Lactic acid production from date juice using *lactobacillus casei ATCC 393* in batch fermentation

Mujtahid Kaavessina[1,*], *Fitriani* Khanifatun[2], and *Saeed M.* Alzahrani[3]

[1]Chemical Engineering Department, Sebelas Maret University, 57126 Surakarta, Indonesia
[2]Educational Staff of Biology, Department of Education, 63202 Ngawi, Indonesia
[3]Chemical Engineering Department, King Saud University, 11421 Riyadh, Saudi Arabia

Abstract. Lactobacillus casei ATCC 393 was employed as a fermentative organism to convert sugars from date juice into lactic acid. Both glucose and fructose in date juice were fermented directly without any pre-treatment. The influences of supplementation of yeast extract and date juice concentration on some fermentation parameters, such as: cell growth rate, sugar conversion, productivity and yield, were investigated using this bacterium in batch fermentation. The results showed that by adding yeast extract about 20g/l in a date juice medium, the maximum specific growth rate of bacteria (μ_m) enhanced from 0.1229 to 0.1819 g/l. Meanwhile, increasing date juice concentration from 86.6942 to 158.9181 and 229.5367 g/l enhanced the μ_m from 0.1819 to 0.2107 and 0.1916 g/l, respectively. It indicated that the optimum value for μ_m is 0.2107 g/l in this concentration range. In the date juice concentration of 158.9181 g/l, the optimum lactic acid can be produced is 117.8301 g/l with yield of 92.685% for 48 h.

1 Introduction

Lactic acid, one of the most important organic acids, and its derivatives has been utilized in many applications such as in the food, textile, pharmaceutical, cosmetic and chemical industries [1]. Even, it became a prime candidate to be developed as a biodegradable polymer. Polymerization of lactic acid obtained poly (lactic acid) which has comparable mechanical properties, transparency, and UV light barrier to many conventional polymer (polystyrene, polyethylene, etc.) [2].

Recently, the global poly (lactic acid) market was expanding rapidly followed by increasing of lactic acid demand. Several factors stimulated this growth such as: sustainability of raw materials and government policy for bio-based and biodegradable product to tackle the waste problem. The global market of lactic acid is predicted to reach 1076.9 thousand tonnes in 2016 [3]. However, the global production of lactic acid is only 120 thousand tonnes in 2006 [4], thus the minimal production growth of lactic acid is 25% per years until 2016 to balance the gap between production and demand.

Lactic acid can be produced through chemical synthesis and microbial fermentation. The fermentation is an effective and attractive method due to produce lactic acid in high purity of one stereoisomer. The high purity of L(+) or D(-) lactic acid can be produced depending on a microbial strain and source of carbon (substrate) [4,5]. The economics of lactic acid fermentation is affected by many factors: raw material, purification, etc. The cost of the raw materials spends approximately 60-80% of the total production cost [5]. Thus, it is important to explore some potential of agriculture product to get cheap and abundantly existing material. It can be summarized that there are three big groups of substrate: sugar, starchy material and lignocellulose.

As well known, sugar was reported as the preferred carbon sources. However, it is very expensive to use as the feedstock for lactic acid fermentation. Date is one of the promising biomass for lactic acid production without complicated pretreatment. Date contains between 70-80 wt% of fermentable sugars, mainly glucose and fructose in a balance ratio which can be consumed directly by lactic acid bacteria [6] Besides that, as reported by Al-Hooti et al. [7] and Al-Farsi et al. [8], date contains some minerals and low range of vitamins. In Arabic countries, a lot of dates are being wasted due to overproduction and poor handling low quality dates. Thus, production of lactic acid from dates is very attractive.

Lactobacillus casei, a genus of facultative anaerobic bacteria, is one of the bacteria that able to convert some sugars to lactic acid. During its growth, it consumes sugars as energy sources and converts to lactic acid. In this work, *Lactobacillus casei* ATCC 393 was employed to produce lactic acid from date juice. We investigated the effect of yeast extract as nitrogen source and initial sugar concentration on lactic acid production in batch fermentation.

* Corresponding author: mkaavessina@staff.uns.ac.id

2 Experimental part

2.1 Materials

Some chemicals such as $MgSO_4$, $MnSO_4$, K_2HPO_4, KH_2PO_4 and $FeSO_4$ were kindly supplied by LOBA Chemie (India). Tween 80 and yeast extract as a fatty acid and a nitrogen source of bacteria, respectively, were purchased from Sigma-Aldrich. Further, all chemicals were used in this investigation without any purification.

2.2 Microorganism

Lactobacillus casei ATCC 393 is a homofermentative bacterium to produce lactic acid. This strain was kindly purchased from ATCC and stored as a freeze-dried inoculum. To revive it, 0.5 ml of a liquid medium was added to the culture with a sterile Pasteur pipette and mixed well. The mixture was then transferred to a test tube containing 5 ml of broth medium and incubated for 24 h on a shaking water bath maintained at 38°C. Further, this mixture was as a parent culture and subsequently kept it in the refrigerator at 10°C.

For inoculation purpose, 2 ml of culture was transferred to 100 ml broth (in 500 ml erlenmeyer) and incubated for 48 h at 37°C. The composition of inoculum broth is: yeast extract (20 g/l), peptone (5 g/l), dextrose (10 g/l), monopotassium phosphate (2 g/l) and polysorbate 80/tween 80 (0.1 g/l). Prior to usage, the pH of broth was adjusted at 6.5 [9].

2.3 Preparation of date juice as a substrate

Date juice was purchased from a local market in Riyadh, Saudi Arabia. The main composition of date juice concentrate is mainly fructose (1000g/l) and glucose (1000g/l). The date juice was then diluted with tap water to desired concentration of glucose and centrifuged at 6000 rpm for 3 mins to separate the cellulosic debris and fibres from the supernatant.

2.4 Fermentation

The batch fermentation was carried out in 100 ml working volume using 250 ml·erlenmeyer without pH control. In this investigation, the effect of nutrient was studied by carried out fermentation in two different media: salted date juice with and without supplementation of yeast extract. Investigation of initial sugar concentration effect was conducted using salted date juice with supplement at various concentration as tabulated in Table 1.

Table 1 List of the date juice samples prepared for this study

Sample name	Concentration of sugar, g/l	Yeast extract, g/l	Salt
DJ_a	86.6942	0	Added
DJ_b	86.6942	20	Added
DJ_c	158.9181	20	Added
DJ_d	229.5367	20	Added

The salt was containing 0.2 g/l $MgSO_4$, 0.03 g/l $MnSO_4$, 0.3 g/l K_2HPO_4, 0.3 g/l KH_2PO_4, 0.02 g/l $FeSO_4$, 1 ml/l Tween 80. Yeast extracts as a nitrogen source was supplemented about 20 g/l. Then, all media were sterilized at 121°C for 15 mins. The fermentation was done by adding culture of *lactobacillus casei* ATCC 393 with inoculum size of 10% (v/v of solution) into each media. For certain time, each sample was analyzed their dry cell weight (DCW) and composition of substrate and product. Table 1 shows the list of date juice samples prepared for this study.

2.5 Characterization

The bio-cell concentration was calculated by measuring the weight of dried cells in 1.5 ml of tested sample at certain time and then converted to the weight of dried cells in 1 liter. The concentrations of glucose, fructose and lactic acid were determined using an Agilent 1260 infinity HPLC, USA. The mixture of acetonitrile in water (3:1) was used as mobile phase during the analysis.

Cell growth of bacteria can be expressed in an equation as follows:

$$\frac{dX}{dt} = \mu X \qquad (1)$$

Where, μ is a specific growth rate of bacteria. The integration of Equation 2can be written:

$$\mu = \frac{\ln (X_t) - \ln (X_o)}{t - t_o} \qquad (2)$$

By analyzing in growth period (log phase), maximum specific growth rate (μ_m) can be obtained as a slope of plotting between ln(X) versus time. The μ_m is a constant value. The yield parameters in fermentation are expressed by $Y_{P/S}$, $Y_{P/X}$ and $Y_{X/S}$, which can be determined by those equations [9]:

$$Y_{p/s} = \frac{\text{Mass of lactic acid formed}}{\text{Mass of substrate consumed}} = \frac{\Delta P}{\Delta S} \qquad (3)$$

$$Y_{p/X} = \frac{\text{Mass of lactic acid formed}}{\text{Mass of new cells formed}} = \frac{\Delta P}{\Delta X} \qquad (4)$$

$$Y_{X/S} = \frac{\text{Mass of new cells formed}}{\text{Mass of substrate consumed}} = \frac{\Delta P}{\Delta S} \qquad (5)$$

3 Results and discussion

The date juice was utilized as a substrate with different supplement and concentration. The Lactobacillus casei ATCC 393 was employed as a fermentative organism for converting sugars to lactic acid. The bio-cells growth

and lactic acid production was presented and discussed here.

3.1 Bio-cells growth

Figure 1 shows the effect of yeast extract and initial sugar concentration of date juice on cell growth in batch fermentation. The growth of Lactobacillus casei showed similar behaviour that can be divided into two regions. First region (0-12 hrs) refers to the growth period and the other refers to the stationary period (12-48 hrs). The lag period was not monitored in this investigation. Probably due to the adaption time was very fast (less than 4 hrs).

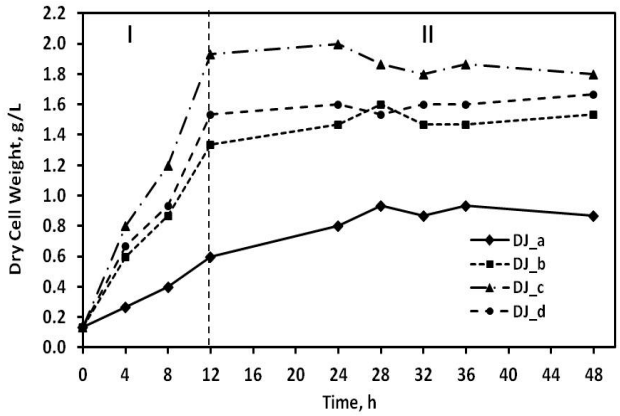

Fig. 1. Effect of yeast extract and initial sugar concentration of date juice on cell growth in batch fermentation.

The effect of yeast extract on bio-cells growth can be seen in comparison between samples of DJ_a and DJ_b (Fig. 1). The presence of yeast extract enhanced cell mass significant. For example at 24 hrs, the cell concentration is 0.87 g/l when grown in medium without supplementation of yeast extract while the cell growth becomes 1.47 g/l when the medium was added with yeast extract. This result was also in agreement with others [11, 12]. The slow cell growth on the date juice may be due to the deficiency of nitrogen in the medium. Some researchers investigated some nitrogen sources (yeast extract, peptone, urea, ammonium sulphate and corn step liquor) and concluded that yeast extract is the best as a nitrogen source than others [11, 13]. Beside as a nitrogen source, yeast extract has also rich in B vitamins.

The effect of sugar concentration in the date juice medium is monitored in samples of DJ_b, DJ_c and DJ_d. It is founf that the cell growth was increasing significantly. For example at 24 hrs, the cell concentration was enhancing from 1.47 g/l to 2.00 g/l when the sugar increased from 86.6942 g/l to 158.9181 g/l. However, the negative effect is monitored when the sugar concentration is at 229.5367 g/l at which the cell concentration tends to decrease to 1.6 g/l. It is possibly due to the high concentration of sugars will restrict the diffusion of sugar and nutrition into the cell. Thus, the cell growth was inhibited. It can be highlighted that the optimum initial sugar concentration is 158.9181 g/l and containing glucose and fructose in a balance ratio to grow the bio-cells.

3.2 Lactic acid production

Figure 2 shows that the lactic acid production is affected by adding yeast extract in the date juice medium. Due to the fermentation entangles the life cycle of *Lactobacillus casei*, the lactic acid production is in accordance with its growth. Figure 2(a) and 2(b) show that the supplemented date juice is more consumable with *L. casei* than that of date juice without yeast extract. Both glucose and fructose were simultaneously consumed. Fig. 2(a) shows that lactic acid production increase slightly, probably due to the bacteria growth was inhibited in date juice with lack of nitrogen. The lactic acid concentration and productivity after 48 hrs were only 17.2578 g/l and 0.3595 g/(l h), respectively. The addition of yeast extract can tackle the deficiency of nitrogen in a medium. Fig. 2(b) shows the lactic acid production can be enhanced significantly become 67.9121 g/l (48 hrs).

The influence of diluted and concentrated date juice on lactic acid fermentation was investigated. The various initial concentrations of sugar are 86.6942, 158.9181 and 229.5367 g/l. As shown in Fig. 3, the final lactic acid concentration improved and also the concentration of sugars was more consumable with an increase initial of sugar. Further, the quantification of date juice fermentation by *Lactobacillus casei* ATCC 393 is summarized in Table 2.

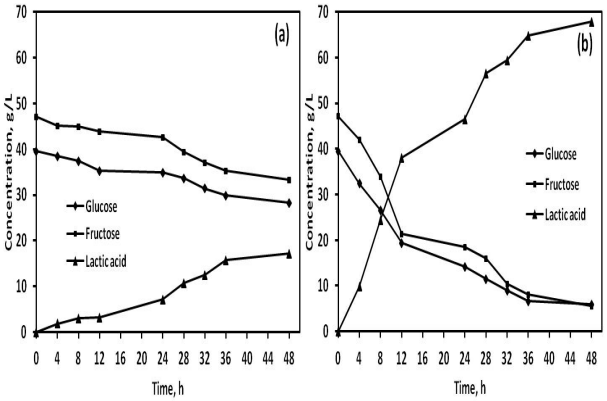

Fig. 2. Effect of yeast extract on lactic acid production in batch fermentation: (a) without yeast extract and (b) with yeast extract (20g/l)

The maximum specific growth rate (μ_m) and maximum quantity of *L. casei* is in DJ_c medium: 0.2107 1/h and 2 g/l, respectively. It could be explained that in this medium, the cells could be growth without any interferences. Nitrogen from yeast extract and some nutrient suited to their growth. Compared with DJ_b and DJ_d medium, the μ_m and maximum quantity of cells were lower than that of DJ_c, although the yeast extract and some nutrient are added in same quantity. It exhibited that the DJ_b medium is not matching with the carbon demand of cells due to the lower initial concentration of sugars. For DJ_d, the higher concentration sugars increased the viscosity of substrate, thus the diffusion of sugars into the cell was inhibited.

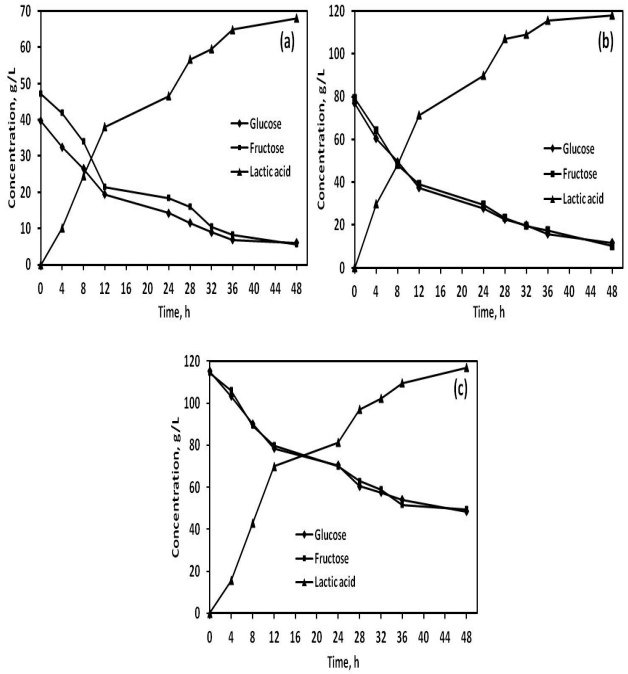

Fig. 3. Effect of initial concentrations of sugar on lactic acid production in batch fermentation: (a) 86.6942, (b) 158.9181 and (c) 229.5367 g/l

The highest total lactic acid production was obtained in the DJ_c medium, 117.8301 g/l for 48 h. Both glucose and fructose in the date juice medium were utilized about 85.0137 and 87.3404 %, respectively. Compared to the DJ_d medium, the total lactic acid produced is 116.8910 g/l and total sugars utilized were about 57 %.

Table 2 The fermentation parameters of lactic acid production from date juice using *Lactobacillus casei* ATCC 393

Parameter	Date juice			
	(DJ_a)	(DJ_b)	(DJ_c)	(DJ_d)
Maximum cells, g/l	0.930	1.600	2.000	1.670
Maximum specific growth rate (μ_m), 1/h	0.123	0.182	0.211	0.192
Initial total sugars [a], g/l	86.694	86.694	158.918	229.537
Residual total sugars (48 hrs), g/l	61.579	11.462	21.523	97.910
Total lactic acid (48 hrs), g/l	17.258	67.912	117.830	116.891
Percentage of utilized glucose (48 hrs), %	28.619	85.023	85.014	57.981
Percentage of utilized fructose (48 hrs), %	29.265	88.251	87.340	56.705
Yield , $Y_{P/S}$ [b]	0.724	0.906	0.927	0.903
pH range	6.5-6	6.5-5.5	6.5-5.3	6.5-5.6
Productivity (48 hrs), g/(l h)	0.359	1.415	2.455	2.435

[a]) Total sugars refer to the summation of glucose and fructose
[b]) $Y_{P/S}$ is the ratio between lactic acid formed and substrate consumed, g/g

Considering the maximum specific growth rate, the bio-cells in DJ_d was lower than that in DJ_c. And also as reported by Yu et al. [14] the presence of lactic acid and concentrated sugars in a medium was also inhibiting the cell growth. It could be predicted that it will take a long time to increasing the conversion of DJ_d till about 80%. Again, this result exhibited that the optimum concentration of date juice is 158.9181 g/l.

The yield of lactic acid was also increased from 72.38% to 90.55% when yeast extract was supplemented in a medium. It could be attributed to be more appropriate of nutritive value was found in supplemented date juice with yeast extract [15] Increasing the concentration of sugars was not significant affected in the yield. It was varying from 90.50% to 92.68 and 90.30% when initial concentrations of sugar were 86.6942, 158.9181 and 229.5367 g/l, respectively.

Table 3 Comparison of lactic acid fermentation in batch from various carbon sources and microorganisms.

C and N sources ($g\ l^{-1}$)	Titer ($g\ l^{-1}$)	Yield (%)	Productivity ($gl^{-1}h^{-1}$)	Strain	Ref.
Date juice w/o YE[a]	86.7	0.72	0.36 (48 h)	*Lactobacillus casei*	This study
Date juice (YE:20g /l)	86.7	0.91	1.42 (48 h)	*Lactobacillus casei*	This study
Date juice (YE:20g /l)	158.9	0.93	2.45 (48 h)	*Lactobacillus casei*	This study
Date juice (YE:20g /l)	229.5	0.9	2.43 (48 h)	*Lactobacillus casei*	This study
Glucose w/o YE	60	0.78	0.67 (48 h)	*Lactococcus lactis*	[19]
Glucose (YE:20g /l)	100	-	2.1 (48 h)	*Lactobacillus casei*	[1]
Wheat hydrolisate (CSL[b]:25g/l)	200	0.92	4.14 (24 h)	*Enterococcus faecalis*	[16]
Glucose w/o YE	112	0.94	5	*Rhyzopus orizae*	[17]
Sugarcane w/o YE	133	0.95	1.66 (72 h)	*Lactobacillus delbrueckii*	[15]
Date juice ($(NH_4)_2SO_4$: 10g/l)	80	-	0.38 (20 h)	*Lactobacillus rhamnosus*	[13]

[a] YE: Yeast Extract
[b] CSL: Corn Steep Liquor

Table 3 summarized the results of lactic acid fermentation conducted in this work and the other reports. As a result of these kinds of substrate, sugars from date juice supplemented by yeast extract can be utilized with higher productivity. Beside yeast extract, the deficiency of nitrogen in medium can be fulfilled with corn steep liquor, ammonium sulphate, urea, etc. [13, 16]. *Rhyzopus orizae*, one species of fungi, showed

its capability to produce lactic acid [17, 18]. From table 3, the capability of microorganisms is not same and they are not always having similar optimum conditions. These conditions are pH, nutrients, temperature, oxygen demand, etc. Therefore, study of the optimum condition during the lactic acid fermentation with different microorganisms is still a major challenge.

4 Conclusions

This investigation result showed that the date juice has a potential to be utilized as a substrate in lactic acid fermentation using *Lactobacillus casei* ATCC 393. Yeast extract is supplemented in a date juice medium to supply the nitrogen. The optimum production of lactic acid is about 117.8301 g/l for 48 hrs with initial sugar concentration of 158.9181 g/l. Moreover, the productivity and yield of lactic acid from date juice were not only high but also cost effective due to the date juice can be utilized directly.

This research was financially supported by Sebelas Maret University through research grant of "Hibah Research Mandatory (RM-UNS) PNBP 2016".

References

1. M. Hujanen, Y.Y. Linko. Appl. Microbiol Biotechnol., **45**, 307-313 (1996).
2. R. Auras, B. Harte, S. Selke, Macromol. Biosci. **4**, 835-864 (2004).
3. Anonym, 2011, Global lactic acid & poly lactic acid (PLA) market by raw materials, types, applications and potential opportunities (forecast to 2016), in http://www.marketsandmarkets.com/ Market-Reports/polylacticacid-387.html.
4. Datta, M. Henry, J. Chem. Technol. Biotechnol, **81**, 1119-1129 (2006).
5. A.K. Yadav, N.K. Bipinraj, A.B. Chaudhari, R.M. Kothari, Starch – Stärke, **63**, 632-636 (2011)
6. N. Nancib, A. Nancib, J. Boudrant (1997)., Bioresour. Technol., **60**, 67-71 (1997).
7. S.N. Al-Hooti, J.S. Sidhu, J.M. Al-Saqer, A. Al-Othman, Food Chem., **79**, 215-220 (2002).
8. M. Al-Farsi, C. Alasalvar, M. Al-Abid, K. Al-Shoaily, M. Al-Amry, F. Al-Rawahy, Food Chem., **104**, 943-947 (2007).
9. R.M. Atlas, *Handbook of Microbiological Media, 4 ed.*, ASM Press, CRC Press, Taylor and Francis Group, 2010.
10. H.S. Fogler, Elements of chemical reaction engineering, 4 ed., Prentice hall international series, Massachusetts, 2008.
11. M. Altaf, B. Naveena, G. Reddy, Food Technol. Biotech., **43**, 235-239 (2005).
12. D. Roy, J. Goulet, A. LeDuy, Appl. Microbiol. Biotechnol., **24**, 206-213 (1986).
13. N. Nancib, A. Nancib, A. Boudjelal, C. Benslimane, F. Blanchard, J. Boudrant, Bioresour. Technol., **78**, 149-153 (2001).
14. L. Yu, X. Pei, T. Lei, Y. Wang, Y. Feng, J. Biotechnol. **134**, 154-159 (2008).
15. B. Calabia, Y. Tokiwa, Biotechnol. Lett., **29**, 1329-1332 (2007).
16. H. Oh, Y.-J. Wee, J.-S. Yun, S. Ho Han, S. Jung, H.-W. Ryu. Bioresour. Technol. **96**, 1492-1498 (2005).
17. A. Tay, S.-T. Yang, Biotechnol. Bioeng, **80**, 1-12. (2002)
18. Z.Y. Zhang, B. Jin, J.M. Kelly, Biochem. Eng. J., **35**, 251-263, (2007)
19. L.S. Cock, A.R. Stouvenel, *Electron. J. Biotechnol.*, **9**, 40-45. (2006)