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Production of lactic acid from date fruit pomace using *Lactobacillus casei* and the enzyme Cellic CTec2

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ABSTRACT

A circular, sustainable bioeconomy is gaining ground across the globe. Green synthesis, which uses renewable feedstock, is becoming a mainstay of the chemical industry. To produce polylactic acid, a biodegradable plastic, researchers are increasingly focusing on low-cost substrates to produce lactic acid. In the United Arab Emirates, one such low-cost substrate with readily available reducing sugar is the date fruit pomace (DFP). It is a solid residue obtained from date fruit processing and contains 35% sugars on a dry basis. It is a potential feedstock for lactic acid fermentation. Here, the effects of media supplements and fermentation parameters on L-lactic acid production by *Lactobacillus casei* were investigated. The lactic acid yield improved when the pH was maintained at 6.2 during incubation and the enzyme Cellic CTec2 was used to release sugar from the pomace. At a controlled pH, a lactic acid concentration of 292 $\pm$ 1.2 g/kg DFP was achieved when 80 g DFP was inoculated with bacteria (3.9×10^{10} colony-forming units) and incubated for 7 days. Upon hydrolysis with 40 FPU/g DFP Cellic CTec2, the sugar content increased to 482 g/kg DFP, and the lactic acid concentration increased to 457 $\pm$ 1.4 g/kg DFP. Using n-butanol, via phase partitioning, 78.9% pure L-lactic acid was extracted from the fermentation broth.

1. Introduction

In response to rising carbon dioxide and greenhouse gas emissions, the chemical industries are on the verge of a paradigm shift from fossil fuel-based production to biorefinery-based production. The feedstock used in biorefinery for the production of biofuels and platform chemicals is renewable sources such as food crops, lignocellulosic waste, by-products of food processing industries, and so on. Over the next few years, biotechnological processes are expected to gain a market share of 5 to 20% for producing various chemical products, particularly organic acids (Yankov, 2022). Lactic acid is one such organic acid that is gaining attention because of its wide spectrum of industrial and biomedical applications (Ahmad et al., 2020). Lactic acid (2-hydroxy propanoic acid) is the most widely occurring hydroxycarboxylic acid produced during fermentation or by chemical synthesis. It is present in various types of food products, such as sauerkraut, yoghurt,

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buttermilk, sourdough breads, and others, both naturally and as a product of *in situ* microbial fermentation. Lactic acid is also a major metabolic intermediate in most living organisms, from anaerobic prokaryotes to humans. It occurs naturally as two optical isomers: D- and L-lactic acid. Because the elevated levels of the D-isomer are harmful to humans, L-lactic acid is the preferred isomer in the food and pharmaceutical industries and has widespread applications (Elsawy et al., 2017). Lactic acid fermentation is also employed at industrial scale to improve shelf life and prepare pre-biotic drinks with enhanced antioxidant activity from fruit juices (Markkinen et al., 2019) and for enhancing the bioaccessibility and bioavailability of fermented foods (Pontonio et al., 2019). The application of lactic acid in the production of biodegradable and biocompatible polylactic acid polymers has driven its market expansion. The main focus of recent research is on improving the lactic acid production from renewable resources, particularly for the development of biodegradable food packaging (Ahmad et al., 2021a). As of 2020, the global lactic acid market is valued at USD 1.1 billion, and it is projected to double by 2025 (Yankov, 2022).

The biotechnological production of lactic acid by fermentation is more advantageous than chemical synthesis because cheap raw materials, such as molasses, starchy waste, cellulosic materials, and other carbohydrate-rich materials, can be used in this process. Moreover, either the pure D- or L-form of lactic acid can be targeted by selecting appropriate microorganisms (Sadaf et al., 2021). The cost of raw materials is one of the major factors to be considered in the production of lactic acid. In addition to the low cost of raw materials, the biotechnological production of lactic acid requires a relatively lower production temperature and energy consumption (John and Nampoothiri, 2007). Therefore, direct fermentation is the most economical method for producing lactic acid. On a commercial scale, lactic acid is primarily derived from renewable and edible resources, such as corn, cassava, sugar cane, and sugar beets (Corbion-Paurac, 2015). The use of edible resources, categorized as first-generation biomass, for biochemical production has been linked to rising food prices. The use of agricultural land for fuel production instead of food is indicated by the term 'food *vs*. fuel' (Mohr and Raman, 2013). As a sustainable response to the debate surrounding the usage of food sources, research is now focused on the utilization of second-generation biomass, such as lignocellulosic waste and other industrial and food wastes. The production of lactic acid has been carried out with a wide range of feedstocks, such as sugarcane press mud, wheat bran (Tirpanalan et al., 2015), loquat kernels, cassava starch, cellulosic date palm wastes, sweet sorghum juice, date juice, and golden syrup from molasses; mixed restaurant food waste; agricultural bioresources such as hydrol, soybean curd residues, and malt; dairy industry waste (Veeranjaneya et al., 2016; Nwamba et al., 2021); starchy food waste (Unban et al., 2019) and macroalgae (Nagarajan et al., 2022a,b), for lactic acid production. Lignocellulosic agricultural waste requires enzymatic saccharification before it can undergo fermentation to convert cellulose into sugars that microorganisms can act upon. Hence, the utilization of waste with readily available sugars is less energy intensive than the lignocellulosic waste.

In the United Arab Emirates (UAE) and other Gulf countries, date fruit is a commonly available biomass with the highest content of available sugars. In the date processing industry, date honey or syrup (also known as dibs in the Middle East) is separated from the fruit by hot water extraction, which produces two types of industrial waste: the seeds and the fibrous material of the fruit pulp, known as date fruit pomace (DFP) or date press cake (Heidarinejad et al., 2018). On an average, date syrup production yields approximately 52%–64% syrup, 17%–24% DFP, and 8%–15% seeds on a dry matter basis (Al-Farsi et al., 2007). Currently, DFP, which is composed of exhausted date flesh with some residual sugars, is either used as animal feed or dumped in landfills (Oladzad et al., 2021). In our previous (Haris et al., 2023) study, a complete characterization revealed that DFP contains approximately 35% soluble sugars in the form of glucose and fructose. These readily available sugars make DFP a potentially better candidate for lactic acid production than other lignocellulosic wastes, as a pre-treatment step for releasing sugars from the structure can be avoided when DFP is used. Evidence from earlier studies has suggested that date fruits, syrup, and waste can be used for lactic acid fermentation (Nancib et al., 2001, 2005, 2009). Date syrup has been fermented with different strains of lactic acid bacteria (LAB), such as *Streptococcus thermophilus* (Bouhadi et al., 2017), mixed cultures of *S. thermophilus* and *Lactobacillus bulgaricus* (Jacob Varghese et al., 2012), *L. lactis* (Nancib et al., 2009), *L. casei* (Nancib et al., 2015; Kaavessina et al., 2017), and *L. acidophilus* and its mixture with *L. casei* (Bushara et al., 2018).

The primary objective of this study was to examine the ability of *L. casei* to produce lactic acid from DFP with and without pH control and investigate the beneficial effects of using Cellic CTec2 in the process.

2. Materials and methods

2.1. Chemicals and reagents

DeMan–Rogosa–Sharpe (MRS) broth was purchased from Neogen Chemicals Ltd (MI, USA). Anhydrous magnesium sulphate ($MgSO_4$), anhydrous potassium phosphate dibasic (K_2HPO_4), anhydrous potassium phosphate monobasic (KH_2PO_4), ferrous sulphate heptahydrate ($FeSO_4.7H_20$), and anhydrous manganese sulphate ($MnSO_4$) were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Tween 80, yeast extract, Cellic CTec2 enzyme with the brand name cellulase blend, L-lactic acid analytical standard ($\geq$98%), D-glucose (high-performance liquid chromatography (HPLC) grade), fructose (HPLC grade), sulphuric acid, n-butanol and deuterium oxide (D_2O) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Preparation of DFP and analysis of proximate components

DFP was obtained from the Al Foah Company-Emirates Date Factory located in Al Saad (Abu Dhabi, UAE). The DFP obtained was dried overnight at 60 °C in an oven, milled, sieved through a 1 mm mesh, and stored in an air-tight container until use. The total solid and ash contents were determined according to the National Renewable Energy Laboratory protocols. The total solid content was determined overnight at 105 °C, and the ash content was determined by heating in a muffle furnace at 575 °C for 3 h (Sluiter et al., 2008). To determine the total solids, the crucibles were baked at 575 °C in a muffle furnace for 2 h. On cooling, the weight of empty crucibles was noted. 2 g of DFP was loaded into a crucible and dried at a temperature of 105 °C overnight (Sluiter et al., 2008). To determine the ash content, the dried crucible is placed in a muffle furnace at 575 °C overnight. The weight of the crucible was noted after cooling in the desiccator. The total solid content and ash content are calculated from the weight difference of the crucibles. The moisture content is 100 minus the total solid content. The volatile matter and fixed carbon contents were estimated according to ASTM D3175 methods (Sait et al., 2012). To determine the volatile matter, 2 g of DFP in a crucible with a lid was placed in a tube furnace heated to 950 °C for 7 min. On cooling down, the volatile matter was calculated from the weight differences of the crucible. Fixed carbon is 100 minus the summation of moisture, ash, and volatile matter.

2.3. Bacterial propagation

Lyophilized Nutrish® *Lactobacillus casei* 431® was purchased from Chr. Hansen Middle East and Africa FZ-LLC (Al-Barsha, Dubai, UAE). The freeze-dried form of the bacterium was activated by culturing in an MRS medium and incubating for 24 h under shaking conditions in a water bath maintained at 38 °C (Kaavessina et al., 2017), with the pH adjusted to 6.2 ± 0.2. The cell concentration was estimated by measuring the optical density at 620 nm (OD_{620}) and correlating the value to the dry cell weights: one OD unit = 0.5 g cell mass/L (Nancib et al., 2015).

2.4. Fermentation

Submerged batch fermentation of dried DFP was conducted in glass bottles with a 50 mL working volume. The fermentation medium was autoclaved at 121 °C for 15 min before inoculation, and fermentation was performed in a shaking incubator (Thermo Scientific, Kansas, USA) at 38 °C and 150 rpm. After incubation, the samples were centrifuged at 4500 rpm for 15 min. The supernatant was filtered using a 0.20-μm syringe filter and analysed for lactic acid and soluble sugars (glucose and fructose) using HPLC.

2.4.1. Effect of supplementation with yeast extract and salts

Initially, the effects of enriching the fermentation media with yeast extract as a nitrogen source and supplementing the media with a salt mixture were investigated. The fermentation of dried DFP was conducted both in the presence and absence of yeast extract. Yeast extract was supplemented at 10, 20, and 30 g/L. To study the effect of supplementation with a salt mixture, fermentation was conducted using biomass supplemented with yeast extract with and without the salt mixture. The salt mixture added was composed of 0.2 g/L $MgSO_4$, 0.3 g/L K_2HPO_4, 0.3 g/L KH_2PO_4, 0.02 g/L $FeSO_4$, 0.03 g/L $MnSO_4$, and 1 mL/L Tween 80.

2.4.2. Effect of biomass, inoculum loading, and incubation time on lactic acid production and optimization with response surface methodology (RSM)

To determine the optimum conditions for maximum lactic acid production, different biomass loading percentages, such as 10%, 20%, and 30% (g DFP per 100 mL of fermentation media) and inoculation with 5%, 10%, and 15% (v/v) *L. casei* in MRS under anaerobic conditions, were studied. Anaerobic conditions were maintained by flushing the bottles with nitrogen gas. Incubation was conducted for 10 days, and samples were collected on the second, third, fifth, seventh, and tenth days. The pH was adjusted to 6.2 ± 0.2. To assess the productivity rate of *L. casei,* the medium was fermented with a mixture of pure glucose and fructose. Bacterial cell concentration was determined by measuring the OD_{620}.

RSM was applied to understand the interaction between various processing parameters, model the fermentation process, and determine the optimum fermentation conditions. Three factors—incubation time, biomass loading, and the inoculum of LAB in the fermentation broth—were considered. Statistical analyses were conducted using the Design Expert Software version 8.0.6. The factors biomass loading (10%, 20%, and 30%) and inoculum loading (5%, 10%, and 15%) had three levels, whereas the third factor, incubation time, had five levels (2, 3, 5, 7, and 10 days). The response for the analysis, i.e., the maximum lactic acid yield, was statistically evaluated using RSM.

2.4.3. Effect of controlled pH on lactic acid production

To determine the optimum reaction parameters, experiments were scaled up and conducted in a 1.5 L fermenter (BioStream, Netherlands) with a working volume of 400 mL, with and without pH control, at 38 °C and an agitation speed of 200 rpm. The pH of the fermentation media was maintained at 6.2 by the continuous addition of 3 M NaOH and 3 M HCl solutions. Samples were drawn at different intervals, and bacterial counts were determined using serial dilution and plating methods.

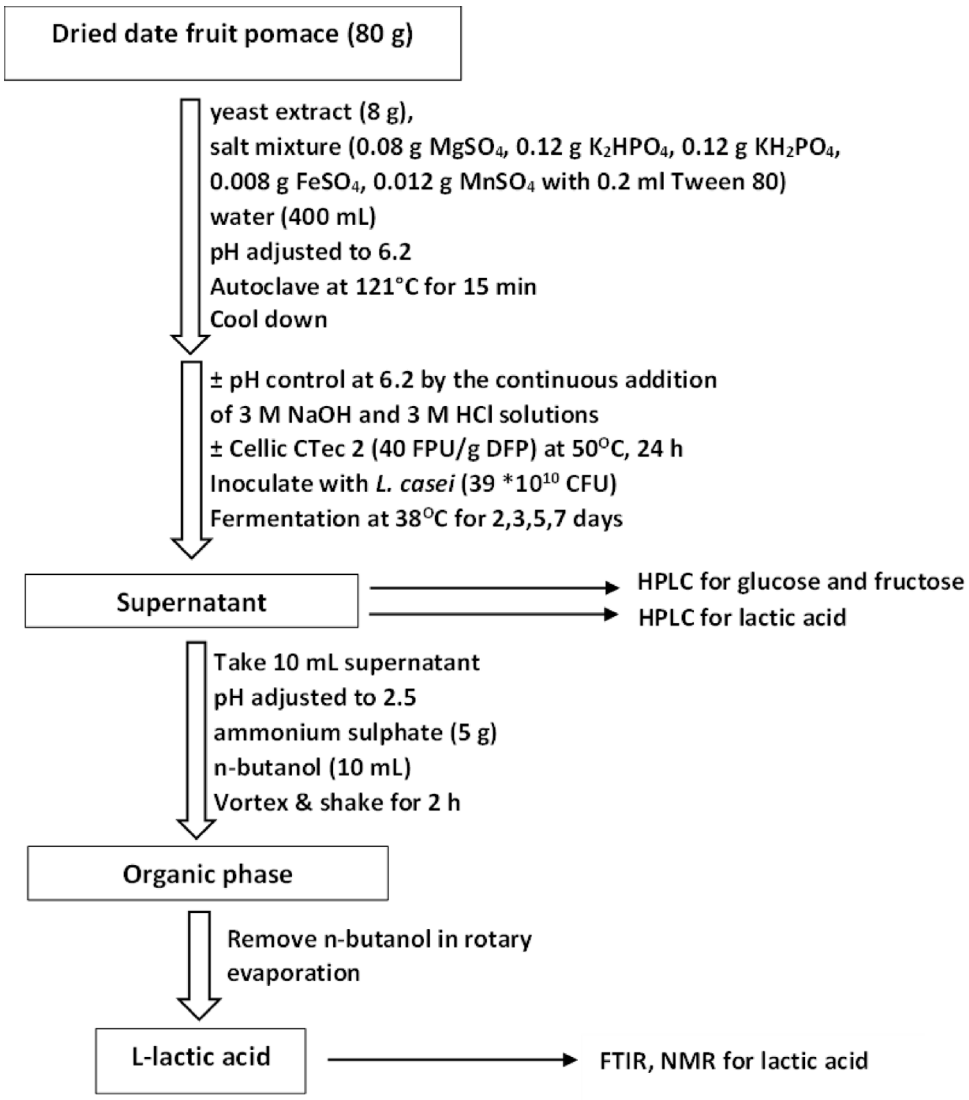

Fig. 1. Schematic diagram for L-lactic acid production.

2.4.4. Effect of enzymes on lactic acid production

The cellulase activity of Cellic CTec2 was determined in a filter paper assay, as described by Adney and Baker (2008). The enzymatic hydrolysis of DFP was conducted at different enzyme dosages of 10, 20, 40, 50, 100, and 200 FPU/g DFP. Enzymatic hydrolysis was conducted in glass bottles with a 50 mL working volume with a biomass loading of 20% on a shaking incubator at 50 °C, 150 rpm for three different periods (2, 24, and 48 h). The enzymatic hydrolysis was stopped immediately after the hydrolysis time by placing the bottles in an ice bath. Upon cooling down, the samples were centrifuged at 4500 rpm for 15 min. On filtering the supernatant with a 0.20-μm syringe filter the glucose and fructose released were quantified using HPLC. To determine the optimum enzyme dosage, enzymatic hydrolysis was followed by fermentation, with and without pH control. A schematic diagram of l-lactic acid production is shown in Fig. 1.

2.5. HPLC analysis of lactic acid and soluble sugars in the fermentation broth

HPLC analysis of lactic acid and soluble sugars in the fermentation broth was performed using a Dionex Ultimate 3000 UPLC instrument (Thermo Scientific, KS, USA). Lactic acid was separated using a Hi-Plex-H column (7.7 × 300 mm, 8 μm, Agilent Technologies, Inc., UK) at 65 °C using 0.005 M H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min, and peaks were detected using a refractive index detector (ERC RefractoMax 520, ERC, Inc., Japan) (Nwobi et al., 2015). The concentrations of soluble sugars, namely glucose, and fructose, were determined using a Rezex RCM Monosaccharide LC

column [Ca^{2+} (8%), 300 × 7.8 mm, Phenomenex, CA, USA] at 75 °C, with Millipore water as the mobile phase at a flow rate of 0.7 mL/min. Sugar concentrations were measured using a refractive index detector.

2.6. Fourier transform infrared spectroscopy (FTIR) and Nuclear magnetic resonance (NMR) of extracted lactic acid

On removing the solids from the fermentation broth by centrifugation, lactic acid was extracted using a phase-partitioning method, as explained in the literature (Kumar et al., 2020). The pH of the supernatant was adjusted to 2.5 using concentrated HCl. Ammonium sulphate (5 g) was dissolved in 10 mL of the supernatant. Next, 10 mL of n-butanol was added, and the mixture was vortexed vigorously and incubated in a water bath at 30 °C for 2 h. The organic phase was separated from the mixture using a separating funnel. The aqueous phase of the mixture was subjected to two additional rounds of n-butanol extraction. The solvent was separated by pooling the organic phase using a rotary evaporator. The dried phase was dissolved in MilliQ water, and the lactic acid concentration was measured using HPLC. To determine the extraction efficiency of 2-butanol, a process similar to that described above was used by replacing n-butanol with 2-butanol. The extracted lactic acid was characterized using FTIR analysis. FTIR analysis was performed using a Spectrum Two™ spectrometer (Perkin-Elmer, MA, USA) in transmission mode, using KBr particles prepared with 0.8 mg of the sample and 80 mg of KBr (Varma and Mondal, 2016). The spectra were obtained in the spectral range of 400–4000 cm^{-1} using the Spectrum software. The NMR samples of the fermentation broth obtained at the 7th day and that of the lactic acid obtained by n-butanol extraction from the fermentation broth, were prepared by dissolving weighted aliquots of lyophilized specimens in 600 μL of D_2O. All ^1H NMR spectra were collected at 600.19 MHz and 293 K with an Avance III Bruker spectrometer (Bruker Corporation, MA, USA) equipped with cryo-probe and *z*-axis gradient system, over a sweep width of 7211.5 Hz. 1D ^1H NMR spectra were digitized over 32 K points with 256/512 scans. All chemical shifts were referred to based on the spectrometer's absolute frequency, calibrated at 293 K in D_2O set at 0 ppm (Cantarutti et al., 2019).

2.7. Statistical analysis

All trials were performed in triplicates and each replicate was analysed once, and the results are expressed as mean ± standard deviation. The means of the fermentation parameters were compared by one-way analysis of variance (ANOVA) and paired *t*-test using the Minitab statistical software package (State College, PA, USA). Differences were considered statistically significant at $p \leq 0.05$.

3. Results and discussion

3.1. Proximate analysis

Proximate analysis revealed that dried DFP had a moisture content of 8.6% ± 0.14% and a total solid content of 91% ± 0.14%. The ash content, volatile matter, and fixed carbon constituted 2.7% ± 0.03%, 28.4% ± 0.2%, and 60.3% ± 0.4% of the total volume, respectively. The DFP used in this study is composed of 40.7% total dietary fibre [38% cellulose, 26% hemicellulose, and 36% lignin (Kamal-Eldin et al., 2020)], 35.3% sugars, 10.6% proteins, 3.2% pectin, and 1.3% fat (Haris et al., 2023).

3.2. Lactic acid fermentation from DFP

3.2.1. Effects of supplementation with yeast extract and salts

To study the effect of supplements, fermentation was conducted with 10% DFP for 5 days. As shown in Fig. 2, the fermentation of dried DFP alone produced only 30.7 g/kg DFP of lactic acid. In the medium supplemented with 10 and 20 g/L yeast extract, the lactic acid concentration increased to 68.1 and 103 g/kg DFP, respectively. However, increasing the concentration of yeast extract to 30 g/L did not further increase the lactic acid concentration. The addition of yeast extract caused an increase in lactic acid production, which shows that a nitrogen source in the form of yeast extract is necessary for the bacteria to grow and facilitate lactic acid production. Supplementing the fermentation media with a nitrogen source (Nancib et al., 2001) and enriching it with essential salts (Nancib et al., 2005; Jacob Varghese et al., 2012) has been shown to promote lactic acid production. Nancib et al. (2001) also showed that yeast extract is a better nitrogen source for lactic acid production than peptone, urea, ammonium sulphate, or corn step liquor. Besides being a nitrogen source, yeast extract is rich in B vitamins (Kaavessina et al., 2017). Upon further supplementation of the fermentation medium with yeast extract and salts, the lactic acid concentration increased to 200 g/kg DFP. This increase resulted from the addition of salts, which made the nutritional content of the fermentation media more appropriate for the bacteria, owing to the provision of essential nutrients for enhancing the lactic acid production potential of the bacteria. The magnesium content in the salt mixture aids the functioning of the enzymes, and manganese acts as an activator of various reactions in bacteria, particularly the transfer of lactic acid produced within bacterial cells across the cytoplasmic membrane for release in the fermentation broth (Jacob Varghese et al., 2012). Moreover, the surfactant Tween 80 is a good source of carbon that creates pores in the bacterial membrane and aids the cellular excretion of lactic acid (Boudjelal and Nancib, 2001).

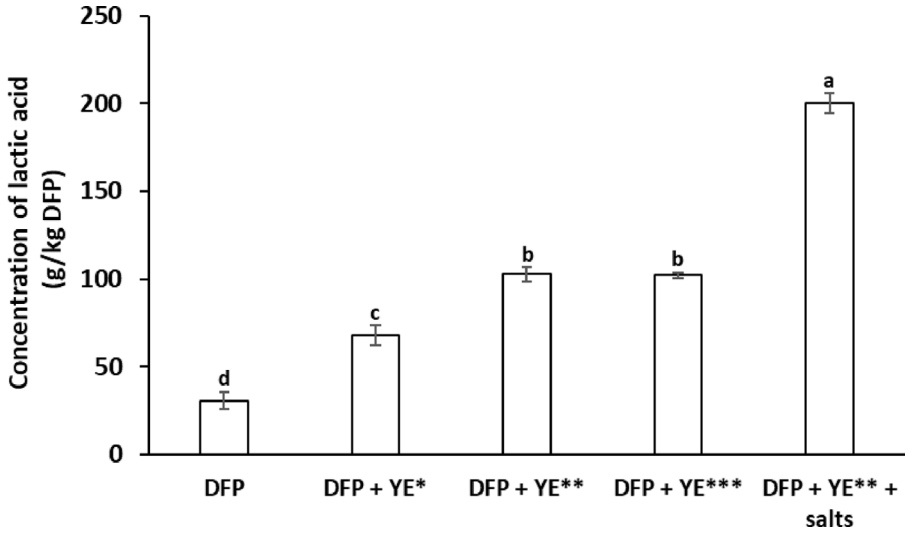

Fig. 2. Concentration of lactic acid produced in samples with date fruit pomace (DFP) alone (DFP) and DFP supplemented with 10 g/L yeast extract (YE) (DFP + YE*), 20 g/L yeast extract (DFP + YE**), 30 g/L yeast extract (DFP + YE***), and 20 g/L yeast extract and salt mixture (DFP + YE** + salts). The values represent the mean ± SD from three runs. Means that do not share a letter are significantly different ($P < 0.05$).

3.2.2. Effects of biomass, inoculum loading, incubation time, and optimization with RSM

To determine the optimum processing parameters, fermentation feed with varying biomass loading and varying inoculum loading for different incubation times were studied. The feed was subjected to fixed yeast extract (20 g/L) and salt supplementation. The bacterial cell concentration was found to be 0.99 g cell-mass/L, with an OD_{620} value of 1.993. As a preliminary investigation, by varying the inoculum load of bacteria, 10% (v/v) bacteria were found to exhibit better lactic acid production. Fig. 3 shows the lactic acid concentrations obtained when 10%, 20%, and 30% DFP were inoculated with 10% bacteria. The maximum concentration achieved was 209 g/kg DFP on the seventh day of incubation with 20% DFP. The fermentation of DFP takes a relatively long time as it contains a high level of lignin (ca 38%) (Kamal-Eldin et al., 2020) and soluble and bound phenolic compounds (Alam et al., 2022) that may inhibit enzyme activity and/or act as a barrier preventing the bacterial enzymes to access the fermentable sugars and cellulose. Upon increasing the DFP to 30%, no increase in lactic acid production was observed under the inoculum load investigated. Upon autoclaving the fermentation media with 30% DFP, the water in the media was absorbed, and the media formed a thick cake, which made the fermentation process similar to solid-state fermentation rather than submerged fermentation, as observed for 10% and 20% DFP loading. This can be attributed to the water-holding capacity of DFP, as reported in our previous study (Haris et al., 2023). The lower water content led to improper mixing. Resultantly, lower accessibility of the bacteria to available sugars in the DFP resulted in a lower lactic acid concentration in all fermentation media with 30% DFP. Increasing the inoculum concentration from 5% to 15% did not cause a significant increase (**Supplementary Data**). Hence, the optimum conditions determined for fermentation were a biomass loading of 20%, inoculum loading of 10%, and incubation for 7 days.

L. casei, a homofermentative bacterium, uses the Embden–Meyerhof–Parnas pathway and produces two moles of lactate per mole of glucose (de Oliveira et al., 2018). Fructose is also subjected to a similar pathway (Cassimiro et al., 2022; Parhi et al., 2021). Fig. 4 shows the glucose and fructose consumption patterns and lactic acid formation in fermentation with optimized parameters of 20% DFP with yeast extract and salt supplementation under inoculation with 10% *L. casei*. On the seventh day, the glucose and fructose were completely consumed. On day 10, the lactic acid concentration was similar to that on day 7, as no residual sugar was available for the bacteria to metabolize. Hence, from 358 g of sugar (glucose + fructose)/kg pomace, 209 g/kg lactic acid was produced with a yield of 0.6 g lactic acid/g sugars. The fructose-to-glucose ratio in DFP is marginally higher than 1, whereas, in date fruit, both sugars are present in a 1:1 ratio. As discussed in our previous study (Haris et al., 2023), this difference can be attributed to the greater concentration of glucose extracted from the syrup than fructose. To assess the lactic acid production potential of *L. casei*, fermentation was conducted using a pure glucose and fructose mixture in the same ratio as that in DFP, and the yield was found to be 1.77 g lactic acid/g sugars, similar to that reported by Nancib et al. (2015). The lower lactic acid production from biomass than pure forms of sugars can be attributed to the presence of fermentation barriers in the lignocellulosic structure of DFP (Sainio et al., 2011). Furthermore, as lactic acid was produced, the pH of the fermentation media eventually reduced from 6.2 to 3.3, which reduced the fermentation potential of the bacteria (Tang et al., 2017).

The experimental data were analysed using the RSM. A quadratic model developed by correlating the processing parameters to the response to maximize lactic acid concentration was found to fit adequately. The model adequacy was

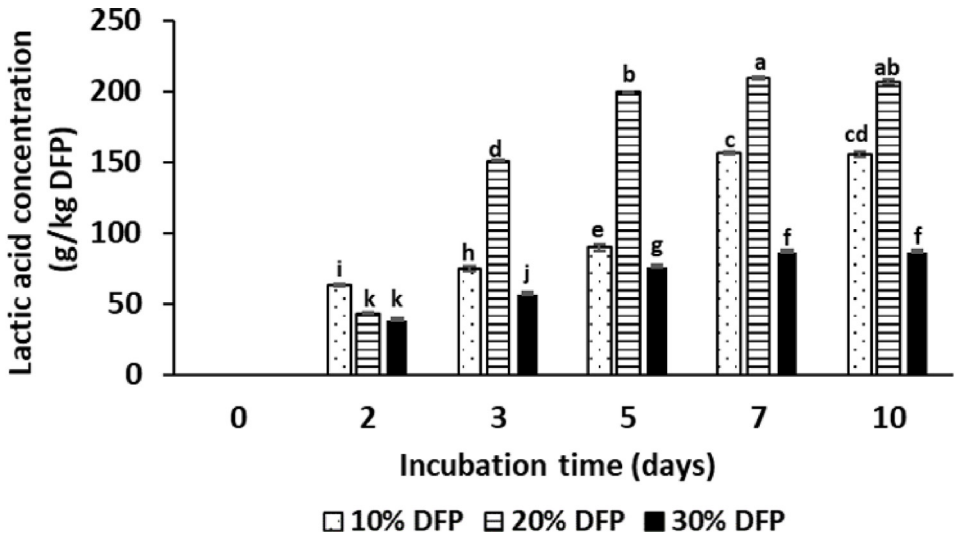

Fig. 3. Concentration of lactic acid produced when 10%, 20%, and 30% date fruit pomace (DFP) were inoculated with 10% bacteria. The values represent the mean ± SD from three runs. Means that do not share a letter are significantly different ($P < 0.05$).

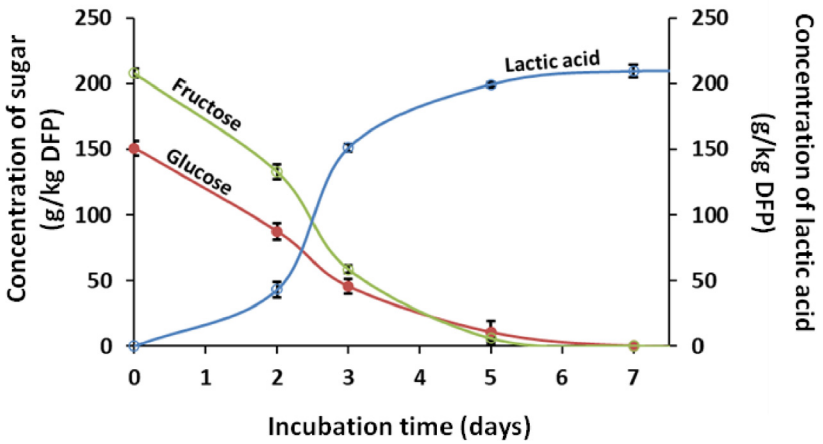

Fig. 4. Concentration of lactic acid, glucose, and fructose in the fermentation media with date fruit pomace (DFP) inoculated with *Lactobacillus casei*. The values represent the mean ± SD from three runs.

assessed using analysis of variance. The ANOVA results of the quadratic model are presented in Table S1. The final model equation in terms of the coded factors is as follows:

Lactic acid concentration (g/kg DFP) = 71.41 + 4.38*A + 15.68*B + 11.76*C + 1.77*A*B + 4.78*A*C + 3.98*B*C − 16.85*A^2 - 16.40*B^2 − 7.55*C^2

where A is biomass loading, B is the incubation time, and C is inoculum loading

The R-Squared value of 0.8341 was in reasonable agreement with the "Adj R-Squared" value of 0.7914. The 'Pred R-squared' value was 0.7336. The difference between the adjusted R-squared and predicted R-squared values was < 0.2, indicating that the model was adequate. The model F-value of 19.55 and *p*-value of *< 0.0001* implied that the model was significant. There was only a 0.01% chance that a "Model F-value" this large could occur because of noise. The values of "Prob > F" were < 0.05, indicating that the model terms are significant. In this case, all three factors (A, B, and C) and interactions A2, B2, C2, and AC were significant model terms and exerted the strongest effect on the lactic acid concentration ($p < 0.05$). "Adeq Precision", which measures the signal-to-noise ratio, had a value of 16.416, indicating an adequate signal. A ratio greater than 4 was desirable. This implies that the model can perform reasonably according to the prediction (Owolabi et al., 2018) and can be used to navigate the design space.

As shown in the **Supplementary Data**, when comparing the predicted and actual experimental values, all data points were uniformly distributed around the mean, indicating that the proposed model was adequate. The adequacy of the model was verified by examining residuals. The residuals and runs were distributed around a straight line, indicating that

Table 1
The lactic acid concentration in the presence and absence of pH control in a lab-scale fermenter.

Incubation time (days)	Lactic acid concentration (g/kg DFP)	
	Without pH control	With pH control
0	n.d.	n.d.
2	119 ± 1.7^{b}	261 ± 0.5^{a}
3	146 ± 0.1^{b}	275 ± 0.8^{a}
5	156 ± 0.1^{b}	279 ± 0.9^{a}
7	171 ± 0.2^{b}	292 ± 1.2^{a}
Yield (g-LA/g-sugars)	0.5	0.8

All values represent the mean ± SD from three runs. n.d. = not detected. Different superscript letters in the column indicate a significant difference ($P < 0.05$). (DFP: date fruit pomace).

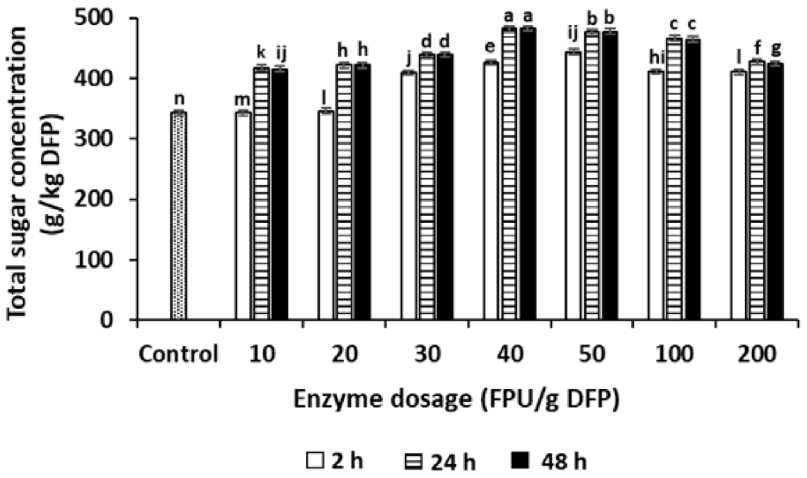

Fig. 5. Concentration of total sugar obtained from the enzymatic hydrolysis of date fruit pomace (DFP) at different enzyme dosages under hydrolysis for varying durations. The control had no enzyme. The values represent the mean ± SD from three runs. Means that do not share a letter are significantly different ($P < 0.05$).

the errors were distributed normally, and outliers were absent (**Supplementary Data**). By applying numerical optimization and based on the desirable criteria, the optimal combination of parameters indicated by the software was 23.04% of biomass loading, incubation time of 8.47 days, and inoculum load of 10% with a desirability factor of 0.968. The response surface plot of optimum lactic acid concentration as a function of biomass loading and incubation time with the actual factor of an inoculum load of 10% is shown in the **Supplementary Data**.

3.2.3. Effects of controlled pH on lactic acid production

Upon scaling up, fermentation was conducted with and without pH control in a lab-scale fermenter. Table 1 shows the lactic acid concentrations in both scenarios. Without pH control, on the 7th day of fermentation, a concentration of 171 g lactic acid/kg DFP was achieved, whereas with pH control, the concentration of lactic acid increased to 292 g/kg DFP with a yield of 0.8 g-LA/g-sugars. When the optimum pH was maintained, the LAB was exposed to optimal conditions, which resulted in a higher yield (Tang et al., 2017).

Samples drawn on different days were serially diluted in peptone water, plated on MRS agar media, and incubated for 48 h. In fermentation without pH control, the bacterial cell count was 9.8×10^{9} colony-forming units (CFUs)/mL on day 1, which was reduced to 6.6×10^{8} CFU/mL on day 7. The presence of bacterial cells on day 7, even at a pH was 3.3, indicated the tolerance level of *L. casei* (Itoh et al., 2012) and lactic acid production was still possible at lower pH levels (Pau et al., 2022). With pH control, the bacterial cell count was 5.5×10^{9} CFU/mL on day 7. Cell growth contributes to lactic acid production, which is affected by the pH of the fermentation broth. A lower pH affects enzyme activity in vivo and thus affects cell growth, as the pH of the medium can change cell permeability by altering the charge distribution on the cell membrane (Thakur et al., 2019a). Maintaining an optimum pH facilitated the survival of bacterial cells; hence, greater lactic acid production was achieved.

3.2.4. Effects of a cellulose-hydrolysing enzyme on lactic acid production

Cellic CTec2 hydrolyses cellulose present in the dietary fibre of DFP to simple sugars. Its cellulase activity was found to be 127.5 FPU/mL (Baral et al., 2021). Fig. 5 shows the total sugar concentration (summation of glucose and fructose concentrations) at different enzyme dosages and varying durations of hydrolysis. Sugar was released at a higher

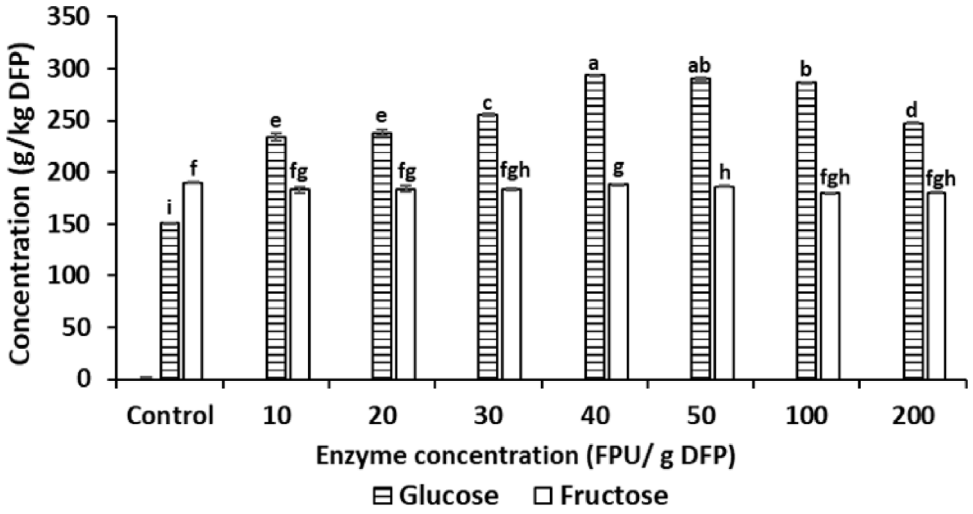

Fig. 6. Concentration of glucose and fructose obtained when date fruit pomace (DFP) was hydrolysed for 24 h at different enzyme dosages. The values represent the mean ± SD from three runs. The different superscript letters in the column indicate a significant difference ($P < 0.05$).

concentration (482 g/kg DFP) when 24-hour enzymatic hydrolysis was conducted with 40 FPU/g DFP. At all dosages tested, 24-hour hydrolysis led to a higher sugar concentration than 2-hour hydrolysis. The 48-hour hydrolysis process yielded almost the same sugar content as 24-hour hydrolysis. Fig. 6 shows the concentration of glucose and fructose released at different enzyme dosages when hydrolysis was conducted for 24 h. Upon increasing the dosage to 40 FPU/g DFP, a steady increase was observed in the glucose concentration. At 40 FPU/g DFP, a 1.4-fold increase in the glucose concentration was observed compared to that in the control sample. The control samples did not contain any enzyme. In the case of fructose, the increase was less compared to that observed in the release of glucose, as Cellic CTec2 converted cellulose present in the DFP fibre to glucose (Rodrigues et al., 2015). Increasing the enzyme dosage to more than 40 FPU/g DFP did not increase the sugar release; hence, the optimum dosage was fixed at 40 FPU/g DFP. After 24-hour enzymatic hydrolysis, the fermentation medium was cooled to 38 °C and inoculated with 10% LAB, and fermentation was conducted for 7 days with and without pH control. Without pH control, a lactic acid yield of 389 g/kg DFP was obtained, as shown in Fig. 7(a). With controlled pH at 6.2, a higher lactic acid yield, 457 g/kg DFP, was obtained, as shown in Fig. 7(b). Using only the indigenous bacteria present in date pulp waste, which is similar to DFP, Ahmad et al. (2021b) obtained a yield of 64 g/kg DFP. Multiple studies have shown the production of L-lactic acid from various biomasses as listed in Table 2. Using *Bacillus coagulans*, Lopez-Gomez et al. (2020) produced lactic acid with a yield of 0.94 g/g from the organic fraction of municipal solid waste. Gullon et al. (2008) obtained a yield of 0.88 g/g apple pomace using *L. rhamnosus*, and Sadaf et al. (2021) obtained a yield of 0.2 g/g bread waste using the strain *L. paracasei*. The efficiency of lactic acid production can be improved by changing the process strategies such as by scaling up and adapting a fed-batch fermentation process (Chenebault et al., 2022); incorporating a recycle stream (Chen et al., 2020b,a) of the fermentation residues to subsequent fermentation cycles; and by immobilizing the lactic acid bacteria (Thakur et al., 2019b; Chen et al., 2020b,a). The economics of the production can also be further improved if the nitrogen source can be replaced with cheaper sources such as protein-rich agricultural hydrolysates as shown by Krull et al. (2020), where they achieved a 23% reduction in total costs. To reduce the waste generated in the studied process, the solid residue resulting from the fermentation can be converted to bio-fertilizer to improve soil properties (Chavez-Rico et al., 2023) or to other value-added products. In our further studies, all such modifications will be incorporated to develop a biorefinery based on DFP for sustainable lactic acid production.

3.3. FTIR and NMR of the extracted lactic acid

To separate lactic acid from the fermentation broth, various down streaming processes such as precipitation, distillation, ultrafiltration, nanofiltration, electrodialysis (Ahmad et al., 2020; Li et al., 2021), and adsorption techniques using ion-exchange resin (Din et al., 2021) are employed. In our study, lactic acid was purified from the fermentation broth using the phase-partitioning method. Prior to phase-partitioning, the fermentation broth was centrifuged to remove solid residues. The pH of the supernatant was adjusted, and the supernatant was mixed with ammonium sulphate and n-butanol to recover lactic acid. A lactic acid recovery of 78.9% was achieved when n-butanol was used as the solvent. When 2-butanol was used, the concentration of lactic acid recovered reduced to 50.7%, suggesting that n-butanol is a better solvent than 2-butanol for extracting lactic acid. Further optimization is required to obtain a higher recovery rate. The extracted lactic acid was characterized by FTIR and compared with the L-lactic acid standard. Both the standard and extracted samples showed -OH bond stretching at the wave number 3390 cm^{-1}, -CH and -CH_3 stretching at 2933 cm^{-1}

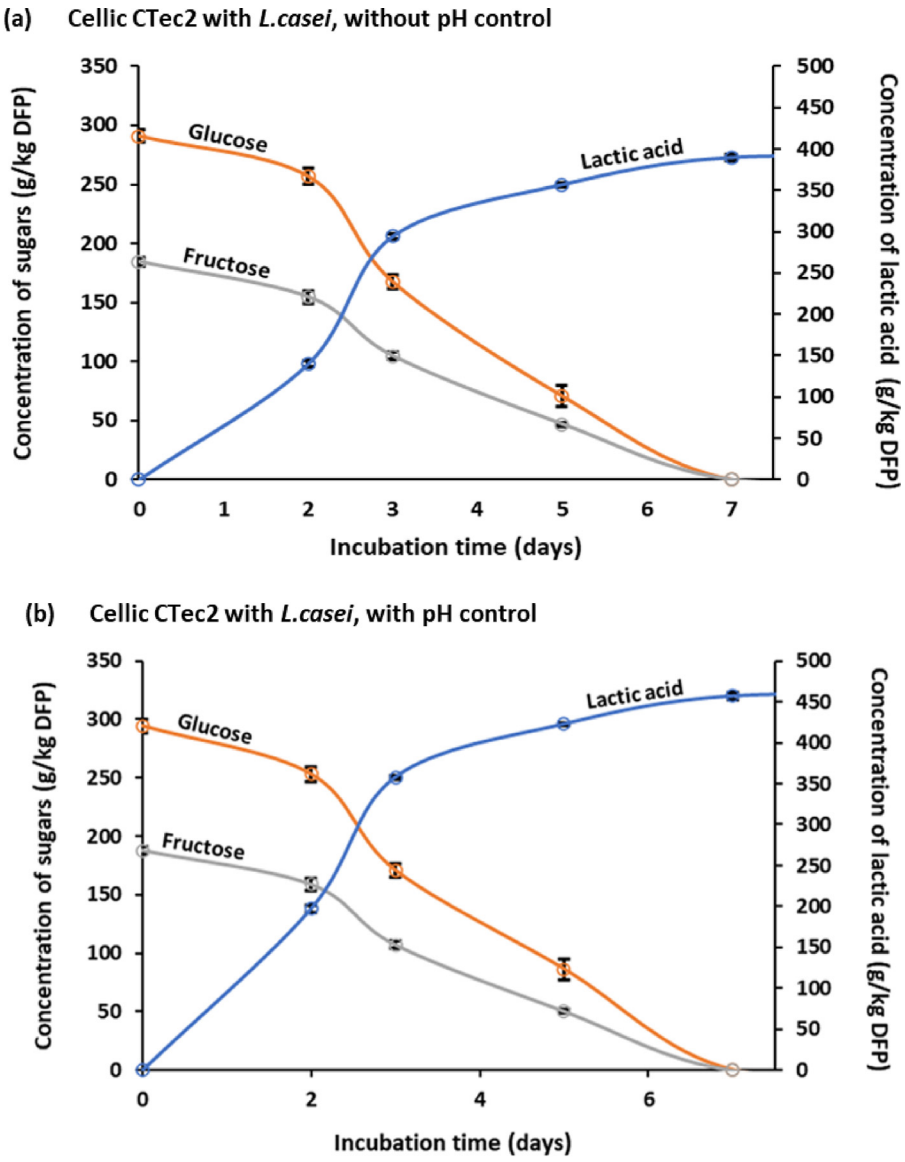

Fig. 7. Concentration of lactic acid, glucose, and fructose (a) during enzymatic hydrolysis followed by the fermentation of DFP with no pH control and (b) during enzymatic hydrolysis followed by the fermentation of DFP with pH control. The values represent the mean ± SD from three runs.

and 2528 cm^{-1}, -C=O stretching vibration at 1716 cm^{-1}, deformation of -OH bond at 1454 cm^{-1} and stretching of -C-C functional group at 1082 cm^{-1}. Similar FTIR spectra were observed by Păucean et al. (2017) for lactic acid in the fermentation media. As the spectra overlapped, common absorption bands were observed, and, hence, similar functional groups were also observed, which confirmed that the extracted product was L-lactic acid.

NMR spectra (**Supplementary Data**) confirm the presence of L-lactic acid and show the lactic acid was partially purified after n-butanol extraction. Both the samples (fermentation broth and extracted lactic acid) showed the presence of methyl (CH_3) and methine (CH) protons of lactic acid at 1.32 ppm and 4.1 ppm, respectively, in agreement with Kumar et al. (2020).

4. Conclusions

In this study, the fermentation parameters for maximum lactic acid production from DFP were assessed experimentally using RSM. The findings indicated that 1 kg of DFP primarily contains 151 g of glucose and 209 kg of fructose and can produce 209 g of lactic acid after enzymatic hydrolysis; lactic acid production can be increased to 457 g when fermentation

Table 2
Lactic acid production from second-generation biomass[a].

No.	Substrate	Microorganism	Yield	Method	Reference
1	Wheat bran	*Lactobacillus pentosus*	0.7 g/g	Enzymatic hydrolysis and fermentation	Tirpanalan et al. (2015)
2	Cellulosic date palm wastes	*Lactobacillus delbrueckii*	0.8 g/g glucose	Separate saccharification and fermentation	Alrumman (2015)
3	Dairy industry waste	*Lactobacillus rhamnosus B103*	0.2 g/g sugar	Fed-batch fermentation	Piassi et al. (2016)
4	Date waste (juice)	*Lactobacillus casei subsp. rhamnosus*	1.7 g/g glucose	Fed-batch fermentation	Nancib et al. (2015)
5	Hydrol, soybean curd residues and malt	*Enterococcus faecalis RKY1*	0.3 g/g	Repeated-batch fermentation	Reddy et al. (2016)
6	Mixed restaurant food waste	Thermophilic *Lactobacillus sp.; Streptococcus sp.*	0.14; 0.7 g/g sugar	Simultaneous saccharification and fermentation	Pleissner et al. (2017)
7	Liquefied cassava starch	*Rhizopus microsporus* DMKU 33	0.93 g/g	Fed-batch fermentation	Trakarnpaiboon et al. (2017)
8	Wasted dates	*Lactobacillus casei*/*Lactobacillus Acidophilus*/Mixed culture	0.97/0.93/0.93 g/g sugars	Batch fermentation	Bushara et al. (2018)
9	Sugarcane molasses	*Clostridium sensustricto*, *Escherichia*, and *Enterococcus*	0.8 g/g	Fed-batch fermentation	Sun et al. (2019)
10	Banana peel & food waste mixture	*Enterococcus faecium*	0.8 g/g sugars	Batch fermentation	Abdel-Rahman et al. (2019)
11	Banana peel	*Lactobacillus delbrueckii*	1.5 g/g sugars	Enzymatic saccharification and fermentation	Martínez-Trujillo et al. (2020).
12	Organic fraction of municipal solid waste	*Bacillus coagulans*	0.94 g/g	Batch fermentation	Lopez-Gomez et al. (2020)
13	Bread waste	*Lactobacillus paracasei*	0.2 g/g	Simultaneous saccharification and fermentation	Sadaf et al. (2021)
14	Date pulp waste	Indegenious microbiota	0.64 g/g	Enzymatic pretreatment and cyclic-mode dark fermentation	Ahmad et al. (2021c)
15	Cassava/rice bran/wheat bran/corn barn	*Lactobacillus plantarum*	1.3/1.8/1.7/1.6 g/g sugars	Enzymatic saccharification and fermentation	Sharma et al. (2021)
16	Food waste	Indegenious microbiota	0.02 g/g	Enzymatic pretreatment and batch dark fermentation	Ahmad et al. (2022a,b)
17	Seaweed/macro algae (Ulva sp., Gracilaria sp., Sargassum cristaefolium) hydrolysate	*Lactobacillus rhamnosus* and *Weissella paramesenteroides*	0.94/0.81/0.85 g/g	Batch fermentation	Nagarajan et al. (2022a,b)
18	Pomegranate juice	*Lactobacillus. paracasei*	0.8 g/g sugars	Batch fermentation	Mantzourani et al. (2022)
19	Cornstover	*Enterococcus mundtii WX1* and *Lactobacillus rhamnosus SCJ9*	0.9 g/g	Enzymatic hydrolysis & Batch fermentation	Klongklaew et al. (2023)
20	Cane molasses	*Lactobacillus delbrueckii NCIM 2025*	0.9 g/g	Continuous fermentation	Gupta et al. (2023)
21	Date fruit pomace	*Lactobacillus casei 431*	0.9 g/g soluble sugars	Enzymatic hydrolysis & Batch fermentation	This study

[a]Second-generation biomass includes lignocellulosic biomass or woody crops, agricultural residues or waste, as well as non-food energy crops grown on marginal land unsuitable for food production. (g/g denotes per g of the substrate).

is conducted under optimal parameters using *L. casei*. The findings indicate that DFP, otherwise considered a waste resource, is a promising low-cost substrate for lactic acid production.

CRediT authorship contribution statement

Sabeera Haris: Conceived and designed the experiments, Performed experiments, Analyzed and interpreted the data, Drafted the manuscript. **Afaf Kamal-Eldin:** Conceived and designed the experiments, Analyzed and interpreted the data, Critical revision and correction of the manuscript, Contributed reagents, materials, and analysis tools, Funding acquisition. **Mutamed M. Ayyash:** Critical revision and correction of the manuscript, Contributed reagents, materials, and analysis tools. **Bart Van der Bruggen:** Critical revision and correction of the manuscript. **Mohamed Mostafa Mohamed:** Critical revision and correction of the manuscript. **Ali H. Al-Marzouqi:** Conceived and designed the experiments, Analyzed and interpreted the data, Critical revision and correction of the manuscript, Contributed reagents, materials, and analysis tools, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analysed during this study are included in this published article and the Supplementary Data.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2023.103151.

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