International Journal of Multidisciplinary Education and Research
www.multieducationjournal.com
ISSN: 2455-4588
Received: 15-05-2026, Accepted: 15-06-2026, Published: 30-06-2026
Volume 11, Issue 2, 2026, Page No. 68-71

Effect of different concentrations of Pandan Leaves (*Pandanus amaryllifolius*) on Total Bacterial Count, Titratable Acidity, and Acceptability

Eka Wulandari, Wendry Setiyadi Putranto, Jajang Gumilar
Department of Livestock Product Technology, Faculty of Animal Husbandry, Padjadjaran University, Jl Raya Bandung, Sumedang, Jatinangor, West Java, Indonesia
Coresponding Author: Eka Wulandari

Abstract
This study aimed to determine the effect of adding pandan leaf extract (*Pandanus amaryllifolius)* with different concentrations on total bacteria, total acid, and yogurt acceptability. The study used a completely randomized design (CRD) with four treatments of pandan leaf extract addition, namely 0%, 5%, 10%, and 15%. The parameters observed included total bacteria, total titratable acid, and acceptability which included color, taste, aroma, texture, and total acceptance. The results showed that the addition of pandan leaf extract had no significant effect (P>0.05) on total bacteria, color, taste, aroma, and texture of yogurt, but had a significant effect (P<0.05) on total acid and total acceptance. Total yogurt bacteria ranged from 8.68–9.41 log CFU/mL, while the highest total acid was obtained in the 10% pandan leaf extract treatment at 1.57%. Increasing the concentration of pandan leaf extract tended to decrease the total acceptance of the panelists. The addition of pandan leaf extract up to 15% does not inhibit the growth of lactic acid bacteria, but affects the sensory acceptance of yogurt.

Keywords: *Pandanus amaryllifolius*, yogurt, total bacteria, total acid, acceptability

Introduction

Milk is a food product that can meet nutritional needs because it contains the protein and minerals the body needs. Milk is then processed into functional foods with additional health benefits as a source of probiotics, such as yogurt (Abbas, 2020) [1]. Yogurt is a fermented milk product using lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophillus* (SNI: 2981, 2009).

The use of spices in Indonesia is believed to provide benefits and increase endurance and is believed to be a "Herbal" medicine by the community, this is because the community believes that the use of herbal medicine has a lower negative impact when compared to the use of chemical drugs (Saleh, *et al*. 2023) [15]. Pandanus leaves (*Pandanus amaryllifolius*) are one of the plants that are often used as a natural green dye and aroma enhancer, this use is due to the pandan leaves are very easy to find throughout Indonesia (Silalahi, 2018) [17].

Pandanus amaryllifolius is a plant often used for its flavonoid, alkaloid, saponin, and tannin content, which are beneficial as antimicrobials (Resmi Aini&Ana Mardiyaningsih, 2016) [13]. Pandan leaves are often used as a natural dye and preservative because the ethyl acetate content in fragrant pandan leaves can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* (Lingling, 2022) [10]. Pandan leaves are expected to reduce the sour smell of yogurt, thereby increasing the level of liking for yogurt with the addition of pandan leaves (Candraningtyastuti, 2016) [4]. The utilization of plant-derived bioactive compounds as natural preservatives is also consistent with SDG 15 (Life on Land), which supports the sustainable use of terrestrial biodiversity. The addition of pandan leaves is expected to affect the physical qualities of yogurt such as Total Bacteria, Total Acid, and Acceptability.

Research methods

1. Making pandan leaf extract

The preparation of pandan leaf extract begins with washing and chopping the pandan leaves into smaller pieces. Next, the pandan leaves are ground using a blender and distilled water is added in a 1:1 ratio until a homogeneous mixture is obtained. The resulting mixture is then filtered to separate any pulp or leaf residue that has not been completely dispersed. The resulting extract is then heated at 80°C for 10 minutes as a thermal treatment to reduce the number of microorganisms still present in the extract and improve the safety of the material before use in the next stage.

2. Making yogurt

Yogurt production refers to the Starbard method (2015) [18] with several modifications. Fresh cow's milk is first added with 5% (w/v) skim milk, then pasteurized at 80°C for 10 minutes. After the pasteurization process is complete, the mixture is cooled to 40°C, then pandan leaf extract is added with concentrations of 0%, 5%, 10%, and 15% (v/v) respectively. A starter culture consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is inoculated at 3% (v/v) of the total volume of the mixture. Next, the mixture is fermented by incubation at 37°C for 18 hours until yogurt is formed.

3. Total Bacteria

The total bacteria in yogurt were determined using the Total Plate Count (TPC) method based on colonies growing on the culture medium (Wulandari *et al*, 2025) [23]. The sample was first diluted in stages using physiological NaCl with a ratio of 1:9. The initial dilution (10^{-1}) was carried out by mixing 1 mL of the sample into 9 mL of physiological NaCl, then continued with serial dilutions until reaching a dilution level of 10^{-8} by transferring 1 mL from the previous dilution into 9 mL of Physiological NaCl (Hidayat, *et al*. 2013) [7].

The medium used was Nutrient Agar (NA). The medium was prepared by dissolving 28g of NA in 1000 mL of distilled water, then sterilized using an autoclave at 121°C for 15 minutes. A 1 mL sample from the dilution was placed into a Petri dish containing approximately 20 mL of NA. Then, the Petri dish was homogenized by moving the Petri dish in a figure-eight shape. After the medium solidified, the dish was incubated in an inverted position at 37°C for 48 hours (Nugroho *et al*., 2018).

Observations were made by calculating the number of bacterial colonies using the calculation formula according to (Maturin and Peeler., 2001) [11] as follows:

$$N = \frac{\sum C}{[(1\ X\ n_1) + (0{,}1\ X\ n_2)]\ X\ (d)}$$

Information

N: Number of colonies per ml
ΣC: Number of colonies on all plates counted
n1: Number of plates in the first dilution counted
n2: Number of plates in the second dilution calculated
d: First calculated dilution

Total Acid

Total titratable acid analysis was performed using a titration method using 0.1 N NaOH solution and phenolphthalein (PP) indicator. A 10 mL sample from each treatment was placed in an Erlenmeyer flask, then 2–3 drops of PP indicator were added. The sample was titrated with 0.1 N NaOH solution until it reached the titration endpoint, which was indicated by the formation of a pink color. The formula for total titratable acid according to Harjiyanti *et al*. (2013) is as follows:

$$Total\ Acid\ \% = \frac{(V\ NaOH\ x\ N\ NaOH\ x\ 90)}{Sample\ volume\ x\ 1000}\ X\ 100\%$$

4. Acceptability

Acceptability testing included color, taste, aroma, texture, and overall acceptability of yogurt with pandan leaf extract at different concentrations. Scores for each test ranged from 1 to 5.

Statistical Analysis

This study was conducted using a completely randomized design (CRD) method consisting of 4 treatments of pandan leaf extract, namely 0%, 5%, 10% and 15%. The data obtained were then analyzed using analysis of variance (ANOVA). Then, further testing was carried out using Duncan's multiple range test (Gasperz, 1995). The acceptability test was analyzed using the Kruskal-Wallis method and the Mann-Whitney further test (Junaidi, 2010).

Results & Discussion

The results of testing the total bacteria in yogurt with the addition of pandan leaves (*Pandanus amaryllifolius*) with various different concentrations on the total number of bacteria and total acid are presented in Table 1.

Table 1: Effect of pandan leaf extract treatment on total bacteria and total acid

Treatment	Total Bacteria (Cfu/mL)	Total Acid (%)
0%	8.80[a]	1.28[a]
5%	8.84[a]	1.33[a]
10%	8.68[a]	1.57[c]
15%	9.41[a]	1.44[b]

Total Bacteria

Based on Table 1. The addition of pandan leaf extract to yogurt did not significantly affect the total bacteria in the resulting yogurt. The addition of pandan leaf extract did not affect microbial activity in the yogurt fermentation process as the concentration of pandan leaves increased. The growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria during the fermentation process was not inhibited by the addition of pandan leaf extract given up to a concentration of 15%. The content of flavonoids, alkaloids, saponins and tannins that act as anti-microbial properties in pandan leaves is thought to be insufficient to inhibit the growth of lactic acid bacteria in the fermentation process in yogurt (Resmi Aini&Ana Mardiyaningsih, 2016) [13]. The addition of pandan leaves to yogurt can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria so that the growth of lactic acid bacteria can develop optimally (Lingling, 2022) [10].

Total Acid

Based on Table 1, the addition of pandan leaf extract to yogurt significantly affected the total acid content of the resulting yogurt. Based on the data in Table 1, the total acid produced increased with increasing concentration of pandan leaf extract, but there was a slight decrease at a pandan leaf concentration of 15%. This can be attributed to the fact that the total acid produced in yogurt is strongly influenced by the fermentation process, which breaks down glucose into lactic acid in yogurt (Imelda *et al*. 2020) [8]. The addition of pandan leaf extract to yogurt can increase total acid because the pH value of pandan leaf extract ranges from 5.00 to 5.80, thus lowering the initial pH of milk before the fermentation process (Wiraguna *et al*. 2015) [22]. Pandan leaf extract can be a nutritional substitute for *L. bulgaricus, L. acidopilus*, and *S. Thermophilus* bacteria in breaking down lactose in milk into lactic acid (Altasya *et al*. 2022) [2].

Acceptability

Table 2: Acceptability table of yogurt with the addition of pandan leaf extract

Variables	Treatment			
	0%	5%	10%	15%
Color	4.25 ± 0.95[a]	3.75 ± 0.95[a]	4.00 ± 0.81[a]	3.10 ± 0.20[a]
Flavor	2.00 ± 1.41[a]	2.00 ± 0.81[a]	1.75 ± 0.50[a]	1.50 ± 0.57[a]
Aroma	4.25 ± 0.95[a]	2.00 ± 0.81[a]	3.5 ± 1.29[a]	3.00 ± 1.63[a]
Texture	4.50 ± 1.00[a]	4.50 ± 1.00[a]	4.00 ± 0.81[a]	3.75 ± 1.50[a]
Total acceptance	3.75 ± 0.50[a]	3.50 ± 1.00[a]	2.75 ± 0.50[ab]	2.25 ± 0.50[b]

Description: (1) Very Dislike, (2) Dislike, (3) Neutral, (4) Like, (5) Very Like

Color

Based on Table 2, the addition of pandan leaf extract up to 15% to yogurt did not significantly affect the level of preference for the resulting yogurt color. The color of

yogurt given pandan leaf extract tended to be greener, this could be due to an increase in chloroplast a and b pigments in chlorophyll, which act as the green colorant in pandan leaves (Puspita *et al*. 2021). This color change did not affect the panelists' level of preference for the color, as evidenced by the panelists' assessments remaining in the range of 3.10–4.25 (Neutral–Very Liked).

Flavor

Based on Table 2. The addition of pandan leaf extract up to 15% to yogurt does not have a significant effect on the taste of the resulting yogurt. The addition of pandan leaf extract tends to decrease the level of taste preference in yogurt. The decrease in the level of taste preference in yogurt can be caused by pandan leaves containing glutamic acid and proline which play a role in providing a savory taste in yogurt (Vaewta & Siree, 2006) [19]. Pandanus leaves (Pandanus amaryllifolius) contain tannin compounds that provide astringent and bitter taste, in addition to containing flavonoids and alkaloids that affect the taste of the resulting yogurt (Sa'adah, *et al*. 2023). This change in taste gives a new impression so that panelists tend to dislike the taste of the resulting pandan yogurt.

Aroma

Based on Table 2. The addition of pandan leaf extract to yogurt did not have a significant effect on the aroma of the resulting yogurt. The addition of pandan leaf extract tends to provide a pandan aroma to yogurt but does not have a significant effect on the panelists' assessment. Pandan leaves have a distinctive aroma influenced by the volatile compounds 2-acetyl-1-pyrroline (2AP), hexanal, phenylacetaldehyde, and β-cyclocitral which tend to be preferred by panelists (Wakte, *et al*. 2010) [20]. The 2-acetyl-1-pyrroline (2AP) compound in pandan leaves tends to provide a distinctive aroma like popcorn and which can increase sensory acceptance. The 2-acetyl-1-pyrroline (2AP) compound in pandan leaves has a very low aroma detection threshold so it is able to provide a strong aroma perception even at small concentrations (Wei, *et al*. 2021) [21]. This distinctive aroma tends to be preferred by panelists so it can increase the acceptability of the resulting pandan yogurt.

Texture

Based on Table 2. The addition of pandan leaf extract to yogurt did not have a significant effect on the texture of the resulting yogurt. The absence of changes in texture in yogurt added with pandan leaf extract was still liked by the panelists. The texture of the resulting yogurt did not change so that it was still in the form of a thick to solid liquid (SNI 2981: 2009). The absence of changes in texture in yogurt is caused by the viscosity of yogurt only caused by a decrease in the pH of milk so that there is a decrease in the solubility of casein compounds contained in milk (Setianto, *et al*. 2014) [16].

Total acceptance

Based on Table 2, the addition of pandan leaf extract to yogurt significantly affected the total acceptance of the yogurt. Higher concentrations of pandan leaf extract tended to decrease panelists' acceptance of the resulting yogurt. This decrease may be due to pandan leaves containing tannins, which impart astringent and bitter notes to yogurt (Sa'adah *et al*. 2023). Furthermore, higher concentrations of pandan leaf extract led to a stronger dominance of the distinctive pandan leaf flavor, thus reducing the yogurt's flavor balance. Although the distinctive aroma of pandan generally enhances a product's sensory appeal, excessively high extract concentrations can potentially produce flavor characteristics that are less favored by panelists.

Conclusion

The addition of pandan leaf extract (*Pandanus amaryllifolius*) at different concentrations did not significantly affect the total bacteria, color, taste, aroma, and texture of the resulting yogurt. However, the addition of pandan leaf extract significantly affected the total acid and total yogurt acceptance. Increasing the concentration of pandan leaf extract tended to increase the total acid up to a concentration of 10%, then experienced a slight decrease at a concentration of 15%. In addition, increasing the concentration of pandan leaf extract tended to decrease the level of panelist acceptance of yogurt, which is thought to be caused by the dominance of pandan flavor and the presence of tannin compounds that provide astringent and bitter sensations. Based on the results of the study, the addition of pandan leaf extract up to a concentration of 15% did not inhibit the growth of lactic acid bacteria, but lower concentrations provided better sensory acceptance levels than higher concentrations.

References

1. Abbas A. Potensi pangan fungsional dan perannya dalam meningkatkan kesehatan manusia yang semakin rentan: Mini review. Jurnal Teknosains,2020:14(2):176-186. https://doi.org/10.24252/teknosains.v14i2.14319
2. Altasya F, Putranto WS, Gumilar J. Pengaruh berbagai konsentrasi pulp buah naga merah pada pembuatan set yoghurt terhadap total bakteri asam laktat, nilai pH, dan total asam. Jurnal Teknologi Hasil Peternakan,2022:3(1):23-32.
3. Badan Standardisasi Nasional. SNI 2981:2009 yoghurt. Badan Standardisasi Nasional, 2009.
4. Candraningtyastuti D. Yoghurt susu kambing dengan penambahan jus daun pandan *(Pandanus amaryllifolius* Roxb.) dan waktu fermentasi [Skripsi]. Universitas Sanata Dharma, 2016.
5. Gaspersz V. *Teknik analisis dalam penelitian percobaan*. Tarsito, 2003.
6. Harjiyanti MD, Pramono YB, Mulyani S. Total asam, viskositas, dan kesukaan pada yoghurt drink dengan sari buah mangga (*Mangifera indica*) sebagai perisa alami. Jurnal Aplikasi Teknologi Pangan,2013:2(2):104-107.
7. Hidayat IR, Kusrahayu, Mulyani S. Total bakteri asam laktat, nilai pH, dan sifat organoleptik drink yoghurt dari susu sapi yang diperkaya dengan ekstrak buah mangga. Animal Agriculture Journal,2013:2(1):160-167.
8. Imelda F, Purwandani L, Saniah S. Total bakteri asam laktat, total asam tertitrasi, dan tingkat kesukaan pada yoghurt drink dengan ubi jalar ungu sebagai sumber prebiotik. Jurnal Vokasi,2020:15(1):1-7.
9. Junaidi. Statistika non-parametrik. Fakultas Ekonomi Universitas Jambi, 2010.

10. Lingling GN. Potensi ekstrak daun pandan wangi (*Pandanus amaryllifolius* Roxb.) sebagai antibakteri pada sediaan gel facial wash. Prosiding Workshop & Seminar Nasional Farmasi,2022:1(1):283-294. https://doi.org/10.24843/WSNF.2022.v01.i01.p23
11. Maturin L, Peeler JT. BAM chapter 3: Aerobic plate count. U.S. Food and Drug Administration, 2001.
12. Nugroho J, Mariani NP, Okarini IA. Respon uji terhadap susu kambing peranakan etawah yang disimpan pada suhu ruang. Jurnal Peternakan Tropika,2019:7(1):21-31.
13. Aini R, Mardiyaningsih A. Pandan leaves extract (*Pandanus amaryllifolius* Roxb.) as a food preservative. Indonesian Journal of Medicine and Health,2016:7(4):166-173.
14. Sa'adah SM, Putri FR, Ibtisam AA, Arrohmah RS, Fitriyah F. Phytochemical analysis of secondary metabolite compounds of Pandanwangi leaf extract (*Pandanus amaryllifolius*). Journal of Natural Sciences and Mathematics Research,2023:9(2):135-142.
15. Saleh MR, Putranto WS, Gumilar J. Pengaruh penambahan minyak jintan hitam (*Nigella sativa*) dengan konsentrasi berbeda pada proses pembuatan yoghurt terhadap jumlah bakteri asam laktat, pH, dan akseptabilitas. Jurnal Teknologi Hasil Peternakan,2023:4(1):12-23.
16. Setianto YC, Pramono YB, Mulyani S. Nilai pH, viskositas, dan tekstur yoghurt drink dengan penambahan ekstrak salak pondoh (*Salacca zalacca*). Jurnal Aplikasi Teknologi Pangan,2014:3(3):110-113.
17. Silalahi M. *Pandanus amaryllifolius* Roxb.: Pemanfaatan dan potensinya sebagai pengawet makanan. Pro-Life,2018:5(3):626-636.
18. Starbard A. The dairy goat handbook for backyard, homestead, and small farm. 1st ed. Voyageur Press, 2015.
19. Vaewta C, Chaiseri S. Free amino acid and reducing sugar composition of pandan (*Pandanus amaryllifolius*) leaves. Agriculture and Natural Resources,2006:40(Suppl. 6):67-74.
20. Wakte KV, Thengane RJ, Jawali N, Nadaf AB. Optimization of HS-SPME conditions for quantification of 2-acetyl-1-pyrroline and study of other volatiles in *Pandanus amaryllifolius* Roxb. Food Chemistry,2010:121(2):595-600. https://doi.org/10.1016/j.foodchem.2009.12.056
21. Wei X, Sun Q, Methven L, Elmore JS. Comparison of the sensory properties of fragrant and non-fragrant rice (*Oryza sativa*), focusing on the role of the popcorn-like aroma compound 2-acetyl-1-pyrroline. Food Chemistry,2021:339:128077. https://doi.org/10.1016/j.foodchem.2020.128077
22. Wiraguna GNP, Wartini NM, Yoga IWGS. Pengaruh metode dan lama curing terhadap karakteristik daun pandan wangi (*Pandanus amaryllifolius* Roxb.). Jurnal Rekayasa dan Manajemen Agroindustri,2015:3(2):109-119.
23. Wulandari E, Utama T, Marlina ET. Physicochemical and microbial evaluation of Kefir produced with sesame seed (*Sesamum indicum* L.) extract as a goat milk substitute. Journal of Advanced Veterinary Research,2025:15(6):801-804.