

Flavor meets function: Red grape juice (*Vitis vinifera* L.) enrichment enhances the microbiological, chemical, and sensory quality of yogurt

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ABSTRACT

Background: One of the main challenges in yoghurt production is the potential for microbiological contamination, especially by Coliform bacteria. Meanwhile, red grapes (*Vitis vinifera* L.) are known to have bioactive content that has the potential to improve yoghurt quality by not only suppressing coliform bacteria, but also increasing Lactic Acid Bacteria (LAB), reducing pH and acid levels, and improving sensory preferences.

Objectives: This study aims to investigate the microbiological, chemical, and organoleptic characteristics of yoghurt after the addition of red grape juice (*Vitis vinifera* L.).

Methods: This study is an exploratory study conducted with four concentrations: K0 (without the addition of red grape juice), K1 (5%), K2 (10%), and K3 (15%). The parameters observed include the number of Coliform bacteria, LAB, lactic acid levels, pH, and organoleptic tests. Data analysis of the total number of LAB, lactic acid levels, the number of coliform bacteria and pH values was carried out descriptively quantitatively. Meanwhile, the results of the organoleptic test were compared between groups using the Kruskal-Wallis's test statistics.

Results: The results showed that K3 produced the highest total BAL of 2.71×10^9 CFU/mL. The lowest number of Coliforms of 3 apm/mL was produced by K2, in accordance with the yoghurt quality standards based on SNI 2981:2009. The highest lactic acid content was recorded in K3 of 1.89%, while the lowest pH value was achieved in K3 with a value of 4.00. Organoleptic tests showed that K2 and K3 gave the highest average values for taste, aroma, and color attributes, with a tendency for consumer preference to increase along with the addition of fruit juice concentration.

Conclusion: The most favorable results were obtained at concentrations of 10% (K2) and 15% (K3), which provided an optimal balance between sensory quality, microbiological safety, and chemical stability of the product. The addition of red grape juice increased the functional value of yoghurt in microbiological, chemical, and organoleptic aspects.

Keywords: Coliform bacteria; food safety; lactic acid bacteria; Red grape juice; yoghurt.

SDGs Relevance: This study supports SDG 3 (Good Health and Well-Being) by improving food safety and quality of functional foods, and SDG 12 (Responsible Consumption and Production) using natural antimicrobial compounds from fruit juices in yoghurt production as an alternative to synthetic preservatives.

Laboratory Affiliation: This research was conducted at the Microbiology and Biochemistry Laboratory, Department of Biology, State Islamic University of Maulana Malik Ibrahim Malang. This facility supports microbiology and chemistry testing on fermented foods.

INTRODUCTION

Yogurt is a dairy product produced through a fermentation process by lactic acid bacteria (LAB), especially *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Bai et al., 2024; Xiong et al., 2023). This product has long been widely known and favored by the public because of its high nutritional content and health



benefits, such as helping the digestive system and acting as a natural probiotic (Şanlıer et al., 2019; Weerathilake et al., 2014). In the fermentation process, LAB will begin to ferment lactose into lactic acid, which will have an impact on the chemical parameters and characteristics of yogurt (García-Burgos et al., 2020; Saleem et al., 2024).

In terms of microbiology, total LAB testing is an important indikator (Yang & Yoon, 2022). LAB is needed in high quantities because it shows the success of fermentation and product stability, and can suppress microbiological contamination (Santos et al., 2025), especially by Coliform bacteria. LAB can contribute to inhibiting the growth of coliforms through competition mechanisms and the production of antimicrobial compounds. In other words, total LAB testing is important to ensure that the yoghurt produced has good functional quality and is safe for consumption (Fayyaz et al., 2020).

In terms of chemistry, the main parameters observed are pH and lactic acid levels (Elizaquível et al., 2011). The function of pH is as an indicator of acidity which is directly related to the metabolic activity of LAB and the final quality of the product. The decrease in pH in yoghurt is caused by the high activity of LAB in yoghurt, so the lower the pH value, the higher the total amount of LAB tends to be (Zhou et al., 2024). During the fermentation process, LAB will ferment substrates such as carbohydrates and glucose so that lactic acid is formed. The formation of this lactic acid causes an increase in acidity and a decrease in pH value (Hidayat et al., 2013). Finally, lactic acid levels play an important role in determining the level of acidity produced during fermentation so that it can also affect the stability and taste of the yoghurt produced (Rasbawati et al., 2019).

To improve the functional and sensory quality of yoghurt, a number of previous studies have explored the use of natural additives such as fruit juice. Gawad Ia & Sayed Em (2014) reported that yoghurt with the addition of fruit juice containing polyphenols had a low level of Coliform contamination. Polyphenol compounds are known to inhibit the growth of pathogenic microorganisms, including Coliform bacteria (Cheng et al., 2012). In addition, polyphenols also support the growth of LAB which can lower the pH value so that pathogenic bacteria are inhibited (Coman et al., 2018). Similar results were reported by previous studies which stated that organic acids and phenolic compounds in fruit juice can accelerate the decrease in pH, increase lactic acid levels (Dimitrellou et al., 2020; Widagdha, 2014), and inhibit the growth of Coliform (Çiftçi & Öncül, 2024).

From an organoleptic perspective, the addition of fruit juice also affects the sensory quality of yoghurt. Research by Covaliov et al. (2024) showed that yoghurt with the addition of red grape juice had a higher acceptance score in organoleptic tests. Consumers tend to prefer products with a distinctive fruity taste and aroma and an attractive appearance. Thus, the addition of red grape juice containing bioactive compounds such as polyphenols, resveratrol, and proanthocyanidins can potentially support the growth of LAB and suppress the growth of coliforms. Meanwhile, the decrease in pH and the increase in lactic acid levels by LAB can ultimately improve the sensory characteristics of the product.

Although there have been many studies examining the addition of fruit juice to yoghurt (Arslan & Bayrakçı, 2016; Bchir et al., 2020; Covaliov et al., 2024; De Souza De Azevedo et al., 2018; Dimitrellou et al., 2020; Karnopp et al., 2017), studies that specifically use local red grape juice (*Vitis vinifera* L.) in various concentrations are still limited. Moreover, there have not been many studies that integrate microbiological (LAB and Coliform), chemical (pH and lactic acid levels), and organoleptic data to identify the optimal concentration in the context of developing functional yoghurt and having a preferred taste. Thus, this study was conducted to provide information on the use of grape juice in graded concentrations with a comprehensive evaluation of the microbiological, chemical, and organoleptic aspects of yoghurt. This study can provide additional evidence that grape juice has natural antimicrobial properties and can suppress Coliform contamination, while improving the sensory quality of the product.

This study supports Sustainable Development Goals (SDGs) point 3: Good Health and Well-being, through the development of safe and healthy functional food products. In addition, it is also relevant to SDG point 12: Responsible Consumption and Production, because it utilizes local resources (red grapes) and increases food added value through fermentation-based innovation. This study aims to analyze the effect of adding red grape juice in various concentrations (5%, 10%, and 15%) on the quality of yoghurt as reviewed from the microbiological (number of BAL and Coliform), chemical (pH and lactic acid levels), and organoleptic (color, taste, aroma, and texture) aspects.

METHODS

This study is a comparative exploration study using a quantitative approach. This study aims to compare the microbiological (total BAL and MPN Coliform), chemical (pH and lactic acid levels), and organoleptic aspects of yoghurt with yoghurt with samples in the form of the addition of red grape juice (*Vitis vinifera* L.) into yoghurt with different concentrations, namely 5% (K1), 10% (K2), and 15% (K3) compared to yoghurt without addition (K0).

The tools used in this study include measuring cups, Erlenmeyer flasks, petri dishes, inoculation needles, micropipettes, bunsen, test tubes, Durham tubes, tube racks, digital scales, aluminum foil, incubators (Memmert), autoclaves (ALP), Laminar Air Flow (Esco), micropipettes, microtips, hotplates (Thermo), bunsen, pH meters (Sasuma) and magnetic stirrers.

The materials used in this study include LSTB (Lauryl Sulfate Tryptose Broth) and BGLBB (Brilliant Green Lactose Bile Broth), MRS (de Man, Rogosa, Sharpe) Agar, BPW (Buffered Peptone Water), NaOH 0.1 N, PP indicator (Phenolphthalein), alcohol, fresh milk, red grape juice (*Vitis vinifera* L.), and commercial yogurt starter Greenfields Plain Yoghurt. The tools and materials to be used in the study were first sterilized using an autoclave. This study began with sample preparation.

The fruit used was ripe and unblemished, then washed with running water and dried. After drying, the fruit was cut using a sterile stainless steel knife to facilitate the blending process. Boiled water (1:1) was added, then the sample was pureed using a blender, then filtered and stored in a clean glass bottle. Meanwhile, the process of making yogurt, namely the basic milk ingredients must be heated for 30 minutes at a temperature of 60-80°C. After being pasteurized and left to stand until a temperature of 40°C, a starter is added as much as 3% (v/v) of the basic milk ingredients used, then the fruit juice that has been made is added. Incubate or ferment the yogurt at a temperature of 37°C for 12 hours in a tightly closed state, after 15 hours remove from the incubator and store in a refrigerator at a temperature of 4°C.

Microbiological tests are carried out with two types, namely the MPN Coliform test and the TPC BAL test. The MPN Coliform test is carried out using LSTB media which is made by dissolving 14.24 g in 400 mL of distilled water, then sterilized. This media is selective for Coliform because it contains Tryptose, lactose, and sodium lauryl sulphate. The yoghurt sample was diluted to 10^{-3} , then 1 mL of each dilution was inoculated into an LSTB tube containing a Durham tube and incubated at 35°C for 24 hours. If gas is formed, the test is continued to the BGLBB tube as a confirmation test. A positive result is indicated by gas in the Durham tube, and the MPN value is calculated from the number of positive tubes. Meanwhile, MRS agar media was used to calculate the total BAL using the Total Plate Count (TPC) method. The sample was diluted in stages from 10^{-1} to 10^{-8} , then planted on a petri dish using the pour plate method. Colonies were counted after 48 hours of incubation at 37°C and expressed in CFU/mL.

Chemical tests were carried out in two stages, namely pH testing and lactic acid level testing. A total of 10 mL of sample was put into the cup, then measured using a digital pH meter that had been calibrated with pH 4.0 and 7.0 buffer solutions. Meanwhile, the lactic acid level test was carried out using the titration method using 0.1 N NaOH and phenolphthalein indicator. A 10 mL sample was added with distilled water, then titrated until the solution turned pink. The volume of NaOH was recorded to calculate the total lactic acid level using a formula based on the titrant volume and the molecular weight of lactic acid.

The organoleptic test was carried out involving 31 panelists. The instruments used in the organoleptic test were empirically validated by Rasch analysis. The results of person and item reliability showed that the panelists were consistent in answering the instruments given, and the instruments used were of good quality.

The data obtained were in the form of the number of coliform bacteria, the total number of BAL, the value of the lactic acid content, the pH value and the results of the organoleptic test which were analyzed descriptively quantitatively. Data on the total number of BAL, the value of the lactic acid content, the number of coliform bacteria and the pH value were compared with SNI (Indonesian National Standard) 2981 in 2008 and compared between groups descriptively quantitatively. Meanwhile, the organoleptic test results were compared between groups with the Kruskal-Wallis test statistics because they did not meet the normality requirements, where the normality requirements of the data obtained were <0.05 . Further testing was carried out by comparing between concentrations using the Mann-Whitney test to see if there were any significant differences.

RESULTS

Based on the estimation test (Table 1), treatment K0 showed the highest number of positive tubes (6 out of 9), followed by K1 (3 tubes), while K2 and K3 each had only 1 positive tube (+). Positive results indicate the presence of coliform bacteria. Then, the results of the confirmation test (Table 2) showed that the addition of red grape juice at a concentration of 10% (K2) and 15% (K3) produced yogurt with the number of coliform bacteria below the maximum limit of <10 APM/mL according to SNI No. 7388:2009.

Table 1. Results of coliform estimation test on yogurt samples with and without the addition of red grape juice

No	Sample code	Serial dilution level								
		10^{-1}	10^{-1}	10^{-1}	10^{-2}	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10^{-3}
1	K0	+	+	+	+	-	+	+	-	-
2	K1	-	-	+	-	-	-	+	+	-
3	K2	-	-	-	-	+	-	-	-	-
4	K3	+	-	-	-	-	-	-	-	-

Table 2. Results of coliform confirmation test on yogurt samples with and without the addition of red grape juice

No	Sample code	Serial dilution level			Number of bacteria (apm/ml)	SNI
		10^{-1}	10^{-2}	10^{-3}		
1	K0	3	2	1	150 apm/ml	Not in compliance
2	K1	1	0	2	11 apm/ml	Not in compliance
3	K2	0	1	0	3 apm/ml	Compliant
4	K3	1	0	0	3,6 apm/ml	Compliant

The results of the total LAB analysis using the TPC method (Table 3) showed that the total LAB in yoghurt with the addition of red grape juice was in the range of 2.01×10^9 to 2.71×10^9 CFU/mL. The highest LAB TPC was obtained in the K3 treatment, namely yoghurt with the addition of 15% red grape juice, with the number of colonies reaching 2.71×10^9 CFU/mL. This value shows that the concentration of red grapes at the K3 level significantly supports the growth of LAB during the fermentation process (Yang et al, 2023). Overall, based on SNI 2981:2009, all samples have met the SNI quality requirements with a minimum number of lactic acid bacteria of 106 CFU/mL.

Table 3. Results of total LAB test on yogurt samples with and without the addition of red grape juice

Sample code	Number of LAB (CFU/mL)	SNI
K0	2.01×10^9	Compliant
K1	2.31×10^9	Compliant
K2	2.60×10^9	Compliant
K3	2.71×10^9	Compliant

The results of the analysis of lactic acid levels (Table 4) in yoghurt K0, K1, K2, and K3 showed values between 0.81% and 1.89%. K0 showed the lowest lactic acid levels (0.81%) as a result of natural fermentation without the influence of additional compounds. The addition of red grape juice increased the acid levels gradually: K1 (1.08%), K2 (1.44%), and K3 (1.89%), along with increasing stimulation of LAB activity. Based on SNI 2981:2009, the lactic acid levels in all yoghurt samples were within the permitted range of 0.5% to 2.0%.

Table 4. Results of the lactic acid level test on yogurt samples with and without the addition of red grape juice.

Sample code	Lactic Acid Content (%)	SNI
K0	0.81	Compliant
K1	1.08	Compliant
K2	1.44	Compliant
K3	1.89	Compliant

Furthermore, the results of the pH test on yoghurt with and without the addition of red grape juice gave a value of 4.6-4.0 (Table 5). The highest pH was owned by K0 which was the control and the lowest pH value was owned by K3 with the addition of 15% red grape juice. This shows that the results are in accordance with the Indonesian National Standard (SNI) 2981:2009 which states that the pH value of yoghurt is 4.0-4.6.

Table 5. Results of pH level tests on yogurt samples with and without the addition of red grape juice

Sample code	pH	SNI
K0	4.60	Compliant
K1	4.47	Compliant
K2	4.25	Compliant
K3	4.00	Compliant

Finally, the average results of organoleptic tests (Table 6) on yogurt with and without the addition of red grape juice showed that there was a significant increase in the level of preference. In the taste attribute, K0, K1, and K3 had significant differences, while K3 was not significantly different from K2. In the color attribute, K0, K1 and K3 had significant differences, while K1 and K2 did not have a significant difference. The test results on the aroma attribute showed that K0 and K1 had the same average value and had significant differences with K2 and K3. In the texture attribute, no significant difference was found because all sig values were >0.05 .

Table 5. Organoleptic test results on yogurt samples with and without the addition of red grape juice

Sample code	Taste	Color	Aroma	Texture
K0	2.84 ± 0.374^a	2.81 ± 0.402^a	3.00 ± 0.000^a	2.68 ± 0.475^a
K1	3.74 ± 0.445^b	3.84 ± 0.374^b	3.00 ± 0.000^a	2.84 ± 0.374^a
K2	4.23 ± 0.425^c	3.90 ± 0.539^b	3.48 ± 0.508^b	2.81 ± 0.402^a
K3	4.35 ± 0.486^c	4.32 ± 0.475^c	3.81 ± 0.402^c	2.71 ± 0.461^a

DISCUSSION

This study aims to evaluate the effect of adding red grape juice with various concentrations on the quality of yogurt comprehensively in terms of microbiological, chemical, and sensory aspects. Overall, the main findings of this study are that a concentration of 10% (K2) provides optimal results and reflects the optimal balance point—effectively increasing consumer safety and acceptance. The 10% concentration contains the lowest coliform count, BAL count, pH, lactic acid levels that meet SNI requirements, and has more preferred sensory components. Meanwhile, a comparison of microbiological, chemical, and organoleptic aspects between yogurts with various concentrations will be discussed further. This study has an important contribution to SDGs Goal 3 (Healthy Lives and Well-Being) and Goal 12 (Responsible Consumption and Production). The results of this study prove the feasibility of using red grape juice as a functional and natural additive in yogurt production.

Reviewed from the microbiological aspect, the findings of the study showed that the higher the concentration of red grapes, the lower the number of coliforms. The decrease in the number of coliforms in the samples, especially K2 and K3 (<10 APM/mL), supports previous findings that polyphenolic compounds in red wine have strong antimicrobial activity (Mandal & Domb, 2024). Compounds, especially resveratrol, anthocyanins, and flavonoids contained in red wine can inhibit Gram-negative bacteria by disrupting cell membranes, binding metal ions, and changing gene expression (De Rossi et al., 2025). In other words, red grape juice functions as a natural antimicrobial agent that improves the microbiological safety of yogurt. At the same time, the increase in the LAB population at higher grape juice concentrations indicates that the compounds contained support the growth of LAB during the fermentation process (Wu et al., 2021). These results not only indicate that the starter culture used is still active and able to adapt well to the environment containing additional ingredients in the form of fruit juice (Pereira et al., 2024), in fact the presence of red grape juice tends to increase the viability and proliferation of LAB. Polyphenols can stimulate LAB growth directly by providing fermentation substrates, or indirectly by changing fermentation

conditions similar to the intestine (Makarewicz et al., 2021). Ranadheera et al. (2017) also emphasized the effectiveness of fruit-based substrates in maintaining probiotic viability in fermented milk products. Meanwhile, from a chemical perspective, increasing lactic acid levels and decreasing pH indicate that red grape juice accelerates the fermentation process. This increase in lactic acid levels is in line with research by Sharma et al. (2022), which states that fruit polyphenols can increase lactic acid production through the carbohydrate metabolism pathway. More simply, phenolic compounds and natural sugars in red grapes are thought to act as a source of energy and support LAB metabolism. Meanwhile, the decrease in pH value is because LAB produces lactic acid during fermentation so that the higher the lactic acid produced, the lower the pH value will be (Santoso et al., 2024).

In sensory analysis, yogurt with 10% and 15% grape juice was preferred in terms of aroma, color, and taste. Ivanov et al. (2023) confirmed the positive effect of polyphenols on yogurt consistency and flavor development. Another finding that panelists preferred 10% (K2) supports the idea that moderate concentrations provide the most desirable sensory experience. Anthocyanins contribute to color enhancement, while grape-derived volatile compounds enhance the aroma profile (Buican et al., 2025). Thus, higher concentrations were less preferred due to their dark color. In addition, the excessive sourness of K3 (15%) may explain the slightly lower taste scores despite the higher LAB content.

Although this study provides a comprehensive evaluation, it has several limitations. First, this study only compared concentrations of 5%, 10%, and 15%, which means it does not cover a wider variety of consumers. Second, the characterization of active compounds in grape juice has not been carried out so that the biological mechanisms of action such as suppressing coliform growth and increasing LAB counts cannot be directly explained. Third, the shelf life of yogurt was not evaluated longitudinally, whereas product stability in storage is an important aspect in commercial products. Fourth, this study did not do replications so that the results provided still have the potential for bias.

CONCLUSION

The addition of red grape juice can improve the microbiological, chemical, and sensory quality of yogurt. Among the samples observed, concentrations of 10% and 15% were able to reduce the number of coliform bacteria, increase the BAL population, reduce pH, and increase lactic acid levels in accordance with SNI. Reviewed from the organoleptic test, the 10% concentration showed the most optimal results in balancing microbial safety (microbiological), acidity (chemical), and consumer acceptance (sensory). The implication is that this finding provides additional evidence that fruit juice has the potential as a natural additive that can increase the functional and commercial value of yogurt. Practically, this finding provides guidance for the food industry to consider yogurt formulation with the addition of 10% red grape juice as a value-added product innovation.

Based on the limitations of this study, there are several suggestions for further research. First, testing using concentrations according to market interest and consumer variation, and testing consumer responses on a wider scale. Second, exploration and characterization of bioactive grape juice, evaluating the stability of the product during long-term storage longitudinally. Finally, testing using an experimental design with a completely randomized design can be carried out to strengthen the research findings.

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