



Research article

Production optimization and properties of carbohydrate-based yogurts utilizing *Dioscorea hispida* Dennst tubers as raw material

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Abstract

Importance of the work: Dairy-based yogurts can trigger lactose intolerance, increasing the demand for carbohydrate-based alternatives for health reasons.

Objectives: To investigate the potential of *Dioscorea hispida* Dennst tubers as raw material for producing carbohydrate-based yogurt.

Materials and Methods: Yogurts were made using *D. hispida* flour and starch, using *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) and *Streptococcus thermophilus* (ST). The production varied the tuber-to-water ratio, lactic acid bacteria and skim milk. Product quality was determined based on the pH, titratable acidity, syneresis and viable cell count. The extended fermentation, storage stability, proximate composition and sensory properties of the products were evaluated.

Results: The optimal flour-based product used a flour-to-water ratio of 1:10, a bacterial mix of LB and ST and 1% skim milk. The starch-based product used a starch-to-water ratio of 1:8, the same bacterial mix and 3% skim milk. Extended fermentation showed significant ($p < 0.05$) variations in titratable acidity (TA) and viable cell counts for yogurt-like product from flour (YLPF), while yogurt-like product from starch (YLPS) had differences in the pH and TA. There was a significant difference in the TA between the two products. Storage at room and cold temperatures (4°C) affected the syneresis, TA and viable cell count for both products, with room temperature storage producing slightly better results. Compared to YLPF, YLPS had higher levels of crude protein, lipid, fiber, reducing sugar and TA. Sensory evaluation revealed that both were inferior to the dairy-based control.

Main finding: *D. hispida* tubers had potential as a substrate for carbohydrate-based yogurts. However, further optimization is required to improve the storage stability and sensory qualities. This study should provide a foundation for developing novel carbohydrate-based products from underutilized tuber crops.

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Introduction

Yogurt, a fermented dairy product, has served as a traditional preserved food for thousands of years. Yogurt fermentation uses lactic acid bacteria, mainly *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. These bacteria convert lactose into lactic acid, which preserves products by creating an acidic environment that inhibits the growth of harmful microorganisms. This inhospitable environment effectively extends product shelf life. The acids also act as mild flavor enhancers, improving the texture and consistency of fermented products (Ayivi and Ibrahim, 2022). Certain yogurt products, such as Greek yogurt, possess probiotic properties. These types of yogurt have attracted attention for their potential benefits for digestive health. These specialized yogurts are enhanced with beneficial probiotic strains, such as *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Lactobacillus casei* (Moineau-Jean et al., 2019). Despite disagreements regarding health claims (Daliri and Lee, 2015), probiotic yogurts are believed to support gut health, enhance immune function and improve nutrient absorption (Khalesi et al., 2019; Merenstein et al., 2024).

Commercially available yogurts are mostly derived from dairy sources. However, the prevalence of allergies and lactose intolerance has increased the demand for non-dairy alternatives. Several studies have documented the utilization of rice derivatives (Plengsaengsri et al., 2021), oat protein concentrate (Brückner-Gühmann et al., 2019), sprouted tigernut tubers (Ogundipe et al., 2021), pea milk (Ma et al., 2021) and a mixture of coconut and soymilk (Kosterina et al., 2020) for yogurt production. However, tuber-based substitutes have not been extensively studied, even though the indigestible carbohydrates in tubers may possess health benefits by promoting beneficial gut bacteria, enhancing digestion and regulating blood sugar levels. These carbohydrates also contain phytochemicals that provide antioxidant effects, enhance gut barrier function and reduce the risk of cardiovascular diseases and diabetes (Xu et al., 2021).

One of the essential tuber types in Asian and African countries belongs to the *Dioscoreaceae* family, which comprises over 800 species. Although the carbohydrate content of *Dioscorea* spp. is lower, it has a higher caloric value than that of cassava. *Dioscorea* spp. also contain sufficient amounts of vitamins A and C (Chandrasekara and Josheph Kumar, 2016). The amounts of potassium and phosphorus were higher than those in other tubers. In addition to being used as a staple

food, *Dioscorea* spp. also plays a vital role in socio-economic, cultural and religious aspects in some African countries (Barton, 2020). *Dioscorea* spp. has long been used as a health supplement in traditional Chinese medicine (Chen et al., 2017) because of its potent antioxidant activity (Mainasara et al., 2021; Chang et al., 2023).

One of the rarely exploited species of the *Dioscoreaceae* family is *Dioscorea hispida* Dennst (Asiatic bitter yam). Our survey in the Leuser Ecosystem Area in Aceh Province shows that the superiority of *D. hispida* over other tuber crops is its ability to grow in all soil types and its resistance to various pests. In addition to its high carbohydrate content (72%), *D. hispida* tubers also contain crude protein (approximately 6%) and minerals (over 1.0%), with phosphorus being the main mineral. They are rich in alkaloids and terpenoids (Saleha et al., 2018). They also contain cyanohydrin, which can decompose into toxic cyanide, rendering them infrequently used. Nevertheless, cyanide can be effectively removed by repeated washing and heating (Estiasih et al., 2022).

Given that *D. hispida* tubers are rich in carbohydrates and active compounds but seldom utilized, this study aimed to explore their potential as a dairy substitute for yogurt production. Fermentation used two forms of tubers, that is, flour and starch. The fermented products were evaluated for pH, titratable acidity, syneresis and viable cells. The storage stability, proximate analysis and organoleptic qualities of the products were further assessed. The findings of this study could enhance the utilization of this plant, thereby increasing its economic value.

Materials and Methods

Samples and microorganisms

Dioscorea hispida (DH) tubers collected from forests in Pekan Biluy, Aceh Besar district, were processed into flour and starch using a method adapted from Aprianita et al. (2014). Fresh tubers were peeled, blended with 1% sodium bisulfite solution in a 1:1 (w/v) ratio and divided into two portions. Half of the slurry was left to precipitate for 24 hr to produce DH flour. The precipitate was filtered using a Buchner funnel with qualitative filter paper connected to a water vacuum system. The precipitate in the funnel was washed thrice with 500 mL of distilled water. To produce DH starch, the other half was squeezed in a clean fine cloth and the filtrate was allowed to

settle. After decanting the solution, the residue was filtered in the same manner as the DH flour. The resulting starch was washed thrice with 500 mL of distilled water. Both flour and starch underwent repeated washing to remove water-soluble toxic compounds (Estiasih et al., 2022). Both flour and starch were dried at 70°C for 24 hr, sieved through a 100-mesh sieve and stored in airtight containers for further use. The lactic acid bacteria (LAB), *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) and *Streptococcus thermophilus* (ST) were obtained from the culture collections of the Milk Processing Laboratory of the Faculty of Agriculture, Universitas Syiah Kuala. LB and ST were cultured on separate plates containing 3% solid DeMan, Rogosa and Sharpe (MRS) medium and incubated at 40°C for 24 hr. A single colony from each plate was propagated in separate 3% liquid MRS media containing 12.5% skim milk and incubated at 40°C for 16 hr. The optical density of the culture was determined at 600 nm (OD600), where an OD600 of 1.0 corresponded to approximately 10⁸ cells/mL (Trabelsi et al., 2013). The cultures were used as inocula for the yogurt fermentation experiments.

Fermentation optimization of Dioscorea hispida flour and starch

Variation in flour- and starch-to water ratios of Dioscorea hispida

In separate Erlenmeyer flasks, three portions of DH flour were mixed with water at ratios of 1:8, 1:10 and 1:12 (w/v) to a final volume of 50 mL. The same procedure was applied to the DH starch. All mixtures were pasteurized at 85°C for 10 min (Ashri et al., 2014). Equal amounts of LB and ST were aseptically added to the mixtures in each container to obtain a total cell density of 10⁸ cells/mL. The inoculated mixtures were then incubated in an incubator at 40°C for 16 hr under static conditions. The pH, syneresis and titratable acidity (TA) were determined as described below.

Variation of lactic acid bacteria

The DH flour-to-water ratios and DH starch-to-water ratios that yielded the optimal parameters were used in the LAB variation experiment. The media were prepared as described previously. Various concentrations of LAB starters, namely LB 10⁸ cells/mL, ST 10⁸ cells/mL and LB+ST (5×10⁷ + 5×10⁷ cells/mL), were added aseptically to the media. The inoculated mixtures were incubated at 40°C for 16 hr under static conditions. The pH, syneresis and TA were determined as described below.

Variation of skim milk addition

The DH flour and DH starch-to-water ratio and the LAB concentration that yielded the optimal parameters were used in the skim milk addition experiment. Media supplemented with 1%, 2% and 3% (w/v) skim milk were prepared as described previously. The media were supplemented with LAB (10⁸ cells/mL) and incubated at 40°C for 16 hr under static conditions. The pH, syneresis and titratable acidity were determined as described below.

The optimal products made using DH flour and DH starch following fermentation optimization are hereinafter referred to as the yogurt-like product from flour (YLPF) and the yogurt-like product from starch (YLPS), respectively.

Extended fermentation experiment

Fermentation of YLPF and YLPS was observed for 32 hr. The media were prepared as described previously. Fermented samples were collected at 8 hr, 16 hr, 24 hr and 32 hr. The pH, syneresis, titratable acidity and viable cells were determined.

Analysis of pH, syneresis, titratable acidity and viable cells

The pH was measured using a pH meter, while syneresis was analyzed by centrifuging samples at 1000×g, 4°C for 10 minutes, calculated as the ratio of supernatant weight to sample weight using Equation 1 (Dönmez et al., 2017):

$$\text{Syneresis} = W_{\text{supernatant}} / W_{\text{sample}} \times 100 \quad (1)$$

where W is the weight in grams.

TA was determined based on NaOH titration to predict the formation of organic acids, particularly lactic acid. A sample in a glass beaker was added with one drop of 1% phenolphthalein and mixed thoroughly. The sample was titrated with 0.1 N NaOH solution until it turned pink. TA was calculated using Equation 2 (Tsegay, 2020):

$$\text{Titratable acidity} = V_{\text{NaOH}} (\text{N}) \times C_{\text{NaOH}} (\text{N}) \times Mr_{\text{lactic acid}} (\text{g/mol}) / (W_{\text{sample}} \times 1000) \times 100 \quad (2)$$

Viable cells were calculated by first combining 1 mL of diluted sample with 20 mL of half-warm sterile MRS medium in a Petri dish. The mixture was well shaken, allowed to solidify and incubated at 40°C for 48 hr. Colony-forming units (CFU) were counted using a colony counter.

Storage experiment

The storage test of YLPF and YLPS was carried out for 7 d at room temperature and 4°C. Sampling was performed daily and changes in pH, syneresis, titratable acidity and viable cells were determined.

Proximate analysis

Proximate analysis of YLPF and YLPS was conducted to measure the content of ash, protein, lipids, crude fiber and reducing sugars (Association of Official Analytical Chemists, 2005). Total ash content was assessed using the ashing method 942.05 (Association of Official Analytical Chemists, 2005), calculated as the weight difference before and after ashing. Crude fiber was determined by removing lipids via Soxhlet extraction based on method 985.29 (Association of Official Analytical Chemists, 2005), followed by acid reflux and neutralization. The fiber content was calculated from weight differences pre- and post-treatment. Protein content (total nitrogen) was measured using the micro-Kjeldahl method with a 5.70 multiplication factor, according to method 920.87 (Association of Official Analytical Chemists, 2005). Lipid content was also assessed by Soxhlet extraction using n-hexane, according to the 948.22 method (Association of Official Analytical Chemists, 2005). Reducing sugars were quantified using the Luff-Schoorl method (Dekker, 1950).

Sensory test

Sensory evaluations were conducted to assess panelists' perceptions of the products, following the procedure described by Poste et al. (Poste et al., 1991). Commercially plain yogurt, with the brand undisclosed, was used as the control sample. The evaluation was conducted with 20 untrained panelists, who assessed the characteristics of color, texture, flavor, aroma and overall acceptance using a hedonic scale from 1 to 5. On this scale, 1 represented 'very like,' 2 'like,' 3 'neutral,' 4 'dislike and 5 'very dislike.' The responses were compiled and averaged.

Statistical analysis

All experiments were conducted in triplicate, and data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). Analysis of variance was used to evaluate the effects of treatments, followed by Tukey's HSD

post hoc test when significant main effects were obtained. Where appropriate, pairwise comparisons between two groups under the same independent variables were conducted using a student's t test. Statistical significance was set at $p < 0.05$.

Results

Optimization involved varying the DS flour/starch-to-water ratio, testing different lactic acid bacteria (LAB) and adding various concentrations of skim milk to induce LAB growth. The optimal results were assessed based on pH, syneresis and titratable acidity. Fermentation of products with optimal parameters was extended to evaluate microbial growth, followed by storage studies. Proximate composition and sensory analysis of the products were conducted to assess their quality.

Variation of the of *D. hispidus* tubers to water ratio

Various tuber-to-water ratios (1:8, 1:10 and 1:12 w/v) were evaluated using DH flour and DH starch. DH flour products had a pH of approximately 4.4, while DH starch products ranged from 3.6 to 3.9. Analysis of variance showed that the ratio did not significantly affect the final pH of both products (Fig. 1A). TA values for DH flour ranged from 0.14% to 0.23%, while those for DH starch ranged from 0.14% to 0.19%. Statistically, TA differed significantly among the DH starch products but not among the DH flour products (Fig. 1B)

Syneresis varied considerably across different ratios of DH flour products. The 1:8 and 1:12 ratios showed high syneresis (26.1% and 41.0%, respectively), while the 1:10 ratio showed negligible syneresis. In contrast, the DH starch products showed lower syneresis (3.5% to 13.7%), with the 1:8 ratio yielding the lowest value. Analysis of variance revealed that tuber-to-water ratios significantly influenced syneresis in both product types (Fig. 1C). Detailed statistical analysis, including Tukey's HSD post hoc test, is available in Supplementary Tables S1– S2.

For subsequent LAB variation experiments, optimal ratios of 1:10 for DH flour-to-water and 1:8 for DH starch-to-water ratios were selected. These ratios were chosen based on their combination of low syneresis and high TA values.

Variation lactic acid bacteria

The LAB variation experiment used LB, ST and a

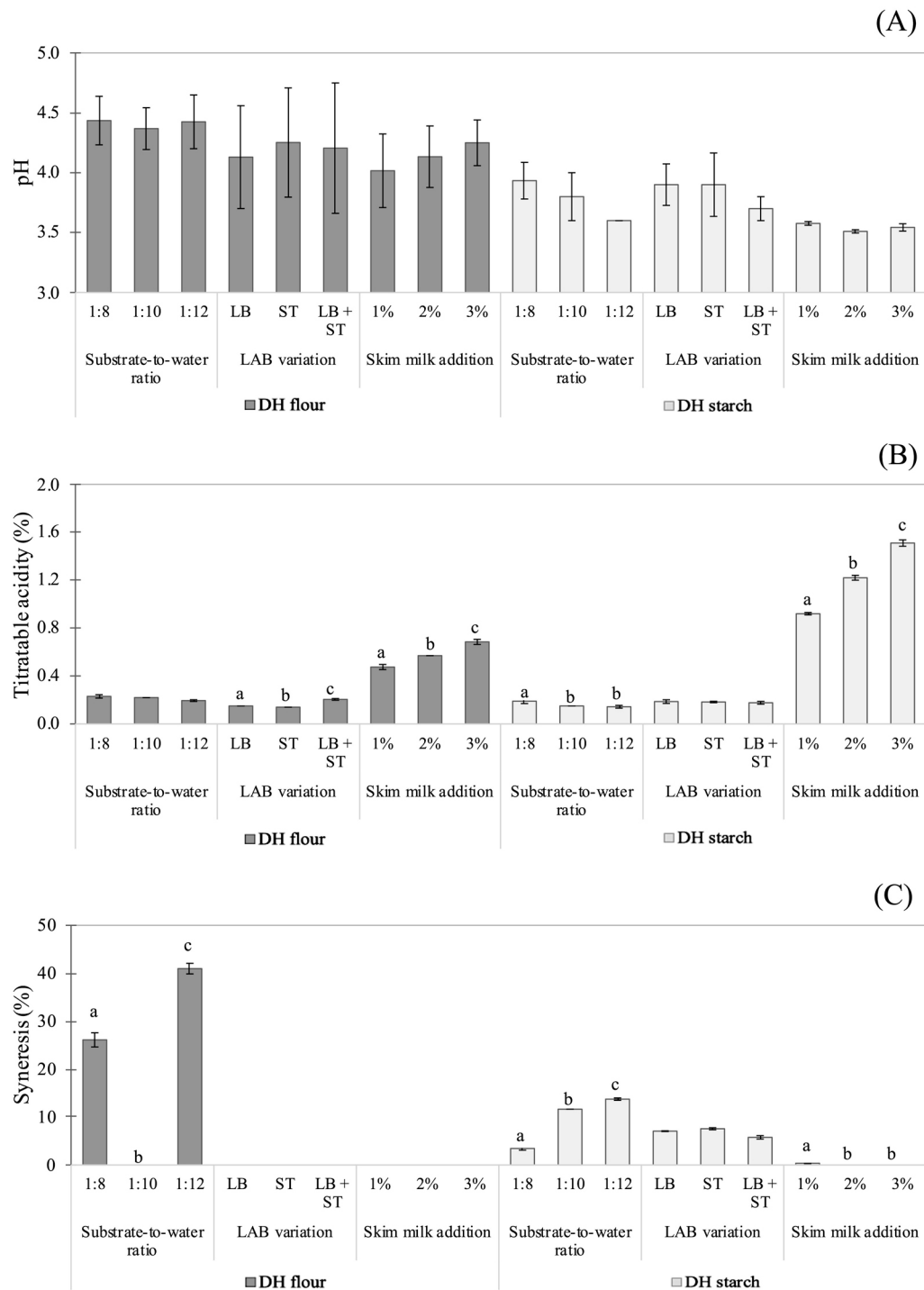


Fig. 1 Effect of substrate-to-water ratio variation, lactic acid bacteria (LAB) variation and skim milk addition using *Dioscorea hispida* flour (DH flour) and starch (DH starch) on: (A) pH; (B) titratable acidity; (C) syneresis. Fermentation was carried out at 40°C for 16 hr; initial lactic acid bacteria = 5.3 log colony-forming units/mL medium. Values = mean±SD (n = 3). Different superscript letters above columns indicate significant differences within the same variable based on Tukey's HSD test ($p < 0.05$).

combination of LB + ST at an initial concentration of 10^8 cells/mL. DH flour products exhibited higher pH values (4.1 to 4.3) than DH starch products (3.7 to 3.9). However, analysis of variance showed no significant differences in pH due to LAB variation in both products (Fig. 1A). For DH flour products, using LB and ST separately resulted in lower TA than when they were combined. The DH starch products showed slightly higher TA (0.18% to 0.19%) than those in the previous experiment. Statistical analysis indicates a significant difference in the TA of DH flour products but not that of DH starch products (Fig. 1B).

DH flour products showed no syneresis, while DH starch products exhibited lower syneresis (5.75% to 7.05%) compared to the previous experiment. The combination of ST and LB yielded the lowest syneresis in DH starch products. Statistically, LAB variations did not significantly affect the syneresis of DH starch products and the effect could not be calculated for DH flour products due to the absence of syneresis (Fig. 1C). Detailed statistical analysis, including Student's t test, is available in Supplementary Tables S1 and S2.

Based on the high TA and low syneresis results, DH flour and DH starch products prepared using a combination of LB and ST were selected for further experiment.

Variation of skim milk addition

Skim milk was added to enhance LAB growth and lactic acid production. The pH of DH flour products made with the addition of various skim milk concentrations ranged from 4.0 to 4.2, while DH starch products had lower pH values between 3.5 and 3.6. Although skim milk lowered the pH, the differences were not statistically significant (Fig. 1A). The addition of skim milk significantly increased the TA of both DH flour and DH starch products. In DH flour, TA increased with skim milk concentration, reaching 0.48%, 0.57% and 0.69% for 1%, 2% and 3% skim milk, respectively. These values were notably higher than in previous experiments. DH starch products exhibited even higher TA levels, ranging from 0.92% (1% skim milk) to 1.51% (3% skim milk), which were 4–8 times greater than in previous experiments. Analysis of variance revealed that adding different concentrations of skim milk significantly increased the TA in both products (Fig. 1B).

Most products showed no syneresis, except for the DH starch product containing 1% skim milk (0.23% syneresis). Analysis of variance showed a significant difference in syneresis due to the addition of different concentrations of skim milk to DH starch products. The effect cannot be calculated in

DH flour products due to non-observable syneresis (Fig. 1C). A detailed statistical analysis is provided in Supplementary Tables S1 and S2.

Based on a combination of TA and syneresis, the optimal parameters for the DH flour product were a 1:10 flour-to-water ratio, a mixture of LB and ST and 3% skim milk. Meanwhile, the optimal conditions for the DH starch product were a 1:8 starch-to-water ratio, a mixture of LB and ST and 3% skim milk. However, the DH flour product with 1% skim milk (Fig. 2A) was chosen (referred to as YLPF), as parameters remained consistent across various skim milk concentrations. The DH starch product with 3% skim milk (Fig. 2B) was selected (referred to as YLPS) because it showed increased TA. Both YLPF and YLPS exhibited texture consistency similar to commercial plain yogurt (Fig. 2C).

Effect of prolonged fermentation time

The prolonged fermentation experiment for YLPF and YLPS was conducted for 32 h, with measurements taken at 8-hour intervals to monitor pH, TA and viable cell counts. Analysis of variance for the prolonged fermentation time revealed product-specific differences. YLPF exhibited significant variations in TA and viable cell counts, while YLPS showed significant differences in both pH and TA (Fig. 3). Further statistical analysis using Student's t test revealed a significant difference in TA between the two products throughout the fermentation period. However, pH values only differed significantly at the 8-hour mark (Supplementary Table S3 and S4).

The pH levels in both YLPF and YLPS exhibited a consistent downward trend throughout the fermentation period. YLPS generally maintained lower pH values than YLPF. Both products experienced a pH decline until the 24-hour mark, after which a slight increase was observed. The final pH values ranged from 3.9 to 4.5, with statistically significant differences between YLPF and YLPS noted only at the 8-hour mark (Fig. 3A).

TA trends differed markedly between YLPF and YLPS. YLPF showed a rapid increase in TA from 0.7% at 8 hr to 1.9% at 16 hr, followed by a decline. In contrast, YLPS showed a steady increase in TA from 1.1% at 8 hr to 1.6% at 32 hr. Significant differences in TA between YLPF and YLPS were observed across all fermentation time points (Fig. 3B).

Viable cell counts in both YLPF and YLPS increased gradually during fermentation. Starting from an initial concentration of $5.3 \log$ CFU/mL, the cells increased by about three orders of magnitude after 8 hr. By the end of the 32-hour

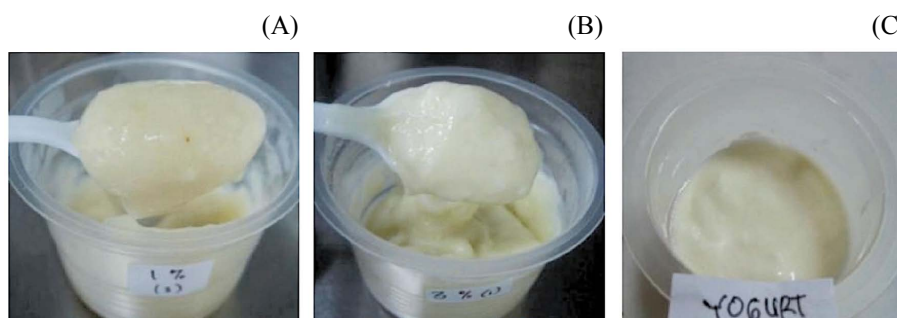


Fig. 2 Optimal products in the optimization of substrate-to-water ratio, lactic acid bacteria variation and addition of skim milk: (A) yogurt-like product from flour (YLPF), made from *D. hispida* flour using *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) + *Streptococcus thermophilus* (ST) with 1% skim milk; (B) yogurt-like product from starch (YLPS), made from *Dioscorea hispida* starch using LB + ST with 3% skim milk; (C) commercial yogurt control.

fermentation period, viable cells in both products exceeded 8 log CFU/mL, with YLPS showing nearly double the cell count of YLPF. There were no significant differences in viable cell counts between YLPF and YLPS across all fermentation time points (Fig. 3C).

Storage experiment

To assess product stability, YLPF and YLPS were stored at room and cold (4°C) temperatures for 7 d, following an initial 16 hr fermentation. Daily monitoring was performed for pH, TA, syneresis and viable cells.

The pH values of YLPF stored at both temperatures ranged from 4.30 to 4.45, while YLPS values ranged from 4.20 to 4.50. Analysis of variance revealed that storage did not affect the pH of YLPF at either temperature, but it did significantly affect YLPS at room temperature. The effect of YLPS storage on pH at cold temperature could not be determined because there was

no observed variation in pH measurements (Fig. 4A).

Syneresis was more pronounced at cold temperatures for both products, with YLPS exhibiting greater effects. Syneresis in YLPF stayed below 1% at room temperature but increased from 9.9% to 28.2% over 7 days in cold storage. Syneresis in YLPS rose slightly (1.60% to 3.40%) at room temperature but dramatically (17.3% to 33.4%) in cold storage. Analysis of variance showed that storage temperatures significantly influenced syneresis in both products (Fig. 4B).

In general, room-temperature storage led to higher TA values than cold storage. TA in YLPF increased from 0.44% to 0.60% at room temperature and from 0.45% to 0.56% at cold temperature. YLPS showed a notable TA increase (0.74% to 1.1%) at room temperature but remained stable (0.76% to 0.82%) in cold storage. Statistical analysis showed that storage temperatures significantly affected TA in both products (Fig. 4C).

Room-temperature storage resulted in slightly higher viable

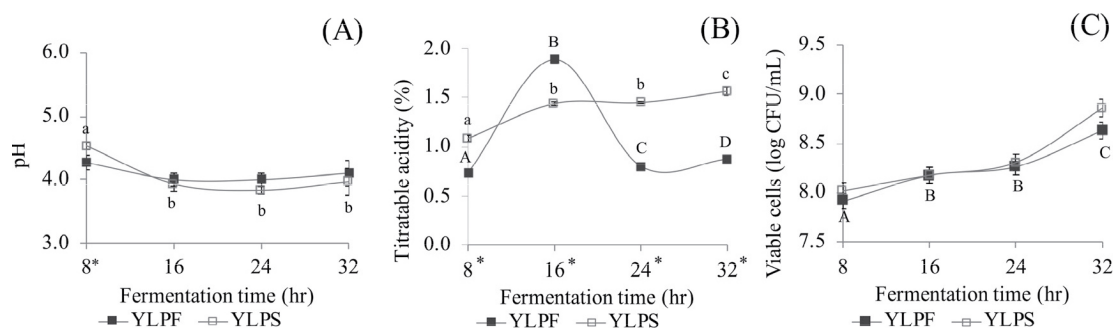


Fig. 3 Effect of extended fermentation time on parameters between yogurt-like product made from flour (YLPF) and yogurt-like product made from starch (YLPS) of *Dioscorea hispida*: (A) pH; (B) titratable acidity; (C) viable cells. Fermentation was performed at 40°C; initial lactic acid bacteria used was 5.3 log colony-forming units/mL. Values = mean±SD (n = 3). Different uppercase and lowercase superscript letters indicate significant differences ($p < 0.05$), within YLPF and YLPS groups, respectively, based on Tukey's HSD test. * = significant ($p < 0.05$) difference between treatments at each point based on student *t* test.

cell counts than cold-temperature storage. For YLPF, counts increased from 7.99 log CFU/mL on day 1 to 8.09 log CFU/mL on day 7 at room temperature, compared to an increase from 7.54 log CFU/mL to 8.05 log CFU/mL in cold storage. YLPS peaked at 7.97 log CFU/mL on day 4 at room temperature, while the highest count at cold temperatures was 8.02 log CFU/mL on day 5. Statistical analysis showed that storage temperature affected the viable cells of both products (Fig. 4D). Detailed statistical analysis results are available in the Supplementary Table S5 and S6.

Proximate analysis of yogurt-like product made from flour and yogurt-like product from starch

The approximate amounts of major components in YLPF and YLPS were analyzed, including ash, crude protein, crude lipid, crude fiber, reducing sugar and TA. The ash content and pH of YLPF were higher than those of YLPS. Conversely, the levels of crude protein, crude lipid, crude fiber, reducing sugar and titratable acidity were greater in YLPS than in YLPF (Table 1). Post-fermentation, the crude protein content of YLPF and YLPS was $0.76 \pm 0.06\%$ and $1.51 \pm 0.40\%$, respectively. The crude lipid contents of YLPF and YLPS were $0.18 \pm 0.08\%$

(0.72% of dry weight) and $0.34 \pm 0.18\%$ (1.35% of dry weight), respectively.

The total carbohydrate content of the products was assessed based on crude fiber and reducing sugars. The crude fiber content in YLPF and YLPS was notably low, at only $0.13 \pm 0.04\%$ and $0.25 \pm 0.04\%$, respectively. Post-fermentation, the reducing sugar contents in YLPF and YLPS were $6.33 \pm 0.57\%$ and $21.83 \pm 0.62\%$, respectively. Furthermore, the TA of YLPS (1.51 ± 0.03) was approximately three times higher than that of YLPF (0.48 ± 0.02).

Sensory evaluation of yogurt-like product made from flour and yogurt-like product made from starch

Sensory tests were conducted to assess untrained panelists' perceptions of the color, texture, flavor, aroma and overall acceptance of YLPF and YLPS. A control of dairy-based yogurt was included, as yogurts made from milk typically provide a significantly better sensory experience than carbohydrate-based products. Analysis of variance revealed significant differences among the tested products in terms of color, texture, flavor, aroma and overall acceptance.

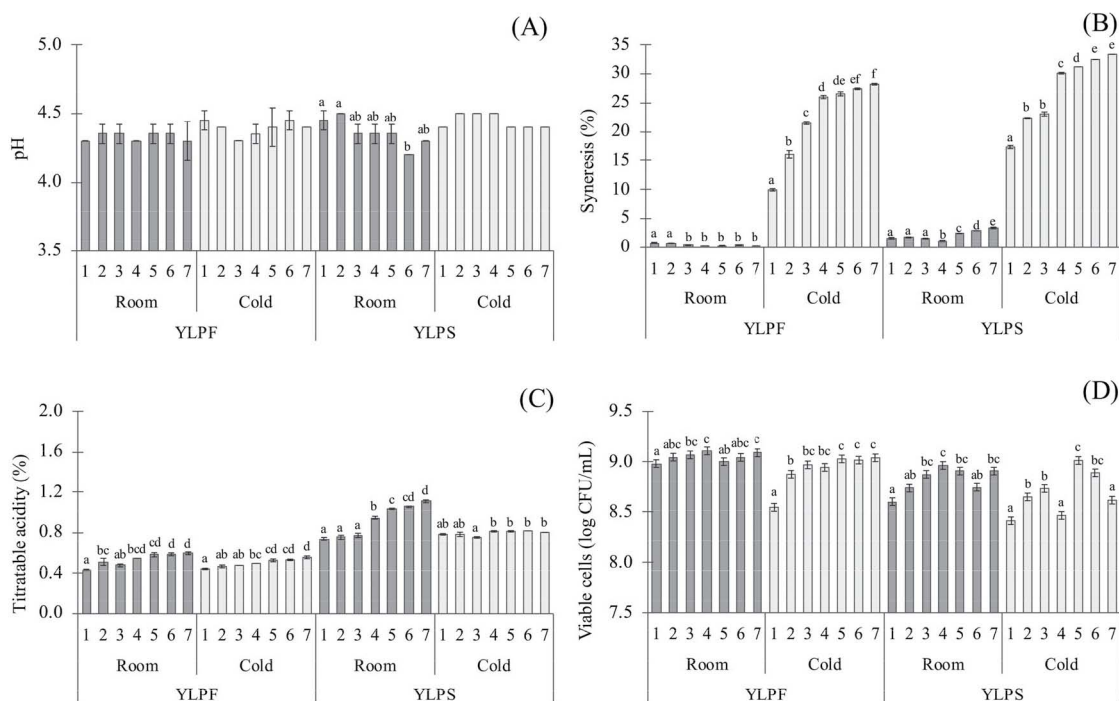


Fig. 4 Effect of temperature and storage time on the parameters of yogurt-like product made from flour (YLPF) or yogurt-like product made from starch (YLPS) of *Dioscorea hispida*: (A) pH of YLPF; (B) pH of YLPS; (C) syneresis of YLPF; (D) syneresis of YLPS; (E) titratable acidity of YLPF; (F) titratable acidity of YLPS; (G) viable cells of YLPF and (H) viable cells of YLPS. Fermentation before storage carried out at 40°C for 16 hr; initial lactic acid bacteria used = 5.3 log colony-forming units/mL; Values = mean $\pm$ SD (n = 3); Different superscript letters above columns within the same group indicate significant differences based on Tukey's HSD test ($p < 0.05$)

Table 1 Proximate composition, reducing sugar content, titratable acidity, and pH of yogurt-like products prepared from *Dioscorea hispida* flour (YLPF) and starch (YLPS) after fermentation at 40°C for 16 hr.

Parameter	Product	
	YLPF (1:10 substrate-to-water ratio, LB+ST, 1% skim milk)	YLPS (1:8 substrate-to-water ratio, LB+ST, 3% skim milk)
Ash (%)	1.78±0.03	0.78±0.19
Crude protein (%)	0.76±0.06	1.51±0.40
Crude lipid (%)	0.18±0.08	0.34±0.18
Crude fiber (%)	0.13±0.04	0.25±0.04
Reducing sugar (%)	6.33±0.57	21.83±0.62
Titrable acidity (%)	0.48±0.02	1.51±0.03
pH	4.02±0.31	3.54±0.03

LB = *Lactobacillus delbrueckii* subsp. *bulgaricus*; ST = *Streptococcus thermophilus*. Values = mean±SD ($n = 3$), expressed on dry weight basis.

Table 2 shows distinct sensory perceptions of YLPF, YLPS and the control yogurt. The control outperformed both YLPF and YLPS in color ratings (1.75±0.97 vs. 2.7±0.57 and 2.7±0.92, respectively). Although significant differences were observed within the group, YLPF and YLPS exhibited similar color perceptions. In terms of texture, YLPS (2.2±0.89) performed better than YLPF (2.7±0.98), yet both were inferior to the control (2.0±0.97). Despite the high standard deviations, significant differences were noted within the group, but not between the control and YLPS or between YLPF and YLPS. Flavor perceptions for YLPF (3.60±1.10) and YLPS (3.35±1.23) were comparable and significantly higher than the control (2.35±0.93). While differences were observed within the group, statistically, YLPF and YLPS showed similar flavor ratings. The aroma of the control yogurt (2.05±0.83) was preferred over both YLPF (3.20±1.01) and YLPS (3.15±0.93). Significant differences in aroma were found within the group, but not between YLPF and YLPS. Overall, the control yogurt received higher total acceptance ratings (1.95±0.51) compared to YLPF (3.15±0.59) and YLPS (2.80±0.77). Differences were observed within the group, but no difference was observed between YLPF and YLPS. Detailed statistical analysis results are available in the Supplementary [Table S7](#) and [S8](#).

Discussion

Based on the findings, the study highlighted the potential of *D. hispida* tubers as raw materials for the production of carbohydrate-based yogurts. An essential step before using *D. hispida* tubers was the pretreatment to remove toxic compounds such as dioscorine, cyanohydrin and dyes. However, these compounds are water-soluble, allowing them to be easily removed by repeated washing (Estiasih et al., 2022).

In the current study, the DH flour and starch were pasteurized at 85°C for 10 min before being used. Apart from sterilizing the substrate, pasteurization induced starch gelatinization, increasing viscosity and preventing phase separation. *D. hispida* starch has a gelatinization temperature of 83.4°C (Ashri et al., 2014). Pasteurization slightly above this temperature promoted starch granule swelling, enhanced water retention, reduced syneresis and produced a more stable texture. In addition, the heating duration affected the degree of gelatinization, with longer times leading to more granules rupturing and releasing amylose into the liquid phase, thereby altering the structural consistency (Xing et al., 2018).

Table 2 Sensory evaluation scores for yogurt-like products prepared from *Dioscorea hispida* flour (YLPF) and starch (YLPS) after fermentation at 40°C for 18 hr. The evaluation was conducted by 20 untrained panelists, with plain commercial yogurt used as the control.

Product	Color	Texture	Flavor	Aroma	Total acceptance
Control	1.75±0.97 ^a	2.00±0.97 ^a	2.35±0.93 ^a	2.05±0.83 ^a	1.95±0.51 ^a
YLPF	2.70±0.57 ^b	2.70±0.98 ^b	3.60±1.10 ^b	3.20±1.01 ^b	3.15±0.59 ^b
YLPS	2.70±0.92 ^b	2.20±0.89 ^{ab}	3.35±1.23 ^b	3.15±0.93 ^b	2.80±0.77 ^b

Scale: 1 = Very like, 2 = Like, 3 = Neutral, 4 = Dislike and 5 = Very dislike.

Values (mean ± SD) within each column followed by different superscript letters are significantly ($p < 0.05$) different based on Tukey's HSD test.

Although not determined in the current study, the carbohydrate composition and structural differences between DH flour and starch were expected to affect the product's parameters. Based on the properties of the flours and starches derived from traditional Indonesian tubers and roots, the starch portion of the various tubers contained higher total starch and non-resistant starch than the flour portion, with the amylopectin-to-amylose ratio in the starch also being higher than that in the flour (Aprianita et al., 2014). In the current study, the starch likely contained simpler, more readily fermentable carbohydrates than the DH flour, facilitating easier utilization by the LAB and affecting acid production. Furthermore, these differences may have impacted the physical structure of the DH flour and starch matrices. A more porous matrix could promote LAB growth, while high viscosity might hinder microbial access to sugars.

Usually, LAB fermentation decreases the pH of products due to the production of organic acids (Ma et al., 2021). In the current study, the pH of the DH starch products was lower than that of the DH flour. However, generally, the DH flour had a slightly higher TA than the DH starch. An exception occurred in the skim milk addition experiment, where the DH starch produced approximately twice as much TA that the DH flour (Fig. 1). Typically, increased acidity leads to a lower pH, as more acids increase the concentration of hydrogen ions. However, pH alone does not give a complete picture of acidity. Products with similar pH may have different TA values if they contain different types or amounts of acids. The DH flour and DH starch might produce different types of acids with varying dissociation strengths, although their specific compositions were not determined. Furthermore, the DH starch products might have contained more acids that lowered the pH but were not easily titrated.

The use of the LB and ST mixture slightly enhanced product parameters by lowering pH, increasing TA and reducing syneresis. In the tuber-to-water ratio experiment, both the DH flour and starch had high syneresis. However, when using the LB and ST mixture, the DH flour products showed no syneresis. In contrast, syneresis in the DH starch products decreased significantly (Fig. 1B). Often, using multiple strains yields better product parameters than from single strains due to synergistic interactions (Chapman et al., 2011). LB and ST are usually used together for dairy-based yogurt production, with ST breaking down lactose and producing pyruvic, formic and folic acids along with carbon dioxide, which stimulates LB growth, while LB converts monosaccharides into lactic acid. In addition, LB produces peptides and amino acids that promote

ST growth. This symbiotic relationship is effective because ST thrives in neutral pH conditions, while LB tolerates acidic environments (Sieuwerdt et al., 2008). However, the absence of lactose in the DH tubers limited the production of lactic acid by LB in the early stage of fermentation. Therefore, experiments involving the addition of skim milk were conducted to improve the fermentation results.

The addition of skim milk further improved the products' parameter values, with the pH values decreasing, accompanied by a significant increase in TA. The DH starch products had TA values twice those of the DH flour products. No syneresis was observed in the DH flour and DH starch products, except in the latter with 1% skim milk addition (Fig. 1C). While the carbohydrate matrix was used as a substitute to avoid lactose intolerance, the skim milk was added in this study as an inducer for ST to produce acids to further induce the LB. Lactose in the supplemented skim milk was expected to be metabolized almost completely during the initial fermentation stage, resulting in minimal or undetectable residual lactose in the final product.

The impact of extended fermentation time up to 32 hr was investigated after optimizing the fermentation conditions. The fermentation showed distinct patterns in pH, TA and viable cell counts. Both products experienced a decline in pH, but at different rates and to different final values. TA trends differed significantly between the two products. YLPF showed a rapid increase followed by a decline, while YLPS showed a steady increase. Viable cell counts in both products increased substantially, with YLPS ultimately having higher counts (Fig. 3). These differences in fermentation patterns suggested that the two products might require different processing or quality control measures.

—This study examined the effects of storage temperature on the products' parameters. It was anticipated that maintaining the products at 4°C would better preserve their quality and ensure their safety than maintaining them at room temperature. However, the results did not support this assumption. Products stored at room temperature had lower syneresis values than those stored at cold temperatures (Fig. 4). These lower values may be attributed to emulsion disruption at low temperatures, which increases syneresis. The addition of emulsifiers may improve product stability. However, most emulsification studies have focused on dairy product emulsions, such as Arab et al. (2023).

Generally, sensory tests of food products measure color, aroma, flavor and texture, which, together, indicate the overall acceptance by consumers. The acceptance ratings

for YLPF and YLPS were significantly lower than those for the control. This discrepancy was understandable, as the control was a commercial dairy-based plain yogurt, whereas the products tested were carbohydrate-based. In the current study, organoleptic analysis could have used plain soy yogurt as a control. However, often, non-dairy products are perceived as inferior to their dairy-based counterparts (Jaeger et al., 2025). Dairy-based yogurt was selected as the control to establish a high benchmark. Additionally, potential assessment inaccuracies could influence the results, as organoleptic tests involved untrained panelists, leading to potentially greater deviation from the mean.

The Student's *t* test analysis revealed significant color differences between the control and both the YLPF and YLPS products, but not between YLPF and YLPS. Both products had significantly lower aroma acceptance than the control. The aroma of the products was attributed primarily to TA, derived mainly from lactic acid. YLPS had approximately three times the TA of YLPF, although the difference was not statistically significant. Increased TA in fermented products can sometimes reduce the aroma due to unfavorable acid production and a lack of ester compounds. Additionally, increased TA alters the pH of the products, which often negatively affects flavor (Ajbli et al., 2025).

Apart from TA, flavor perception in yogurt is also influenced by other components. For example, reducing sugars contribute to sweetness, while higher protein and lipid contents enhance flavor and texture scores by improving firmness and creating a denser mouthfeel. Generally, increased lipid levels result in a creamier texture (Desai et al., 2013; Leeward et al., 2023).

Texture is influenced by factors such as the insoluble material content. For example, increased crude fiber can reduce homogeneity and affect sensory assessments (Ajbli et al., 2025). While fiber composition was not determined in the current study, differences in crude fiber content can impact gelatinization during heating, influencing yogurt viscosity and texture (Leeward et al., 2023). These factors may explain YLPS acceptance, which was only slightly lower than the control, with no significant texture difference between the two (Supplementary Table S7 and S8).

Improving the sensory attributes of carbohydrate-based yogurts, such as YLPF and YLPS, is a major challenge. To address this, a combination of yeast and LAB can be used to improve the production of organic acids and volatile compounds, thereby enhancing the aroma (Freire et al., 2015). Incorporating fruit and vegetable extracts may increase flavor acceptance (Januário et al., 2017). Texture improvements can

be achieved through enzymatic modification (Ercili-Cura et al., 2015), physical treatment (Ciron et al., 2012; Aslan and Dogan, 2018) and polymer addition (Lal et al., 2006). Careful selection of fortification materials can enhance both nutritional value and sensory parameters (Gahruie et al., 2015). In addition, safety measures are crucial, as contamination during production stages is a primary concern, with yeast and mold contamination being more common in traditionally prepared yogurt (Wang et al., 2025). In the current study, visual assessment indicated mold development in YLPF and YLPS products stored at room temperature after 5 d (data not shown).

The current study refrained from labelling the resulting yogurt as probiotic because the probiotic status of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* used in the study is still being debated, particularly regarding their survival in the digestive system. One study suggested these bacteria may not persist for long in the human intestine due to poor acid and bile resistance and a lack of genes necessary for gut survival (Meybodi et al., 2020). However, being carbohydrate-based and rich in indigestible fibers, the yogurt may still benefit gut bacteria by promoting their growth and activity, potentially improving overall health (Xu et al., 2021).

To have probiotic benefits, the yogurt products require enrichment with additional proven probiotic strains such as *L. acidophilus*, *B. bifidum* and *L. casei*. Further investigations are needed to determine their efficacy, accounting for strain-specific effects, dosage, gastrointestinal survival and host interactions. The investigations should involve *in vitro* studies, animal trials and human clinical trials. Thus, additional studies are necessary to translate these findings into practical applications.

In conclusion, this study highlighted the potential of *D. hispida* tubers as a possible substrate for carbohydrate-based yogurt. The findings revealed that using a mixture of LAB strains was advantageous and that the addition of a low concentration of skim milk at the early stage of the fermentation helped the LAB to improve product parameters. Longer fermentation times did not yield consistently better results. Although 7 d of storage maintained high viable cell counts, syneresis occurred, particularly in cold-temperature storage. Challenges persist in improving the sensory attributes and reducing contamination. Future research should address these issues by reducing contamination, increasing product stability during storage, enhancing flavor and texture and exploring the addition of probiotic properties. These efforts should contribute to the development of *D. hispida*-based products that meet consumer expectations and industry standards.

Conflicts of interest

The authors declare no conflicts of interest.

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