Isolation of yeast and ethanol production from papaya (*Carica papaya*) and grape (*Vitis vinifera*) fruits

Parameswari K, Hemalatha.M, Priyanka.K and Kishori B*

Abstract-The present study was carried out to isolation of yeast and production of ethanol using papaya (*Carica papaya*) and grape (*Vitis vinifera*) pulps with isolated yeast. Using selective medium, the yeast strains were isolated from fruit pulps and differential tests were applied for identification of the yeast. Percentage of yield of ethanol and effect of different nutrient supplements for the production of ethanol was studied using isolated yeast as synthetic medium is control. The reducing sugar was also estimated after 72 hrs saccharification, the amount of reducing sugar increased significantly (14.6 g/100g) in papaya fruit than grape fruit pulp (10.3 g/ 100g) and it is evident that high amount of carbohydrate levels. The fermented papaya yielded (6.20%) more ethanol content than grape pulp (3.83%). Addition of carbohydrate supplement, sucrose and nitrogen supplement of soya meal produced high yield of ethanol with yeast in papaya (11.45% & 9.16 %) than grape pulp (7.61% & 6.03%). The present study revealed that the fully ripened papaya fruit is a good source for ethanol production and it is possibility of ethanol production from our locally available fruits using simple, cheap, and adaptive technology with biochemically characterized yeast isolated from fruit pulps.

Index Terms- Ethanol, papaya (*Carica papaya*), grape pulps (*Vitis vinifera*), *Saccharomyces cerevisiae.*

1 INTRODUCTION

Over the past few decades there is an increase in the demand for ethanol production not only for its use as feedstock of chemical manufacturing but also it has been considered as a potential alternative source of liquid fuel for automobiles [1]. Usually ethanol production is accomplished by chemical synthesis of petrochemical substrates but, due to depleting reserves and competing industrial needs of petrochemical feed stocks, there is global emphasis on bioethanol production using fermentation process [2]. In fermentation process, ethanol can produced with various substrates, among sugar cane molasses is the main raw material for ethanol production but the increased cost and short supply it is not able to producing required quantity. Cellulosic materials are other cheaper raw materials for the ethanol production. Due to presence of many steps in cellulose conversion it is also expensive. In order to meet the demand and cost effective for ethanol production in fermentation technology, researchers are concentrating on inexpensive raw materials.

In this aspect many investigators have studied the production of ethanol using *Saccharomyces cerevisiae* with different raw materials such as agricultural wastes [3], municipal wastes [4], the fruit wastes like papaya [5], [2], [6] banana peels [7], [8], [9], mangoes [10], pineapple [11] and grapes [12], [13], [6]. Hence, for the present study fully ripened *Carica papaya* (papaya) and grapes (*Vitis vinifera*) pulps were selected for isolation and production of ethanol using isolated yeast *Saccharomyces cerevisiae*. The study was also conducted with addition of carbohydrate and nitrogen supplements sucrose and soya meal

Author: Dr. B. Kishori, Asst. Professor Dept. of Biotechnology
Sri Padmavati Mahila Visvavidyalayam
Tirupati – 517 502.
Email: kktinku@rediffmail.com

respectively.

2. MATERIALS AND METHODS:

2.1 Collection and extraction of fruit samples

Fruit samples of papaya and grapes were collected randomly from the local market of different areas in Tirupati, Andhra Pradesh. The fruits were peeled and removed the seeds. The samples were blended aseptically, sieve the fruit pulps using clean sterile cheese-cloth and transferred into containers, stored in the refrigerator for further studies.

2.2 Isolation of yeast

Using Rose Bengal agar medium yeasts were isolated separately from collected fruit pulps papaya and grapes with chloramphenicol as described by [14]. Yeast malt (YM) agar used for sub culturing and maintenance of isolated strains at 280C.

2.3 Morphological investigations and biochemical tests for identification of yeast

Yeast isolates were identified based on the morphological and physiological studies [15] and species was identified by reference [16], [17] Yeast isolates were identified on the basis of standard biochemical tests like carbohydrate fermentation test, carbohydrate catabolism, triple sugar iron agar, catalase and urease test. Other additional tests such as starch formation, urea hydrolysis, cycloheximide (0.01% or 0.1%), Diazonium blue-B (DBB) test will be performed and preserved the isolated yeasts.

Saccharification was carried out on the sample using Yeast strain for the period of 72 hrs and the total reducing sugar was determined using DNS. The values were determined after 24 hr, 48 hr and 72 hrs of saccharification.

2.4 Fruit extract preparation and fermentation process.

Papaya and grape pulps were collected, weighed and blended aseptically. Clean sterile cheese-cloth was used to sieve the fruit pulps, which was then distributed into 500 ml conical flasks (each 100g/100 ml) in four different experimental set with addition of supplements. The pH of media were adjusted to 5.0 and closed using stopper with non-absorbent cotton wool and aluminum foil to ensure they were air-tight as to provide anaerobic conditions. These were pasteurized by boiling water bath for 15 min and were cooled to inoculate 1% of Yeast strain for ethanol production. After inoculation, the flasks were tightly covered with aluminum foil, which would restrict but not eliminate gas exchange. Then flasks were incubated at 30 ± 0.5ºC for three days. At the end of fermentation, the alcohol was recovered by simple distillation using laboratory distillation unit.

2.5 Analytical Method

At third day, the fermented samples were estimated for ethanol and sugar concentrations spectrophotometrically. Ethanol was determined by measuring optical density at 600nm after standard distillation. The total reducing sugar content was analyzed by optical density measurement at 520 nm by dinitrosalicylic (DNS) method [18]. Ethanol was determined with oxidation with acid dichromate solution [19] and absorbance was measured at 660 nm.

3. RESULT AND DISCUSSION:

Yeast strains were isolated from papaya and grape fruit pulps were identified based on colony characteristics and biochemical tests. The colony characteristics of isolated yeast strains, white and creamy texture, ovoid microscope shape and pseudomycelium, found to belong *sacharomyces* [20], [21] (Table 1). The biochemical analysis of isolated yeast strains from papaya and grape fruit pulps showed positive to catalase, fermentation of carbohydrate and triple sugar iron agar tests and negative to urease, Starch hydrolysis, carbohydrate metaboloism and Diazonium blue-B (Table 2).

The amount of reducing sugars obtained from fruit pulps were shown in Fig. 1. The amount of reducing sugars was low in fresh papaya waste (7.3g/100g) and grape pulp (5.57g/100g). The values were significantly ($p < 0.05$) increased after 72 hr saccharification, but the amount of reducing sugars in papaya fruit was more (14.6 g/ 100g) than grape fruit pulp (10.3g/100mg). The percentage of ethanol yield was significantly ($p < 0.05$) higher in yeast isolate of papaya pulp than yeast isolate of gape pulp after 72 hrs (Table 3). Also addition of carbohydrate supplement sucrose and nitrogen supplement soya meal were carried out in the papaya, grape fruit pulps along with synthetic medium as control. The highest amount of ethanol yield was recorded when yeast isolate from papaya was used for fermentation after 72 hrs. with addition of supplements, sucrose and

nitrogen supplement of soya meal. The yield of ethanol increased significantly with sucrose and soya meal supplement in papaya fruit pulp than grape pulp (Table 3).

In the present study, Yeast strains were isolated from papaya and grape fruit pulps on YM agar medium and 4 isolates were obtained. Previous studies have shown that the yeast are commonly associated with sugar rich samples such as leaves, flowers, sweet fruits, tree exudates, grain, roots, insects, dung, soil [22]. Due to readily availability of carbohydrates in synthetic media, it produced more amount of ethanol with both cultures. Apart from synthetic medium the rate of ethanol production was higher in papaya pulp than grape. The results of this work have shown that papaya agro waste could serve as raw material for the production of alcohol. The present investigation has revealed the processing of wastes and more production of ethanol by *S. cerevisiea* was agreement with other reports [23], [2], [5], [24]. The production of ethanol from papaya fruit is more than the grape pulp [5], [12], [25], [26]. The result of this study indicated that yeasts, isolated from papaya fruit pulps showed good fermentation process with agri waste products.

Table 1: Morphological characteristics of the yeast strain isolated from papaya and grape pulp

Characteristics	Isolate from Papaya	Isolate from Grape
Surface	Smooth	Smooth
Shape	Spherical/ oval	Round/oval
Colour	Creamy white	Creamy white
Pesudo mycellum	Present	Present
True mycellum	Absent	Absent

Table 2: Biochemical analysis of the yeast strain isolated from papaya and grape pulp

S.No	Biochemical Tests	Yeast isolate from Papaya	Yeast isolate from Grape
1.	Catalase test	Positive	Positive
2.	Urease test	Negative	Negative
3.	Carbohydrate catabolism	Negative	Negative
4.	Fermentation of carbohydrate	Positive	Positive
5.	Starch hydrolysis	Negative	Negative
6.	Triple sugar iron agar test	Positive	Negative
7.	Cyclonhexamide (0.1%)	Negative	Negative
8.	Diazonium blue-B	Positive	Negative

Fig 1: Effect of saccharification on the amount of reducing sugar 0-72 hrs in papaya and grape pulp:

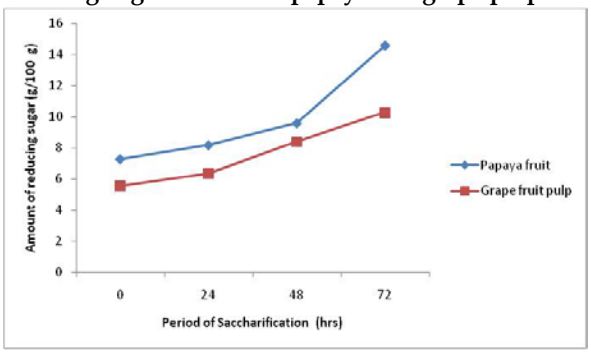

Table-3: Amount of ethanol produced by isolated yeast using different substrates with additional supplements

Substrate	Ethanol yield (%)	
	Yeast isolate from papaya pulp	Yeast isolate from grape pulp
Papaya fruit pulp	5.23 ± 0.07	4.27 ± 0.06
Grape fruit pulp	4. 35 ± 0.04	3.16 ± 0.11
Synthetic medium	7.90 ± 0.01	7. 02 ± 0.14
Papaya fruit pulp + Sucrose	11.45 ± 0.05	9.16 ± 0.02
Grape fruit pulp + Sucrose	7.61 ± 0.61	6.03 ± 0.05
Synthetic medium + Sucrose	13.21 ± 1.21	10.04 ± 0.03
Papaya fruit pulp + soya meal	8.34 ± 0.05	6.04 ± 0.13
Grape fruit pulp + soya meal	6.47 ± 0.04	5.52 ± 0.16
Synthetic medium + soya meal	10.42 ± 0.21	7.04 ± 0.06

4. CONCLUSION

Processing of waste using yeast was produced ethanol. Due to higher amounts of sugars in papaya pulp ethanol yield is more and addition of sucrose as additional supplement in the media produced higher amount of ethanol. Ethanol can be used as renewable energy source and it can produce from different agro-wastes successfully.

ACKNOWLEDGEMENT

The authors wish to thank Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, A.P. for providing facilities to carry out the work.

REFERENCES

[1] J.B. Ahmeh, A.A. Ahmadm, and C.O. Ikediobi, "Ethanol production from Corn-cob wastes and Grass-straw," Nig. J. Biotechnol. Vol. 6, pp. 110-112, 1988.

[2] BC Akin-Osanaiye, HC Nzelibe, AS Agbaji, " Ethanol production from Carica papaya (Pawpaw) fruit waste," Asian J. Biochem. Vol. 3, no. 3, pp. 188-193, 2008.

[3] K. Schugerl, "Agricultureal wastes: A sources of bulk products," J. Chem. Engg. Technol, vol. 17, pp, 291.

[4] M. Green and G. Shelef, "Ethanol fermentation of acid hydrolysate of municipal solid waste," Che. Engg. J, vol. 40, pp. B25-B28.,1989.

[5] BC Akin-Osanaiye, HC Nzelibe, AS Agbaji, "Production of ethanol from Carica papaya (pawpaw) agro waste: effect of saccharification and different treatments on ethanol yield," Afr. J. Biotechnol, vol. 4, no.7, pp. 657-659, 2005.

[6] K Janani., M Ketzi., S Megavathi. D Vinothkumar. And N.G Ramesh Babu, "Comparative studies of ethanol production from different fruit wastes using Saccharomyces cerevisiae," Int. J. Innvat. Res. Sci.J. Engg. Technol, vol. 2, no. 12, pp. 7161-7167, 2013.

[7] S. Joshi, S. R. Dhopeshwarker, U. Jadav, R. Jadav, L. D'souza, and D. Jayaprakash, "Continuous Ethanol Production by Fermentation of Waste Banana Peels Using Flocculating Yeast,"Indian J. of Chem. Tech, vol. 40, pp. 325.

[8] K. Manikandan, , V. Saravanana, and T. Viruthagiri, "Kinetics on ethanol production from banana peel waste using mutant strain of Saccharomyces cerevisiae," Ind J. Biotechnol, vol. 7, pp. 83 – 88, 2008.

[9] R.Shyam Kumar, I. Ganesh Moorthy, R. Rajeswari and H. Harikrishnan, "Utilization of waste ripe Banana, and peels for Bio ethanol production using *Saccharomyces cerevisiae*," J. Biosci. Res.,. Vol. 2, no. 2, pp. 67-71, 2011

[10] V. Reddy and V. Reddy, "Production of ethanol from mango (*Mangifera indica* L.) fruit juice fermentation," Res. J. Microbiol, vol. 2, no. 1, pp. 763-769, 2007.

[11] S. Muttara, and D.J. Nirmala, "Production of alcohol and acetic acid fro pineapple waste," J. Water Quality Bull, vol. 7, no. 2, pp. 76-82, 1983.

[12] K. Pramanik and D.E. Rao, "Kinetic study of ethanol fermentation of grape waste using Saccharomyces cerevisiae yeast isolated from toddy," J. Institution of engineers (I), vol. 85, pp. 53-58, 2005.

[13] M.S. Asli, "A study on some efficient parameters in batch fermentation of ethanol using Saccharomyces cerevesiae SCI extracted from siahe sardasht pomace," Afr. J. Biotechnol, vol. 9, no. 20, pp. 2906-2912, 2010.

[14] B. Bhadra, S.R. Rao, , N.N.Kumar, P.Chaturvedi, P.K.Sarkar, and S. Shivaji, "Pichia cecembensis sp. nov. isolated from a papaya fruit (*Carica papaya* L., Caricaceae)," FEMS Yeast, Res vol. 7, pp. 579–584., 2007.

[15] AV Martini, and A.Martini, "A Taxonomic Key for the Genus Saccharomyces," Systematic and Applied Microbiology. Vol.16, no. 1, pp. 113-119, 1993.

[16] JA Barnett, R. Payne and D. Yarrow., Yeasts, "characteristics and identification," 3rd edn. Cambridge Univ. Press, Cambridge UK. 2000

[17] C.P. Kurtzman, and C.J. Robnett, "Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences," Antonie Van Leeuwenhoek , vol. 73, pp. 331–371.,1998.

18] DL. Miller, " Use of Dinitrosaliylic acid as reagent of reducing sugars," Anal. Chem. Vol. 31, pp. 426-428, 1959.

[19] Jr A Caputi, M Ueda and T.Brown., "Spectrophotometric determination of ethanol in wine," American. J. Enol. Viticul. Vol. 19, pp. 160-165.,1968.

[20] J Lodder., "The yeasts: A Taxonomic study," NorthHoll and Publishing, Amsterdam. 1971.

[21] T. Boekhout, and C.P. Kurtzman, "Principles and methods used in yeast classification, and an overview of currently accepted yeast genera," In K. Wolf (Ed.), Non-conventional Yeasts in Biotechnology: a Handbook (pp. 1-81). Berlin: Springer Verlag., 1996.

[22] M.W.Miller, , H.J. Phaff and H.E. Snyder. Jan, "On the occurrence of various species of yeast in nature," in: Mycopathologia, Vol. 16, 1, , pp. 1-18., 1962.

[23] P Gunasekaran, K.Chandraraj, "Ethanol fermentation technology-Zymomonas mobilis," Cur Sci., vol. 77, pp: 56–68., 1999.

[24] O. Obire, R. Ramesh Putheti, A. A Dick and R. N. Okigbo, "Biotechnology Influence for the Production of ethyl Alcohol (Ethanol) from Waste Fruits," E. J. of Sci. Technol, vol. 3, no. 3, pp. 17-32., 2008.

[25] N Sharma, KL Kalra, HS Oberoi, S Bansal, "Optimization of fermentation paramet ers for production of ethanol from kinnow waste and banana peels by simultaneous saccharification And fermentation," Ind J Microbiol, vol. 47, pp. 310–316., 2007.

[26] S Bhushan, K Kalia, M Sharma, B Singh, PS Ahuja, "Processing of AP for bioactive molecules," Crit. Rev. Biotechnol. Vol. 28, no. 4, pp. 285-296.