Advances in Bioresearch
Adv. Biores., Vol 9 (2) March 2018: 99-104
©2018 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.2.99104

Advances in Bioresearch

ORIGINAL ARTICLE

Lactic acid production from wasted dates in Saudi Arabia using Single Culture *Lactobacillus casei* ATCC 393 and *Lactobacillus acidophilus and mixed culture*

[1,2*]**Mahmoud H. Bushara,** [1]**Fahad Alkoaik,** [3]**Ahmed Abasaeed,** [1]**Ronnel Fulleros**
[1]Department of Agricultural Engineering, College of Food and Agriculture Sciences King Saud University, Riyadh, Kingdom of Saudi Arabia
[2]Department of Agricultural and Biological Engineering, Faculty of Engineering, University of Khartoum, Khartoum, Sudan
[3]Department of Chemical Engineering, College of Engineering, King Saud University, Riyadh, Kingdom of Saudi Arabia
*E-mail: mhmoud008@gmail.com/Tel. (mobile):+249911757437 (Corresponding author)

ABSTRACT
Production of palm dates in the Kingdom of Saudi Arabia (KSA) is estimated to be over 1 million metric tons annually, which in turn produce a 20% of dates residue annually. In accordance to that, this study aimed at making use of the wasted date to produce lactic acid through the fermentation process, using single culture Lactobacillus casei (ATCC393) and Lactobacillus acidophilus (CICC 6088) and mixed culture of both strains. To investigate the effect of single and mixed culture on the productivity of lactic acid, the fermentation was carried out by using date syrup of 41g/L of sugar (glucose and fructose) for 72 hr of fermentation at a 37° C, within a pH between 5 to 7, where 34, 36 and 33 g/l were produced at the highest concentrations of lactic acid at 60hr,with productivities of 0.56, 0.60 and 0.55 g/l.hr, respectively. It was found that the single culture CICC 6088 achieved the highest productivity (0.6 g/l.hr) of lactic acid comparing with ATCC 393 and Mixed culture.

Keywords; *Lactic acid production, wasted dates, Lactobacillus casei ATCC93, Lactobacillus acidophilus CICC 6088, Mixed culture.*

Received 21.09.2017 Revised 11.12.2017 Accepted 23.02.2018

How to cite this article:
Mahmoud H. Bushara, F Alkoaik, A Abasaeed, R Fulleros. Lactic acid production from wasted dates in Saudi Arabia using Single Culture *Lactobacillus casei* ATCC 393 and *Lactobacillus acidophilus and mixed culture*. Adv. Biores., Vol 9 [2] March 2018.99-104.

INTRODUCTION

The Kingdom of Saudi Arabia is one of the leading countries in dates' production worldwide. In various regions of the kingdom, about 28 million trees are planted that produce more than 450 kinds of dates [1, 2]. The Kingdom of Saudi Arabia is the world's third largest date's producer [3], with a cultivated area estimated as 156901ha, and a total production of over 1 million Metric Tons (MT) in 2013 [4]. Kaavessina, [5] reported that less than 60% of dates produced in the kingdom have been used, thus, leaving large quantities of unused dates behind. Thus, there is an urgent need to find suitable means and processes to add value to this wasted date. Date and their products have many uses, they are consumed, as additives and sweeteners in many industries. Their syrup, can be used as a sweetener for human consumption and microbial fermentation to ethanol, vinegar and single cell protein production [6]. Chemical and biological methods are used to open new opportunities for date producers. In Saudi Arabia, management of agricultural wastes, most of which comes from date's production is a major problem; this waste provides a renewable source for many applications. It has been suggested to use low quality date to produce ethanol [7]. Algeria and Tunisia produced ethanol, citric acid and lactic acid from date waste [8, 9]. Lactic acid has significant applications in the pharmaceutical, cosmetics and food industries [10]. In addition to its use in the synthesis of biodegradable polymers, lactic acid can be regarded as a feedstock for the green chemistry of the future [11]. The continuous demand for lactic acid has been increasingdue

to its increased applications in preparation of biodegradable polymers, medical sutures, and green solvents [12, 13]. The market capacity has reached around 800,000 tons in 2013, also the global polylactic acid capacity increased to around 320,000 tons [14]. Currently, research efforts are focused on discovering new and effective nutritional sources. New fermentation techniques provide a practical means of achieving both high substrate conversion and high productivity. Lactic acid production has been studied with various raw materials, such as barley, wheat and corn, and media plays a vital role in the improvement of such process [15]. Although several substrates were used for lactic acid production by bacterial fermentation, wasted dates are minimally explored. Due to limited studies on wasted date in Saudi Arabia (compared to the high international demand for lactic acid). This study aimed to produce lactic acid from wasted dates in Saudi Arabia using single culture *Lactobacillus casei* (ATCC393) and *Lactobacillus acidophilus* (CICC 6088) and mixed culture of both strains, and their effect in lactic acid productivity.

MATERIALS AND METHODS

Preparation of date syrup

The wasted dates were supplied from a date factory belongs to college of food and agriculture sciences, King Saud University Riyadh, Saudi Arabia. Dates were thoroughly cleaned manually to remove dust and foreign materials. Date fruit is composed of the fleshy part and the seed. The seeds were separated by manual splitting, and a mill was used for cutting dates to small parts. Two litters of distilled water were added to one kilogram of dates (V/W=2/1). The mixture was heated at 80^{o}C for 2 hr with continuous stirring. The date syrup was centrifuged at 3000 rpm for 15 min to separate the cellulosic debris. After filtration, the date juice was observed to have mainly fructose 59% and glucose 40% with accordance to HPLC analysis .Then the syrup was sterilized for 15 min at 121^{o} C, and kept in refrigerator at 5^{o} C.

MRS medium and Inoculum preparation microorganism

The composition of the MRS (Man, Rogosa and Sharpe) medium used for each bacteria strainas shown in Table 1. Sterilization of the medium components was performed by autoclaving at 121°C for 15 minutes.Numerous microorganisms either bacteria or fungi are capable of producing a large amount of lactic acid from carbohydrates and sugar. *Lactobacillus casei* (ATCC 393) and *Lactobacillus Acidophilus* (CICC 6088) are homofermentative bacteria strains that produce lactic acid. The pre-mentionedbacteria strains were purchased fromtwo locations, ATCC 393 from (ATCC Company, USA)and CICC 6088 from (CICC Company, China). To activate the bacteria, the entire pellet was dissolved with 1ml of MRS media which was then divided equally into two tubes. Each of the divided mixture was then added to two tubes already containing 4.5ml of MRS. Finally, each tube contains 5ml of the mixture. Both tubes were incubated and shacked for 24 hours at 37^{o}C and 200rpm. For inoculum preparation, five tubes of MRS media were prepared and inoculated with 1ml of the prepared mixture. The tubes were incubated and shacked for 24 hours at 37^{o}C and 200rpm. The stock (parent culture) was kept in the refrigerator at 8^{o}C.

Table1; MRS media composition for each strain (ATCC and CICC companies)

MRS for ATCC 393	MRS for CICC 6088
10 g/l Peptone	10 g/l Peptone
10 g/l Beef extract	10 g/l Beef extract
5 g/l Yeast extract	5 g/l Yeast extract
5 g/l Sodium Acetate	5 g/l Sodium Acetate
2 g/l Na_2HPO_4	2 g/l K_2HPO_4
0.1 g/l $MgSO_4.7H_2O$	2 g/l $MgSO_4.7H_2O$
0.05 g/l $MnSO_4.H_2O$	0.05 g/l $MnSO_4.H_2O$
1 g/l Tween-80	1 g/l Tween-80
20 g/l Dextrose	20 /l Dextrose

Production medium and Experiment set-up

A date syrup of 41g/l of sugar (glucose and fructose) was used as the substrate. The medium was sterilized at 121 °C for 15 min, and supplemented with salt solution (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 FeSO4 and 1 ml/l tween 80), yeast extract (as a nitrogen source to supplement about 20 g/l).All Lactic acid fermentations were performed as batch culture. The fermentation experiments were performed in 250 ml Erlenmeyer flasks with 100 ml working volume. Then, flasks containing the production medium of sugar concentration was inoculated with a portion of the microorganism culture of (*Lactobacullus casei* (ATCC 393), *Lactobacullus acidophilus* (CICC 6088) and

mixed culture of both. The mixed culture was obtained as a 50% of (ATCC 393) and 50% of (CICC 6088). A 20% inoculum grown in the MRS medium of *lactobacillus* (ATCC 393, CICC 6088 and mixed culture) was used in all fermentation processes. The fermentation was conducted between pH 5 to 7 by using pH meter. The pH was maintained manually by adding 5 N NH_4OH solution each 6hours. The production of lactic acid was carried out at a fixed temperature of shaker at 37°C. The agitation rate was 200 rpm, for 60 hr. Three replicates of each experiment were done.

Bio-cell growth and Lactic acid and sugar concentration measurement

Using a spectrophotometer (UV-1201, Japan) at a wavelength of 620nm to determine cell growth. One millilitre of culture was centrifuged at 7000 rpm for 5 min using (Centrifuge 5415 D, USA). The product of fermentation was a mixture containing Lactic acid, unconverted substrate, broth and salts. Lactic acid and sugar concentration were determined using a High-Performance Liquid Chromatography system (HPLC Agilent model 1260 infinity, USA, equipped with RI, UV detector at 210nm), in which an injection volume of sample was set at 5 µl. Analytical guard column (4 x 80 mm), and the column temperature that adjusted at 40^{o}C was diluted with 1mM H_2SO_4 as a mobile phase at a flow rate of 0.8 ml/min.

RESULTS AND DISCUSSION

Bio-cells growth

The cultivation of *Lactobacillus. casei* (ATCC 393), *Lactobacillus. acidophilus* (CICC 6088) and Mixed culture (L. ATCC 393 L. CICC 6088) were performed to investigate the kinetics of cell growth in fermentation process as it is shown in Figure 1. The cells grew exponentially until it reached its maximum growth,the maximum density was achieved after 24 hrfor the three cultures, and therewas no significant difference between them in the growth.

Sugar Utilization and Lactic acid production

To study the feasibility of using wasted dates as carbon source for lactic acid production, the main sugar in the date syrups were glucose and fructose according to the HPLC analysis. Figures 2 and 3 show the utilization of glucose and fructose, respectively for the three bacteria strains. The case of glucose in Figure 2 displays the utilization of glucose from 16 g/l of initial concentration by the single culture ATCC 393 and CICC 6088 and the mixed culture at 24 hr. Also, fructose (of 25 g/l initial concentration) was 100% utilized by, ATCC 393, CICC 6088 and mixed culture at 60, 66 and 66 hr, respectively as shown in Figure 3. It is obvious that in sugar utilization, the glucose was consumed easily and quickly by the three bacteria strains comparing with fructose. This reveals that our all selected bacteria strains (ATCC 393, CICC 6088 and mixed culture)prefer glucose than fructose within our specific conditions that prepared for fermentation processes.

Figure 4 shows the variation of lactic acid production with time during the fermentation process. The highest concentration of lactic acid was observed to be 36 g/l,obtained by CICC 6088. While ATCC 393 and mixed culture obtained 34 and 33 g/l of lactic acid concentrations,respectively.From Figure 4, it was noted that after 60 hr the concentration of lactic acid production of the three strains started declining, due to absence of sugar.Productivity (expressed as product concentration divided by fermentation time), is shown in Table 2. These results showed that the mixed culture system was ineffective comparedto single culture regarding the productionof lactic acid. The results in this study agrees with other reports, such asSerna et al, [16] who reported that the Highproduction of lactic acid was obtained at 14 and 32 g/l during 48 hr, using a strain of *Lactococcuslactis subs lactis* and glucose concentrations of 20 and 60 g/l, respectively. Also, Jacob et al., [17] Used mixed culture from low value dates syrup at initial total sugar concentration of 56.8 g/l and achieved lactic acid of 49 g/l.

Table2; Production and productivity of lactic acid by single culture ATCC 393 and CICC 6088 and Mixed culture at 60 hr.

Kinetic Parameters	*ATCC 393*	*CICC 6088*	Mixed Culture
Maximal Lactic acid concentration, (g/l)	34	36	33
Maximal Lactic acid productivity, (g/l.hr)	0.56	0.60	0.55

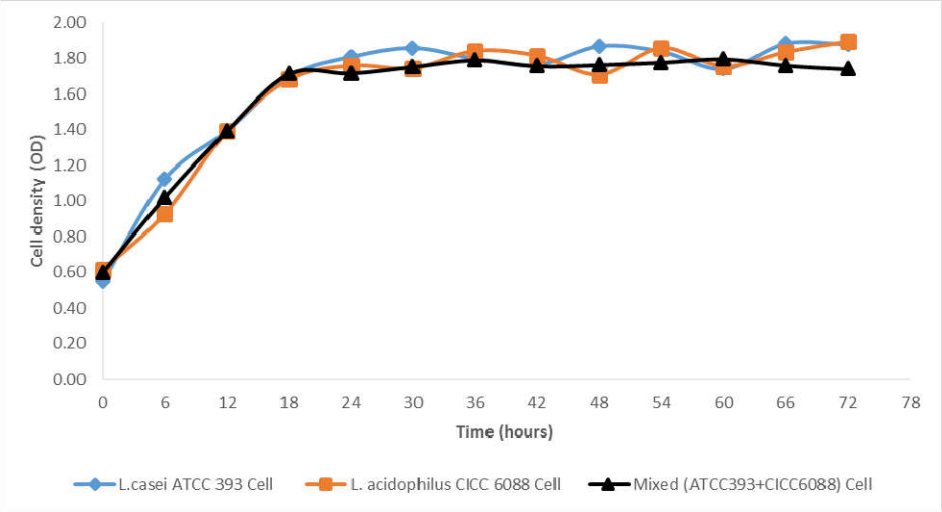

Figure1. Cells growth during fermentation.

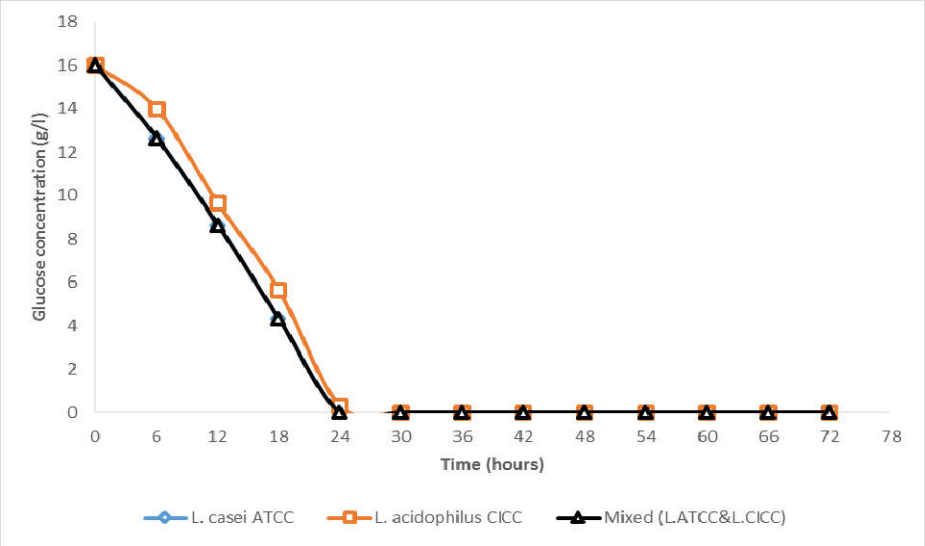

Figure. 2. Variation of glucose utilization with time.

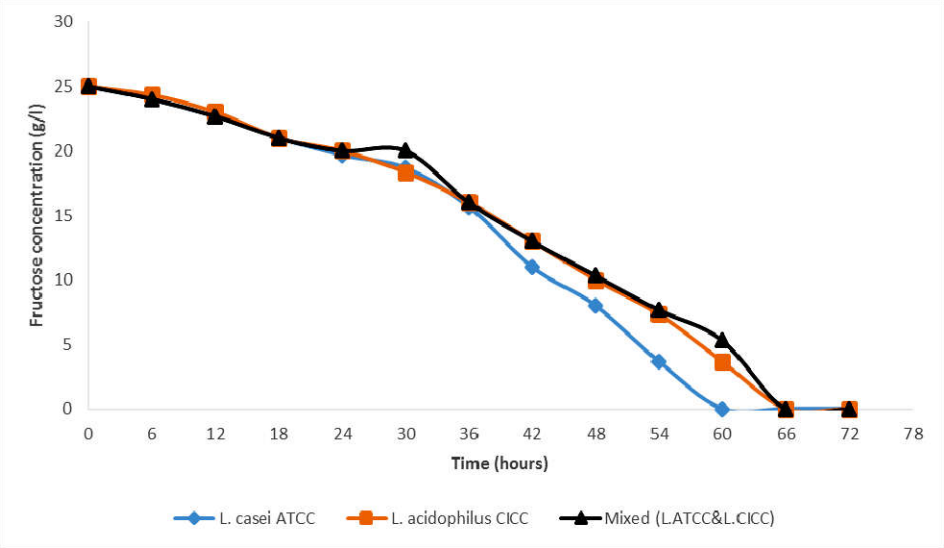

Figure 3. Variation of fructose utilization with time.

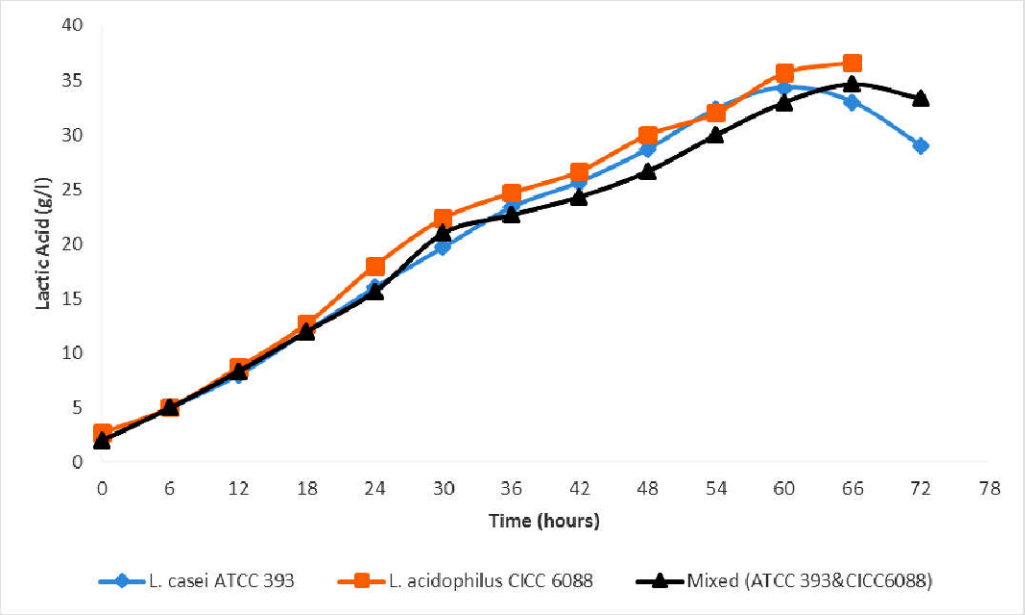

Figure 4. Concentration of lactic acid production for three bacteria with time during fermentation

CONCLUSION

In this study,lactic acid was produce from wasted dates using single culture *Lactobacillus casei* (ATCC 393) and *Lactobacillus acidophilus (CICC 6088) and* mixed culture.The investigations achieved 34, 36 and 33 g/l of lactic acid, respectively from 41 g/l of sugar (date juice). The study findings demonstrated that wasted dates are possible to substitute most of the new dates without a decrease in lactic acid production. Also from this study, it appears that the valuation of date's syrup by biological means (fermentation) to produce lactic acid has certainly huge advantages.

ACKNOWLEDGMENTS

This research was financial supported by Research Center, College of Food and Agriculture Sciences at King Saud University.

REFERENCES

1. Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., & Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. Food Chemistry, 104(3), 943-947.
2. Al-Fredan, M. A. (2013). Peroxidase activity in male and female plants of date palm (Phoenix dactylifera L.) growing in Al-Hassa, Saudi Arabia in vitro. El-MiniaSci Bull, 24(1), 37-55.
3. FAO (2013). "FAO Statistical Yearbook, Agricultural Production Statistics Division." 26.
4. FAO (2014). "FAO Agriculture statistical Year book." 27.
5. Kaavessina, M. (2013). Synthesis and characterization of biodegrable poly (lactic acid) and its engineering application Ph.d dessertation, King Saud University.
6. Barreveld, W. H. (1993). Date palm products. Agricultural services bulletin no. 101. FAO, Rome.
7. Zohri, A. N. A., and Mostafa, M. (2000). Ethanol production from dates in Saudi Arabia on industrial scale. Mycobiology, 28(2): 76-81.
8. Acourene, S, and A. Ammouche (2012). Optimization of ethanol, citric acid, and α-amylase production from date wastes by strains of *Saccharomyces cerevisiae, Aspergillus niger*, and *Candida guilliermondii*. Journal of industrial microbiology & biotechnology 39(5):759-766.
9. Nancib, A., Nancib, N., Boubendir, A., and Boudrant, J. (2015). The use of date waste for lactic acid production by a fed-batch culture using Lactobacillus casei subsp. rhamnosus. Brazilian Journal of Microbiology, 46(3): 893-902.
10. Göksungur, Y., and Güvenç, U. (1997). Batch and continuous production of lactic acid from beet molasses by Lactobacillus delbrueckii IFO 3202. Journal of chemical technology and biotechnology, 69(4): 399-404.
11. Gao, C., Ma, C., and Xu, P. (2011). Biotechnological routes based on lactic acid production from biomass. Biotechnology advances, 29(6): 930-939.
12. Datta, R., S.-P. Tsai, Patrick Bonsignore, Seung-Hyeon Moon and James R. Frank (1995). "Technological and economic potential of poly (lactic acid) and lactic acid derivatives." FEMS Microbiology Reviews, 16(2): 221-231.
13. Payot, T., Chemaly, Z., and Fick, M. (1999). Lactic acid production by Bacillus coagulans—kinetic studies and optimization of culture medium for batch and continuous fermentations. Enzyme and Microbial Technology, 24(3): 191-199.

14. Rmir,(2014). Research and Markets Industry Report, (2014). Global and China Lactic Acid and Derivative Industry Report, 2014-2016
15. Oh, H., Wee, Y. J., Yun, J. S., Han, S. H., Jung, S., and Ryu, H. W. (2005). Lactic acid production from agricultural resources as cheap raw materials. Bioresource technology, 96(13): 1492-1498.
16. Serna Cock, L., and Rodríguez de Stouvenel, A. (2006). Lactic acid production by a strain of *Lactococcus lactis* subs lactis isolated from sugar cane plants. Electronic Journal of Biotechnology, 9(1): 0-0.
17. Jacob Varghese, B., K. Bui, Naomi Divall, Alexander Egan, Ali Kermani, Adam Lodge and Daniela Vollmer. (2012). "Lactic acid Production by a Mixed Culture of Lactic bacteria based on low value dates syrup and their metabolic Uses." J Metabolic Synd 1(116): 2167-0943.1000116.