20-35°C that can grow well at 30-37°C and optimum pH 6,5 (Wardani et al., 2017). *L. plantarum* Dad-13 has many beneficial effects

on body health. Research conducted by Tari et al. (2016) showed that the addition of probiotic *L. plantarum* to yoghurt with purple sweet potato extract supplementation has the potential to reduce diarrhea and free radicals. *L. plantarum* Dad-13 can reduce cholesterol, lactose levels in yogurt products, relatively resistant at pH 3.0 and can grow at bile salt levels of 0.5% (Tari et al., 2020). *L. plantarum* Dad-13 has the highest antibacterial activity against pathogenic bacteria compared to other indigenous probiotic bacteria from Indonesia, such as *Lactobacillus plantarum* MUT-7, *Lactobacillus plantarum* MUT-13, and *Lactobacillus paracasei* SNP-2 (Rahayu et al., 2015).

Probiotic products that can be consumed daily as complementary foods are jams. Jam is a semi-solid food product with a thick texture and usually made from fruit with the addition of sugar and thickeners to form a gel-like structure (Agustina and Handayani, 2016). Jam can also be made from another ingredients other than fruit, such as chocolate and peanuts. According to Cozentino et al. (2022) chocolate-based products are suitable

vehicles for probiotics, preserving the viability of different bacteria strains because of its polyphenols that can act as prebiotics that can cause the growth of beneficial bacteria. In addition, the substitution of peanuts in jam serves as an enhancement of nutritional value and flavor that can attract the consumer.

Peanut chocolate jam can be developed as a probiotic food product. However, the processing of jam involves heating process that can affect the amount and quality of probiotics cell so the addition of probiotics is done after the jam reaches a certain temperature. Previously, research on snake fruit jam products was conducted by Purnasari et al. (2015) related to the additional of encapsulated *L. plantarum* BSL and *L. plantarum* 2C12 at a jam temperature of 50°C after heating, while the research of Lestari et al. (2019) on low-sugar pineapple fruit jam products added encapsulated probiotic *L. acidophilus* ATCC 314 at 40°C after heating. Both results showed that the microencapsulation was able to increase the resistance of probiotics to heat during processing.

According to Kamil et al. (2021) food products that are categorized as probiotic foods must retain the viability of 6-7 log CFU/g or mL at the end of the expired date. The processing of jam involves heating which can affect the number of probiotic cells in the product and the metabolic processes that take place. Therefore, this research aims to determine the effect of temperature addition of powdered probiotic *L. plantarum* Dad-13 on peanut chocolate jam towards total lactic acid bacteria (LAB), sublethal injury, pH value, and total acids. This research may provide beneficial information to the optimal temperature of probiotic powder *L. plantarum* Dad-13 addition on peanut chocolate jam.

Materials and Methods

Materials

The materials used were microencapsulated *L. plantarum* Dad-13 probiotic powder obtained from Food and Nutrition Culture Collection at Universitas Gadjah Mada which has been encapsulated in skim milk using the method of Kamil et al. (2020), cocoa powder, peanut, oil, sugar, salt, and lecithin. The chemicals used in this research were analytical reagent grade.

Methods

This research was conducted in November–Desember, 2023 at the Laboratory of Chemistry and Food Nutrition and the Laboratory of Food Engineering and Agricultural Products, Faculty of Animal and Agriculture Sciences, Diponegoro University, Semarang.

The process of making peanut chocolate jam refers to Halik et al. (2023). All ingredients are weighed including probiotic powder (3 g), peanuts (10 g), cocoa powder (20.7 g), refined sugar, oil, vanilla, lecithin, and salt. Peanuts are then roasted and mashed using a blender. Cocoa powder, refined sugar, oil, and crushed peanuts are put into a heat-resistant bowl to be heated over boiling water (double pan method) at a heating temperature 80°C while being stirred evenly for 5 minutes. Vanilla and lecithin were added into the bowl then stirred until the mixture is homogeneous. Turn off the stove and wait until the peanut chocolate jam mixture has the temperature according to the treatment that

refers to Purnasari et al. (2015) with modification that is T1 (30°C), T2 (40°C), T3 (50°C), and T4 (60°C), then add *L. plantarum* Dad-13 probiotic powder and mixed until homogeneous. Last step, the probiotic peanut chocolate jam was put into a cup or container and the parameter test can be done.

Total Lactic Acid Bacteria (LAB) was measured using de Man Rogosa Sharpe Agar (MRSA) medium with Total Plate Count (TPC) method (Nugroho et al., 2023), sublethal injury was calculated using de Man Rogosa Sharpe Agar (MRSA) medium with the addition of 3% NaCl with Total Plate Count (TPC) method (Purnasari et al., 2015), pH value was measured using pH meter (Rasbawati et al., 2019), and total acids was analyzed using the titration method (Usman et al., 2018).

The experiment used a Completely Randomized Design with 4 treatment combinations and 5 replications. The obtained data were statistically analyzed using one-way Analysis of Variance (ANOVA) with a significance level of 5% and followed by Duncan's Multiple Range Test (DMRT) if there are difference between treatments. Statistical analyses were carried out in IBM SPSS Statistics 26.0 application.

Results and Discussion

Total Lactic Acid Bacteria (LAB)

Table 1 shows that the different temperature of additional powdered probiotic *L. plantarum* Dad-13 on peanut chocolate jam had a significant effect ($p < 0.05$) on total lactic acid bacteria (LAB). The result showed that total lactic acid bacteria decreased as the temperature of *L. plantarum* Dad-13 probiotic powder addition increased. This indicates that heat exposure can affect the growth of bacterial cells and their growth rate. According to Ayuti et al. (2016) temperatures that are too high or too low can slow down and stop the growth and metabolism of bacteria so that cell components become inactive and lactic acid bacteria cells inevitably die.

Table 1. Total Lactic Acid Bacteria (LAB) in peanut chocolate jam with different temperatures of *L. plantarum* Dad-13 addition

Treatment	Total LAB on MRSA (Log CFU/g)	Total LAB on MRSA with NaCl 3% (Log CFU/g)
T1 (30°C)	8.04 ± 0.02 ^c	8.00 ± 0.02 ^c
T2 (40°C)	8.01 ± 0.02 ^c	7.96 ± 0.01 ^c
T3 (50°C)	7.89 ± 0.03 ^b	7.78 ± 0.02 ^b
T4 (60°C)	7.67 ± 0.05 ^a	7.42 ± 0.07 ^a

*Different superscript within the same column indicates a significant difference ($p < 0.05$)

Figure 1 shows that the different temperature of additional powdered probiotic *L. plantarum* Dad-13 on peanut chocolate jam had a significant effect ($p < 0.05$) on survival rates. Survival rate is the percentage or proportion of probiotic microorganisms that are alive and can remain actively metabolizing after being exposed to various conditions (Qi et al., 2019). Survival rate is the main criteria in determining the effectiveness of probiotics because it indicates the survival rate of healthy probiotics that can actively metabolize (Kocabay and Cetinkaya, 2020). The R^2 value of 0.9979 or close to 1 is obtained from the polynomial pattern. The polynomial

pattern showed that the temperature treatment of adding *L. plantarum* Dad-13 affects the survival rate so that the equation $y = -0.4338x^2 + 1.2681x + 98.617$ can be used to predict the survival rate value at higher temperatures.

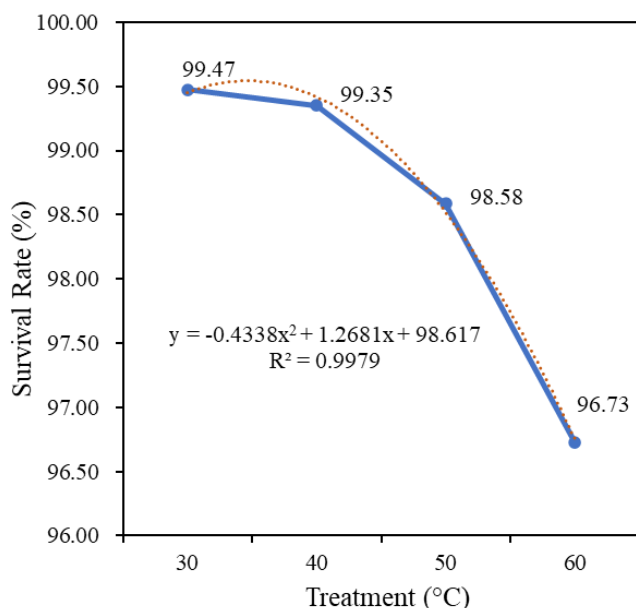


Figure 1. Survival rate in peanut chocolate jam with different temperatures of *L. plantarum* Dad-13 addition

Good probiotic products has probiotic cells 6-7 log CFU/g. Anindita and Anwar (2021) stated that a good probiotic product must contain 6-7 log CFU/g live probiotic cells when consumed to be able to colonize and provide health effects. The results of this study are by the good probiotic product, which range from 8.04-7.67 log CFU/g and survival rate 99.47-96.73%. This may be influenced by the use of probiotics in microencapsulated form with skim milk coating that can play role in maintaining stability from damage caused by processing involving hot temperatures, storage, and bile salts (Jati et al., 2015). Research conducted by Yulianto et al. (2022) also showed the addition of *L. plantarum* Dad-13 on black and white glutinous rice tapes could increase the amount of LAB up to 2 log cycles from 6 to 8 log CFU/g, which qualifies minimum LAB content for probiotic food.

Sublethal Injury

Figure 2 shows that the different temperature of additional powdered probiotic *L. plantarum* Dad-13 on peanut chocolate jam had a significant effect ($p < 0.05$) on sublethal injury. The amount of sublethal injury increased as the temperature of the addition of probiotic powder *L. plantarum* Dad-13 increased. According to Kamil et al. (2023) sublethal injury is where the bacterial cell is metabolically active but unable to grow in selective culture media. A significant increase in injured cells can occur as they suffer damage to their structure. Sublethal injured cells experience damage to the cell membrane, cell wall, DNA, RNA, ribosomes, enzymes and eventually experience death due to damage of functional components of the cell (Saimah et al. 2016). The mechanism of bacterial death due to heat exposure is the cells will experience injury that identified by loss of permeability and increased sensitivity to several toxic compounds (Kurniati et al., 2020). The R^2 value of

0.9977 or close to 1 is obtained from the polynomial pattern. The polynomial pattern showed that the temperature treatment of adding *L. plantarum* Dad-13 affects the sublethal injury so that the equation $y = 0.0339x^2 - 0.0992x + 0.1094$ can be used to predict the sublethal injury value at higher temperatures.

Processing that involves heat can reduce the level of cell activation and disrupt their metabolic processes, causing death cell. According to Budiarti et al. (2020) the heating process with high temperatures can have an impact on injury and damage the growth of the number of bacteria. The addition of probiotic powder *L. plantarum* Dad-13 at 50°C and 60°C showed a significant increase in injured cells. This can occur because as the temperature increases, the number of healthy cells decreases. The addition of probiotic powder at increasingly high jam temperatures causes *L. plantarum* Dad-13 bacteria to be divided into injured and healthy cells. However, too much heat can cause the injured cells to decrease as some of the injured cells die, leaving only the healthy cells to survive (Purnasari et al., 2015).

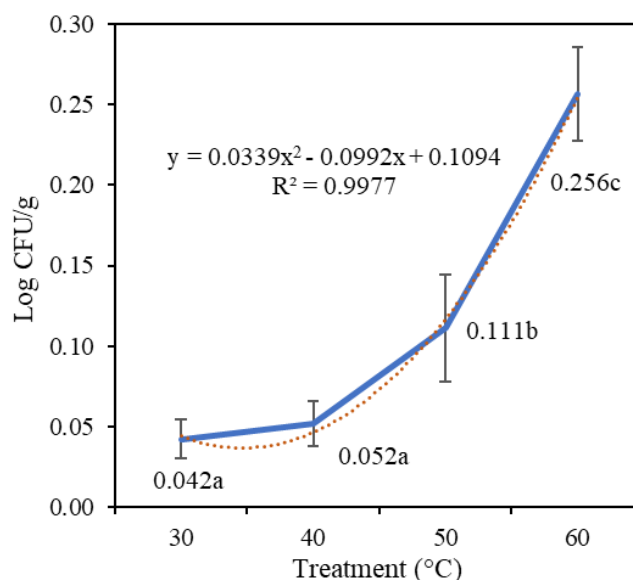


Figure 2. Sublethal Injury in peanut chocolate jam with different temperatures of *L. plantarum* Dad-13 addition. Different superscripts indicate significant differences ($p < 0.05$)

Table 2. pH value in peanut chocolate jam with different temperatures of *L. plantarum* Dad-13 addition

Treatment	pH value
T1 (30°C)	7.20±0.16
T2 (40°C)	7.24±0.15
T3 (50°C)	7.22±0.11
T4 (60°C)	7.30±0.15

Results are mean±standard deviation

pH value

Table 2 shows that the different temperature of additional powdered probiotic *L. plantarum* Dad-13 on peanut chocolate jam had no significant effect ($p > 0.05$) on pH value. The pH value had no significant effect because the lactic acid bacteria on the peanut chocolate jam are in a small amount around 7.67-8.04 log CFU/g. This may be caused by the lactic acid bacteria in *L. plantarum* Dad-13 probiotic powder are in a lag phase.

According to Risna et al. (2022) lag phase is where the bacteria still adjust to the new environment, so the growth is slow and the metabolic process has not been maximized. In addition, the process of making peanut chocolate jam did not involve the incubation process so that the bacteria did not have enough time to produce a metabolic product optimally.

Temperature is one of the factors that can affect bacterial growth, so the addition of probiotic powder to media with a certain temperature can impact the metabolism of lactic acid-producing bacteria. The higher temperature of probiotic powder addition causes the bacteria to have difficulty metabolizing, resulting in a low amount of acid and an increasing pH value (Ayuti et al., 2016).

Table 3. Total acids in peanut chocolate jam with different temperatures of *L. plantarum* Dad-13 addition

Treatment	Total Acids (%)
T1 (30°C)	0.09±0.01
T2 (40°C)	0.09±0.01
T3 (50°C)	0.08±0.01
T4 (60°C)	0.08±0.01

Results are mean±standard deviation

Total Acids

Table 3 shows that the different temperature of additional powdered probiotic *L. plantarum* Dad-13 on peanut chocolate jam had no significant effect ($p>0.05$) on total acids. This is influenced by lactic acid bacteria that have not metabolized optimally so that they only produce low levels of acid. According to Safitri et al. (2016) acid production in probiotic products is influenced by the growth rate of bacteria and their metabolic ability to break down carbohydrates as substrates.

The total acids had no significant effect because LAB could not metabolize optimally due to the absence of the incubation process so that the bacteria were still adapting to their environment. According to Pranayanti and Sutrisno (2015) the process of remodeling or metabolism in producing energy is influenced by the substrate and incubation time. However, too high amount of substrate in the media will also inhibit the growth of LAB because cells will lose water due to the high osmotic pressure difference that inhibits the breakdown of substrates into organic acids and reduces the total acid value (Effendi and Parhusip, 2021).

Conclusion

Based on the results of the research, it can be concluded that the different temperatures of *L. plantarum* Dad-13 probiotic powder addition on peanut chocolate jam decrease total lactic acid bacteria and survival rate, but increase the sublethal injury on lactic acid bacteria cells. The higher the addition temperature of probiotic powder *L. plantarum* Dad-13, the lower the total LAB and the higher the sublethal injury of LAB cells. The best treatment was addition of probiotic powder *L. plantarum* Dad-13 at 30–40°C because it has the highest total amount of LAB in peanut chocolate jam.

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