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Research Article

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A Preliminary Attempt of Ethanol Production from Fig (*Ficus carica*) and Date (*Phoenix dactylifera*) Fruits using *Saccharomyces cerevisiaes*

Ramar Krishnamurthy[1], David Adedayo Animasaun[2]*, Rajashekhar Shivappa Ingalhalli[1], Nikunjkumar Devechandbhai Ramani[1]

[1]C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, Surat (Dist) Gujarat, India
[2]Department of Plant Biology, University of Ilorin, P. M. B. 1515, Ilorin, Nigeria
*Corresponding Author E-mail: animasaun.ad@unilorin.edu.ng

ABSTRACT

Research for new, cheap and abundant agro-raw material for ethanol production remains a contemporary issue in the quest for safe and renewable energy globally, particularly with reference to bio-fuel production. This study evaluates the potentials of Ficus carica (Fig) and Phoenix dactylifera (Date palm) fruits as possible alternative raw materials to food grains for ethanol production. Fig and date palm fruit pulps were fermented in flask on a rotary shaker using Saccharomyces cersvisiaes (NCIM. 3288) obtained from National Collection of Industrial Microorganisms (NCIM) Pune, India. pH and Ethanol yield of the reacting media were determined every 24 hr till 120 hr. At 120 hr, distillation of ethanol from the pulp syrups was carried out at two temperature regimes (80-88, 78-82^{o}C) and the distilled ethanol purified by sodium hydroxide reaction (NaOH). The results showed that pH decreases as fermentation time increase and ethanol yield increased with increase in fermentation time till 96 hr when optimal yield was attained and subsequently decrease in ethanol yield beyond 96 hr to 120 hr. Both ethanol quantity determination by fermentation (0.95w/v for fig and 1.23w/v for Date fruits) and distillation (8.76% for fig and 10.64% for date fruits) revealed that date fruit yielded more ethanol than fig fruit fermented syrup. However, ethanol obtained and purified from the fruit pulps showed that fig fruit produced purer ethanol than date pulp. The study concluded that both fig and date fruits could be utilised as potential alternative raw material to food grains for bioethanol production.

Keywords: *bioethanol, distillation, ethanol yield, fermentation, Saccharomyces cersvisiaes.*

INTRODUCTION

As the debate on renewable and clean energy particularly with respect to bio-fuel production continues, the search for new substrates for ethanol production is imperative. Lately, fuel prices are rising along with increase in the demand globally. Consequently, other energy sources like biofuel, and solar energy are increasingly being used as alternative energy sources to mitigate the emission of green house gas and curb global warming. Biofuel are biodegradable, non-toxic and usually free of aromatic and sulphur compounds[1].

Fuel-ethanol production is one of the fastest growing energy industries globally and in the recent time, European Union has given priority to produce bio-ethanol from agricultural residues to reduce fossil oil consumption by 20 % [2]. Similarly, about 30% of the gasoline in the United State is reportedly blended with bio-ethanol[3]. To save premium motor sprit (PMS), the Government of India seeks to implement addition of 10 % ethyl alcohol to gasoline for the full utilization and combustion of gasoline fuel. According to Ward and Singh[4], bio-ethanol blended with gasoline can reduce vehicle carbon dioxide emissions by 90%. Currently, about 80 % of total world ethanol production is obtained from the fermentation of simple sugars by yeast[5]. *Saccharomyces cerevisiae* is species of yeast that has been used

in brewing industry for production of alcoholic beverages for years; also, it is routinely used in production of ethanol by fermentation of molasses. The yield of ethanol from microbial fermentation reportedly depends upon the type of yeast strain, physiology of the yeast, media composition and other environmental parameters like pH, temperature etc.[6, 7].

Basically, cereals, corn in particular have been used as raw material in ethanol production[8, 9]. However, there is constraint in the supply of alcohol corresponding to demand using corn solely as the raw material. Considering the role corn plays in global food security and its utilization in animal feed formulation there is need to diversify the source of bioethanol production. To this end, other cereals have been exploited in industrial bioethanol production, meanwhile, these alternative cereals are reported to be associated with a number of problems are likely to reduce their ethanol yield[10, 11, 12].

Abundance and economies of biological raw materials are critical factors that determine its exploitation for ethanol production. Availability of molasses from sugarcane has made it material of choice for ethanol production in Brazil, while corn and sorghum are mostly used in USA and Canada, but cereal sources are more relevant in solving global food security problems than production of bioethanol[13]. Therefore, there is a pressing need for research into fermentability of ethanol from non-edible agricultural sources such as non-edible fruits and seeds of forest species to reduce the pressure on cereals bioethanol production[14].

The present study attempts to use cheap and readily available sugar sources from pulp of the fruits of *Ficus carica* (Fig) and *Phoenix dactylifera* (Date palm) for ethanol production using *Saccharomyces cerevisiae*. The study also seeks to estimate the amount of ethanol produced during fermentation and distillation processes of these natural sugar sources.

MATERIAL AND METHODS

Materials

The seeds of *Ficus carica* (Fig) and *Phoenix dactylifera* (Date palm) used for this research were sourced from a local market in Mahuva village, Surat District of Gujarat State of India. The yeast (*Saccharomyces cerevisiae* NCIM strain No. 3288) in active culture form was obtained from National Collection of Industrial Microorganisms (NCIM) Pune. NCIM is located within the National Chemical Laboratory (NCL), Pune, in western India with mandate of repository for isolation, preservation and distribution of industrially important cultured microorganism for scientific and industrial purpose. The sucrose use for the experiment was obtained from BDH Limited, Poole in England while other reagents were obtained from SD Fine CHEM Ltd, Mumbai and HIMEDIA Chemicals, Bangalore, both in India.

Methods

Preparation of plant materials

The plant samples were identified at Department of Botany, Navsari University of Agriculture, Navsari, Gujarat, India. The experiment was carried out in the Biotechnology Laboratories, C.G.Bhakta Institute of Biotechnology, Uka Tarsadia Univeristy, Bardoli in Surat District of Gujarat, India. The samples were air dried for about 20-25 days, after which they were gently crushed using pestle and mortar to separate the fruit pulp from the seeds (Fig. 1a & b). The fruit pulp obtained after crushing was stored in a cool, dry cupboard until used.

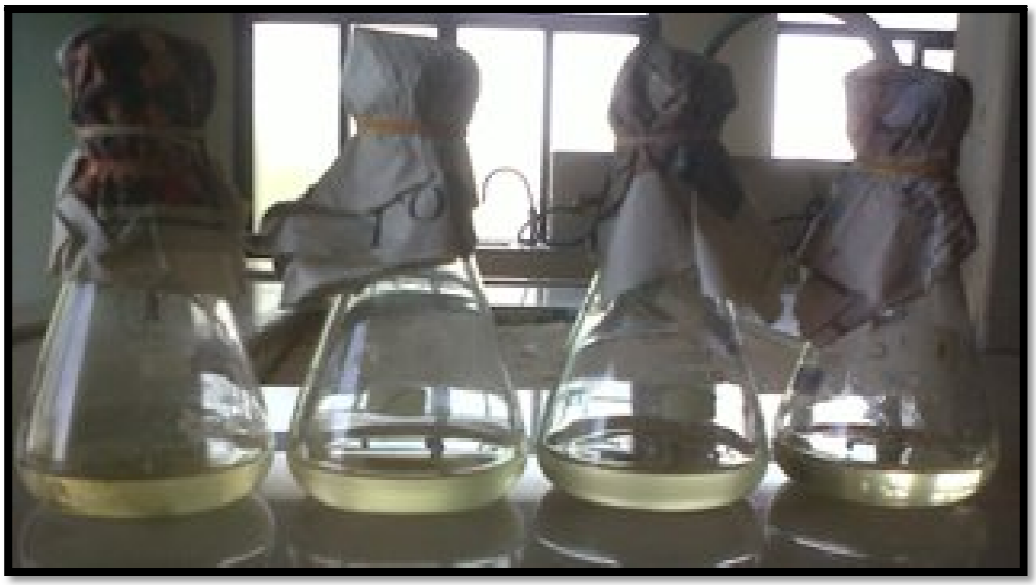
Fig.1a: Dry pulp of fig fruit

Fig.1b: Dry pulp of date fruit

Maintenance of organism

The yeast strain *Saccharomyces cerevisiae* NCIM strain No. 3288 for the fermentation process was maintained on yeast media by inoculating wire loop of the culture in to the yeast extract/peptone/dextrose agar (YPDA), sealed with sterile mineral oil at room temperature (25^0C) and kept for growth till three days when ready for use (Image 1)

Image 1: showing the growth of the yeast *Saccharomyces cerevisiae* NCIM strain no. 3288



Fermentation and ethanol estimation Process

20 g of pulp mash of *F. carica* and *P. dactylifera* were measured into 500 ml flasks using WENSAR high precision balance (Model 9001) with three replicates for each species. The fermentation procedure as described by Igwe *et. al.*[14] was adapted with slight modifications. *S. cerevisiae* taken by loop wire were first dissolved in nutrient agar (NA) broth and put in incubator at 37^0C for 24 hr before used. Yeast preculture was prepared according to the method described by Zhan *et. al.* [15] and the cell concentrations of the preculture were checked by A_{600} values of Spectrophotometer. The A_{600} values ranged between 2.00-2.40. Warm water was added to the pulp in the flask to enhance fermentation. 5 mL of yeast was added to each of the flasks, made up to 500 ml and the content was thoroughly agitated using a rotary shaker. The flasks were properly covered with several layers of sterile aluminum foil to create favourable condition for the anaerobic reaction. Fermentation was allowed for 120 hours. pH of the fermenting system was adjusted to 6 at the onset of fermentation and determined at interval of 24 hr using Electronic pH meter (CL 54+ by Toshcon Industries Pvt Ltd, Hardwar, India), sugar consumption was estimated by titration of ferricynanide [16] and ethanol production of the system were determined every 24 hr using ethanol assay by dichromate colorimetric method as described by Wang [17] and Lakhawat *et. al.*, [18].

Distillation Process

After 120 hr (on the fifth day when it was observed that fermentation process had stopped), the resultant syrup from the fermentation of the fruit pulps were filtered separately into 500 ml distillation flasks. Glass chips were added into the flasks to reduce side swerving of the filtrates. The distillation experiment was set up and distilled ethanol was collected at 80-88 ^{0}C for the first round of distillation and at 78-82 ^{0}C for the second round of distillation. Raw percentage ethanol yield was determined. The ethanol obtained after second distillation was purified by addition of sodium hydroxide to the distilled and kept overnight to obtain ethanol of 90 -96% purity.

Statistical Analysis

The data obtained from the experiment was in three replicates and the mean values were determined using Microsoft Excel for Microsoft Window Operating System.

RESULTS

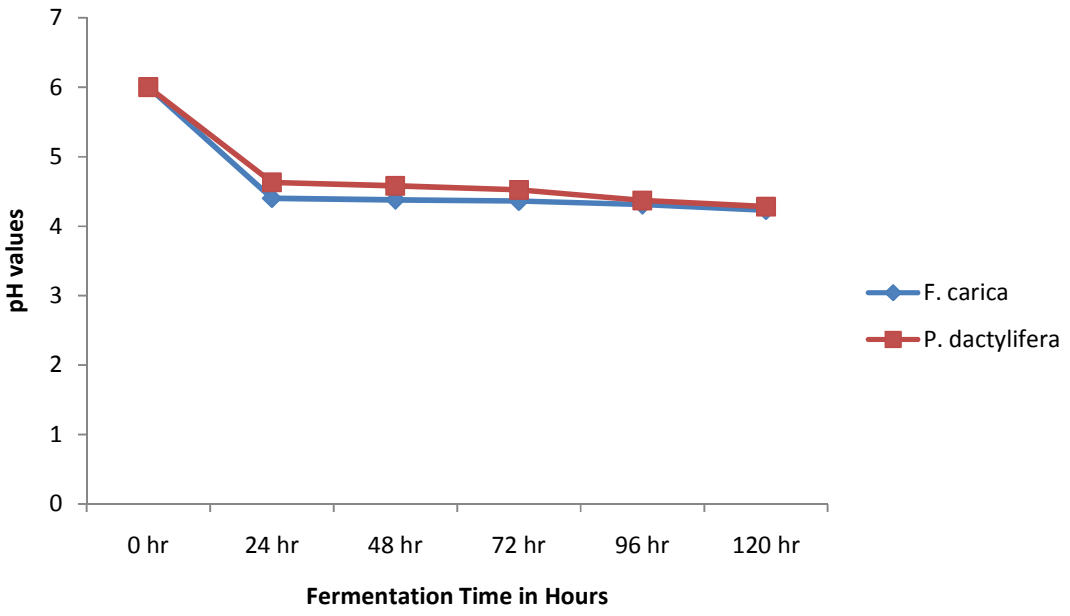
The determination of reducing sugar by titration of ferricyanide resulted in colour change as it reaches end point. The colour changes in dichromate method analysis are shown in Image 2a and 2b.

Image 2a: shows color formation in dichromate method of fig sample

Image 2b: shows color formation in dichromate method of date sample

The pH value of fermentation media was adjusted to 6 at the beginning of the fermentation. The pH values of the fermenting syrup determined at 24 hr interval showed that as fermentation time increases, the acidity of the medium increases. The pattern of pH change in the fermentation medium for the two samples was similar in trend. The trend in pH changes for the fermentation process is presented in Fig 2.

Fig 2: Showing pH values of *F. carica* and *P. dactylifera* during fermentation process



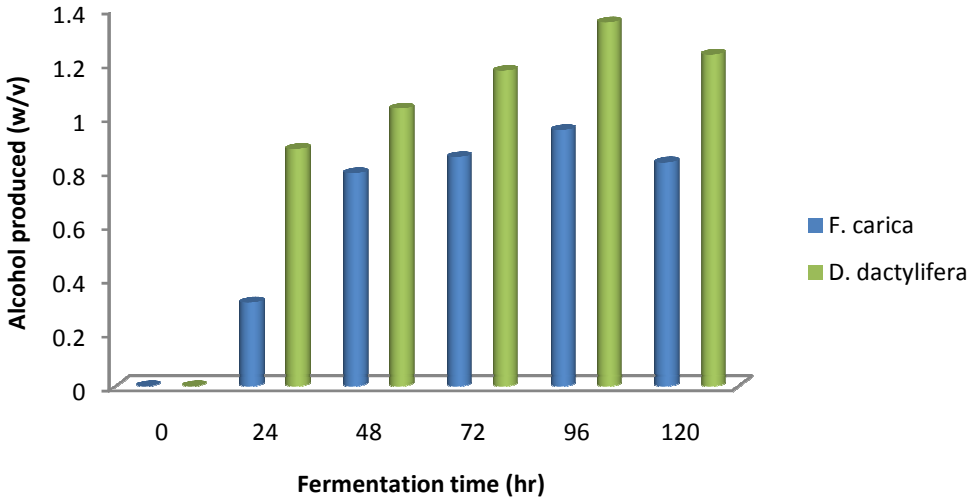
The sugar content present at the starting of fermentation was 13.40 and 11.10w/v for *F. carica* and *P. dactylifera* media respectively. The amount of sugar present in the fermenting syrup gradually decreased by every 24 hours up to 6.20 w/v in fig and 4.10 w/v in date medium at 120 hr. As the sugar content decreases from 24 -20 hrs, sugar consumption increases (Table 1).

Table 1: Sugar content and consumption in the fig and date media in different time of fermentation

Time (hr)	Sugar contents (w/v)		Sugar consumption (w/v)	
	F. carica	*P. dactylifera*	*F. carica*	*P. dactylifera*
0	13.40	11.10	0.00	0.00
24	11.10	6.90	2.30	4.23
48	7.50	5.20	5.90	5.90
72	7.10	4.10	6.30	6.00
96	6.70	3.60	6.70	7.50
120	6.20	3.45	7.20	7.62

The alcohol production from the fig sample progressively increased from 0.31 w/v at 24 hr of fermentation to a peak value of 0.95 w/v at 96 hr but a decline in ethanol yield was recorded at 120 hr (0.85 w/v). Although ethanol yield was higher in date fermentation (0.88 w/v at 24 hr) which also peaked at 96 hr with ethanol yield of 1.23 w/v it then reduces on further fermentation at 120 hr to 1.23 w/v (Fig.3).

Fig.3: Comparison of ethanol production between fig and date fruit at different times of fermentation



Results obtained from the distillation process showed that date fruit fermented syrup yielded more ethanol than fig fermented syrup at the two temperature regimes of distillation. The percentage purity of ethanol obtained after reflux distillation of the fermented syrups of the two samples was found to be 93.3 and 92.6% for *F. carica* and *P. dactylifera* respectively (Table 2).

Table 2: Distillation product and percentage yield of ethanol from 250 mL of fermented syrups of *F. carica* and *P. dactylifera*

Sample	Fermented syrup	1st Distillation	2nd Distillation	% ethanol yield	% purity
	Staring Vol (mL)	80 – 88 (°C)	78 – 82 (°C)		
F. carica	250.00	27.60	21.90	8.76	93.3
D. dactylifera	250.00	33.45	26.60	10.64	92.6

DISCUSSION

Research and product optimization in bioethanol production is a continuous process, as more biological materials are being explored for ethanol production. *S. cerevisiae* used in this study for fermentation process of the tested substrates yielded ethanol. The decrease in pH of the reactor systems occurred because ethanol fermentation process generates CO_2 (carbon iv oxide), which increases the acidity of the fermenting syrup as fermentation time increases and thereby causing decline in pH values of the media. At the 0 hr the pH of the two media was the same but differ though slightly as the reaction proceed and proportional to the amount of sugar utilization in the media. Similar pH reduction in ethanol fermentation of pearl millet was reported by Wu *et. al.*,[12] and Lakhawat *et. al.*,[18] who worked of microbial fermentation of *Vitis lanata*.

The level of sugar in the reactors decreased with the progress of the fermentation process. The difference in the sugar content and surface area of the starting materials for the fermentation process could account for slight difference in sugar consumption pattern of the systems. Less amount of sugar in date mash and larger surface area possibly increase enzymatic turnover and sugar metabolism by the microbial activity. Although sugar metabolism by the yeast was progressive from 24 hr till 96 hr after which sugar consumption reduced, since fermentation process generates equal moles of CO2 and ethanol. Reduction in sugar consumption signified weight loss in CO_2, which consequently indicated reduced yield in ethanol production after 96 hr of fermentation. A number of workers had reported that weight loss from escaped

CO_2 in a fermentation process could be used to monitor fermentation process[19,20]. In determining the optimum fermentation time, it was suggested that monitoring of CO_2 weight loss during a shaking-flask fermentation process can be a convenient way to predict ethanol yield and determine the end point of a fermentation process[12].

The alcohol yield from the tested plant materials was progressive and peaked at 96 hr, thereafter decreased. The decrease in ethanol yield was corresponding to decrease in sugar in the media. Fermentation beyond 96 hr was not sustainable, this suggests that at 96 hr, most of the glucose in the mash has been consumed and other fermentable sugars such as maltose, dextrins and maltotriose were already hydrolyzed and utilized in rapid ethanol yielding phases of the fermentation process. Therefore, the ethanol fermentation for fig and date fruits used for the study essentially ends at 96 hr for optimum ethanol yield, beyond this; the ethanol produced is possibly reconverted to glucose in the system thereby reducing the yield of ethanol. This finding was in agreement with earlier workers who had earlier observed ethanol yield reduction beyond optimum fermentation time in biological material studied[21, 22].

From the results, the overall yield of ethanol fermentation from date fruit mash is higher than ethanol yield form fig mash, even though, sugar content was slightly higher in fig than in date pulps. The reason for this may likely be dissimilarities in chemical and physical properties of the tested fruit meshes which affect sugar consumption and consequently the ethanol yield, also, free α-amino nitrogen (FAN) contents has being indicated to affect ethanol yield in plant material[23]. However, ethanol obtained from distillation of syrups from fig was purer that ethanol obtained from date mash.

CONCLUSION

The research into bioethanol from sources that has less food dependency, high availability and comparatively good fermentation efficiency is a way to revolutionize bioethanol production in the future. A large amount of renewable biomass could be utilized and converted to ethanol using environment friendly approach. The present work evaluated ethanol yield from *F. carica* (fig) and *P. dactylifera* (date) fruits using yeast fermentation method. The optimum fermentation time for higher yield for ethanol in this study was 96 hr and more ethanol product was obtained from date fruit than fig fruit mash at optimum fermentation time. The ethanol yield from the studied fruits may further be enhanced for fermentation efficiency by using fermentor rather than rotary shaking flask method. The study concluded that fig and date fruit could be utilized in production of ethanol and therefore could serve as alternative feedstocks to food grains in the production of fuel ethanol.

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