Valorization of Sweet Potato Peel Biomass for Lactic Acid Production in Solid-state Fermentation and Control of Abiotic Bacteria in Goat Meat

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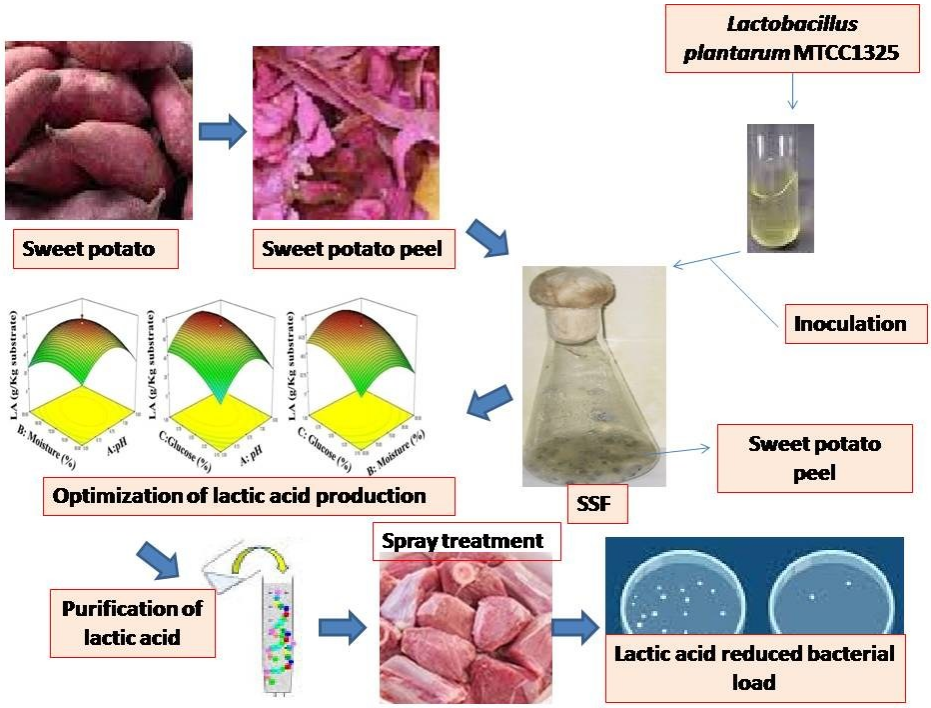
GRAPHICAL ABSTRACT



Valorization of Sweet Potato Peel Biomass for Lactic Acid Production in Solid-State Fermentation and Control of Abiotic Bacteria in Goat Meat

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Sweet potato peel, a lignocellulosic residue, was used as a sugar source for lactic acid production in solid-state fermentation. The dried sweet tuber peels were heated at 80, 90, and 100 °C for 15, 30, and 60 min. They were steamed three times, first at 68.9 KPa for 15 to 60 min, then at 86.2 KPa for 15 to 60 min, and lastly at 103.4 KPa for 15 to 60 min. Compared with the 15 min treatment, the steam treatment significantly improved the reducing sugar content after 60 min from 190.4 ± 2.2 to 245.4±3.5 mg/g biomass. Enzymatic hydrolysis afforded 29.5 g/L total sugars, including 22.7 g/L glucose, 3.5 g/L disaccharides, 0.1 g/L arabinose, and 3.2% xylose. The pretreated substrate was used as a solid medium to produce lactic acid in solid-state fermentation via Lactobacillus plantarum MTCC1325. Central composite rotatory design (CCRD) was used to optimize lactic acid production to improve the lactic acid yield. Fermentation of sweet potato peel hydrolysate by L. plantarum yielded 85.6 g lactic acid/kg substrate, which was an overall fourfold increase compared with that of the unoptimized medium. Compared with the untreated control, goat meat treated with 1.25% to 5% lactic acid presented a reduced aerobic bacteria count ($p<0.001$). These studies imply that the sweet potato peel substrate is a promising biomass for the production of lactic acid in the food industry.

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Keywords: Sweet potato peel; Solid substrate; Lactic acid medium; Abiotic bacteria; Food protection; Solid-state fermentation

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INTRODUCTION

Lactic acid (LA) is applied in several industrial processes. One of the major industrial applications is in the food industry, which accounts for approximately 24% of the total LA demand (Reroof *et al*. 2021). LA has several functional properties, including reducing the pH of meats to inhibit the proliferation of spoilage bacteria; improving the preparation of candies and chocolates; reducing sucrose inversion in candy production; enhancing chocolate flavor; and regulating the pH of pickles, dairy, beverages, and baked goods (Krishna *et al*. 2018). LA exists as two different optical isomers: D(−) lactic acid and L(−) lactic acid (Ameen and Caruso 2017). D(−) lactic acid isomers exceeding >100 mg/kg body mass/day are highly harmful to human health. In contrast, L-lactate dehydrogenase (L-LDH) is an enzyme in the body that metabolizes L(+) lactic acid. In the pharmaceutical, cosmetic, and food industries, the L(+) lactic acid isomer, which is synthesized to different degrees, is useful. Generally, the industrial-scale production of

LA is achieved *via* chemical synthesis using a chemical precursor (petroleum), resulting in a racemic mixture of LA. In another way, the scale-up of LA is achieved by microbial fermentation, and pure D(−) or L(+) lactic acid is produced (Alves De Oliveira *et al*. 2018). Currently, >90% of the total production of LA is achieved through sugar fermentation because of its reduced cost, being more environmentally friendly, having low process temperatures, and yielding high-purity LA. Moreover, improvements are still needed in various aspects, such as enhancing bioprocess performance, preventing raw materials from competing with food production, and reducing raw material costs. Hence, promising approaches are needed for the effective utilization of agricultural residues to improve the LA yield. The bioprocesses involve the hydrolysis of organic waste materials, followed by microbial fermentation, and fractionation of lactic acid. The LA can be effectively utilized in the production of films, such as polylactic acid, as well as in the production of organic fertilizers and in the generation of green energy (Thongchul 2013).

Lactic acid has been classified as generally regarded as safe (GRAS) product and is recommended for use in food products. The antibacterial activity of lactic acid is mediated by various mechanisms, including its ability to lower the cytoplasmic pH and generate reactive oxygen species, resulting in physical disruption of bacteria and hence immediate bacterial reduction on meat surfaces (Mols *et al*. 2010). The application of lactic acid on the meat surface by spraying or other methods could reduce the bacterial load. The antimicrobial effect of lactic acid may vary based on the bacteria colonized on the meat surface, the microbial load, and the type of bacteria (Barcenilla *et al*. 2022; Marcelli *et al*. 2024).

Despite the demand for LA in the global market, it has not yet taken off because of the issue of attaining sustainable and affordable raw materials from reliable sources that ensure maximum production of LA. Therefore, biotechnological approaches are critical for minimizing production costs, maximizing production rates, and making this market attractive to investors. An important approach that would improve biotechnological advances is the optimization of bioprocess conditions and control methods for bioprocesses. Sweet potato (*Ipomoea batatas* (L.) Lam.) is an important crop that grows in the tropics, warm temperate regions, and subtropics and is cultivated for consumption. The use of sweet potato tubers and cassava for the biosynthesis of products has previously been reported (Forkum *et al*. 2025; Xu *et al*. 2022). However, the application of sweet potato tubers in LA production has not been reported. Sweet potato peel is a readily available substrate with a low cost and can be considered an alternative carbon source to produce LA in SSF. The SSF process generally provides higher yield than submerged fermentation. The use of this feedstock could reduce environmental pollution and greenhouse gas emissions (Aboyeji *et al*. 2020).

Bioprocess control is an effective tool for ensuring the management of bioprocess systems, as it is highly regulated by interactions among the chemical, physical, and biological conditions of the biochemical processes within microorganisms and the fermentation environment. The bioprocess performance of SSFs is generally affected by physiochemical (pH, aeration, temperature, moisture, and particle size), biological (solid substrate type, inoculum size, and type of bacteria), and mechanical factors (mixing or a fixed bioreactor) (Manan and Webb 2017; Alarjani *et al*. 2024a). In SSF, relative humidity and temperature tend to fluctuate significantly due to heterogeneity in the environment. This is one of the most problematic process issues in SSF and affects mass transfer and excess heat generation, which negatively affects the growth of bacteria and

product yield. In SSF, several agricultural waste materials have been employed to produce products. LA is produced from pineapple waste (Idris and Suzana 2006), date juice (Choi *et al*. 2014), hardwood pulp hydrolysate (Hama *et al*. 2015), corn stover (Hu *et al*. 2015), brown rice (Okano *et al*. 2017), and food waste (Van Dyk *et al*. 2013; Girotto *et al*. 2015). The yield of the fermentation process depends on the selection of substrate, microbial source, and process variables. In addition, fermentation process and productivity vary based on the nutritive value of the available substrate.

Response surface methodology has been employed for the optimized production of lactic acid through the use of food waste (Anagnostopoulou *et al*. 2022), paneer whey substrates (Tripathi *et al*. 2015), and cassava peels **(**Zakariyah *et al*. 2021). The aim of this work was to use sweet potato peel as a low-cost substrate to produce LA by *Lactobacillus plantarum* MTCC1325 in solid-state fermentation.

EXPERIMENTAL

Substrate and Analysis of Cellulose Components

Sweet tuber peel was used as the substrate to produce LA in the SSF. Sweet tubers were collected from the vegetable market. The material was peeled and dried for one week. The mixture was subsequently heated in a hot air oven at 80 °C until a constant weight was obtained. The dry matter content of the sweet tuber peels was assayed. The composition of the sweet tuber peels was assayed by determining free-reducing sugars (Miller 1959). The amounts of cellulose (Updegraff 1969), carbohydrates (Hedge and Hofreiter 1962), starch (Hansen and Moller 1975), and hemicelluloses (Gao *et al*. 2014) were assayed.

Pretreatment Methods

The dried sweet tuber peels were ground using a mechanical grinder, and the biomass was transferred to an ice-cold water bath for 24 h with continuous agitation at room temperature to completely extract the sugars. The mixture was subsequently washed with distilled water and filtered through cheese cloth, and the remaining plant biomass was initially dried at 40 °C for 2 days, followed by drying at 50 °C for two days. The samples were then ground with a mechanical grinder and stored at 25 °C. A total of 10 g of milled sweet potato peel powder was mixed with 100 mL of double distilled water in a 500-mL Erlenmeyer flask. The mixture was boiled for 60 min in a water bath. In addition, it was heated at 80, 90, and 100 °C for 15, 30, and 60 min. They were steamed three times, first at 10 lbs for 15 to 60 min, then at 12.5 lbs for 15 to 60 min, and lastly at 15 lbs for 15 to 60 min. The amount of reducing sugars in the peels was determined (Alarjani et al. 2024b; Arokiyaraj et al. 2024; Sathya et al. 2024).

Enzymatic Hydrolysis of Lignocellulose Materials

The pretreated lignocellulose material was subjected to enzymatic hydrolysis using a previously described protocol (Premjet et al. 2022). Briefly, at a volume of 500 mL for the reaction mixture, 2 g of pretreated biomass, 0.1 M citrate buffer (pH 4.8), and 0.2 mL of 1% sodium azide (w/v) were added. The enzyme concentrations for cellulase (Bestzyme, Jinan, Shandong Province, China) and Accellerase (Sigma Aldrich, St. Louis, MO, USA) were 20 and 30 U/g dry biomass, respectively. The mixture was placed on a

shaker incubator for 24 h at 37 °C and stirred continuously (150 rpm). The hydrolysate solution (2 mL) was collected after every 6 h for fermentable sugar analysis.

Microorganism

Lactobacillus plantarum (MTCC1325) is a homofermentative lactic acid-producing bacterium that is used to produce L(+)-lactic acid. The strain was cultured on deMan, Rogosa, and Sharpe (MRS) agar media and stored in slants. It was subcultured every two months and stored at 2to8 °C.

Inoculum

A loop culture of the bacterial strain from the MRS agar slant was inoculated into an Erlenmeyer flask (250 mL) containing 50 mL of MRS broth medium. The mixture was subsequently incubated for 12 h at 37 °C in a shaker incubator at 100 rpm. It was used as an inoculum for SSF.

Fermentation of Sugar and Production of Lactic Acid in Submerged Fermentation

Sweet tuber peel powder (1%) was used as the substrate to produce LA in submerged fermentation. The submerged fermentation was carried out in 250-mL Erlenmeyer flasks with a 50 mL working volume. Approximately 50 mL of mineral salt medium was added to the Erlenmeyer flask, and 0.5 g of sweet tuber peel (1%, w/v) powder was added. It was mixed and inoculated with 1% (v/v) inoculum (0.5 mL) of an exponentially growing bacterial isolate. The medium pH was maintained at 6.2±0.1, and fermentation was carried out for 96 h at 30 °C at 125 rpm in an orbital shaker incubator. The cultures were withdrawn at regular intervals (12, 24, 36, 48, 60, 72, 84, and 96 h) and centrifuged at 5000×g for 10 min, and the clear supernatant was used as the source of LA.

Solid-state Fermentation and Lactic Acid Production

A total of 2 g of pretreated sweet tuber peel powder was added to a 100-mL Erlenmeyer flask and moistened with sodium phosphate buffer (pH 6.1, 0.05 M). The initial moisture content of the substrate was set to 75%, and the samples were inoculated with 1% inoculum. To the solid substrate, $CaCO_3$ was added at 1 g/2 g substrate as the buffering agent. The solid medium was sterilized for 30 min and cooled. The mixture was inoculated with inoculums and mixed thoroughly. The culture flasks were incubated for three days, and LA production was assayed.

Optimization of Bioprocess Variables for Lactic Acid Production

To analyze the effects of supplemented glucose on lactic acid production, fermentation was carried out with various concentrations of glucose (0.25, 0.5, 0.75, 1, and 1.25%). The initial moisture content of the medium for microbial culture is one of the important parameters in the SSF. To screen the initial moisture content of the medium for lactic acid production, the substrate was moistened with sodium phosphate buffer (pH 6.0) at various concentrations (60, 70, 80, and 90%). To analyze the effect of pH, the pretreated substrate was moistened with buffer at various pH values (5.0, 5.5, 6.0, 6.5, and 7.0). To analyze the effect of fermentation time, the experiment was performed for 12 to 72 h. The SSF was performed as described earlier. The experiment was performed with $CaCO_3$ (1 g/2 g) as a buffering agent.

Optimization of Lactic Acid Production *via* Statistical Methods

Statistical culture medium optimization was performed *via* three variables. The culture medium pH (5.5 to 8.0), moisture content (65 to 85%), and glucose content (0.1 to 1.0%) were optimized *via* a central composite rotatory design (CCRD). The CCRD model comprises 20 experimental runs, and the significance variation between the means was analyzed against the difference at the 1% level of significance using Design Expert software (version 8.0). The experiments were performed in duplicate, and the mean value was used for data analysis. The experimental results obtained from the CCRD design were analyzed *via* a regression equation from Design Expert software using the following second-order quadratic equation:

$$Y_i = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x^2{}_i + \sum \beta_{ij} x_i x_j \quad (1)$$

where Y_i is the response variable, $x_i x_j$are the independent variables, β_0 is the offset term, $\beta_i$is thelinear coefficient, $\beta_{ii}$is the quadratic coefficient, and $\beta_{ij}$is the interaction coefficient.

Preparation of Lactic Acid Solution

The culture filtrate (20 mL) was loaded on anion exchange resin (Amberlite IRA-100), and the anionic lactate ions became bound to the anion exchange resin. The matrix was packed in a column (20 cm×2.5 cm), and the unbound sample was washed. The bound lactic acid was subsequently eluted, and the amount of lactic acid was determined as described previously (Kumar *et al*. 2019). The concentrated lactic acid was diluted with double distilled water at 1.25%, 2.5%, 3.75%, and 5% (v/v). The pH values of the final solutions were 2.46, 2.32, 2.21, and 2.04 for 1.25%, 2.5%, 3.75%, and 5% lactic acid, respectively. Approximately 250 mL of lactic acid stock was prepared and applied through a manual sprayer. The bacterial load was determined before and after application of the lactic acid solution (Arrioja-Bretón *et al*. 2020).

Effect of Lactic Acid on Bacterial Load Control in Goat Meat

A sterile swab was used for microbiological examinations, and analysis was performed at 0 h (control, before spraying), 1 h, 24 h, 7 days, and 15 days. The goat meat was aseptically cut, and approximately 75±5 g was weighed. A sterile swab was used for sampling, and it was placed in a test tube containing 10 mL of buffered peptone water. The samples were diluted 10-fold and spread on plate count agar. The mixture was incubated for 48 h at 37 °C, and the developed colonies were counted *via* an automated colony counting unit. The results are expressed as log CFU per cm^2 (Marraiki *et al*. 2020; Balasubramanian *et al*. 2021).

Statistical Analysis

Analysis of variance (ANOVA) was used to determine the significance level. All experiments were performed in triplicates and the data are expressed as mean±standard deviation. The p-value <0.01 was considered as statistically significant.

RESULTS AND DISCUSSION

Proximate Analysis of Lignocellulose Biomass

The dry matter content of the sweet tuber peels was 89.3%. The lignin content of the biomass was 24.8%. The cellulose content was 18.3%, and a low hemicellulose content (13.2%) was observed. The total carbohydrate content was 68.3% (% dry weight). It consists of 17.3% cellulose, 10.4% free sugars, 20.5% starch, and 16.9% hemicelluloses. The increased amount of carbohydrates determined in this study makes sweet potato peels an alternative substrate for LA production. The carbohydrate content ranged between 75.07% and 87.04%, and the fat content ranged from 0.83±0.06% to 2.2±0.2%. The moisture content ranged from 5.77±0.07 to 7.17±1.27, and the ash content varied from 4.46±0.09 to 16.96±0.33%. The lignin, cellulose, and hemicellulose contents observed in the current study were greater than those of other cassava varieties. In cassava, the lignin, hemicellulose, and cellulose contents are in the ranges of 9.0 to 16.0%, 41.0 to 65.0%, and 5.5 to 15.0%, respectively (Kayiwa et al. 2021). Moreover, the composition of lignocellulose varies depending on the source, and lignocellulose consists of 20 to 40% hemicellulose, 40 to 60% cellulose, and 10 to 24% lignin (Putro et al. 2016). Pagana et al. (2014) reported that sweet potato residue consists of 4.4% protein, 18.5% ash, 19% soluble starch, and 20.5% simple sugars.

Reducing Sugar Content of Pretreated Sweet Tuber Peels

To identify a suitable pretreatment methodology, six different pretreatment methods were analyzed. The steam treatment resulted in better results than did the heat treatment. Table 1 shows the amount of reducing sugars in the pretreated sweet potato peel powder. The pretreatment of lignocellulosic biomass before enzyme hydrolysis is a prerequisite for lactic acid production. The pretreatment method reduced the strength, compactness, and crystalline nature of the material, thereby aiding in the hydrolysis of the lignocellulosic biomass to simple sugar molecules. Enzymes utilize this pretreated lignocellulosic biomass and converted as sugars within short period of time. Enzymatic hydrolysis is a biological strategy to degrade lignocellulosic biomass effectively (Li et al. 2022). The reducing sugar content of thermally treated sweet tuber peels was increased. The results are shown in Table 1. The heat treatment at 80 °C increased the reducing sugar content after 15 min (140.2±11.2 mg/g biomass), and it improved after 60 min (202.1±10.2 mg/g biomass). Similarly, the 100 °C treatment resulted in 164.3±3.9 mg/g biomass after 15 min and 229.4±2.6 mg/g biomass after 60 min. The steam treatment improved the reducing sugar content, and the amount of reducing sugars was 190.4±2.2 mg/g biomass after 15 min. This value increased significantly after 60 min of treatment (245.4±3.5 mg/g biomass).

The steam pretreatment method used in the current study improved the reducing sugar content (245.4 ± 3.5 mg/g biomass) significantly after 60 min with 12.5 lbs pressure ($p<0.01$). The reducing sugar content was improved at higher incubation temperature (100 °C) than lower incubation temperature (80 °C). In addition, incubation time also influenced the reducing sugar content in the medium. The amount of reducing sugar level was high after 60 minutes treatment either heat or steam treatment. The pretreatment method was useful to improve the reducing sugar content in the medium, which improved the lactic acid yield. The results observed in this work were similar to those of a previous report and pretreatment at 15 psi for 60 min (Chugh et al. 2023). Steam pretreatment is the recommended strategy because it disrupts most hemicellulose

and lignin from the strongly crystalline structure, thereby reducing the amount of cellulose that is more accessible to enzyme digestion (Varga et al. 2004).

Table 1. Reducing Sugar Content after the Thermal Pretreatment of Sweet Tuber Peels for Various Durations

Treatment	Reducing Sugar Content in Peels (mg/g Biomass)			
	0 h	15 min	30 min	60 min
Heating (80 °C)	40.1 ± 2.5	140.2 ± 11.2	178.5 ± 3.3	202.1 ± 10.2
Heating (90 °C)	42.5 ± 3.6	159.1 ± 2.2	189.5 ± 4.4	216.2 ± 7.8
Boiling (100 °C)	45.3 ± 1.8	164.3 ± 3.9	190.5 ± 2.9	229.4 ± 2.6
Steaming (10 lbs)	51.2 ± 3.9	183.5 ± 2.89	207.2 ± 1.8	218.5 ± 2.8
Steaming (12.5 lbs)	55.9 ± 1.5	187.5 ± 4.81	210.4 ± 2.9	220.5 ± 1.2
Steaming (15 lbs)	59.3 ± 2.2	190.4 ± 2.2	218.5 ± 1.7	245.4 ± 3.5
Untreated control	15.2 ± 0.52	124.5 ± 5.92	149.3 ± 4.92	152.6 ± 4.92

Enzymatic Hydrolysis Increased the Availability of Sugars

The enzymatic hydrolysis of sweet tuber peels presents various advantages over chemical hydrolysis. Enzymatic hydrolysis afforded 29.5 g/L total sugars, including 22.7 g/L glucose, 3.5 g/L disaccharides, 0.1 g/L arabinose, and 3.2% xylose. The amount of lignin in the biomass was low after enzymatic hydrolysis, and the hydrolyzed substrate can be utilized directly for LA production, which reduces the cost of bioprocessing. Pretreatment methods, such as chemical (acid or alkali, or both), thermal (moist heat treatment), and enzymatic hydrolysis using amylases or cellulases, are frequently used for the saccharification of biomass. This pretreatment method allows improved product formation, maximum yield, and an increased rate of glucose utilization (Soni *et al*. 2023).

Lactic Acid Production in Submerged Fermentation

Lactic acid production was performed for 96 h. Peak production was observed after 12 h of incubation in submerged cultivation (10.35 ± 1.4 g/L), and the sugar content was 7.1 ± 0.5 g/L. The amount of sugar significantly decreased ($p<0.01$) at this incubation time, and the selected bacterial strains utilized a carbon source for LA production. The peak production of LA was observed within 12 h, and LA production was increased up to 96 h incubation (Fig. 1). L (+) lactic acid production is reportedly associated with the initial glucose concentration in the medium. In this study, the strain *L. plantarum* utilized glucose from the medium and converted into lactic acid during initial growth phase. Gonçalves *et al*. (1991) reported a maximum amount of lactic acid production when the initial glucose concentration was 200 g/L; however, maximum growth was obtained with 100 g/L glucose. *Lactobacillus plantarum* can survive at low pH values; however, the acidification of the culture medium in the case of high accumulation of lactic acid causes an inhibition byproduct that is not generally considered for improved production of lactic acid. Calcium carbonate is recommended as a neutralizing agent and for regulating the pH of the culture during fermentation (Yang *et al*. 2015).

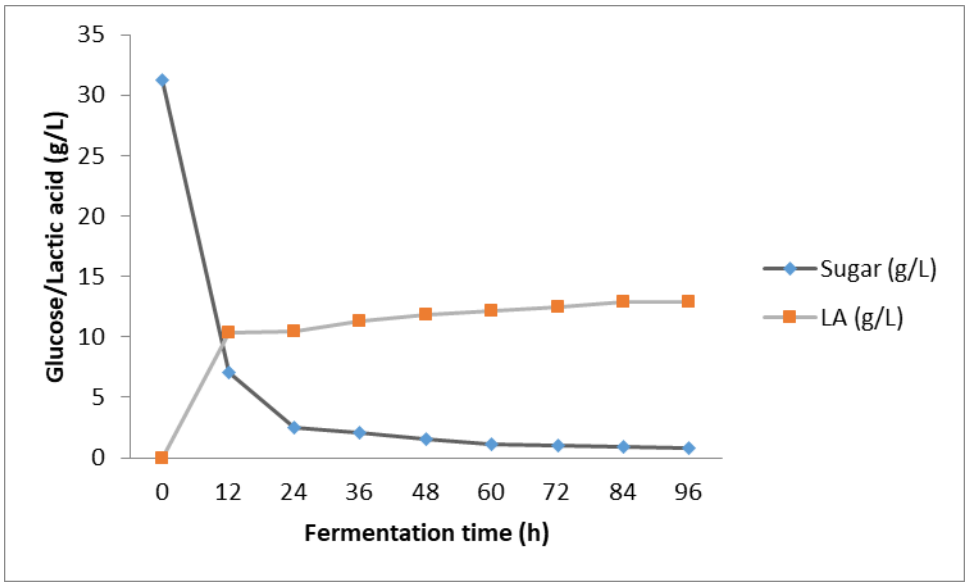
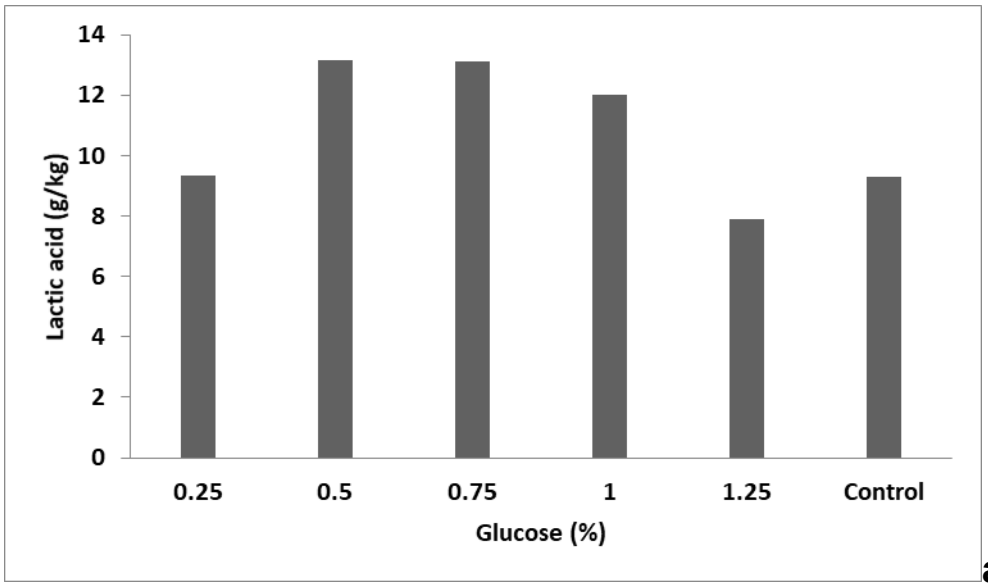


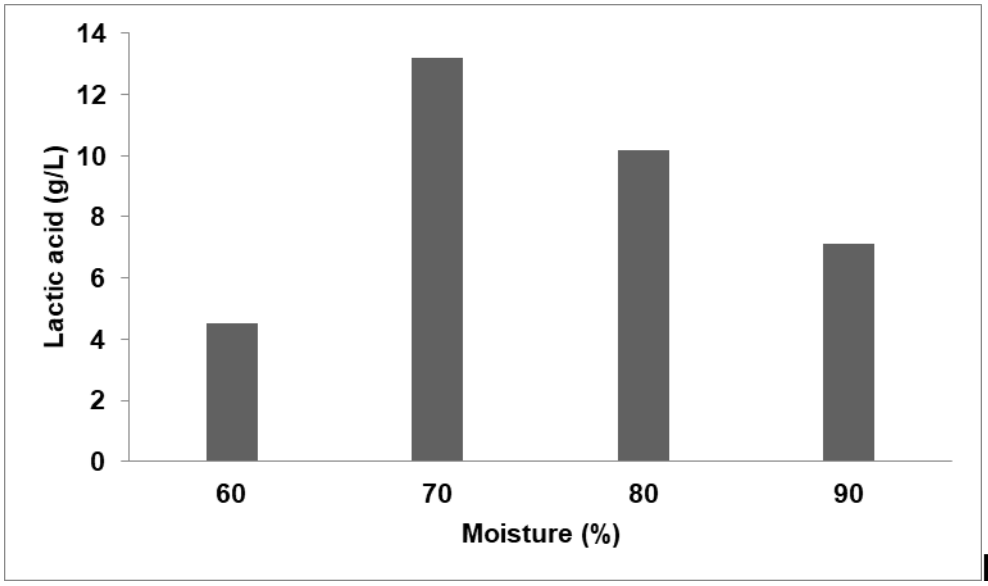
Fig. 1. Fermentation of sugar and production of lactic acid in submerged fermentation by *Lactobacillus plantarum*. The results represent the means of three different experiments. LA: Lactic acid

Screening of Independent Variables for Lactic Acid Production

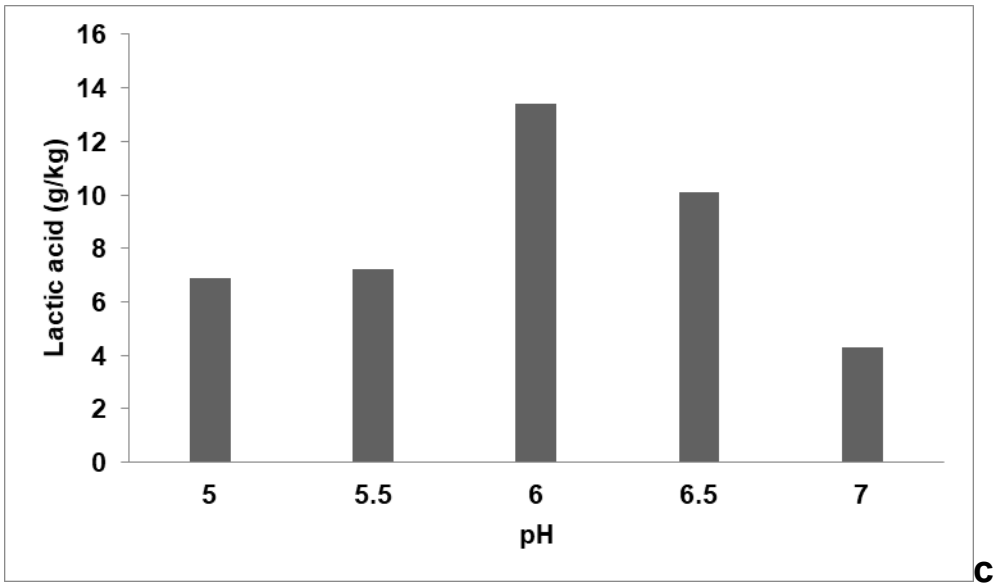
Figure 2a shows that with increasing concentrations of glucose in the culture medium, lactic acid biosynthesis increased. Lactic acid production was greatest (13.1 ± 1.3 g/kg) in the 0.75% glucose-supplemented sweet tuber peel medium ($p<0.01$). Figure 2b shows that with increasing moisture content of the culture medium, lactic acid production increased. At 70% moisture, lactic acid production reached a maximum (13.2 ± 2.3 g/kg), and a further increase in moisture content in the medium affected lactic acid biosynthesis ($p<0.01$). In SSF, the initial moisture content of the medium significantly influences growth and indirectly affects product formation. Bacteria require approximately 70% moisture for better growth, whereas fungal culture requires 20 to 70% moisture content. The current results clearly revealed that 70% was optimal for improving the production of lactic acid in SSF, and these findings were similar to those of previous studies. Figure 2c shows that with increasing pH of the culture medium in the SSF, lactic acid biosynthesis increased to pH 6.0 (13.4 ± 0.9 g/kg). Lacic acid production decreased near neutral pH (6.5) and neutral pH values (10.1 ± 0.9 g/kg and 4.3 ± 0.5 g/kg, respectively) ($p<0.01$). *L. plantarum* optimally grows at acidic pH values between 4.0 and 7.0, which was consistent with the results of this study. To optimize the incubation time for lactic acid production, fermentation experiments were performed for 72 h. Lactic acid production was time dependent, and maximum production was achieved after 36 h (17.4 ± 2.1 g/kg) and declined after 72 h of incubation (12.8 ± 1.9 g/kg) (Fig. 2d) ($p<0.01$). The biosynthesis of lactic acid was compared with that reported in previous studies, which revealed that the lactic acid produced in the present study was relatively high. Lactic acid bacteria, such as *L. rhamnosus* (Ye *et al*. 2014), *L. delbrueckii* (Nakano *et al*. 2012), *L. plantarum* (Okano *et al*. 2009), and *L. paracasei* (Nakano *et al*. 2012), reportedly survive at low pH values between 5.5 and 7.0 and produce significant amount of lactic acid.


14
12
10
8
6
4
2
0
Lactic acid (g/kg)
0.25
0.5
0.75
1
1.25
Control
Glucose (%)

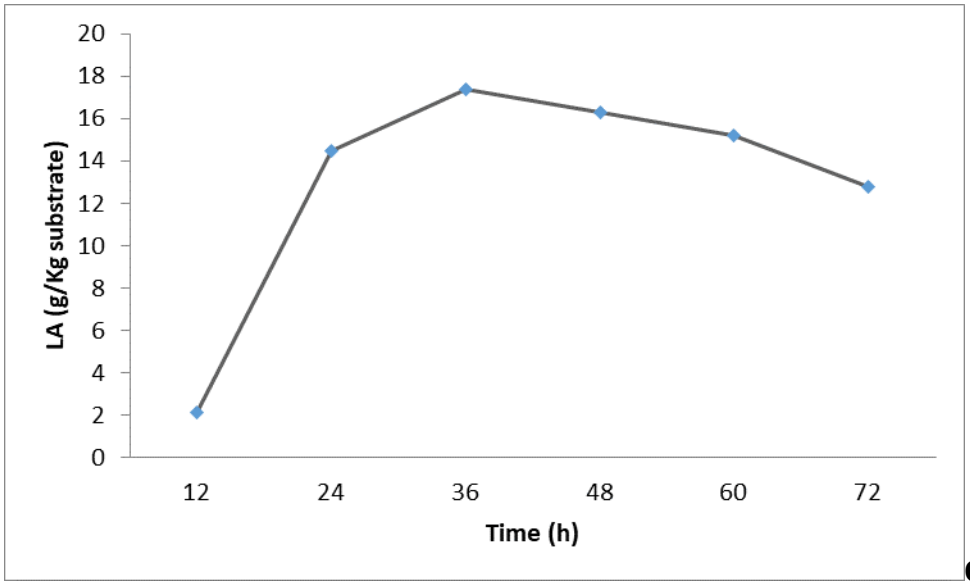
a


14
12
10
8
6
4
2
0
Lactic acid (g/L)
60
70
80
90
Moisture (%)

b



c



d

Fig. 2. Effects of glucose (a), moisture (b), pH (c), and fermentation period (d) on lactic acid production during solid-state fermentation. The pretreated substrate (sweet tuber peel) was hydrolyzed *via* cellulose and used as the substrate. The error bar represents the standard deviation.

Optimization of Lactic Acid Production Improved Yield

After 36 h of fermentation, *L. plantarum* MTCC1325 produced 12.1 ± 1.1 g lactic acid/kg substrate in the preoptimized culture medium. Under optimized culture conditions (run 13), the same LAB strain produced a maximum of 79.6 g/kg lactic acid. Lactic acid production was significantly improved by supplementing glucose in SSF. Compared with the other models, the quadratic model was shown to be the "best fit model" for lactic acid production, with the maximum F value. By adjusting the three selected process variables (pH, moisture, and glucose) at various concentrations, the applied CCRD model improved lactic acid production (Table 2). As shown in Table 2, the selected process variables (glucose, pH, and moisture) were found to influence lactic acid production. Lactic acid production increased with increasing sugar concentration to

0.756% and pH 6.75, as shown in the response surface graph. Moreover, further enhancement of sugar content and the initial pH of the medium significantly decreased lactic acid production in the SSF. Analysis of variance (ANOVA) was applied to compare the predicted and optimum response (*Y*) values to analyze whether the selected polynomial expression could optimally predict the response (lactic acid production) (Table 3). Figure 2 shows the 3D response surface plots for the optimal levels of each selected variable for improved lactic acid production in solid-state fermentation. The 3D plot revealed a potential influence on *L. plantarum* MTCC1325 growth and lactic acid production. Compared with pH and moisture, the amount of glucose in the culture medium significantly influenced lactic acid production. The 3D plots revealed that the moisture content of the medium and pH had less of an effect on lactic acid production. The addition of glucose to the culture medium significantly improved lactic acid production ($p<0.01$). The lactic acid production rate decreased below and above the optimal glucose concentration and moisture level. Hence, the above findings (Fig. 3a and 3b) revealed that moisture and glucose significantly influence lactic acid production.

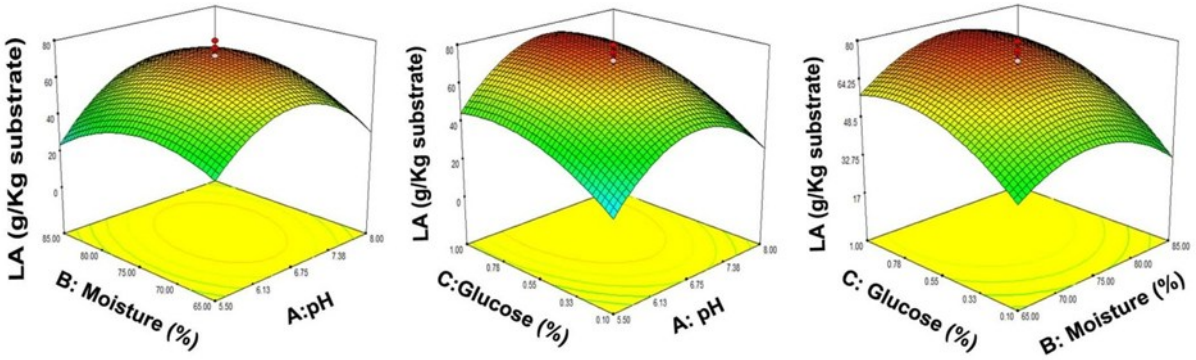


Fig. 3. 3D interactions between pH moisture (a), pH and glucose (b), and moisture and glucose (C)

The CCRD model F value of 15.85 implies that the model was statistically significant. A value of "Prob > F" less than 0.0500 indicates that the model terms were significant. In this model, C (moisture), A2, B2, and C2 were significant model terms. The reduction in the selected ranges may improve the model design. The "lack of fit F value" of 6.84 implies that there was an 8.30% chance that a lack of fit F value could occur because of noise. The R2 value was 0.978, and the adjusted R2 value was 0.981. The differences between the predicted R2 values and adjusted R2 values were small, revealing good agreement between the model values. The adequate precision ratio of the model was >4 (10.863), indicating an adequate signal. The coefficient estimate was positive for pH, moisture, and glucose concentration, and the maximum coefficient estimate (13.25) was observed for glucose concentration. The optimum concentrations were pH 6.89, moisture 75.3%, and glucose 0.90%. Under these optimum culture conditions, lactic acid production improved (85.6 g/kg substrate). The predicted response and actual lactic acid production were very similar. The actual lactic acid yield (predicted) (86.1 g/kg substrate) was very close to the experimental value (84.2 g/kg substrate), supporting the validity of the selected model. Bacteria require an optimum pH value (external and internal proton concentrations) for growth and metabolism. Bacteria have the potential to maintain the intracellular pH at a constant rate, and more energy is

consumed if the external pH continuously fluctuates. In the current study, a decrease in lactic acid production at higher glucose concentrations after the optimum level was reached was also detected, which may be due mainly to the substrate inhibition effect. The production of lactic acid increased with increasing initial moisture content and acidic pH. The RSM has been used to optimize variables for lactic acid production by Lactobacillus rhamnosusand Lactobacillus acidophilus (Jafarpour et al. 2021). Food waste has been utilized to produce lactic acid, and optimized process conditions were optimized via RSM (Anagnostopoulou et al. 2022). RSM has been used to improve lactic acid production by optimizing physico-factors and nutrients (Ha et al. 2020; Tefaraet al. 2024).

Table 2. Central Composite Rotatory Design for the Production of Lactic Acid in Solid-state Fermentation

Run	pH	Moisture (%)	Glucose (%)	LA (g/L)
1	5.5	65	1	34.2
2	5.5	65	0.1	3.14
3	6.75	75	0.55	61.49
4	5.5	85	1	18.42
5	6.75	58.1821	0.55	39.2
6	6.75	75	-0.2068	17.3
7	8	85	0.1	13.7
8	6.75	75	0.55	67.81
9	6.75	75	1.30681	70.42
10	4.64776	75	0.55	0.98
11	6.75	91.8179	0.55	28.2
12	8	65	1	21.9
13	6.75	75	0.55	79.6
14	6.75	75	0.55	76.1
15	6.75	75	0.55	74.9
16	8	85	1	60.2
17	8	65	0.1	18.3
18	6.75	75	0.55	71.9
19	5.5	85	0.1	7.92
20	8.85224	75	0.55	2.4

Table 3. Analysis of Variance for Lactic Acid Production by *L. plantarum* MTCC1325

Source	Sum of Squares	df	Mean Square	F Value	p Value Prob > F
Model	14572	9	1619.1	15.85	< 0.0001
A-pH	204.1	1	204.1	1.99	0.1878

B-Moisture	1.291	1	1.291	0.012	0.9127
C-Glucose	2398.7	1	2398.7	23.48	0.0007
AB	249.7	1	249.7	2.44	0.1489
AC	9.1	1	9.1	0.089	0.7712
BC	62.3	1	62.3	0.610	0.4526
A^2	9114.4	1	9114.4	89.2	< 0.0001
B^2	2756.9	1	2756.9	26.99	0.0004
C^2	1510.8	1	1510.8	14.7	0.0032
Residual	1021.4	10	102.1		
Lack of Fit	810.4	5	162.0	3.84	0.0830
Pure Error	210.9	5	42.1		
Cor Total	15593.4	19			

Effect of Lactic Acid on Total Bacteria in Goat Meat

The bacterial load on lactic acid-treated goat meat is shown in Table 1. Compared with the untreated control, goat meat treated with 1.25%, 2.5%, 3.75%, or 5% lactic acid presented reduced aerobic bacteria counts (Table 4). The bactericidal activity of lactic acid-treated meat (5% lactic acid) was greater than that of 1.25% lactic acid-treated meat. The difference between the bacterial count of lactic acid-treated goat meat at 1 h post-spraying was 0.19 log units for 1.25%, 0.26 units for 2.5%, 1.11 units for 3.75%, and 2.04 units for 5% lactic acid. A reduction in the native microbial load of <1 log has been observed earlier when lactic acid treatment was used (Naveena *et al*. 2006; Harris *et al*. 2012). In the current study, a reduction of <1 log was observed after 1 h, 24 days, 7 days, and 15 days of treatment with 3.75% and 5% lactic acid, respectively. The reduction in bactericidal activity was concentration dependent, and this result was similar to that of a previous report. Rodriguez-Melcon *et al*. (2017) reported that increasing the lactic acid concentration increased the antimicrobial activity. The decrease in microbial count in lactic acid-treated meat is believed to be due to the oxidative stress caused by lactic acid on the bacterial plasma membrane, leading to an increased level of superoxide radical generation in the cytoplasm. The generated free radicals damage bacterial cells and cause cell death (Desriac*et al*. 2013). The increased lactic acid concentration in the medium resulted in a high cytoplasmic pH, which inhibits the metabolic activity of bacteria. The results obtained in the current study and those of previous reports show that some similarities and variations were also observed. The variation in previous studies might be due to differences in methods of sampling, selected lactic acid concentrations, treatment times, modes of treatment, handling methods, incubation temperatures, and methods of microbiological analysis (Capita *et al*. 2002; Ben Braïek and Smaoui 2021; Kaveh *et al*. 2023).

Lactic acid has been used as a natural preservative agent in meat storage, which reduced bacterial spoilage, and maintains desirable properties. Lactic acid preserve odour, taste, and texture of meat during storage; however, excessive lactic acid application may affect sensory properties (Premj et *et al*. 2022). The increased concentration of lactic acid reduced meat hardness during storage. In addition, lactic acid treatment accelerates meat tenderization after two days in beef muscle and increased level of collagen content. In the present study, 1.25 to 5% LA acid spray was used to control

bacterial growth. Rodríguez-Melcón *et al.* (2017) reported that 4% lactic acid effectively reduced microbial load and preserve sensory property and color in beef during storage.

Table 4. Lactic Acid Treatment of Goat Meat at Various Concentrations and Analysis of the Bacterial Population

Time	Bacteria (log CFU per cm^2)					p-Value
	Control	1.25% LA	2.5% LA	3.75% LA	5% LA	
0 h	3.12 ± 0.12	3.09 ± 0.04	3.02 ± 0.09	3.15 ± 0.14	3.27 ± 0.25	0.078
1 h	3.29 ± 0.23	2.91 ± 0.16	2.76 ± 0.05	2.04 ± 0.18	1.23 ± 0.05	<0.0001
24 h	3.39 ± 0.18	2.98 ± 0.25	2.53 ± 0.17	2.07 ± 0.06	1.28 ± 0.16	<0.0001
7 days	3.48 ± 0.11	3.01 ± 0.04	2.64 ± 0.13	2.19 ± 0.14	1.49 **± 0.04**	<0.0001
15 days	3.51 ± 0.17	3.12 ± 0.19	2.65 ± 0.16	2.24 ± 0.27	1.52 ± 0.18	<0.0001

The results are log CFU per cm^2 in goat meat

CONCLUSIONS

1. Sweet potato peel was utilized as a low-cost culture medium for the production of lactic acid in solid-state fermentation. The valorization of this waste could reduce environmental pollution and may contribute to a circular bioeconomy.
2. The average reducing sugar content improved after steam treatment (15 KPa) after 60 min. The pretreatment methods reduced the lignin and hemicellulose contents of the sweet potato peel.
3. The supplementation of glucose (0.90%), optimum pH value (6.89), and moisture content (75.3%) improved the lactic acid yield in central composite rotatory design. The lactic acid yield was improved threefold after optimization compared with that of the unoptimized medium.
4. Goat meat was sprayed with 1.25to5% lactic acid and presented a reduced aerobic bacteria count at higher lactic acid concentration. The bactericidal effect of lactic acid was dose dependent.

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