Chapter 2

Lactic acid production using date juice
2.1 Introduction:

Lactic acid is a major commodity chemical for the food and chemical industry, mainly produced by various homo fermentative lactic acid bacteria. Traditional production of lactic acid and downstream processing has been shown to reach as much as 70% of its total manufacturing cost (Stanbury et al, 1995).

The emergence of new market for lactic acid application, like biodegradable thermoplastics has intensified the search for the cost effective production systems. A tremendous reduction in lactic acid production cost is therefore a prerequisite to enable lactic acid derived lactide (polymeric lactic acid) to penetrate the plastic industry. Low cost renewable materials like whey, molasses, starch, hemicellulose, lignocellulose and cellulosic feed stocks have gained major interest as a sugar source for production media in lactic acid fermentation (Hofvendahl and Hagerdal, 2000). All the above reported substrates require pretreatment either to remove heavy metals and other impurities or to release sugars that can be utilized by the microorganisms (Sreenath et al, 2001a; Sreenath et al, 2001b; Roukas, 1998; Patel et al, 2004; Rivas et al, 2004; Wee et al, 2004).

Commercial use of agricultural residues and other biomass resources have been proposed through the novel concept of a “Green Biorefinery”. A green biorefinery envisages the biotechnological, chemical and physical fractionation and application of biomass residues to produce valuable and commercially marketable products and services; namely organic acids, proteins, enzymes, biodegradable plastics, alcohol, fertilizers and fuels (Danner et al, 2000).

In simultaneous saccharification and fermentation (SSF) studies using agricultural resources, it is necessary to hydrolyze them into fermentable sugars through enzymatic treatment. However, this might result in the extra cost for lactic acid production. Akerberg and Zacchi (2000) observed that the major contributor to the operational cost in the hydrolysis step was the cost of the enzyme mixture.
2.2 Palm date:

Dates are among the sweetest of fruits: Up to 70 % of their weight may be sugar. They provide an important source of carbohydrates. In the dry, desert like regions dates have been cultivated for more than 4,000 years. Crowning the tops of towering palm trees, dates grow in heavy clusters of oblong brown fruits as many as 200 in a cluster that weighs up to 25 pounds. The fruit is oblong, 1 to 3 inch (2.5-7.5 cm) long, dark-brown, reddish or yellowish-brown when ripe with thin or thick skin, thick sweet flesh (astringent until fully ripe) and a single, cylindrical, slender and very hard seed grooved on one side (Morton, 1987).

Dates develop through basically 4 stages named by their Arabic denominations; kimri, khalaal, rutab and tamr (Fig. 2.1). Hababauk is the term used for the female flower and is the period just after pollination when the young fruit is still creamy white before gradually turning green at the kimri stage. At the kimri stage there is a rapid increase in size, weight, and reducing sugars; it is the period of highest acidity and moisture content (up to 85 %). All factors level off at the end of this stage when the fruit starts to turn yellow (or red according to variety). At this point the date seed could already germinate and the fruit is physiologically mature. At the khalaal stage (Fig. 2.2 A) weight gain is slow but sucrose content increases, moisture content goes down, and tannins will start to precipitate and lose their astringency. With (normally) the tips of the fruit starting to turn brown, the rutab stage (Fig. 2.2 B) sets in which is characterized by a decrease in weight due to moisture loss, a partial (the degree depending on the variety) inversion of sucrose into invert sugar and a browning of the skin and softening of the tissues. The moisture content goes down to about 35 % and the dates at this stage are sold as fresh fruit. Only when the dates are left to ripen further on the palm tree they turn into tamr (Fig. 2.2 C), characterized by moisture content at which the date is self-preserving. The upper limit of moisture for the date to be self-preserving lies at around 24-25 % (Barreveld, 1993).

2.2.1 Composition and quality:

Dates are grouped into three varieties: soft, semi-soft and dry or bread dates. The soft types have a soft flesh, high moisture and relatively low sugar content and are the most perishable. Semi-soft dates have firm flesh, low moisture and high sugar
Lactic acid production using date extract

Hababauk
(1 week
creamy white)

Kimri
(5 weeks)

Kimri
(9 weeks,
green

Kimri,
(17 weeks
turning yellow to

Khalal,
(19-25 weeks,
yellow to red)

Rutab,
(26-28 weeks,
turning brown)

Tamr,
(29 weeks)

Figure: 2.1 Formation and ripening of dates

Figure: 2.2 Three major stages of maturity (A) Khalal, (B) Rutab and (C) Tamr
<table>
<thead>
<tr>
<th></th>
<th>Fresh</th>
<th>Dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>142</td>
<td>274-293</td>
</tr>
<tr>
<td>Moisture</td>
<td>31.9-78.5</td>
<td>7.0-26.1</td>
</tr>
<tr>
<td>Protein</td>
<td>0.9-2.6</td>
<td>1.7-3.9</td>
</tr>
<tr>
<td>Fat</td>
<td>0.6-1.5</td>
<td>0.1-1.2</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>36.6</td>
<td>72.9-77.6</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.6-4.5</td>
<td>2.0-8.5</td>
</tr>
<tr>
<td>Ash</td>
<td>0.5-2.8</td>
<td>0.5-2.7</td>
</tr>
<tr>
<td>Calcium</td>
<td>34 mg</td>
<td>59-103 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>350 mg</td>
<td>63-105 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>6.0 mg</td>
<td>3.0-13.7 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>?</td>
<td>648 mg</td>
</tr>
<tr>
<td>Vitamin A (β carotene)</td>
<td>110-175 mcg</td>
<td>15.60 mg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>?</td>
<td>0.03-0.09 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>?</td>
<td>0.10-0.16 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>4.4-6.9 mg</td>
<td>1.4-2.2 mg</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>?</td>
<td>10-17 mg</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>30 mg</td>
<td>0</td>
</tr>
</tbody>
</table>

Table: 2.1 Food value per 100 g of edible portion of dates

a. Moisture

At the natural stages of development, the date goes from one extreme of moisture content (85% at the early kimri stage) to another (5-10% in dry desert dates). In between there are several levels of importance, i.e. about 50-60% for sweet khalaal, about 35-40% for rutab, around 24% for entering the zone of self-preservation, and about 20% at which a large amount of dates are marketed because they are safe to store but have still retained a pliable and attractive texture.

b. Sugars

For practical purposes all sugars in dates consist of a mixture of sucrose (C\textsubscript{12}H\textsubscript{22}O\textsubscript{11}), glucose (C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}) and fructose (C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}) of which the latter two are the derivations of sucrose after inversion. The relative amounts of sucrose, glucose and fructose are determined mainly by characteristics of different varieties, but it can be said that most dates belong to the invert sugar type, i.e. at the stage at which they are consumed, most if not all sucrose has been inverted into glucose and fructose by the enzyme invertase.
c. Proteins and fats

Both substances occur in small amounts in the date flesh. Fat is mainly concentrated in the skin (2.5-7.5%) and has more physiological importance in the protection of the fruit than contributing to the nutritional value of the date flesh (0.1-0.4%). Palmitic, capric and caprylic acid were identified as the major free fatty acids in the date flesh followed by linoleic, lauric, myristic acid and a number of others. Proteins occur in date fruit in the range of 1-3% and though their amino acid pattern is favorable to human needs, the amounts are too small to be considered an important nutritional source. When extracting dates for sugar, proteins may create turbidity in the juice and have to be removed.

d. Crude fibers (non-soluble solids)

These are usually connotated with the insoluble, non-nutritive portion of the date flesh, and mainly composed of cellulose, hemicellulose, lignins and ligno-cellulose, and insoluble proteins. During the ripening process these substances are gradually broken down by enzymes to more soluble compounds to render the fruit more tender and soft.

e. Vitamins and minerals

Dates at the stage of maturation in which they are normally and mostly consumed, contain vitamins A, B1, B2 and niacin in reasonable amounts, but no significant amounts of the other vitamins, notably vitamin C. Dates are the good source of potassium, calcium and iron, and a fair amount of chlorine, copper, magnesium, sulphur and phosphorus.

f. Enzymes

Enzymes play an important role in the conversion processes that take place during formation and maturation of the date fruit and the activities of four of them are of particular interest to final product quality:

1. **Invertase:** Responsible for the inversion of sucrose into glucose and fructose and related to texture and softness.

2. **Polygalacturonase** and **pectinesterase:** Both convert insoluble pectic substances into more soluble pectins, contributing to softness of the fruit.
3. **Cellulase:** Breaks down cellulose into shorter chain substances with increasing solubility and eventually leading to glucose, thus decreasing fiber content.

4. **Polyphenol oxidase:** Responsible for biochemical changes of polyphenols to which the tannins belong; they are important in non-oxidative browning reactions of the date.

Enzyme activity normally takes place in solution or moist atmosphere; the optimum temperature range usually falls between 30 and 40°C, over and below which the activity will decrease (for instance invertase at 50°C, loses 50% activity and 90% at 65°C after 10 minutes). Prolonged storage of dates under refrigeration or freezing is based mainly on the slowing down of enzyme activity (Barreveld, 1993).

g. **Other chemical substances**

Although over 95% of the constituents of the date flesh are contributed by the above mentioned compounds quantitatively; there are a number of substances that even in minute quantities have a decisive influence on the final quality of the date.

1. **Polyphenols:** Include the tannins which on a dry weight basis may constitute up to 3% of the date flesh. One of their main effects in the maturation process is when they change from a soluble form (astringent in taste) to an insoluble form (tasteless), probably resulting from a combination with protein. Tannins are also believed to play a role in the darkening of dates at the post-harvest stages. The enzymatic browning reaction is now more attributed to the more simple polyphenols such as flavans, whilst the more complex tannins play a role in non-enzymatic oxidative browning.

2. **Organic acids:** A number of organic acids such as citric-, malic- and oxalic acid, have been isolated from date flesh as contributors to flavour, though generally during maturation the acid content tends to go down. Most common pH values for dates range from 5.3 to 6.3.

3. **Pigments:** Pigments of various nature have been reported: carotenoids, anthocyanins, flavones, flavonoles, lycopene, carotenes, flavoxanthin and lutein in some fresh Egyptian dates (Barreveld, 1993).
2.2.2 Medicinal uses:

The date fruit, because of its tannin content, is used medicinally as a cleansing agent and astringent in intestinal troubles. In the form of syrup or paste, is administered as a treatment for sore throat, colds, bronchial catarrh. It is taken to relieve fever, gonorrhea, edema, liver and abdominal troubles. And it is said to counteract alcohol intoxication. The seed powder is an ingredient in a paste given to relieve chills and fever (Morton 1987).

2.2.3 Products through fermentation:

The group of products derived using date as a fermentation medium have in common that they are produced by microbial conversion of date. In most cases the target product is the metabolic by-product of this microbial conversion like alcohol from sugar or acetic acid from alcohol. However, in a few instances the target product is the microbial biomass itself such as baker’s or fodder yeast. Another distinction can be made between products in which the other components of the date also play a role in the final aroma, colour and general quality of the target product like date wine or vinegar that require lower purification, whilst others are required in a pure form as in the case of distilled alcohol or citric acid.

a. Organic acids

The most known and widespread is acetic acid in the form of household vinegar. In the date producing countries date vinegar is well known. The principles of manufacture are much similar as that for wine making, i.e. both products can be made (and used) at the household (or cottage) level incorporating the flavours of the date juice and secondary fermentation products. Like for grape vinegar very acceptable household date vinegar can be made by inoculating a strain of *Acetobacter* into a date wine supplemented with some nutrients (urea or malt) and allowed to stand with an access to oxygen (for instance a barrel on its side with the bung hole left open).

With respect to other potential organic acids which are quite numerous as compared to the product lists for other sugars, for date juice or date syrup most attention in research and project development has been given to citric acid. The reason probably is that citric acid would have an assured market potential for the soft drink...
and food industry. Roukas and Kotzekidou (1991) produced citric acid from date syrup while production of vinegar was experimented by Mehaia and Cheryan (1991).

b. Others

Antibiotic oxytetracyclin has been produced using Streptomyces rimosus (Abou-Zeid et al, 1993) from date juice. Dates have also been used to produce single cell protein and biomass to use as dairy starter culture and baker’s yeast (Kamel, 1979; Khan et al, 1995; Nancib et al, 1997; Nancib et al, 1999).

2.3 Materials and methods:

2.3.1 Organisms and inoculum development:

Lactic acid producing organism was isolated from buttermilk obtained from local market of Anand city, Gujarat, India. A local isolate of homo fermentative, lactic acid producing bacterial strain has been identified as Gram positive Lactobacillus sp. by morphological and biochemical tests according to Bergey’s Manual of Determinative Bacteriology (Holt et al, 1994) and referred as KCP01. The organism was maintained in 50 % glycerol as pure culture and stored below 0 °C. From the stock, 100µl culture was transferred to a sterile Lactobacillus MRS Broth (Hi-Media, Mumbai, India) and incubated at 37 °C for 24 hr under static condition for inoculum preparation. Cells were harvested in a sterile centrifuge tube (15 ml) by centrifugation at 9,000 g for 10 min. The pellet obtained was resuspended in sterile distilled water to adjust the optical density of 1.0 at 660 nm. 1 ml of thus prepared inoculum was then transferred to 100 ml of production media.

2.3.2 Media preparation:

Two different varieties of dates available in the local market were used in the present study. One of them was more resembling to the ‘Black’ semi soft variety of dates and other was ‘brown’ colored and semi soft variety. 40 gm of seedless dates were weighed and minced in distilled water and final volume was made up to 300 ml. This juice was boiled for 10 min so as to flocculate the undissolved suspended matter that settled rapidly upon cooling to room temperature. This was followed by filtration to obtain clear date juice (Samuel and Lee, 1980). Crude juice of dates was further diluted with distilled water to have final concentration of 5 gm% reducing sugar and
used as production media with the supplemented nutrients except the recommended concentration of sugar source of MRS medium (composition in gm per liter of distilled water: peptone 10, beef extract 10, yeast extract 5, K2HPO4 2, sodium acetate 5, tri-sodium citrate 2.15, MgSO4 0.2, MnSO4 0.05 and tween-80 1.0). The medium was autoclaved at 121 °C for 15 min after adjusting the pH to 6.5. Medium pH was left uncontrolled during fermentation. Different sugars at 5 % concentration were also used as a carbon sources in the same manner.

2.3.3 Analyses:

During the fermentation samples were harvested at 0 hr, and at every 24 hr interval then after. Cell count was done from the samples after preparing appropriate dilutions and plating on MRS agar plates and was expressed as colony forming unit CFU/ml. The remaining broth was centrifuged and the clear supernatant was subjected to further analysis. Reducing sugar from date extract was estimated by dinitro salicylic acid method (Miller, 1951).

Lactic acid was estimated by colorimetric method described by Kimberley and Taylor (1996). Working solutions of lactic acid (100 μg/ml) was taken as an aliquot of 0.5 μg to 3.0 μg and the final volume was made to 0.5 ml with distilled water. 3.0 ml of concentrated sulfuric acid was added in cold condition to prevent the evaporation loss. The tubes were covered to prevent the moisture loss during boiling, and were incubated in boiling water bath for 10 min and then allowed to cool down to room temperature. 50 μl of 4.0 % (v/v) CuSO4 was added followed by addition of 100 μl of 1.5 % (v/v) ethanolic para-phenyl phenol and further incubated at room temperature for 30 min. Purple color obtained was read at 570 nm.

Sugars were identified by ascending paper chromatography using the solvent system; butanol: ethanol: water in the ratio of 40:11:19 (v/v). Sugars were identified as brown spots by spraying the chromatogram with silver nitrate reagent (2.5 ml of saturated AgNO3 in 500 ml acetone), dried and dipping in NaOH reagent (16 gm NaOH in 800 ml of 0.5 N methanol). Glucose, fructose and protein were estimated colorimetrically whereas free fatty acids were estimated by titrimetric method (Sadasivam and Manickam, 1996).
2.4 Results and discussion:

2.4.1 Organism:

Lactic acid producing organism isolated from butter milk showed medium, round, semi transparent and smooth colony with entire margin. Staining and microscopic observation revealed it as Gram positive rods arranged singly and in pairs (Fig. 2.3). Based on the biochemical tests according to Burgey’s manual, it was identified to be a member of genus *Lactobacillus* (table 2.2).

Figure: 2.3 Gram staining and morphological characters of *Lactobacillus* KCP01

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia production</td>
<td>Negative</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>Negative</td>
</tr>
<tr>
<td>Motility</td>
<td>Positive</td>
</tr>
<tr>
<td>Catalase</td>
<td>Negative</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>Positive</td>
</tr>
<tr>
<td>Glucose</td>
<td>Positive</td>
</tr>
<tr>
<td>Galactose</td>
<td>Positive</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>Negative</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>Negative</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Negative</td>
</tr>
<tr>
<td>(\text{CO}_2) production</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table: 2.2 Results of biochemical tests for genus *Lactobacillus*

*Lactobacillus* strain KCP01 was checked for its lactic acid production potential based upon its utilization of various sugars supplied along with MRS medium at 5.0 gm% (w/v) concentration (Fig. 2.4). Higher production of lactic acid was obtained (20.80 gm/lit) with lactose followed by fructose (13.3 gm/lit) and sucrose (11.92 gm/lit). Lower production was achieved with glucose (7.96 gm/lit) than with lactose and still lesser with galactose (7.70 gm/lit), maltose (4.0 gm/lit) and negligible with pentose sugar xylose (1.24 gm/lit).
Lactic acid bacterium *Lactobacillus* sp. KCP01 has ability to utilize and ferment various sugars. It showed maximum production on lactose sugar since it was isolated from butter milk, a natural source rich in lactose. It was surprising that it gave three times lesser production with glucose. This can be attributed to the fact that the organism is well adapted to utilize lactose. Lactose was followed by fructose and sucrose in terms of lactic acid production. Organism preferred xylose—the pentose sugar the least since it resulted in only 1.24 gm/lit lactic acid.

### 2.4.2 Nutrient composition of dates:

Laboratory analysis of both the date varieties obtained from local market showed approximately 40 gm of total sugar per 100 gm of dates of which 46.72 % (18.68 gm) was fructose and 53.27 % (21.30 gm) was glucose in brown dates (table 2.3). This was qualitatively confirmed by the paper chromatography that showed presence of both the sugars with traces of sucrose.

<table>
<thead>
<tr>
<th>Type of Analysis</th>
<th>Black Date</th>
<th>Brown Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>21.75 gm</td>
<td>21.30 gm</td>
</tr>
<tr>
<td>Fructose</td>
<td>17.63 gm</td>
<td>18.68 gm</td>
</tr>
<tr>
<td>Sucrose</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total Sugar</td>
<td>39.40 gm</td>
<td>40.00 gm</td>
</tr>
<tr>
<td>Free Fatty Acid</td>
<td>3.07 gm</td>
<td>2.71 gm</td>
</tr>
<tr>
<td>Protein</td>
<td>7.29 gm</td>
<td>6.254 gm</td>
</tr>
</tbody>
</table>

Table: 2.3 Composition of fresh dates (per 100 gm). ND: not detected
Palm dates are rich in nutrients when compared with the alternative raw materials like starchy and cellulosic substrates that must be prior treated with enzyme or by other means to release the sugars in fermentable form for the organisms (Sreenath et al, 2001 a and b; Rivas et al, 2004; Kurbanoglu and Kurbanoglu 2003; Patel et al, 2004; Wee et al, 2004). Dates are reported to contain 36.6 gm of total sugar/100 gm fresh date (Morton, 1987). Based on analysis, black date contained 39.40 gm and brown date contained 40.00 gm of total sugar per 100 gm of fresh dates. Among that major portion was contributed by glucose followed by fructose with traces of sucrose as revealed by biochemical analysis and paper chromatography. All sugars in dates consist of a mixture of sucrose, glucose and fructose of which the later two are the derivatives of sucrose after inversion. Sugar present in most of the dates belong to the invert sugar type i.e. at the stage at which they are consumed, most if not all sucrose has been inverted into glucose and fructose by the enzyme invertase (Barreveld, 1993).

2.4.3 Lactic acid production using date extract:

Black date juice alone when diluted to have 5 gm% sugar and used for fermentation, gave very low production of lactic acid (2.65 gm/lit). Black date juice along with the nutrient supplements resulted in maximum lactic acid production of 8.64 gm/lit. pH of the medium was dropped to 3.75 after 48 hr of fermentation. Similarly with juice of brown variety of date the production was 10.3 gm/lit with final pH of 3.76 and residual sugar being 40.59 gm/lit (Fig. 2.5).

Biochemically dates are nutritive for the growth of organisms. Since, date juice alone could not result into considerable lactic acid production, to further increase the lactic acid production, the date juice was supplemented with the salts and protein as per MRS medium. This is because lactic acid bacteria are fastidious and have complex nutrient requirements (Chopin, 1993). Many research studies have employed the raw agricultural substrates along with either basal (only mineral salts) or complete supplement media (salts and protein) for higher production of lactic acid (Patel et al, 2004; Sreenath et al, 2001a and b; Kurbanoglu and Kurbanoglu, 2003). In the present study glucose has been replaced in the nutrient composition of the MRS medium by the addition of crude date juice.
The supplementation had a positive effect and resulted in four fold increase in the lactic acid production (from 2.65 to 8.64 gm/lit).

2.4.4 Effect of pH and temperature on lactic acid production:

Initial pH of the medium was set ranging from 4.5 to 7.0. The fermentation was carried out without further pH control and under static condition since lactic acid bacteria, especially *Lactobacillus* are micro-aerophilic. Initial pH 7.0 gave the maximum production, i.e. 16.48 gm/lit after 48 hr of incubation. pH below 7.0 i.e. 6.0 and 6.5 gave 13.17 and 14.58 gm/lit lactic acid production, respectively (Fig. 2.6). The values that gave maximum production were selected for further optimization of temperature to have the effect of two factors simultaneously.

pH range was selected on the basis of the fact that lactic acid bacteria have their optimum growth pH between 5.5 - 6.5. On the basis of results obtained, three pH values were selected; 6.0, 6.5 and 7.0 and were employed for the temperature optimization. This was performed to check the simultaneous effect of pH (6.0, 6.5 and 7.0) and temperatures (30 °C to 60 °C) on the lactic acid production using previously described media with black date juice under static condition.

The results showed pH 7.0 and temperature 40 °C as the optimum for lactic acid production using KCP01. At pH values 6.0 and 6.5 lesser production was observed regardless of temperature than at pH 7.0. At temperature above 40 °C, lactic acid production was severely inhibited with the increase in the temperature.
The maximum lactic acid production obtained was 15.1 gm/lit at pH 7.0, 40 °C. At the same temperature and initial medium pH at 6.0 and 6.5, 12.36 gm/lit and 13.35 gm/lit lactic acid production, respectively was observed. At temperatures above 40 °C i.e. 50 °C and 60 °C lactic acid production was severely inhibited as lactic acid production was only 6.3 and 2.72 gm/lit with pH 6.0, 8.3 and 4.11 gm/lit for pH 6.5 and 7.17 and 3.76 gm/lit with pH 7.0 (Fig. 2.7). Brown date juice was fermented with the same formulated medium and the organism. This gave little higher production of lactic acid i.e. 17.15 gm/lit.
2.5 Conclusion:

From the fermentation data obtained, it was observed that the difference between fermentation of juices of both the variety of dates by the strain *Lactobacillus* KCP01 is not significant and the organism can ferment sugars present in both the date juices very well. The growth pH that was lowered down to 3.5 was also not suitable for the growth of lactic acid bacteria, which is evident from the decrease in the CFU count. *Lactobacillus* strain KCP01 can easily grow on and ferment date sugars to lactic acid. The larger amount of unutilized sugar at the end of fermentation can be
made fully consumed by the organisms if the pH of the medium is controlled. Production can be increased by the optimization of the medium components and scaling up at laboratory scale. The sugar which is still not utilized can also become important starting material for microbial utilization leading to fuel or feed production.
2.6 References:


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