

Effect of pandan (*Pandanus amaryllifolius*) leaf extract addition on the shelf life of stirred yogurt

Eka Wulandari, Dicky Tri Utama, Lilis Suryaningsih

Department of Livestock Product Technology, Faculty of Animal Husbandry, Padjadjaran University, Jl Raya Bandung Sumedang, Jatinangor, West Java, Indonesia

Corresponding Author: Eka Wulandari

Abstract

Stirred yogurt is one of the many fermented milk products developed as food functional through addition material natural containing compound bioactive. Pandanus leaves (*Pandanus amaryllifolius*) are known own flavonoid content, compounds phenolics, tannins, and potential alkaloids play a role as antioxidants and antimicrobials so that expected can maintain yogurt quality during storage. Research This aim for evaluate the effect of storage time to pH value, total bacteria, and number bacteria sour lactate in *stirred yogurt* added with 10% extract pandan leaves. Research use Design Random Complete (RAL) with Onefactors namely storage time on days 1, 6, and 10. The parameters observed includes pH, total bacteria, and number bacteria sour lactate. Data analyzed use analysis variety (ANOVA), then to be continued with Duncan's test at the level 5% significance. Research results show that the storage period influential significant ($P < 0.05$) against all observed parameters. The pH value increased in a way significant from 4.19 on day 1 to 4.60 on day 10. In contrast, total bacteria decrease from 8.63 to 7.16 log CFU/mL, while amount bacteria sour lactate decrease from 7.60 to 7.06 log CFU/mL during storage. Although happen decrease, amount bacteria sour lactate at the end storage Still is above 10^7 CFU/mL so Still fulfil minimum recommended range for fermented milk products cultured live. Results study show that addition of 10% extract pandan leaves have potential applied as material experience in development *stirred yogurt* with quality fixed microbiological awake during storage cold.

Keywords: *Pandanus amaryllifolius*, stirred yogurt, storage cold, bacteria sour lactate, pH, total bacteria

Introduction

Yogurt is one of the product Food the most fermentation consumed in the world because own mark nutrition tall as well as give various benefit health. Products This produced through milk fermentation using bacteria sour lactate (BAL), especially *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which transform lactose become sour lactate so that produce distinctive taste, good texture, as well increase Power save products. In Addition as a source of protein, calcium, vitamins, and minerals, yogurt is also known as food functional Because contain microorganisms capable life give effect positive to health channel digestion, improve system immune, as well as potential lower risk various degenerative disease (Wulandari, *et al.*, 2024) ^[10]. Therefore that, innovation yogurt products continue develop along increasing awareness public to consumption food healthy and natural.

Development industry food moment This show existence change preference consumer to product *clean label*, namely food that uses material addition experience as replacement material synthetic. Use extract plant as source compound bioactive the more Lots developed Because besides can increase characteristics sensory products, also have the potential give activity antioxidants and antimicrobials (Saleh, *et al.*, 2023) ^[7]. Various study has report that addition extract herbal plants in yogurt or drink other fermentations such as kefir are capable of increase mark functional product without reduce quality fermentation. Therefore that, exploration material experience local as Fortifying yogurt is one of the strategies in produce product more food healthy, safe, and have mark added (Dwiki *et al.*, 2024) ^[3].

One of plant potential local developed is fragrant pandan (*Pandanus amaryllifolius* Roxb). Plants This has long been used as material flavoring experience Because has a distinctive aroma that comes from from compound volatiles, especially 2-acetyl-1-pyrroline. In addition provide a preferred aroma consumer, pandan leaves also contain various compound bioactive such as flavonoids, phenolics, alkaloids, tannins, saponins, and carotenoids which are known own activity antioxidant, antimicrobial, antidiabetic, and anti-inflammatory. Content compound phenolic the capable hinder growth microorganisms certain and catch radical free so that potential maintain quality food during storage. (Herman *et al.*, 2011) ^[4].

Application pandan extract in yogurt products is not only expected capable enhance aroma and taste, but can also maintain quality microbiological during storage. Compounds phenolics and flavonoids in pandan extract is suspected capable pressing growth microorganisms rot without hinder growth bacteria sour lactate as a starter culture. With Thus, fortified yogurt pandan extract has potential have a longer shelf life Good at a time maintain the number of BAL at a level that meets the requirements standard food fermentation.

Shelf life is one of the important parameters in determine yogurt quality (Semeru, *et al.*, 2024). During storage cold, various change physicochemical and microbiological Keep going ongoing consequence activity metabolism bacteria that are still continue although in faster rate low. Change the covering change pH value, decrease amount total bacteria, as well as decrease viability bacteria sour Lactate. BAL viability is a very important parameter Because related direct with benefit functional yogurt. Yogurt products

generally expected still contain bacteria sour lactate life in minimum count of 10^6 – 10^7 CFU/mL up to the end of its shelf life for benefits probiotics still can obtained consumers.

Various study about addition pandan leaves in yogurt have Lots focused on characteristics sensory, activity antioxidants, as well as characteristic physicochemical products. For example, fortification tea pandan leaves reported influence a number of characteristics physicochemical and aroma of yogurt, while other studies show that pandan extract is capable increase activity yogurt antioxidants. However Thus, research about change quality microbiological yogurt containing pandan extract for storage cold, especially related pH changes, total bacteria, and number bacteria sour lactate, still relatively limited.

Based on condition mentioned, it is necessary study for evaluate stability quality fortified yogurt pandan extract for storage. Information the important as base scientific in development of functional yogurtbased material experience local who have quality microbiological Good as well as an adequate shelf life.

Study This aim for analyze the effect of storage time cold to pH value, total bacteria, and number bacteria sour lactate in stirred yogurt added extract Pandanus leaves (*Pandanus amaryllifolius*). Research results This expected can give information scientific about potential extract pandan leaves as material experience in maintain quality microbiological stirred yogurt during storage as well as support development fermented milk products functional based source Power biological local.

Research Methods

Material

Material main used in study This includes fresh cow's milk obtained from farm cow dairy in Bandung Regency, skim milk powder, commercial yogurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, fresh pandan leaves (*Pandanus amaryllifolius* Roxb.) obtained from traditional markets in the Regency Sumedang, and aquades.

Material For analysis microbiology includes Nutrient Agar, de Man Rogosa Sharpe Agar, 0.85% physiological NaCl solution, 70% alcohol, and other ingredients supporters other

Tool

Equipment used includes blender, scales analytical balance, stainless steel pan, digital thermometer, incubator, refrigerator, digital pH meter, autoclave, laminar air flow, colony counter, micropipette, sterile petri dish, test tube reaction, glass measuring, Erlenmeyer, and equipment laboratory microbiology other.

Making Extract Pandan Leaves (Siti Juariah, *et al* 2022) [8]

Fresh pandan leaves sorted for separate damaged leaves, then washed using running water until clean, Leaves cut into pieces along ± 2 cm then weighed as much as 100 g. Next leaf blended with addition of 100 mL of distilled water sterile (ratio 1:1, w/v) for 3 minutes until homogeneous.

The blender results are filtered use two layers of sterile muslin cloth for get extract pandan leaves extract Then saved in bottle sterile at 4°C and used on the same day for prevent decline activity compound bioactive.

Making Stirred Yogurt

1 L of fresh cow's milk is added with 5% (w/v) skim milk then... homogenized until all over material late perfect. Mix pasteurized use Low Temperature Long Time (LTLT) method at 85 °C for 30 minutes while stirred slowly. After pasteurization finished, the milk is cooled until reach temperature 42°C. 3% (v/v) yogurt starter was added in a way aseptic, then incubated at 42°C for 6–8 hours until formed coagulation and pH reaches around 4.5. After fermentation finished, stir the yogurt slowly until obtained texture homogeneous (stirred yogurt). Next added extract pandan leaves as many as 10% (v/v) and homogenized for ± 2 minutes use mixer sterile. Product Then packed to in a plastic cup sterile with a capacity of 100 mL and stored at room temperature refrigeration ($4 \pm 1^\circ\text{C}$).

Study use Design Random Complete (RAL) with One factor namely the storage period consisting of on three level, namely: P1 = storage day 1, P2 = storage day 6, P3 = storage 10th day. Each treatment repeated as much as six times, so that obtained 18 units test.

pH Measurement

The pH value is measured using an existing digital pH meter calibrated use buffer solution pH 4.00 and pH 7.00 before used. A total of 20 mL of yogurt sample was added to in glass cup sterile, then pH meter electrode dipped until reading stable. Measurement done as much as three times for every samples and results stated as average value.

Total Bacteria Analysis

Total bacteria determined use Total Plate Count (TPC) method based on SNI 2897:2008. A total of 1 mL of yogurt sample was added to in 9 mL of physiological NaCl solution sterile so that obtained 10^{-1} dilution. Dilution tiered done up to 10^{-7} . A total of 1 mL of suspension from appropriate dilution entered to in sterile petri dish, then added approximately 15–20 mL of sterile Nutrient Agar (PCA) at a temperature of $\pm 45^\circ\text{C}$ using pour plate method. The media is homogenized with movement rotate form number eight, then incubated at 37 °C for 48 hours. Colonies that grew between 30–300 colonies counted using a colony counter. The results are stated as log CFU/ mL.

Total Bacteria Analysis Sour Lactate

Amount bacteria sour lactate analyzed using de Man Rogosa Sharpe Agar (MRSA) media. Dilution sample done as in the total bacterial analysis. A total of 1 mL of suspension inoculated to in sterile petri dish use method pour plate, then Sterile MRSA media was added. Cup incubated in a way anaerob using anaerobic jar at temperature 37°C for 48 hours. Colonies typical BAL is calculated on a plate that has amount colony 30–300 colonies and results stated as log CFU/ mL.

Data analyzed use Analysis of Variance (ANOVA) at the level 95% confidence ($\alpha = 0.05$). If ANOVA results show significant effect ($P < 0.05$), then to be continued with Duncan's Multiple Range Test (DMRT) For know difference between treatment.

Discussion

Effect of Storage Time on the pH of Stirred Yogurt Added Extract Pandan leaves

Research result show that the storage period give influence significant ($P < 0.05$) against pH value of added stirred

yogurt extract pandan leaves 10% (Table 1). The pH value increased in a way gradually from 4.19 on day 1 to 4.40 on day 6 and reaching 4.60 on day 10. Based on Duncan's

further test, each storage period Show real difference to mark pH.

Table 1: Effect of shelf life on pH, total bacteria and total BAL in yoghurt with 10% pandan addition

Treatment	pH	Total Bacteria (CFU/ mL)	Total Lactic Acid Bacteria (CFU/ mL)
Day 1	4.19 ^a	8.63 ^a	7.60 ^a
Day 6	4.40 ^b	7.34 ^b	7.34 ^b
Day 10	4.60 ^c	7.16 ^b	7.06 ^c

Improvement pH value indicates that level the acidity of yogurt increases decline during storage. This result different with phenomenon *post-acidification* which is generally occurs in yogurt, namely decrease in pH due to production sour lactate that is still ongoing during storage cold. Difference the indicates that there Is other factors that influence activity metabolism bacteria sour lactate in added products extract pandan leaves (Arya *et al.*, 2024) ^[1].

One of possibility reason increasing pH is decline activity metabolic bacteria sour lactate consequence storage at room temperature low ($4 \pm 1^\circ\text{C}$). In conditions said, the activity enzymes that play a role in fermentation lactose become sour lactate experience decline so that production sour organic become the morelow. Besides that, during storage bacteria enter phase stationary until phase death consequence limitations substrate and accumulation metabolites, so that ability produce acid also decreases.

Besides influence temperature storage, increase in pH in research This allegedly related with existence compound bioactive in extract Pandan leaves. Pandanus leaves are known contain various metabolit secondary such as flavonoids, compounds phenolics, tannins, alkaloids, saponins, and terpenoids which have activity antimicrobial and antioxidant compounds the can hinder activity enzymatic microorganisms through damage membrane cells, protein denaturation, and disturbance to nutrient transport system. If activity bacteria sour lactate experience decline, then production sour lactate during storage will also be reduce so that pH value tends to increase.

Besides that, compound phenolic in pandan extract is also suspected interact with milk protein, especially casein, through bond hydrogen and interaction hydrophobic. Interaction the can increase capacity buffering *capacity* of the yogurt system so that change hydrogen ion concentration free become moresmall. As a result, the pH value can increase although Still there is a number of sour Organic in products. Some study report that interaction between polyphenols plants and milk proteins can influence characteristics physicochemical product fermentation, including pH stability during storage.

Research result This show that although pH value increases in a way significant during storage, quantity bacteria sour lactate Still is above 10^7 CFU/mL to day 10. This is show that addition extract pandan leaves no cause death of starter culture drastic, but possibility more play a role in modulate activity metabolism bacteria so that production sour during storage become morelow. With Thus, extract pandan leaves have potential functioning as material natural capable maintain stability microbiological *stirred yogurt* without remove viability bacteria sour lactate.

Effect of Storage Time against Total Bacteria

Storage time also provides influence significantly ($P < 0.05$) on total bacteria. The total number of bacteria decrease from

8.63 log CFU/mL on day First to 7.34 log CFU/mL on day sixth and 7.16 log CFU/mL on day tenth. Based on Duncan's test, the number of bacteria on the 6th and 10th day did not different real, but both of them different real compared to day First.

Total bacterial decline is common phenomenon happen during storage cold. Temperature low cause activity metabolism microorganisms decrease so that rate growth bacteria become very slow. Besides that, during storage happen limitations nutrients, accumulation metabolit results fermentation, as well as increasing pressure environmental *stress* that causes part cell bacteria experience death.

Other suspected factors play a role is existence compound bioactive in extract Pandan leaves. The flavonoid, phenolic, tannin and alkaloid content Is known capable bother integrity membrane cell bacteria, inhibit activity enzymes, as well as reduce ability microorganisms for developing (Mastuti, 2026) ^[6]. Therefore that, besides effect temperature low, decrease amount bacteria may also be influenced by activities antimicrobial pandan extract.

Although experience decrease, total number of bacteria at the end storage Still is above 10^7 CFU/mL, indicating that product Still own population high microbes as characteristics typical food fermentation. This is Indicates that pandan extract no cause decline population microbes in a way drastic, but rather only hinder growth during storage.

Effect of Storage Time to Amount Bacteria Sour Lactate

Amount bacteria sour lactate (BAL) experienced decline significant ($P < 0.05$) during storage, namely from 7.60 log CFU/mL on day First to 7.34 log CFU/mL on day sixth and 7.06 log CFU/mL on day tenth. Decline This show that the storage period influence viability of starter culture in yogurt. During storage cold, bacteria sour lactate experience various condition stress, such as temperature low, decrease availability nutrients, changes pressure osmotic, as well as accumulation metabolit results fermentation. Conditions the cause activity metabolism and division cell become the morelow so that amount bacteria life Keep going decreased. Although Thus, the number of BAL on the 10th day was still reach more from 7 log CFU/mL, so that Still fulfil recommendation international for food fermentation containing live cultures (minimum 10^6 – 10^7 CFU/mL).

Interestingly, the decline number of BALs in progress relatively slow. This is show that addition extract pandan leaves 10% no hinder growth of starter culture significant. Some study show that compound phenolic plant can nature selective , namely more effective hinder microorganisms rot compared to bacteria sour lactate which has tolerance more Good to condition sour and compound antimicrobial natural .Besides that , research latest report that fermentation pandan leaves with bacteria sour lactate precisely increase content compound phenolics and flavonoids without hinder BAL growth , indicating existence compatibility between

pandan and bacterial culture sour lactate (Junping *et al.*, 2024) ^[5]

Research result This support development stirred yogurtbased material experience local. Addition extract pandan leaves no only provides a distinctive aroma, but also has the potential maintain stability microbiological during storage. On the other hand, research previously about yogurt with fortification more pandan leaves Lots focus on characteristics physicochemical, color, and properties sensory, whereas information about viability bacteria sour lactate during storage Still limited. Therefore that, research This give information new about change population microbes during storage in fortified stirred yogurt extract pandan leaves (Cut *et al.*, 2016) ^[2]

Conclusion

Storage period influential significantly ($P < 0.05$) on pH, total bacteria, and number of bacteria sour lactate in stirred yogurt with addition of 10% extract pandan leaves (*Pandanus amaryllifolius*). During 10 days of storage, pH increases from 4.19 to 4.60, while the total bacteria and bacteria sour lactate decreased. However, the number of bacteria sour lactate on day 10 still reached 7.06 log CFU/mL, so fulfil minimum standards for fermented milk products cultured live. This result show that addition of 10% extract pandan leaves have potential used as material experience in development stirred yogurt functional Because capable maintain viability bacteria sour lactate during storage.

Reference

1. Arya Dwiki Ramadhan, Lulu'u Wilda Maslachah, Dinda Amallia Kurniati Nurjannah, Triana Setyawardani, June Sumarmono, Irfan Fadhlurrohman, *et al.* Physicochemical and Organoleptic Properties of Yogurt with Fortification Pandan Tea (*Pandanus amaryllifolius* Roxb.). Journal Scientific Animal Husbandry Halu Oleo, 2024;6(4):336–344.
2. Cut AM, Yusdar Zakaria, Nurliana. Effects Temperature and Shelf Life to Activity Antimicrobial Fermented Milk with *Lactobacillus casei*. Agrippet, 2016;16(1).
3. Dwiki Ramadhan A, Wilda Maslachah L, Amallia Kurniati Nurjannah D, Setyawardani T, Sumarmono J, Fadhlurrohman I, *et al.* Physicochemical and Organoleptic Properties of Yogurt with Fortification Pandan Tea (*Pandanus amaryllifolius* Roxb.). Journal Scientific Farm Halu Oleo, 2024;6(4):336–344.
4. Herman Hindarso, Sheila Margareta, Swita Dewi Handayani, Nani Indraswati. Extraction Phenolic Compounds of *Pandanus amaryllifolius* Roxb. as Antioxidants Experience. Widya Teknik, 2011;10(1):20–30.
5. Junping Zhou, Zhen Feng, Mingzhe Yue, Ziqing Chang, Junxia Chen, Mengrui Wang, *et al.* Innovative enhancement of flavor profiles and functional metabolites composition in *Pandanus amaryllifolius* through lactic acid bacteria fermentation. Food Chemistry: X, 2024;24:101964.
6. Mastuti Widianingsih. Narrative Literature Review: Activities Antibacterial Robusta Coffee Leaves (*Coffea canephora* Pierre ex A. Froehne). Borneo Journal of Biology Education, 2026;8(1):12–23.
7. Saleh MR, Putranto WS, Gumilar J. The Effect of Addition of Oil Caraway Black (*Nigella sativa*) With Concentration Differences in the Process of Making Yogurt Amount Bacteria Sour Lactate, pH, and Acceptability. Journal Animal Product Technology, 2023;4(1):12–23.
8. Siti Juariah, Junia Wiranda, Harni Sepryani. Effectiveness Test Extract *Pandanus amaryllifolius* (Fragrant Pandan Leaves Roxb) To Growth Bacteria *Streptococcus mutans*. Journal of Indonesian Medical Laboratory and Science, 2022;3(1):81–96.
9. Sumeru HA, Rina Gaida Latifah, Fajriana Shafira Nurruysyda, Safri Ishmayana. Influence Initial Treatment, Variation Temperature Heating and Inoculation to Viscosity of Yogurt. Padjadjaran Chemistry, 2024;3(1):23–29.
10. Wulandari E, Lilis Suryaningsih, Dicky Tri Utama. The Effect of Addition of Black Cumin Oil (*Nigella sativa*) With Different Concentrations in The Yoghurt Production Process on Ph, Viscosity and Water Holding Capacity. Himalayan Journal of Agriculture, 2024;5(1):1–7.