

CORRELATION BETWEEN STARTER CULTURE BACTERIAL DENSITY AND THE QUALITY OF PURPLE BROWN RICE-BASED YOGURT

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ABSTRACT

Background. This study aimed to determine the optimal bacterial cell density in the initial starter culture for fermentation of plant-based yogurt produced from purple brown rice supplemented with soy milk.

Material and methods. The relationship between bacterial density and the quality of the final product was examined in terms of both physicochemical and sensory properties. The initial starter cultures had bacterial densities of 10^7 , 2×10^7 , 4×10^7 , 6×10^7 , 8×10^7 , and 10^8 cfu/mL, which were used for fermentation.

Results and discussion. The optimal bacterial density was found to be 6×10^7 cfu/mL. Yogurt produced at this density exhibited the highest water-holding capacity (96.60%) and firmness (3.88 g-force), resulting in a soft texture with minimal syneresis. The brightness L and a redness value in the CIELAB scales were 43.49 and 1.65, respectively, corresponding to the peak sensory scores. The viable cell count reached at least 2.14×10^8 cfu/mL; °Brix, lactic acid, protein, total sugar, and reducing sugar contents were 11.64, 0.63, 9.26, 19.98, and 16.28 g/100 g dry matter, respectively – values consistent with the requirements for marketable yogurt products. Although bioactive components such as anthocyanins and phenolics were present in relatively low concentrations (0.07 g and 1.08 g TAE per 100 g dry matter, respectively), their presence indicates the product's antioxidant potential.

Conclusion. The study demonstrated that an initial microbial density of 6×10^7 cfu/mL in the starter culture yields a product with the most desirable balance of fermentation efficiency and yogurt quality.

Keywords: bacterial density, fermentation, organoleptic, physicochemical, purple brown rice

INTRODUCTION

The global plant-based dairy market has experienced significant growth, driven by increasing consumer awareness of health, environmental sustainability, and ethical considerations. According to Dhakal et al. (2023), the global market for plant-based yogurt was valued at \$1.6 billion in 2019 and is projected to reach \$2.89 billion by 2026. The distribution of plant-based dairy alternatives

has been expanding for over a decade and continues to rise, primarily due to lactose intolerance, milk allergies, and the growing preference for lower-calorie, nutrient-rich options (Alcorta et al., 2021). Plant-forward diets have further accelerated the demand for plant-based yogurt as a nutritious and inclusive alternative to traditional dairy products (Montemurro et al., 2021). The

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market share of plant-based products derived from milk is expected to increase from 7.4% to over 18.5% in the coming years. A wide variety of non-dairy alternatives are produced from soybean, almond, coconut, rice, and oats, with soy-based products being the most commonly consumed (Pua et al., 2022).

Yogurt, whether traditional or plant-based, is widely recognized as a functional food due to its unique nutritional value and health-promoting properties. Both types share several health benefits, including improved digestibility and reduced bloating, largely attributed to the activity of bacterial α -galactosidase, which breaks down oligosaccharides during fermentation (Harper et al., 2022; Kumar et al., 2015). Unlike conventional yogurt made from cow's milk, plant-based yogurt is produced from aqueous extracts of legumes, grains, and oilseeds, which impart distinct compositional and nutritional characteristics (Boukid et al., 2023; Grasso et al., 2020; Silva et al., 2020). Plant-derived yogurts also offer additional advantages, as they are naturally free of cholesterol and contain bioactive compounds such as arabinose, tocopherol, and sterols, which possess antioxidant, anti-inflammatory, and anti-diabetic properties (Wang et al., 2025; Zhai et al., 2025). Among the promising raw materials for plant-based products, purple brown rice stands out as a valuable ingredient. It is favored by health-conscious consumers for its natural anthocyanin content, particularly in regions where rice is a dietary staple (Yamuangmorn and Prom-u-Thai, 2021). This rice variety is a potent source of antioxidants that help protect the body from oxidative stress and inflammation. It is also rich in amino acids, dietary fiber, vitamins, and essential minerals, making this naturally gluten-free grain a wholesome and healthful choice for modern diets (Xiong et al., 2024).

Fermenting plant-derived products with lactic acid bacteria represents a promising strategy to enhance their nutritional and functional properties (Zannini et al., 2018). Probiotic foods containing live beneficial bacteria offer numerous health benefits, including improved gut health and immune function (Kechagia et al., 2013). Fermentation not only enhances digestibility and sensory qualities but also generates new bioactive compounds with anti-inflammatory, anti-obesity, and antioxidant effects (Anumudu et al., 2024). As consumer demand continues to grow, the fermentation of novel plant-based ingredients is expected to play a central

role in the development of next-generation functional foods (Abbaspour, 2024). One of the key factors influencing the quality of fermented yogurt products is the microbial concentration in the starter culture. This factor determines the fermentation rate and directly affects gel structure formation, flavor development, color characteristics, and overall product stability (Yang et al., 2023; Anwar et al., 2025). However, there remains a lack of systematic studies evaluating the impact of starter culture inoculum levels on the physicochemical, microbiological, and sensory properties of yogurt-like products. Therefore, the present study aimed to elucidate the role of microbial density in the yogurt starter culture on the overall quality attributes of the final product and to identify the optimal bacterial concentration for the fermentation stage in producing nutritious plant-based yogurt from purple brown rice.

MATERIALS AND METHODS

Materials

Purple brown rice of the purple ST variety (Soc Trang, Vietnam) was purchased from Shrimp Rice Co., Ltd (Vietnam). Soybeans were supplied by Tam Nong Co., Ltd (Vietnam).

The commercial yoghurt starter culture, consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains, was obtained from Hanh Phuc Co., Ltd (Vietnam).

All other analytical-grade chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (China).

Sample procedure

Soy milk preparation: soybeans were rinsed to remove impurities and soaked for 8 hours to facilitate the removal of the seed coat. After soaking, the soybeans were blended using a Blue Stone BLB-6035 blender. The ratio of soybeans to water was 125 g to 600 mL (Ny et al., 2015). The blended mixture was then filtered, and the filtrate (soymilk) was collected.

Fermentation substrate: Purple brown rice (100 g) was sorted and rinsed thoroughly. After draining, the rice was cooked for 40 minutes using a TC-NK6D rice cooker (China). Water was added at a 1:4 ratio (w/v). The cooked rice was then blended into a puree and passed through a 0.5 mm sieve to collect the filtrate, which served as the rice extract.

Yogurt procedure: A mixture of soymilk and purple brown rice extract was prepared at a 1:1 (v/v) ratio and pasteurized at 85°C for 15 minutes. The total soluble solids content was adjusted to 14°Brix using sucrose, and the mixture was then cooled to 43°C. The yogurt starter culture was added to initiate fermentation, which was carried out at 43°C until the pH level reached 4.6. The resulting yogurt was stored at 4–6°C for 24 hours before further evaluation.

Experimental design

The experiment was conducted using a completely randomized design. During the fermentation stage, the commercial yogurt starter culture was added at different bacterial densities of 10^7 , 2×10^7 , 4×10^7 , 6×10^7 , 8×10^7 , and 10^8 cfu/mL. After fermentation, each yogurt sample was collected for further analysis and quality assessment.

Physical properties analysis

Color analysis: The color parameters (L, a, b) of yogurt samples were measured using a colorimeter (Konica Minolta CR400).

Texture analysis: The firmness (g-force) of the yogurt samples was measured using a texture analyzer (CT3, Brookfield, U.S.A.). Measurements were performed at 6–8°C using a TA-MP cutting blade, with the following settings: target value = 3.0 mm, trigger load = 2 g, and test speed = 5 mm/s.

Water holding capacity (WHC): Phase separation was determined by centrifugation, following the method of Zannini et al. (2018). Approximately 10 g of yogurt was placed into a centrifuge tube and centrifuged at 4,000 rpm for 20 minutes at 4°C. After centrifugation, the supernatant was carefully decanted, and the remaining gel was weighed. The WHC was calculated using the following equation (1):

$$WHC (\%) = \left(1 - \frac{W_o - W}{W} \right) \cdot 100 \quad (1)$$

Where: W is the weight of the sample after centrifugation, and W_o is the initial weight of the sample.

Total dissolved solids (°Brix): The total dissolved solids were determined using a hand-held refractometer with a measurement range of 0–32 °Brix.

Microbiological determination

The total lactic acid bacteria (LAB) count (TLC) of yogurt samples was determined using MRS agar. Diluted samples were spread on MRS agar plates and incubated at 37°C for 48 hours (Haskito and Padaga, 2019). The number of LAB colonies was then enumerated using the following equation (2):

$$LAB = \text{num of colonies} \times \frac{1}{\text{dilution factor}} \quad (2)$$

Chemical properties analysis

Lactic acid determination: Lactic acid was determined according to the method described by Borshchevskaya et al. (2016), based on a yellowish-green iron (III)–lactate complex after reaction with FeCl_3 . The absorbance of the complex formed between 50 μL of sample and 2 mL of 0.2% FeCl_3 solution was measured at 390 nm. Lactic acid concentration was calculated using a standard calibration curve: $y = 31.1360x + 0.2709$ ($R^2 = 0.9999$), where y denotes the absorbance and x denotes the protein concentration (g/mL) in the tube.

Total sugar and reducing sugar content (g/100 g of dry matter): These were measured using the DNS method with some modifications. The method is primarily based on the oxidation of the carbonyl (C = O) group by 3,5-dinitrosalicylic acid, forming an orange-red complex in an alkaline medium (Nielsen, 2017b). Briefly, 1 mL of the sample was placed in a test tube and mixed with 2 mL of DNS reagent. Standard glucose solutions and samples were mixed thoroughly, then divided into two portions for the determination of reducing sugar (without hydrolysis) and total sugar content (after 3 hours of hydrolysis in boiling water). Subsequently, 7 mL of distilled water was added before measuring absorbance at 575 nm. The total sugar concentration was calculated using a standard glucose calibration curve: $y = 23.885x + 0.126$ ($R^2 = 0.9999$), where y denotes the absorbance and x corresponds to the lactic acid concentration (g/mL) in the tube.

Total protein content (g/100 g of dry matter): This parameter was determined using a modified Lowry method (Nielsen, 2017a). Briefly, 0.1 mL of the solution (prepared by digesting 1 g of sample with 10 mL concentrated H_2SO_4) was mixed with 0.1 mL of 2 N NaOH and heated in boiling water for 10 minutes.

After cooling, 1 mL of a reagent (prepared from Na_2CO_3 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and $\text{C}_4\text{H}_4\text{O}_6\text{KNa} \cdot 4\text{H}_2\text{O}$) and 0.1 mL of Folin–Ciocalteu reagent were added. The mixture was left to stand at room temperature for 30 minutes, and the absorbance was then measured at 750 nm. Protein content was calculated using a standard calibration curve: $y = 0.0041x + 0.0118$ ($R^2 = 0.9999$), where y represents the absorbance, and x corresponds to the lactic acid concentration (g/mL) in the tube.

Bioactive compounds analysis

Anthocyanin determination (g/100 g dry matter): Anthocyanins, expressed as cyanidin-3-glucoside, were quantified based on their color change in response to pH variation. Samples were diluted in two buffer solutions: 0.025 M KCl buffer (pH 1.0) and 0.4 M CH_3COONa buffer (pH 4.5). Absorbance was measured at 520 nm and 700 nm using a spectrophotometer (Gabriela et al., 2010). Anthocyanin content was calculated using Equation (3):

$$\text{Anthocyanin content} = (A \times 449.2 \times V \times F) / (\epsilon \times l) \quad (3)$$

Where: $A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})$ at pH 1.0 – $(A_{520 \text{ nm}} - A_{700 \text{ nm}})$ at pH 4.5; F = dilution factor; V = volume of extract (L); l = path length of cuvette (1 cm); ϵ = 26,900 (molar absorptivity)

Total Phenolic compounds (g TAE per 100 g of dry matter): phenolic content was determined using the Folin–Ciocalteu method, which forms a blue phosphomolybdenum complex upon reaction with phenolic compounds (Sumaiyah et al., 2015). Briefly, 150 μL of the sample was mixed with 450 μL of 5% (w/v) Na_2CO_3 solution and 1.2 mL of distilled water in a test tube. Then, 100 μL of Folin–Ciocalteu reagent was added, and the mixture was allowed to react for 1.5 hours. Absorbance was measured at 750 nm. The phenolic content was calculated using a standard tannic acid curve (TAE): $y = 0.0021x + 0.0064$ ($R^2 = 0.9999$), where y represents the absorbance and x denotes the protein concentration (g/mL) in the tube.

Sensory characteristics

The sensory properties of the product were evaluated in terms of color, flavor, taste, and overall acceptability. This assessment was performed using the Quantitative Descriptive Analysis (QDA) method. Panelists

were instructed to evaluate each yogurt sample based on these attributes, using a descriptive scale from 1 to 5, where 1 indicated “poor” and 5 indicated “excellent” (Thuy et al., 2012). Overall preference level was further assessed using a Hedonic scale.

Data analysis

Data were analyzed using Statgraphics Centurion XVI software (USA.). The LSD test was applied to identify differences among treatment means at a 5% significance level ($P = 0.05$). Microsoft Excel was used for data computation and graphical presentation.

RESULTS AND DISCUSSIONS

Influence of the bacterial concentration in the added starter yogurt on the microbial count of the yogurt product

The results showed that the initial bacterial concentration in the starter yogurt had a significant effect on the bacterial count of the final product. The highest viable cell count (3.7×10^8 cfu/mL) was obtained when the starter yogurt contained 8×10^7 (cfu/mL), which differed significantly from the other concentrations tested. However, when the inoculum level was increased further to 10^8 cfu/mL, the bacterial count in the final product decreased.

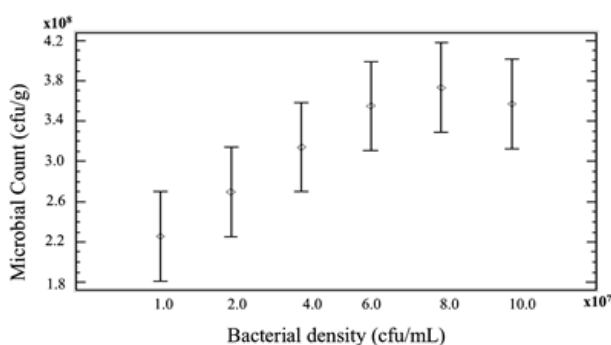


Fig. 1. The microbial count of the yogurt product at different initial bacterial densities

Under identical volume and nutrient conditions, only an optimal inoculum level can yield the maximum biomass. A low inoculum level prolongs the bacterial growth phase, whereas an excessively high

inoculation rate rapidly depletes available nutrients, leading to early cell senescence (Hoang et al., 2024). To ensure the health benefits of plant-based fermented dairy alternatives, the final product should contain a minimum viable cell count of 10^6 – 10^7 cfu/g (Shorti and Baba, 2013). In this study, all purple rice-based yogurt formulations exceeded 10^8 cfu/g (Fig. 1).

A key characteristic of yogurt is the presence of live microorganisms responsible for fermentation, which directly influence the fermentation time. Generally, a higher microbial cell density shortens the fermentation time needed to reach the target pH (Célia et al., 2022). The fermentation duration varies widely between dairy and plant-based yogurt matrices. In cow's milk yogurt, the presence of readily metabolizable lactose and the buffering capacity of casein result in a relatively stable fermentation time of approximately 4–7 hours at 42–45 °C (Almghawesh et al., 2022; de Souza et al., 2024). By contrast, plant-based substrates such as soy, oat, or cereal-based matrices show a broader fermentation range (2–12 hours), depending on their sugar composition, protein structure, and antinutritional factors that can inhibit microbial activity (Harper et al., 2022; Dhakal et al., 2023). For instance, soy-based yogurt can reach the target pH within 2–2.5 hours with optimized starter cultures, whereas other plant formulations may require more than 8 hours (Almghawesh et al., 2022). Oat-based systems often take up to 12 hours to achieve the desired acidity and gel consistency (Mary-Liis et al., 2023). In the present study, the fermentation process required approximately 7 hours (405 minutes) for the yogurt to reach a pH of 4.6, which aligns with previous studies.

Influence of the bacterial concentration in the added starter yogurt on °Brix and lactic acid in the product

The results shown in Fig. 2a indicate that the inoculated bacterial concentration influenced the final °Brix of the product, with higher bacterial cell densities leading to a decrease in °Brix. When the bacterial concentration was 10^7 cfu/mL, the total soluble solids content (°Brix) was 12.23; however, increasing the inoculated bacterial concentration to 6×10^7 – 10^8 cfu/mL resulted in °Brix values of approximately 11.53–11.64, though the difference was not statistically significant at the 5% level. Lactic acid bacteria utilize sugars and other available nutrients in the milk matrix for growth and fermentation, producing lactic acid as a by-product (Thomas, 2018). As the inoculated bacterial count increases, more sucrose is metabolized, leading to a reduction in total soluble solids (Hee and Myung, 2019).

Conversely, lactic acid content was directly proportional to the inoculated bacterial cell density. The highest lactic acid concentration (0.66 g/100 g dry matter) was observed when the starter culture was inoculated at 10^8 cfu/mL. At bacterial concentrations of 10^7 , 2×10^7 , 4×10^7 , 6×10^7 , 8×10^7 cfu/mL, the corresponding lactic acid contents were 0.60, 0.61, 0.62, 0.63, and 0.64 g/100 g dry matter, respectively.

Lactic acid bacteria (LAB) are characterized by their strong ability to produce lactic acid through the metabolism of various mono- and disaccharides. Lactic fermentation refers to the microbial conversion of sugars into lactic acid, typically by LAB. During yogurt fermentation, changes in pH and lactic acid concentration vary depending on the bacterial cell density.

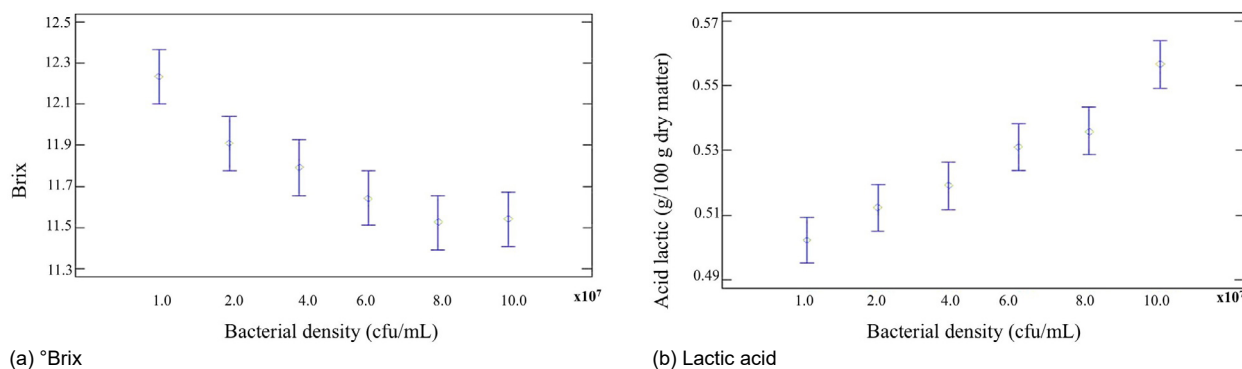


Fig. 2. °Brix and lactic acid content of the product at different initial bacterial densities

According to Nguyen et al. (2022), soy milk inoculated with LAB at concentrations of 0.1% and 0.5% showed an increase in acidity and a corresponding decrease in pH over time during fermentation. Different inoculation levels lead to distinct acid production rates and pH changes. At 0.1% and 0.5% inoculation levels, LAB exhibited vigorous growth, and lactic acid production proceeded more rapidly. At these concentrations, a marked decline in pH was observed within the 6–12 hour fermentation period, corresponding to the most active phase of microbial metabolism. This can be attributed to the exponential growth phase of the microorganisms: as the bacterial population increases and reaches its peak, the pH drops to around 4.2–4.6. After 12 hours, further pH decline becomes negligible. This finding aligns with the study by Ny et al. (2015), which reported minimal pH decrease after 16 hours of fermentation, with the most significant drop occurring between 4 and 16 hours. Several studies have also identified that the optimal pH for high-quality fermented milk products ranges from 4.2 to 4.6 (Nguyen and Do, 2020). Furthermore, different inoculation levels result in varying lactic acid yields. Most LAB strains are capable of producing between 0.5% and 1.5% lactic acid, while some can produce up to 3% (Le, 2018). However, excessive inoculation can reduce osmotic pressure, inhibit microbial cell activity, promote the formation of undesirable by-products, and ultimately decrease the acid-producing capacity of *Lactobacillus* during yogurt fermentation (Le and Ngo, 2020).

In plant-based yogurts, the interaction between microbial density, °Brix, and lactic acid production plays a decisive role in determining product quality. As LAB proliferate, they consume available sugars, resulting in a decline in °Brix values and an increase in lactic acid, which progressively lowers pH (Rui et al., 2019). While sufficient sugar availability supports high microbial density and stable gel formation, excessive °Brix may accelerate acidification, leading to over-souring and structural instability. Conversely, insufficient °Brix can limit microbial growth, resulting in weak acidification and poor texture (Walther et al., 2022; Xu et al., 2024). Therefore, identifying the optimal bacterial inoculum level is essential to balance the interplay between lactic acid formation and °Brix levels, ensuring better alignment between flavor development and textural stability.

Influence of the bacterial concentration in the added starter yogurt on the product's chemical properties

The results showed that the protein content of the product was influenced by the bacterial concentration in the added starter yogurt, with statistically significant differences observed at the 5% level. As shown in Figure 3a, the protein content increased with higher inoculated bacterial concentrations, reaching a maximum of 9.37 g/100 g dry matter when the starter culture was supplemented at 2×10^7 to 8×10^7 cfu/mL. However, this increase was not statistically significant compared to the other supplemented samples ($p > 0.05$).

Similarly, the total sugar content of the yogurt was affected by the bacterial concentration in the starter yogurt, with significant differences at the 5% level. In contrast, the reducing sugar content was not significantly affected ($p > 0.05$). As shown in Figure 3b, the total sugar content increased with higher bacterial concentrations, reaching its peak (20.14 g/100 g dry matter) at 10^8 cfu/mL. Between 6×10^7 cfu/mL and 10^8 cfu/mL, however, this increase was not statistically significant ($p > 0.05$). Figure 3c shows that the reducing sugar content fluctuated slightly between 15.82 and 16.66 g/100 g dry matter.

The starter yogurt was fermented from a mixture of purple rice extract and soy milk. A higher inoculum concentration implies a greater volume of starter added during fermentation, thereby increasing the nutritional content of the final product. Studies by Barbosa et al. (2020) and Deziderio et al. (2023) reported that the protein content of plant-based yogurts did not differ significantly between the substrate and the fermenting agent. Moreover, microbial biomass itself contributes to protein content. Therefore, an increase in the inoculated bacterial concentration naturally enhances the protein content of the yogurt. This can be explained by the metabolic activity of LAB, which converts sugars into lactic acid. The resulting acidification promotes protein coagulation and gel formation, improving the apparent protein content of the gel matrix by enhancing water-holding capacity and reducing syneresis (Rui et al., 2019; Walther et al., 2022). However, when microbial density becomes excessive, intensified proteolytic activity can occur. LAB and adjunct cultures secrete proteases and peptidases that degrade plant proteins into peptides and

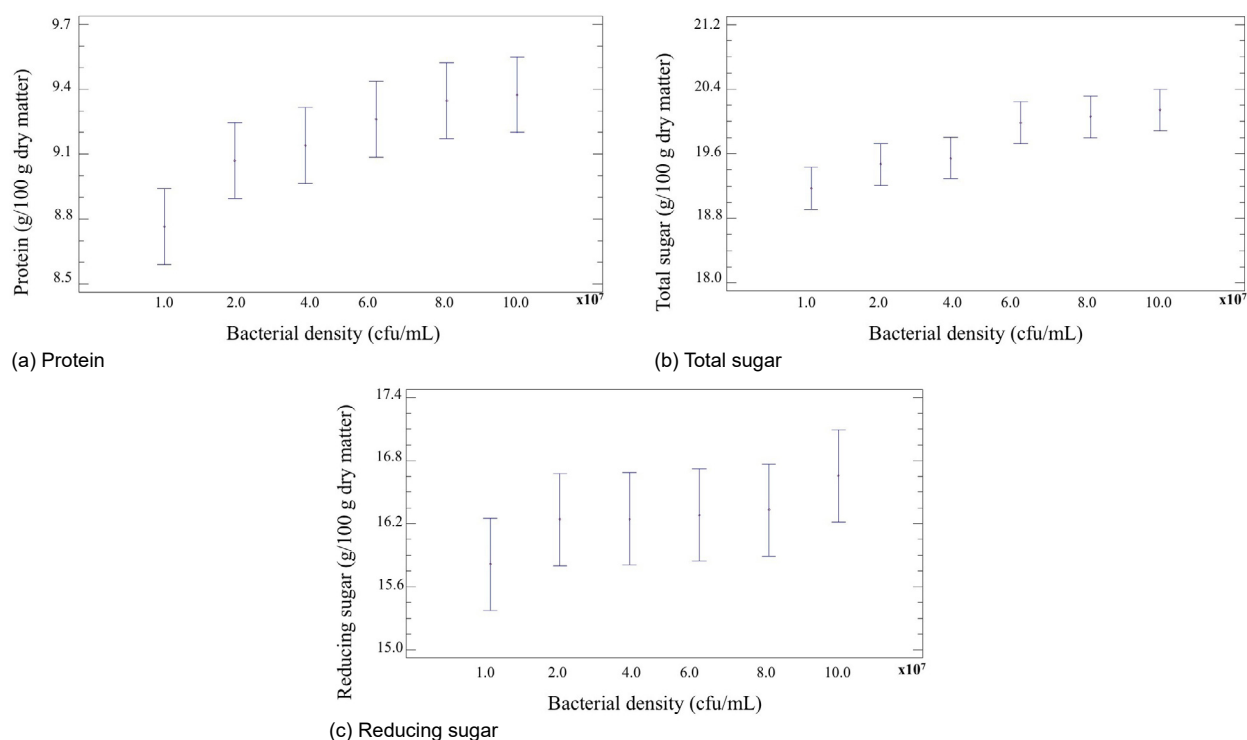


Fig. 3. Chemical compositions at different initial bacterial densities

free amino acids. While this can improve digestibility and bioactive peptide release, it also reduces the measurable intact protein fraction. Excessive proteolysis may weaken the gel network, soften the texture, and increase water separation (Walther et al., 2022; Xu et al., 2024).

Additionally, yogurt fermentation involves the conversion of sugars into lactic acid, and the primary fermentation substrates in this study were purple rice and soybeans. Lactic acid bacteria can produce enzymes that hydrolyze starch into sugars, which are then metabolized during fermentation (Barbosa et al., 2020). As bacterial biomass increased (Fig. 1), the total sugar content in the product varied noticeably with the inoculum level. However, when the bacterial concentration exceeded 8×10^7 – 10^8 cfu/mL, the total sugar content increased only slightly and without statistical significance. According to Bui et al. (2019), beyond a certain threshold, additional sugar content does not enhance biomass yield or fermentation efficiency and therefore does not increase reducing sugar levels. At low initial bacterial concentrations, microorganisms

require an adaptation period before reaching optimal density and initiating fermentation (Hoang et al., 2024). Conversely, excessively high inoculum levels can raise production costs and negatively affect product quality (Giang et al., 2022).

Influence of the bacterial concentration in the added starter yogurt on bioactive compounds remaining in the product

The results showed that the phenolic and anthocyanin contents in yogurt made from purple glutinous brown rice and soy milk increased to an optimal value and then gradually decreased with higher inoculated bacterial cell densities. Specifically, the phenolic content increased from 1.01 g TAE/100 g dry matter to 1.08 g TAE/100 g dry matter as the inoculated bacterial density rose from 1×10^7 to 6×10^7 cfu/mL. However, further increasing the inoculation level to 8×10^7 – 10^8 cfu/mL did not result in a significant difference compared to 6×10^7 cfu/mL. A similar trend was observed for anthocyanin, which peaked at 0.07 g/100 g dry matter at 6×10^7 cfu/mL.

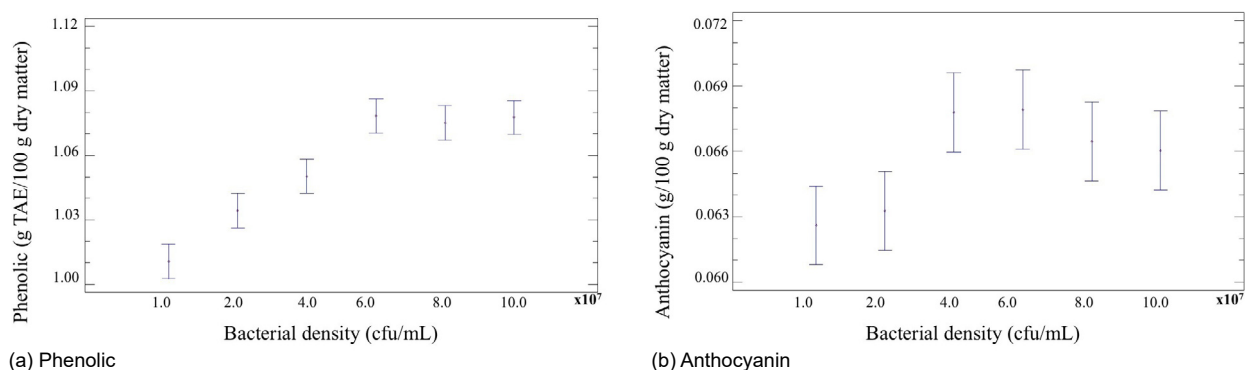


Fig. 4. Bioactive compounds remained in yogurt at different initial bacterial densities

Purple brown rice, the main substrate, is naturally rich in anthocyanins. The rice was gelatinized and finely ground before fermentation, which reduced phase separation and provided an accessible carbon source for bacterial growth (Ignat et al., 2020). Soy milk also contributed essential macro- and micronutrients. Lactic acid bacteria (LAB) can secrete enzymes that break down carbohydrates, thereby enhancing the bioavailability of bioactive compounds, especially water-soluble compounds like anthocyanins and phenolics as microbial populations proliferate (Dinh et al., 2024; Shi et al., 2024). However, when the inoculation level becomes excessively high, the decrease in osmotic pressure can inhibit microbial cell activity (Le and Ngo, 2020). Additionally, rapid fermentation occurs, resulting in the production of large amounts of lactic acid (Hoang et al., 2024). When the acidity of the fermentation medium rises beyond the optimal range, bacterial metabolism slows, reducing enzymatic activity and consequently the metabolic conversion rates (Huynh et al., 2020).

Influence of the bacterial concentration in the added starter yogurt on water holding capacity and firmness of the product

The results in Fig. 5a demonstrate that the inoculated bacterial cell density influenced the firmness of the purple brown rice yogurt. The yogurt's firmness ranged from 3.88 to 4.05 g-force when the starter culture was inoculated at concentrations of 6×10^7 to 1×10^8 cfu/mL, with no statistically significant differences observed among these levels. However, a low inoculation density reduced firmness, with a value of 2.00 g-force at

10^7 cfu/mL. Firmness is a critical parameter in yogurt quality assessment, as it directly affects sensory perception. According to Thuy et al. (2019), increasing the inoculated bacterial density leads to higher product firmness, attributed to the faster bacterial growth rate and increased lactic acid production, which promote the formation of a firmer coagulated gel structure.

Firmness is widely recognized as one of the most important texture parameters, particularly for fresh and minimally processed foods (Chen and Opara, 2013). In sensory science, firmness strongly correlates with consumer perception of freshness and textural acceptability, serving as a link between instrumental measurements and oral processing cues such as chewing effort (Liu et al., 2017). Peleg (2019) emphasizes that the traditional Texture Profile Analysis (TPA) "firmness" value remains a key indicator; however, it should be interpreted alongside other complementary parameters to capture the full complexity of food texture. Water holding capacity (WHC) is one such parameter closely associated with firmness.

The results in Figure 5b show that increasing the inoculated bacterial cell density enhanced the water-holding capacity (WHC) of purple brown rice yogurt up to an optimal point, after which it declined. The highest WHC was observed at an inoculation level of 6×10^7 cfu/mL (96.60%), with no statistically significant difference compared to treatments inoculated with 2×10^7 – 10^8 cfu/mL ($p > 0.05$), which yielded WHC values of 95.96%, 96.52%, 95.66% and 95.34%, respectively.

The inoculation of different bacterial concentrations during yogurt production influences physical

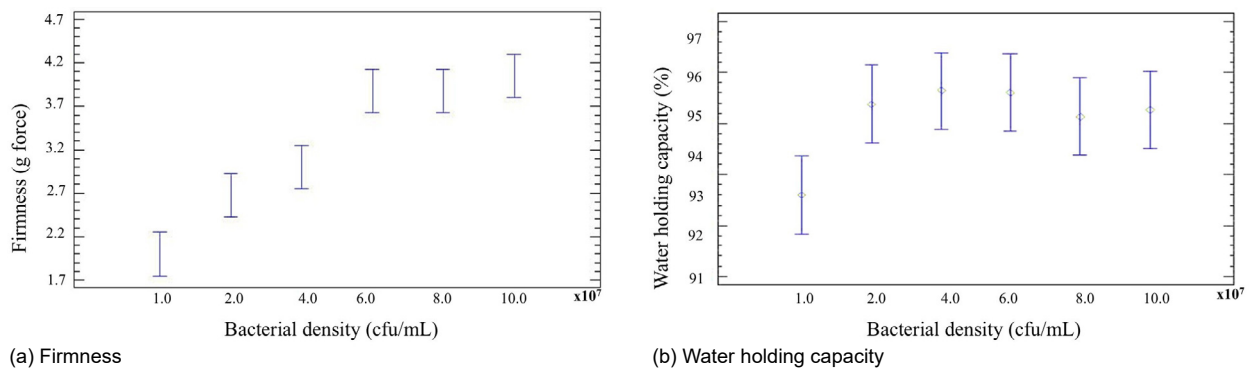


Fig. 5. Yogurt's texture and WHC values at different initial bacterial densities

properties such as firmness and WHC, depending on cell density. At lower inoculation levels, the reduced WHC may be explained by the limited inhibition of casein fiber contraction within the product's microstructure. The presence of fat globule membrane fragments may also interfere with casein contraction during gel formation (Le et al., 2011). Conversely, increasing the inoculated bacterial density enhances the levels of membrane proteins and lipids derived from soy, which can strengthen water retention and improve the cohesion of the curd matrix during yogurt fermentation (Chenebault et al., 2022).

Influence of the bacterial concentration in the added starter yogurt on the color of the product

The results showed that the yogurt's color parameters varied with bacterial inoculation levels. The highest lightness value (L^*) was recorded at 44.73 when the inoculated bacterial density was 8×10^7 cfu/mL, while

the lowest L^* value (43.10) occurred at 2×10^7 cfu/mL. As shown in Figure 6b, the redness value (a^*) peaked at 1.89 with an inoculation level of 4×10^7 cfu/mL and reached the lowest value (1.41) at 2×10^7 cfu/mL.

Bacterial inoculation levels significantly influenced the color attributes of the yogurt. Higher concentrations of lactic acid bacteria accelerated the fermentation process, leading to increased lactic acid production and a rapid drop in pH. The acidification promotes the conversion of anthocyanins into their purple-colored forms, thereby increasing the a^* value (Duong et al., 2023; Ho and Vu, 2024)

Influence of the bacterial concentration in the added starter yogurt on the sensory perception of the product

Sensory evaluation results indicated that the highest color score (4.97) was obtained when the yogurt was inoculated with 6×10^7 cfu/mL of bacterial culture. At this inoculation level, the yogurt exhibited

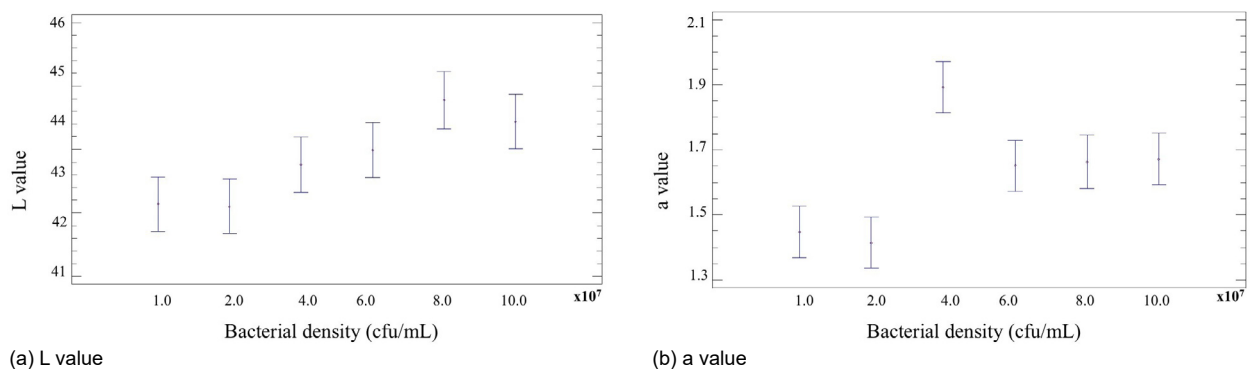


Fig. 6. Yogurt's color reflection at different initial bacterial densities

a distinctive aroma of purple rice and soy milk (4.86) and a rich, well-balanced sweet–sour taste derived from the combination of purple brown rice and soy milk (4.97). Additionally, the product achieved a high overall acceptability score (8.3).

The structure of the yogurt product was comprehensively analyzed based on key physicochemical parameters, including firmness (Texture Profile Analysis) and water-holding capacity (WHC). To enhance the reliability of the structural evaluation, a sensory assessment was also performed to reflect the panelists' practical perception of the yogurt's texture. The results revealed a correlation between instrumental measurements and sensory parameters. At an inoculation level of 6×10^7 cfu/mL, the yogurt exhibited a homogeneous texture without syneresis, achieving the highest score (4.97). This finding was consistent with the firmness index reported earlier, which showed no statistical difference from the maximum value.

Table 1. Effect of bacterial density on the sensory attributes of the product

Bacterial density (cfu/mL)	Color	Aroma	Taste	Texture	Preferred level
10^7	3.33 ^{*a}	3.08 ^a	3.08 ^a	2.99 ^a	6.99 ^a
2×10^7	2.91 ^a	2.49 ^a	2.91 ^a	2.5 ^a	7.11 ^b
4×10^7	3.08 ^a	3.08 ^a	2.72 ^a	3.08 ^a	6.41 ^a
6×10^7	4.97 ^b	4.86 ^b	4.97 ^b	4.97 ^b	8.30 ^c
8×10^7	2.66 ^a	2.91 ^a	2.99 ^a	2.99 ^a	6.61 ^{ab}
10^8	2.61 ^a	3.11 ^a	3.05 ^b	2.91 ^a	6.47 ^{ab}

Values are presented as means of triplicate tests. Values with different superscript letters in each column are statistically significantly different.

In yogurt production, a certain level of acidity is required to achieve proper protein coagulation. At higher bacterial concentrations, more lactic acid is produced, enabling the yogurt to reach the target acidity in a shorter time. However, excessive acidification can negatively affect flavor, texture, and consistency, and may lead to consumer discomfort (Nguyen and Nguyen, 2018).

CONCLUSION

The findings of this study indicate that an initial bacterial cell density of 6×10^7 cfu/mL in the starter culture produces the most desirable results in yogurt fermentation. At this inoculation level, the final product demonstrated optimal physicochemical and sensory characteristics. These results suggest the potential for further research aimed at developing novel and sustainable fermented products from locally available raw materials.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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