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Nitrogen and Salt Supplementation of Oil Palm Trunk Juice and Its Optimization Conditions to Enhance Lactic Acid Production by *Lactobacillus rhamnosus* TISTR 108

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Abstract

The optimal conditions of lactic acid fermentation of undiluted oil palm trunks (OPTs) juice by *L. rhamnosus* TISTR 108 were investigated. The conditions with and without nutrient supplementation were studied. In the condition of no nutrient supplementation, fermentation at 10 % inoculum, 40 °C and pH of 6.5 could enhance growth and lactic acid production. The highest lactic acid yield and productivity were obtained at 0.78 g g^{-1} and 1.36 g $l^{-1}h^{-1}$, respectively. However sugars were found to remain in fermentation broth and the fermentation time was prolonged to 72 h. In the condition of nutrient supplementation, 10 g l^{-1} of yeast extract, 5 g $l^{-1}$of peptone and salts were added into the fermentation process. It was found that the fermentation time was shortened to 21 - 54 h and the sugars were completely consumed. The highest lactic acid yields and productivities were achieved at 0.82 - 0.85 g g^{-1} and 2.47 - 3.83 g $l^{-1}h^{-1}$, respectively.

Keywords: Lactic acid, fermentation, oil palm trunk, OPT, *Lactobacillus rhamnosus*

Introduction

Lactic acid and its derivatives are widely used in many industrial applications like the food, pharmaceutical, leather, textile and chemical industries. It also could contribute to a cleaner environment for polylactic acid (PLA) production. Lactic acid production can be manufactured both by chemical synthesis and microbial fermentation. Chemical synthesis always produces a racemic DL-lactic acid, whereas the stereo specific lactic acid, D(-) or L(+)-lactic acid, can be obtained by microbial fermentation [1]. Currently, approximately 90 % of lactic acid is produced by lactic acid bacteria because of the significant advantage over chemical synthesis, which can use cheap raw material, such as molasses, starchy waste, cellulosic, and other carbohydrate rich materials [2-4]. Old oil palm trunks (OPTs) are the agricultural wastes left in fields after replanting. Its fibrous materials can be used in many industries, such as wood, gypsum board, wood-cement composites, pulp and paper making, animal feed, and other related useful products. The sap squeezed from old OPTs contains glucose up to 85.2 g l^{-1}, and other sugars, such as sucrose, fructose, galactose, xylose, and rhamnose, were found in low concentrations. In addition, oil palm sap contains amino acids, organic acids, minerals, and vitamins [5,6]. A number of factors, including the fermentation processes [7-9], microorganisms [10-12], and the complex nutritional requirements, such as carbon, nitrogen, vitamin and mineral sources [13,14], play significant roles in efficient lactic acid production. Moreover, the lactic acid yield and productivity are affected by the optimal growth conditions, such as temperature, incubation period, pH, osmotic pressure, oxygen, and other high stress factors [15,16]. Although OPT juice is rich in carbohydrates, their utilization may be limited due to low protein content, at 2.18 g l^{-1} (this study). Different nitrogen sources have been studied

for lactic acid production and the increase of yeast extract will increase lactic acid production linearly. Most studies have been directed towards yeast extract supplementation, even though it has a high cost, as it could lead to the highest lactic acid concentration, due to a wide range of growth factors including amino acids, vitamins, specific minerals, fatty acids, purines, and pyrimidines [16,17]. Furthermore, lactic acid bacteria have a limited ability to synthesize B-vitamin and amino acids [18].

Previous studies used a low concentration of sap squeezed from old OPT as a nutrient source for lactic acid production [5,6]. In this study, the fermentation of lactic acid using OPT juice as a cheap raw material substrate with a concentration as high as possible was emphasized. Studies in the optimization of conditions and nutrients supplementation for lactic acid fermentation were investigated. Undiluted OPT juice and *L. rhamnosus* TISTR 108 were utilized.

Materials and methods

OPT juice and inoculum preparation

The sap squeezed from oil palm trunks (OPTs), or OPT juice, was used in this study. Palm trees were cut into small pieces. Juice was extracted by using a sugar cane press. OPT juice was then centrifuged at 4000 rpm for 40 min at 4 °C and stored at −18 °C for further analysis. The total sugars and total soluble protein of the OPT juice were analyzed.

Bacterium of *Lactobacillus rhamnosus* TISTR108 strain was used for lactic acid fermentation. For inoculum preparation, 100 ml of MRS medium, containing 20 g glucose, 10 g bactotryptone, 10 g yeast extract, 2 g K_2HPO_4, 5 g $CH_3COONa.3H_2O$, 0.2 g $MgSO_4.7H_2O$, and 5 mg $MnSO_4.4H_2O$ per liter was used [6]. The pH was adjusted to 7.0 with NaOH or HCl diluted solution. The medium was sterilized at 121 °C for 15 min. The fermentation was performed under a controlled temperature of 40 °C for 18 h, and the rotation speed was 150 rpm. The inoculum bacterial cell was adjusted to a constant number using the OD technique of 620 nm before being used as a fermentation starter.

Media and OPT juice fermentation in 2 l fermenter

Batch fermentation was performed in a 2 l stirred tank fermenter with a working volume of 1.6 l under anaerobic conditions. The fermentation study began with the use of undiluted OPTs juice as a main substrate without nutrient addition. The medium was sterilized at 110 °C for 10 min; 10 % v/v of preseed was then inoculated into the fermentation medium. pH was automatic controlled at 6.0 with 6 N $Ca(OH)_2$ solution. The temperature and agitator speed were maintained at 40 °C and 200 rpm, respectively. Fermentation parameters including initial OPT juice dilutions (OPT juice: water = 4:0, 3:1, 2:2, 1:3), inoculum sizes (5, 10, 15 % v/v), pHs (5.0, 5.5, 6.0, 6.5, 7.0) and temperatures (30, 37, 40, 43 °C) were initially determined for the optimal condition of lactic acid production. The optimized condition was then utilized in further experiments. The supplementations (yeast extract, peptone and salts solution) were performed under the optimized conditions. The modified medium was based on the study by Timbuntam [19]. The medium comprised of undiluted OPT juice, 10 g l^{-1} yeast extract (YE), 5 g l^{-1} peptone and salts solution (1.5 g $CH_3COONa.3H_2O$, 1.5 g KH_2PO_4, 1.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, and 0.05 g $MnSO_4.4H_2O$) [19]. The control fermentation was carried out using 100 g l^{-1} glucose, 10 g l^{-1} yeast extract, 5 g l^{-1} peptone, and salts, as mentioned. The fermentation was performed in anaerobic conditions. All experiments were done in duplicate.

Analysis

The cultured samples were taken at 0, 3, 6, 9, 12, 15, 18, 21, 24, 30, 33, 36, 42, 48, 54, 60 and 72 h. The viable cells were measured by spreading the samples on MRS agar plates and incubated in an anaerobic jar at 40 °C. Cultured broth was heated at 70 °C for 30 min, centrifuged (10000 g, 10 min), and filtered through 0.45µm. Lactic acid and total sugars concentrations were analyzed using HPLC system with an Animex HPX-87H column, and a 300×7.8 mm (Bio-Rad) with a refractive index detector (Waters, USA). The eluent 0.005 M H_2SO_4, a flow rate of 0.6 ml min^{-1}, and a column temperature of 50 °C were used. Lactic acid yield ($Y_{P/S}$) was expressed as gram lactic acid produced per gram total

consumed sugars (g g^{-1}) or gram per liter (g l^{-1}). The lactic acid productivity (Q_P, g $l^{-1}h^{-1}$) was determined as the ratio of lactic acid concentration (g l^{-1}) to the fermentation time (h). Residual sugars (g l^{-1}) referred to the total sugars in the fermentation broth.

Results and discussion

Influence of initial OPT juice concentration

Lactic acid fermentation of OPT juice by *L. rhamnosus* TISTR 108 was evaluated without nutrient supplementation. Four dilution conditions with the ratio of OPT juice to water at 4:0, 3:1, 2:2 and 1:3 were performed in order to obtain the optimal sugar levels from varying concentrations of 101.8, 78.2, 53.2 and 26.9 g l^{-1}, respectively. The results are summarized in **Table 1** and **Figure 1**. The maximum lactic acid yield of 0.75 g g^{-1} and maximum productivity of 1.19 g $l^{-1}h^{-1}$ were obtained when the highest initial sugar levels of the OPT juice was approximately 101.8 g l^{-1}. The yield and productivity were significantly higher ($p < 0.05$) than that of the initial OPT juice concentration of 78.2, 53.2 and 26.9 g l^{-1}, where lactic acid yields of 0.73, 0.65 and 0.65 g $g^{-1,}$ and productivities of 0.99, 0.38 and 0.28 g $l^{-1}h^{-1}$ were obtained, respectively. Maximum growth was reached at values of 1.70×10^{11}, 5.0×10^{10}, 5.30×10^{9} and 1.59×10^{9} cfu ml^{-1} at cultivation times of 30, 33, 60 and 60 h for the OPT juice concentrations of 101.8, 78.2, 53.2 and 26.9 g l^{-1}, respectively. The use of higher OPT juice concentrations resulted in the better growth of *L. rhamnosus* TISTR 108, and the shorter length of time it took to reach the stationary phase. Sugars were rapidly consumed during the log phase of cell growth in correspondence with decreasing sugars levels and growth. However, sugars were found not to be completely utilized during the late fermentation (72 h) by *L. rhamnosus* TISTR 108. Although *L. rhamnosus* NBRC 3863 was reported to resist glucose inhibition [7], high level of sugars remaining during fermentation may be due to growth limitation from insufficient amounts of some nutrients, e.g. nitrogen and minerals [20]. With other sources of sugar, such as sugarcane juice, sugarcane molasses, and sugar beet juice, efficient fermentation to lactic acid by *Lactobacillus delbruskii* was reported [21].

Influence of optimal conditions on undiluted OPT juice fermentation without supplementation

In **Figure 2**, without supplementation, the increased inoculum size was found to accelerate growth and lactic acid production by *L. rhamnosus* TISTR 108. The maximum cell growth reached 9.7×10^{10}, 1.70×10^{11}, and 4.50×10^{11} cfu ml^{-1}, at 42, 30, and 27 h fermentation time, with 5, 10, and 15 % inoculum, respectively. The highest lactic acid yield, and productivity (**Table 1**) were 0.66, 0.75, 0.69 g g^{-1}, and 0.86, 1.19, 1.34 g $l^{-1}h^{-1}$, with 5, 10, 15 % inoculum, respectively. The increase of inoculum size shortened the time of maximum lactic acid production, because higher cell numbers were obtained ($p \leq 0.05$). However, with 15 % inoculum, a significant decrease ($p \leq 0.05$) in lactic acid yield was observed, in comparison with 10 %. Residual sugars remained in fermentation medium concentrations of 47.1, 49.8, and 43.5 g l^{-1}, with 5, 10, and 15 % inoculum sizes at the cultivation times of 42, 33, and 30 h. After that, a constant in lactic acid production was observed, which may be because of insufficient amounts of nutrients in OPT juice and product accumulation. The study of Djukic-Vukovic *et al.* [22] suggested that, at 5 %, initial inoculum provided higher lactic acid concentration and viable cells number in comparison with 2 and 10 %. Some studies also reported that higher inoculum concentrations could reduce fermentation time [22,23].

Previous reports showed that *L. rhamnosus* exhibited the highest lactic acid yields and productivities at 38 to 45 °C [15,16,23,24]. However differences in optimum temperature may have resulted from substrate complexity, which differed in carbon, nitrogen, vitamin, and mineral supplementations, that caused limitation of kinetic patterns of lactic acid production and sugar consumption. The effect of temperature on lactic acid production by *L. rhamnosus* TISTR 108 on undiluted OPT juice without supplements is shown in **Figure 3** and **Table 1**. The maximum lactic acid was 32.26, 38.82, 41.40, and 39.95 g l^{-1} when the temperature was carried out at 30, 37, 40, and 43 °C, respectively. At 40 °C gave the highest lactic acid yield and productivity of 0.75 g g^{-1} and 1.21 g $l^{-1}h^{-1}$, respectively. The lowest lactic acid yield and volumetric productivity achieved at 0.69 g g^{-1} and

0.52 g $l^{-1}h^{-1}$, respectively, were quite low when the fermentation temperature was performed at 30 °C. At 43 °C, the maximum lactic acid yield and productivity was decreased to 0.72 g g^{-1} and 1.07 g $l^{-1}h^{-1}$, respectively. Bacterial growth was also affected by temperature; on increasing temperature, maximum viable cell number was found to be 9.85×10^{9}, 3.95×10^{10}, 1.72×10^{11}, and 9.90×10^{10} at 60, 33, 33, and 36 h fermentation time at 30, 37, 40, and 43 °C, respectively. Residual sugar concentrations of 56.79, 50.13, 48.78, and 48.71 g l^{-1} remained at temperatures of 30, 37, 40, and 43 °C at cultivation times of 60, 33, 33, and 36 h, in which the maximum lactic acid was achieved, respectively. However the residual sugars still remained in culture medium at the end of the fermentation time of 72 h. This was because, at higher temperatures and high OPT juice concentrations, a certain amount of reducing sugars was hardly converted to lactic acid and there was an increase in by-product formation [23,25].

Table 1 Fermentation parameters on lactic acid production by *L. rhamnosus* TISTR 108 using undiluted OPT juice without supplementation as a basal substrate.

Parameters	Lactic acid (g l^{-1})	$Y_{P/S}$ (g g^{-1})	Q_P (g $l^{-1}h^{-1}$)	Residual sugars (g l^{-1})	Fermentation time[a] (h)	Cells growth[b] (cfu ml^{-1})
Initial sugars of OPT juice (g l^{-1})[c]						
101.8	40.3A	0.75A	1.19A	49.8A	33	1.70×0^{11}A
78.2	36.8B	0.73B	0.99B	29.4B	36	5.0×0^{10}B
53.2	28.9C	0.65C	0.38C	10.7C	72	5.3×0^{9}C
26.9	17.9D	0.65C	0.28D	0.8D	60	1.59×0^{9}D
Inoculum (% v/v)[d]						
5	37.1C	0.66C	0.86C	47.1B	42	9.75×0^{10}C
10	40.3B	0.75A	1.19B	49.8A	33	1.70×0^{11}B
15	41.3A	0.69B	1.34A	43.5C	30	4.50×0^{11}A
Temperature (°C)[e]						
30	32.26D	0.69A	0.52D	56.79A	60	9.85×10^{9}D
37	38.82C	0.72B	1.13B	50.13B	33	3.95×10^{10}C
40	41.40A	0.75C	1.21A	48.78C	33	1.72×10^{11}A
43	39.95B	0.72B	1.07C	48.71C	36	9.9×10^{10}B
pH[f]						
5.0	36.63E	0.66E	0.85E	48.12C	42	9.34×10^{9}D
5.5	39.71C	0.69D	1.07C	45.93D	36	1.87×10^{10}C
6.0	41.40B	0.75B	1.21B	48.78B	33	1.72×10^{11}B
6.5	46.01A	0.78A	1.36A	44.57E	33	3.64×10^{11}A
7.0	38.28D	0.73C	1.05D	50.75A	36	1.78×0^{10}C

Note: the fermentation was conducted in a 2 l fermenter with a 1.6 l working volume and an agitation speed of 200 rpm; [a] the time for maximum lactic acid; [b] the maximum viable cells; the experiment was performed at; [c] 40 °C, pH 6.0, 10 % inoculum, [d] 40 °C, pH 6.0, the initial sugar concentration ≈ 101.8 g l^{-1}, [e] pH 6.0, 10 % inoculum, the initial sugar concentration ≈ 102 g l^{-1}, [f] 40 °C, 10 % inoculum, the initial sugar concentration ≈ 102 g l^{-1}; A, B, C, D, E are grouping information using the Tukey Method, A is significantly higher than B, C, D and E respectively, at $p \leq 0.05$.

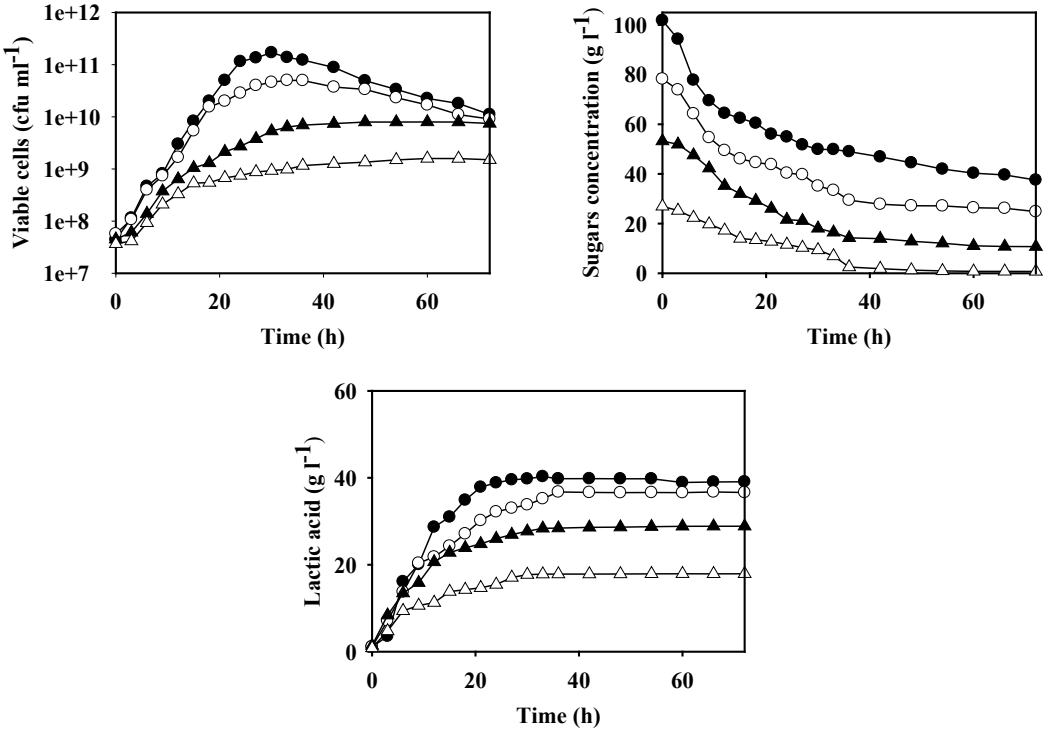

Figure 1 Profiles of growth and lactic acid production from various initial OPT juice concentrations without supplementation by *L. rhamnosus* TISTR 108. The batch fermentation was performed at pH 6.0,10 % inoculum, an agitation speed of 200 rpm and a temperature of 40 °C, with a ratio between OPT juice to water of 4:0 (●), 3:1 (○), 2:2 (▲) and 1:3(Δ).

pH was varied from 5.0 to 7.0 at 0.5 intervals. Profiles and kinetic parameters of lactic acid fermentation by *L. rhamnosus* TISTR 108 using undiluted OPT juice without supplements are summarized in **Table 1** and **Figure 4**. At pH 6.5, the maximum lactic acid was achieved at 46.01 gl^{-1}, at 33 h of cultivation, with a yield of 0.78 g g^{-1}, and 1.36 g $l^{-1}h^{-1}$ of volumetric productivity. At pHs lower or higher than 6.5, the decrease in lactic acid production and growth of *L. rhamnosus* TISTR 108 were observed. The lactic acid yields and productivities were also decreased. Lactic acid concentrations of 36.63, 39.71, 41.40, 46.01, and 38.28 gl^{-1} were obtained at 42, 36, 33, 33, and 36 h cultivation at pHs of 5.0, 5.5, 6.0, 6.5, and 7.0, respectively. At lower pH levels, lactic acid production may be decreased, because of the formation of byproduct, such as acetic and ethanol [26]. The highest viable cell of 3.64×10^{11} cfu ml^{-1} was achieved at pH 6.5. Decreasing pH below 6.5 significantly decreased maximum cells ($p \leq 0.05$). It was found that the tested pH of 6.0, 5.5, and 5.0 resulted in maximum cells of 1.72×10^{11}, 1.87×10^{10}, and 9.34×10^{9} cfu ml^{-1}. At pH 7.0, the maximum cell achieved at 1.78×10^{10} cfu ml^{-1} was not significantly different from that of pH 5.5 (1.87×10^{10}), but was significantly lower in pH 6.0 ($p \leq 0.05$). It can be concluded that the lactic acid production depends on the cell growths during undiluted OPT juice fermentation by *L. rhamnosus* TISTR 108. The high lactic acid productivity may have been caused by the use of $Ca(OH)_2$ as a neutralizing agent which was more effective than the monovalent (Na^+) cation [27].

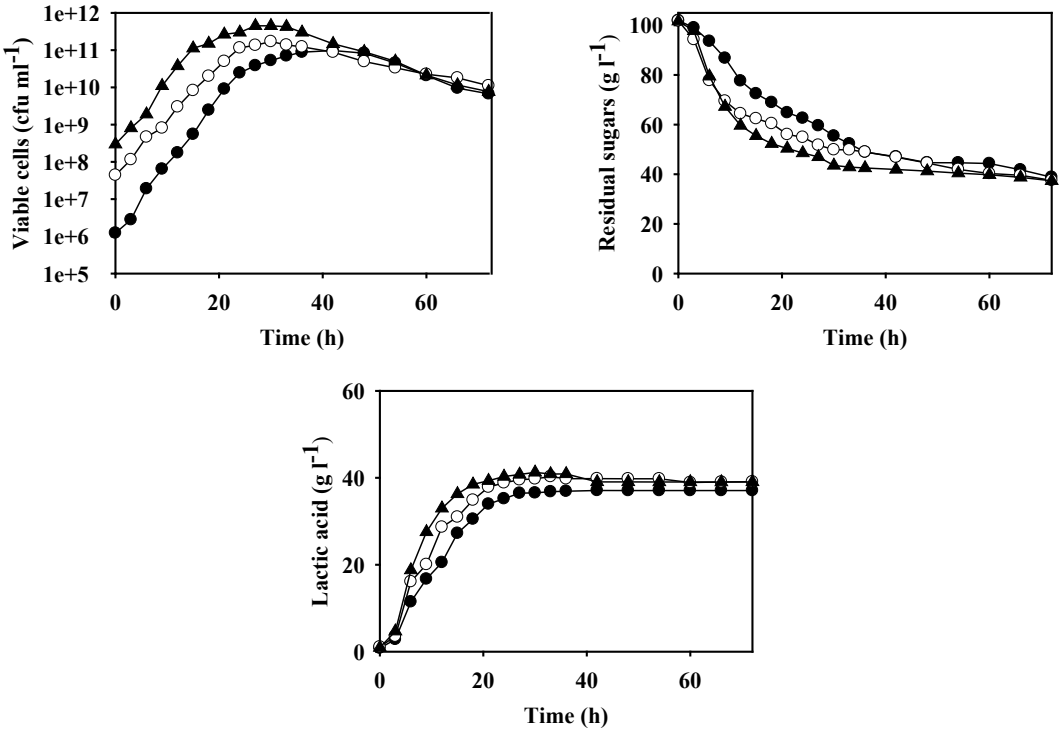

Figure 2 Effect of inoculum size on cell growth and lactic acid production during undiluted OPT juice fermentation by *L. rhamnosus* TISTR 108 without supplements at pH 6.0, an agitation speed of 200 rpm and a temperature of 40 °C, with an inoculum size of 5 % (●), 10 % (○) and 15 % (▲).

Even though these optimized conditions, including pH, temperature, inoculum size and fermentation time, could increase lactic acid production on undiluted OPT juice without supplements by *L. rhamnosus* TISTR 108, it still had a large amount of residual sugars in the fermentation broth, and prolonged the fermentation time to complete sugar consumption. This may be due to the insufficient amount of nutrients in undiluted OPT juice, or the loss of some nutrients during medium sterilization.

Effect of nitrogen and salts supplementation on undiluted OPT juice for lactic acid production

The effects of nitrogen and mineral salts supplementations on undiluted OPT juice for the production of lactic acid by *L. rhamnosus* TISTR 108 was tested between the addition of 10 g l^{-1} yeast extract and 5 g l^{-1} peptone (YE+P), the addition of 10 g l^{-1} yeast extract, 5 g l^{-1} peptone and salts solution (YE+P+T), and the addition of salts solution (T). The control experiment was performed using glucose with the addition of nitrogen and salts, as mentioned in the section on material and methods.

In **Table 2** and **Figure 5**, the supplementation of YE+P, YE+P+T, and T could increase the lactic acid yield and productivity of 0.85, 0.82, and 0.65 g g^{-1} and 2.47, 3.86, and 1.16 g $l^{-1}h^{-1}$, respectively. The maximum lactic acid concentration achieved was not significantly different ($p \leq 0.05$) to YE+P (82.69 g l^{-1}) and YE+P+T (82.10 g l^{-1}) supplementation, but they were significantly higher ($p \leq 0.05$) than T supplementation (63.38 g l^{-1}). In comparison to the control, where a pure sugar (glucose) was used as a main carbon source, lactic acid concentration was significantly high ($p \leq 0.05$) with the value of 94.09 g l^{-1}, and sugar was completely utilized (0.59 g l^{-1}). However, the maximum time for highest lactic acid was shortened in nitrogen and salts supplementation. In correlation, the remaining sugar was almost completely consumed at 33, 21, and 54 h in YE+P, YE+P+T, and T supplementation. In the condition

where trace amounts of sugars remained at the end of fermentation, this may suggest that some sugar in the OPT juice was hardly utilized by *L. rhamnosus* TISTR 108 [23]. The maximum yield of YE+P+T was obtained at 21 h, whereas YE+P supplementation reached the highest yield at 33 h; thus, a higher productivity was found in YE+P+T. Addition of YE+P and YE+P+T could not only shorten the lag time but also increase the lactic acid fermentation. Furthermore, the addition of salts also increased lactic acid concentration, but still prolonged the lag and fermentation time in comparison with non supplements on undiluted OPT juice. This may confirm the deficiency of some nutrients, such as nitrogen sources, in undiluted OPT juice.

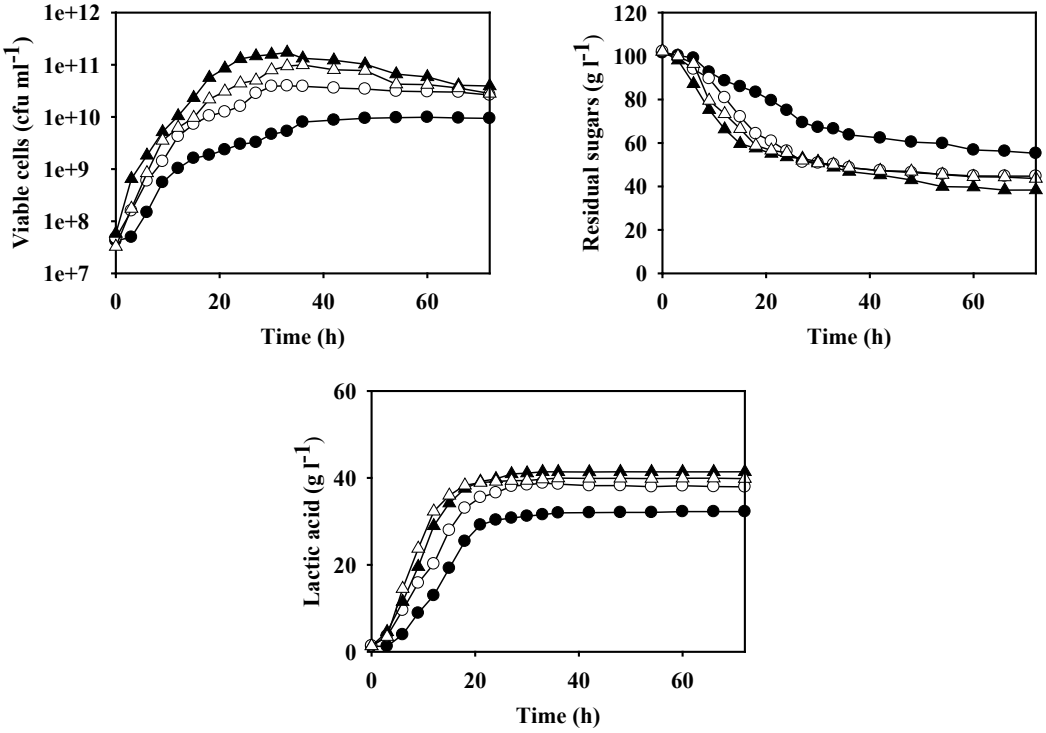

Figure 3 Effect of temperature on cell growth and lactic acid production during undiluted OPT juice fermentation by *L. rhamnosus* TISTR 108 without supplements at pH 6.0, a 10 % inoculum, an agitation speed of 200 rpm and temperatures of 30 °C (●), 37 °C (o) , 40 °C (▲) and 43 °C (Δ).

According to the bacterium growth, viable cells were increased in all supplementations. The highest viable cells were achieved at 1.86×10^{12}, 2.68×10^{12}, and 5.76×10^{11} cfu ml^{-1} at 30, 21, and 42 h with YE+P, YE+P+T, and T supplementation, respectively. This also proved that the maintained cell could also produce lactic acid and, in some cases, the time to achieve maximum cells was shorter than the highest lactic acid production time.

It could be concluded that yeast extract, peptone, and salt supplementations were essential for lactic acid fermentation by *L. rhamnosus* TISTR 108 when undiluted OPT juice was used as a substrate under sterile conditions. In comparison with the control, 100 g l^{-1} glucose supplemented with the mentioned nitrogen and salts produced 94.09 g l^{-1} of lactic acid yield and a productivity of 4.44 g $l^{-1}h^{-1}$ at 21 h cultivation, and glucose was completely consumed during fermentation. Although undiluted OPT juice contained 2.18±0.41 g l^{-1} of total soluble proteins (data not shown), it was much lower than the requirement in the control, which contained 10 g l^{-1} yeast extract and 5 g l^{-1} peptone. Thus, to achieve the highest yield and productivity of lactic acid by *L. rhamnosus* TISTR 108, the supplements of nitrogen and

salts were needed. In comparison with the control, a lower yield and productivity in this study were obtained, because some production inhibition occurred during fermentation using undiluted OPT juice. Therefore, high levels of nitrogen supplements were used. In further study, the suitable amounts of these nutrients should be emphasized in order to improve economic cost and obtain the highest lactic acid yield and productivity.

Table 2 Influence of nutrient supplementations on lactic acid production by *L. rhamnosus* TISTR 108 on undiluted OPT juice.

Nutrient supplementation	Lactic acid ($g\ l^{-1}$)	$Y_{P/S}$ ($g\ g^{-1}$)	Q_P ($g\ l^{-1}h^{-1}$)	Residual sugars ($g\ l^{-1}$)	Fermentation time[a] (h)	Cells growth[b] ($cfu\ ml^{-1}$)
YE+P	82.69B	0.85B	2.47C	5.43A	33	1.86×10^{12}B
YE+P+T	82.10B	0.82C	3.83B	2.65C	21	2.68×10^{12}A
T	63.38C	0.65D	1.16D	4.40B	54	5.76×10^{11}D
Control	94.09A	0.93A	4.44A	0.59D	21	1.21×10^{12}C

Note: YE, P and T represent yeast extract, peptone, and salts, respectively; the control experiment was performed using 100 g l^{-1} glucose, supplemented with yeast extract, peptone, and salts, as indicated in the section on materials and methods; the fermentation was conducted in a 2 l fermenter with a 1.6 l working volume at 40 °C, pH 6.5, 10 % inoculum, an initial sugar concentration ≈ 101 g l^{-1} and an agitation speed of 200 rpm; [a] the time for maximum lactic acid; [b] the maximum viable cells; A,B,C,D are grouping information using the Tukey Method, A is significantly higher than B, C and D respectively, at $p \leq 0.05$.

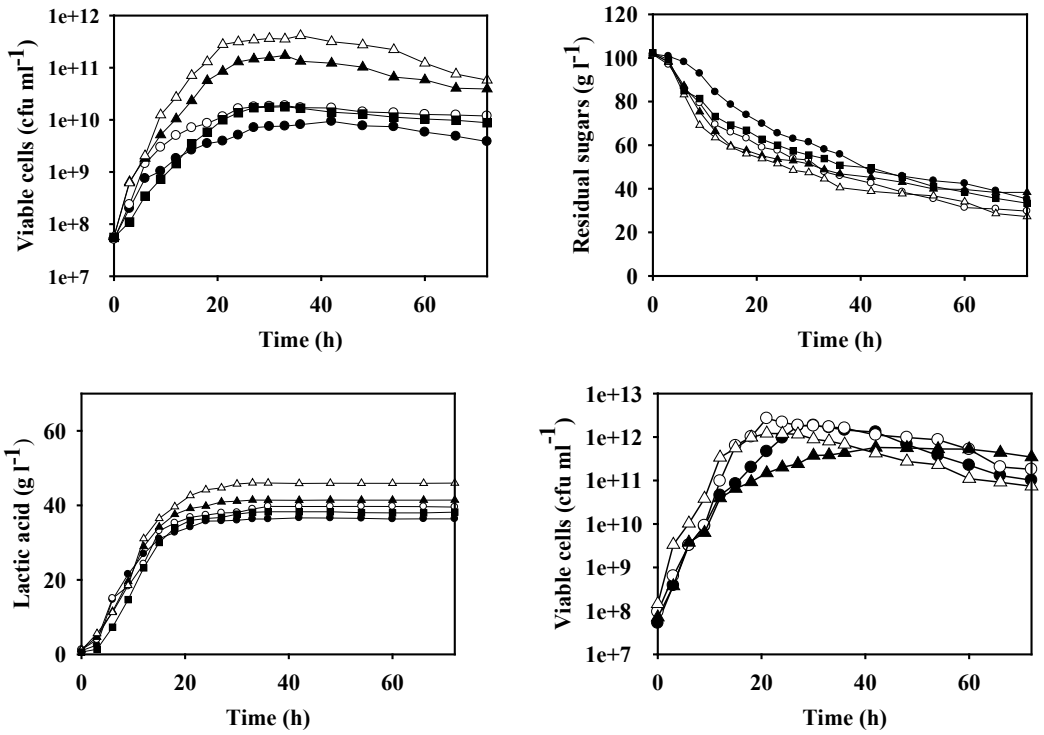

Figure 4 Effect of pH on cell growth and lactic acid production during undiluted OPT juice fermentation by *L. rhamnosus* TISTR 108 without supplements at temperature 40 °C, 10 % inoculum, agitation speed of 200 rpm and pH of 5.0 (●), 5.5 (o) , 6.0 (▲), 6.5 (Δ) and 7.0 (■).

With the addition of nutrients, the higher nutrient concentrations generally had a positive effect on the lactic acid production [24,28]. Yeast extract was the best supplement for efficient lactic acid production, due to the amino acid and vitamin requirement for the growth of *L. casei* subsp. *rhamnosus*, but yeast extract above 20 g l^{-1} had no significant increase in growth [20]. Cell growth showed different increasing trends with the increase of carbon-nitrogen ratios. If the nitrogen supply is short, the cell cannot produce the necessary enzymes for metabolism. If the nitrogen supply is too much in the form of ammonia, the cell growth will be inhibited, because the toxicosis of some key enzymes [11].

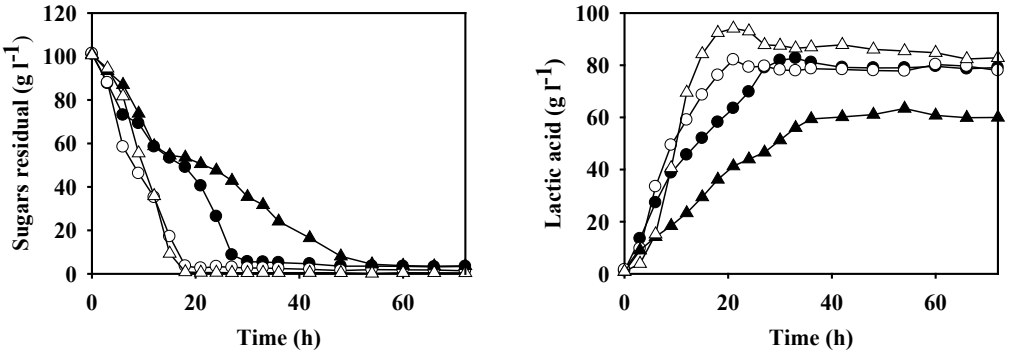

Figure 5 Effect of nutrient supplementations on cell growth and lactic acid production during undiluted OPT juice fermentation by *L. rhamnosus* TISTR 108 at a temperature of 40 °C, 10 % inoculum, an agitation speed of 200 rpm, pH 6.5 and supplemented with yeast extract + peptone (YE+P) (●), yeast extract + peptone + salts (YE+P+T) (o), salts (T) (▲) and control (Δ).

Conclusions

OPT juice from old OPTs contain a large amount of carbon and nutrient sources. These properties are suitable for being utilized as a lactic acid fermentation substrate by *L. rhamnosus* TISTR 108. Efficient lactic acid fermentation of undiluted OPT juice could be performed under the optimal conditions, with yeast extract, peptone and salts supplementation. In the optimized conditions obtained in this study, high lactic acid productivity could be achieved by complete fermentation and reduction of the fermentation time. This suggests that undiluted OPT juice could be potentially used for production of highly concentrated lactic acid on a large scale in the future.

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