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# Fermenting Saudi Wasted Dates by Using *Lactobacillus casei* (ATCC 393), *Acidophilus* (CICC 6088) and the Mixed-Culture Bacteria to Produce Lactic Acid

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## Abstract

The Kingdom of Saudi Arabia produces over one million metric tons annually, which returns a 20% of wasted dates annually. Lactic acid and its derivatives are widely used in food, pharmaceutical and textile industries. There has been an increase in lactic acid production because it is used as a raw material to produce polylactic acid, a polymer that is used as a special medical and environmental friendly biodegradable plastic. This study aimed to use wasted dates to produce lactic acid by single culture *Lactobacillus casei* (ATCC 393), *Lactobacillus acidophilus* (CICC 6088) and the mixed culture using batch fermentation. The investigation results showed that the maximum concentration of lactic acid for ATCC 393, CICC 6088 and the mixed culture are 87, 84 and 84 g/l respectively. For single CICC 6088 and the mixed culture, the total percentage of glucose and fructose utilized was found to be 100%; 76%, respectively, whereas in the case of the single culture ATCC 393, the total percentage of glucose and fructose were 100% and 72%, respectively. With regard to lactic acid concentration, and sugar consumption, the results revealed that the single culture ATCC 393 produced the optimum lactic acid of 87 g/l for 48 hr with initial sugar concentration of 90 g/l.

## Keywords

Lactic Acid, Culture, Lactobacillus, Wasted Dates and Fermentation

## 1. Introduction

The annual production of palm dates in the Kingdom of Saudi Arabia (KSA) is estimated to be over 1 million metric tons in 2013 [1], which in turn produces a 20% of wasted dates annually. Wasted dates are very rich in nutriments, sugars such as: glucose, fructose and sucrose, vitamins and minerals although, they are less appreciated, less commercialized and less competitive [2] [3]. Adding also to that, the dates have already been the subjects of many tests in other countries. They are used as fermentation substrate for several metabolites production such as citric acid, ethanol and even biomass production [4] [5]. Lactic acid or its derivatives are indeed widely used by pharmaceutical industry, cosmetics and food processing [6]. 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 [7]. At present, approximately 90% of the world production of lactic acid is made by bacterial fermentation and the rest is produced synthetically by the hydrolysis of lactonitrile. However, lactic acid has two optical isomers: L-lactic acid and D-lactic acid, and the physical property and biodegradability of PLA are highly dependent on the optical purity of L-isomer [8]. Produced of Lactic acid from Lactic acid bacteria (LAB) have received a wide interest because of their high growth rate and product yield. However, LAB has complex nutrient requirements because of their limited ability to synthesize B-vitamins and amino acids [9]. Although LAB have been used for lactic acid production on an industrial scale, their inability to ferment a wide range of sugars and requirements for complex media have disserved the use of PLA (Poly Lactic Acid) as a renewable plastic. This becomes more critical as we attempt to replace petroleum-based plastics with renewable PLA-based plastics, which would require production of the base chemical in large quantities as the feedstock for fermentation. Biocatalysts are currently being developed to reduce the production cost of optically pure lactic acid isomers that include *Lactobacillus* [10]. Presently, 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. Although several substrates were used for production of lactic acid by bacterial fermentation, wasted dates were minimally explored. Due to little researches on Saudi wasted dates, this study aimed to use *Lactobacillus casei* (ATCC 393), and *Lactobacillus acidophilus* (CICC 6088) and mixed culture from both strains to produce lactic acid from wasted dates.

## 2. Materials and Methods

### 2.1. Microorganism

The strains of *Lactobacillus casei* (ATCC 393) and *Lactobacillus acidophilus* (CICC 6088) are homofermentative bacteria that produce lactic acid. These strains were kindly purchased from (ATCC company, USA) and (CICC company, China) and stored as freeze-dried substances. The ATCC 393 and CICC 6088 were prepared from ATCC medium and CICC mediumas shown in **Table 1**. The inoculum was obtained from cultures which incubated for 24 hr on a shaking water bath maintained at 37˚C.

**Table 1.** Medium composition for each strain (ATCC and CICC).

| Medium for ATCC 393 | Medium 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 $MgSO_{4.7}{\cdot}H_2O$ | 2 g/l $MgSO_{4.7}{\cdot}H_2O$ |
| 0.05 g/l $MnSO_4{\cdot}H_2O$ | 0.05 g/l $MnSO_4{\cdot}H_2O$ |
| 1 g/l Tween-80 | 1 g/l Tween-80 |
| 20 g/l Dextrose | 20 g/l Dextrose |

## 2.2. Inoculum Preparation

The inoculum was prepared by transferring 5 ml of culture to a 250 ml Erlenmeyer flask containing 100 ml of liquid (ATCC and CICC) Medium for pre-culture. The flask was subsequently incubated at 37˚C and 200 rpm for 24 hr.

## 2.3. Dates Juice Extraction

The substrate used was obtained from wasted dates. The wasted dates or sorting date discards are a part of the palm tree fruit, which were assessed invalid for human consumption because it has been stored for solong time. The wasted dates were supplied from a date factory belongs to the college of food and agriculture sciences at King Saud University, Riyadh. The dates were thoroughly cleaned manually from dust and other substances. Date fruit is composed of the fleshy part and the seed. The seeds were separated by manual splitting, then two liters of distilled water were added to one kilogram of dates (V/W = 2/1). Then, the mixture was heated at 80˚C for 2 hr with continuous stirring. The resultant dates syrup was centrifuged at 3000 rpm for 15 min to separate the cellulosic debris. After filtration, the dates juice was observed to have mainly 60% of fructose and 40% of glucose, with accordance to High-Performance Liquid Chromatography (HPLC) analysis. With temperature adjusted at 40˚C was diluted with 1 ml $H_2SO_4$ as a mobile phase at a flow rate of 0.8 ml/min.

## 2.4. Production Medium

The medium consisted of dates syrup as a substrate of 90 g/l of total sugar (36 g/l of glucose and 54 g/l of fructose) used as the substrate. The medium was sterilized at 121˚C for 15 minutes. After cooling, the medium was supplemented with salt solution containing (0.2 g/l of $MgSO_4$, 0.03 g/l of $MnSO_4$, 0.3 g/l of $K_2HPO_4$, 0.3 g/l of $KH_2PO_4$, 0.02 g/l of $FeSO_4$ and 1 ml/l tween 80), yeast extracted as a nitrogen source to supplement about 20 g/l.

## 2.5. Experiment Setup

The batch fermentation cultivations were performed in 250 ml-Erlenmeyer flasks with a working volume of 100 ml. The pH value of the cultures was maintained manually between pH 5 to 7 by adding a 5 N of $NH_4OH$ solution each 6 hours, and a fixed temperature of shaker was maintained at 37˚C with an agitation speed of 200 rpm. Flasks containing the production medium of sugar concentration were 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). Aninoculum (with a percent of 20%) grown in the medium of lactobacillus (ATCC 393, CICC 6088 and mixed culture) was used in all fermentation processes.

### 2.6. Analytical Methods

The cells concentration was estimated by measuring the optical density at a wavelength of 620 nm. Lactic acid, glucose and fructose concentrations were determined by High-Performance Liquid Chromatography system (HPLC Agilent model 1260 infinity, USA, equipped with RI, UV detector at 210 nm), in which an injection volume of sample was set at 5 μl. Analytical guard column (4 × 80 mm) with temperature adjusted at 40˚C was diluted with 1 ml $H_2SO_4$ as a mobile phase at a flow rate of 0.8 ml/min.

## 3. Results and Discussion

### 3.1. Bio-Cells Growth

The cells growth during fermentation for the strains ATCC 393, CICC 6088 and the mixed culture are shows in Figure 1. The cells reached a stationary growth after 24 hours for the three cultures. Hence, there were insignificant differences between them in the growth.

### 3.2. Sugar Utilization and Lactic Acid Production

In this part, a comparison was conducted between sugar utilization and lactic

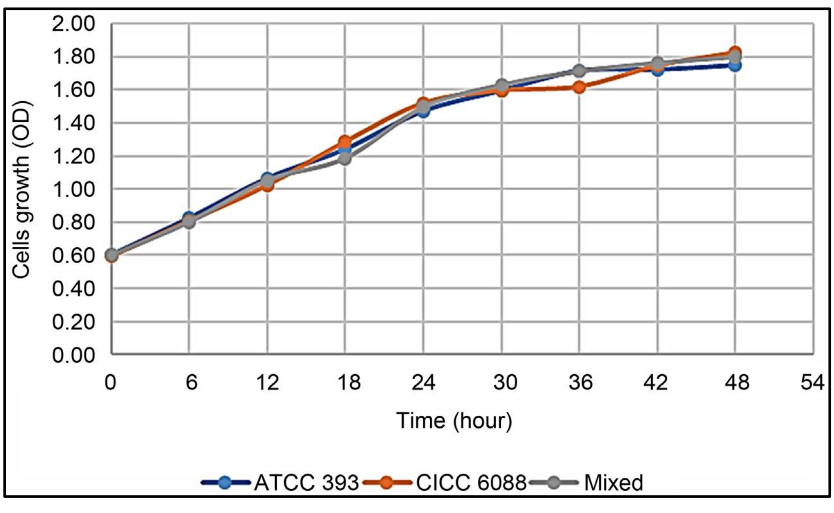


Figure 1. Cells growth during fermentation.

acid production with single and mixed culture during fermentation. The utilization of glucose and fructose from dates juice has been achieved by a single ATCC 393, CICC 6088 and a mixed culture of these strains. The results are shown in Figure 2 and Figure 3, supported with Table 2. According to the acquired results, ATCC 393, CICC 6088 and the mixed culture were observed to be unequal in their capacity to use glucose and fructose. Where, glucoseutilization has started from 36 g/l of initial concentration by the single culture ATCC 393 and CICC 6088 and the mixed culture, and consumed all glucose at 30 hr. As for fructose, the residual concentration decreased with time during the fermentation. But, at 48 hrs, it was observed that the highest concentration of residual fructose was obtained in ATCC 393 fermentation with as a 15 g/l, while the lowest concentration was found in CICC 6088 and mixed culture fermentations which were 13 g/l. The total amounts of fructose utilized were 72%, 76% and

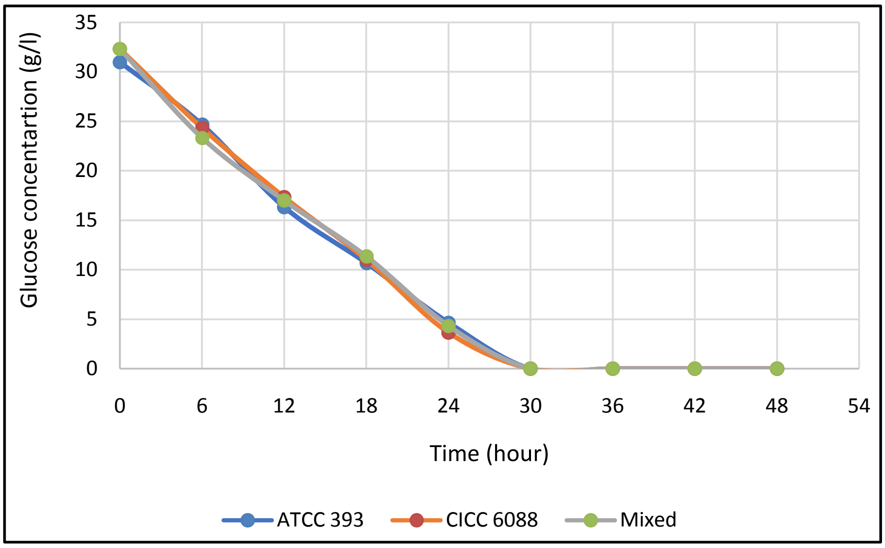


Figure 2. Variation of glucose utilization with time.

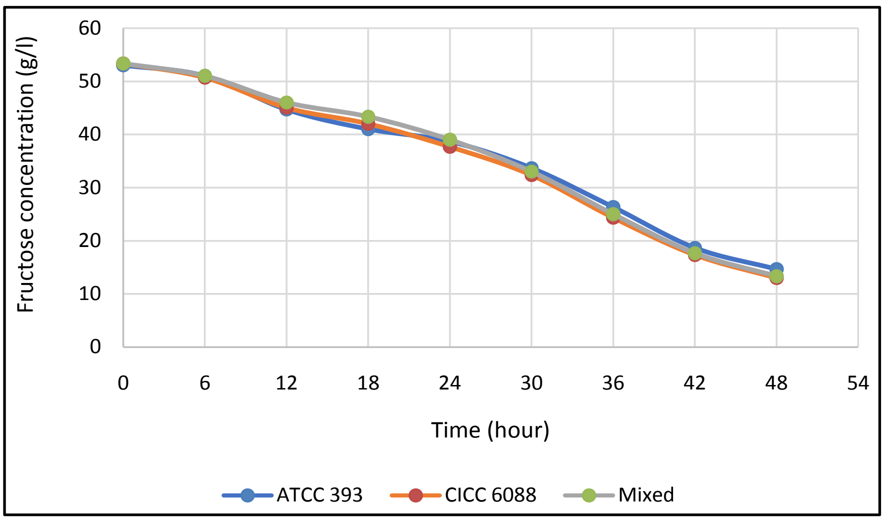


Figure 3. Variation of fructose utilization with time.

76% in fermentation with ATCC 393, CICC 6088 and the mixed culture, respectively. It was noted that in sugar utilization, the glucose was consumed early by the three cultures when comparing with fructose. This reveals that the selected bacteria strains (ATCC 393, CICC 6088 and mixed culture) prefer glucose than fructose based on our specific conditions that prepared for fermentation processes.

Production of Lactic acid (as shown in **Figure 4**) has increased with fermentation time. It is worth to mention that, the highest concentration of lactic acid which was 87 g/l has been obtained with ATCC 393 at 48 hr, while in case of CICC 6088 and the mixed culture; they only produced 84 g/l as their highest concentration of lactic acid. These results assured that the single culture ATCC 393, CICC 6088 and the mixed culture were more effective compared to Kaavessina *et al.* [11] in which only 67.9 g/l of lactic acid was obtained from 86.69 g/l of date juice. Also, Choi *et al.*, [12] reported 87.9 g/l achieved from date juice at 74.4 g/l of glucose and 82.3 g/l of fructose concentrations. On the other hand, Nancib *et al.* [6] obtained lactic acid of 60.3 g/l from dates syrup at 50 g/l of glucose and 40 g/l of fructose concentrations. Thus, this study was considered have good results relative to the studies which were devoted to investigate the production of lactic acid with regard to sugar utilization and lactic acid production.

**Table 2.** Kinetic parameters of single culture ATCC 393 and CICC 6088 and the mixed culture of fermentation from dates juice.

| Kinetic Parameters (48 hr) | ATCC 393 | CICC 6088 | Mixed Culture |
|---|---|---|---|
| Maximal Lactic acid concentration, (g/l) | 87 | 84 | 84 |
| Maximal Lactic acid productivity, (g/l·hr) | 1.81 | 1.75 | 1.75 |
| Maximum Cells growth (OD) | 1.75 | 1.83 | 1.8 |
| Percent glucose utilized (%) | 100 | 100 | 100 |
| Percent fructose utilized (%) | 72 | 76 | 76 |

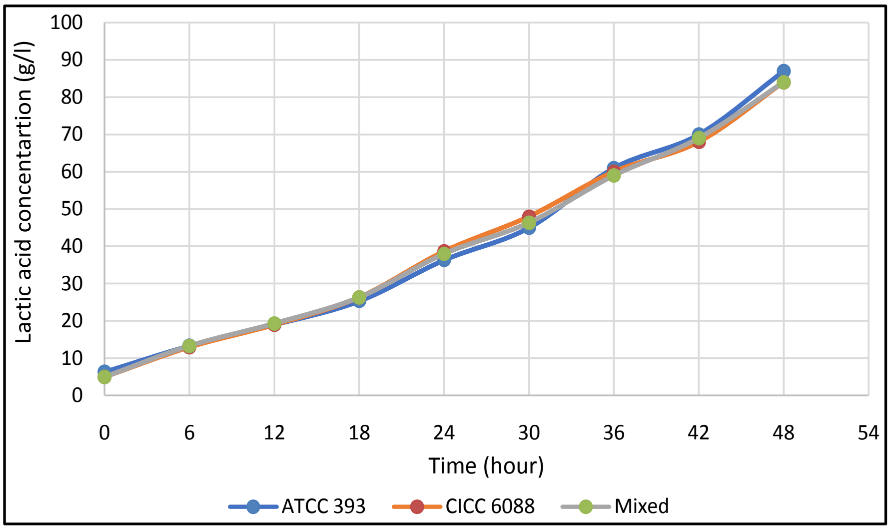


**Figure 4.** Concentration of lactic acid production with time during fermentation.

## 4. Conclusion

The study was intended to use wasted dates to produce lactic acid by single culture *Lactobacillus casei* (ATCC 393), *Lactobacillus acidophilus* (CICC 6088) and the mixed culture through batch fermentation. The investigation results showed that the highest concentration of lactic acid was 87 g/l archived by ATCC 393, While CICC 6088 and the mixed culture produced 84 g/l as their highest concentrations. The summarized conclusion of the study is that the single culture ATCC 393 has achieved the optimum lactic acid concentration (which was 87 g/l) for 48 hrs with initial sugar concentration of 90 g/l with regards to lactic acid concentration and sugar consumption. Hence, upon the study findings, it is worthy to note that wasted dates could be an attractive substrate for the lactic acid production by fermentation.

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