

Short Communication

Production of wine from Tamil Nadu traditional rice varieties

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Article history

Received: 2 January 2014

Received in revised form:

21 May 2014

Accepted: 27 May 2014

Keywords

Rice wine

Saccharomyces cerevisiae

Enzymatic pretreatment

Anaerobic fermentation

Abstract

An experiment was conducted to study the preparation of wine using locally available different rice varieties viz., CR1009, ADT 36, and IR 20. The standard yeast strain such as *Saccharomyces cerevisiae* was used for wine preparation using enzymatic pretreatment method, to obtain maximum reducing sugars. Among the different pretreatment methods, enzymatic pretreatment with Diastase α -amylase was selected for hydrolysis in wine preparation as it shows the highest release of reducing sugars. The chemical analysis of wine prepared with *Saccharomyces cerevisiae* at 5 per cent inoculums level. The chemical analysis and organoleptic evaluation showed that efficient wine can be prepared using standard yeast strain *Saccharomyces cerevisiae* at 5 per cent inoculums level with alcohol content.

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Introduction

Rice (*Oryza sativa* L.) a native to South East Asia is one of the leading food crops of the world and is second only to wheat in terms of annual production for food use. It is the main staple food for about the 60 % of the world's population rice is predominantly an Asian crop, 95 % of it is being produced and consumed in the south East Asian countries extending from Indo-Pakistan sub-continent to Japan. Rice is the most important food crop of India, nearly three-fourth of the people in the country subsist on it. India has the largest area under rice in the world but ranks second in production after China. It is grown in India under diverse agro-climatic conditions including irrigated, upland and lowland conditions. In India, rice is grown in an area of 449.72 lakh hectares, with a production of 905.5 lakh tons. India is the second largest exporter of rice and the export being 67.66 lakh tons in the last year (Anonymous, 2003).

Starch is the major constituent of rice and makes up to 90 % of rice in dry weight. Considerable attention has been given to the production of different beverages from various sugary substrates such as starchy materials viz. rice, wheat, barley etc. Most biological processes concerned with the conversion of starchy materials into alcoholic beverages have three steps, liquefaction of starch, enzymatic saccharification and fermentation (Suresh *et al.*, 1999). The well-known fermented rice foods in liquid form are rice wine rice beer and rice vinegar.

Wine is fermented beverage of cereals, fresh fruits, etc. Wine from rice is produced after saccharification of starch by microbes, enzymes (especially,

commercial α -amylase) etc. The wine is a complex mixture of organic and inorganic substances like carbohydrates, proteins, amino acids, ethyl alcohol, organic acids, inorganic acids and micronutrients etc. The quality of wine depends on the composition of rice. The wine quality differs with rice varieties and also with different yeast strains.

Since, from ancient times rice wine is popular in various parts of world and also in some part of India. The rice wine has been developed from very primitive Thai rice wines to highly sophisticated Japanese Sake which itself developed from very primitive beverage. Even the Korean beverages yakju and takju were originally were originally made from rice which are ancient beverages popular among the common people (Park *et al.*, 1977). Chinese rice wines are traditional alcoholic beverages in China, with more than 14,000 years of history and are popular in China particularly in Southern part of the country. The present study was taken up with rice varieties CR 1009, ADT 36 and IR 20, with enzymatic pretreatments and *Saccharomyces cerevisiae* for wine production.

Materials and Methods

Selection of Locally Available Rice Varieties and selection of Microorganism for Wine Production An investigation on rice wine production carried out in the Microbiology Department, Refsyn Bioscience Pvt., Ltd., and Pondicherry using different varieties of rice, to hydrolyse fermentable sugars with enzymatic pretreatment method, and anaerobic fermentation using *Saccharomyces cerevisiae*. The pure culture of *Saccharomyces cerevisiae* was obtained from NCIM

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3090, Pune. The culture was maintained on Potato Dextrose Agar slant. The culture was preserved at 4°C and used in all further study. In the present investigation popular varieties of rice of Tamil Nadu were selected for the study. ADT 36, CR1009 and IR 20.

Wine production

An investigation on rice wine production carried out in the Department of Microbiology, Refsyn Bioscience Pvt., Ltd, and Puducherry during June to Aug. 2013 using different varieties of rice, to hydrolyse fermentable sugars with enzymatic pretreatment method, and anaerobic fermentation using *Saccharomyces cerevisiae*, the pure culture of *Saccharomyces cerevisiae* was obtained from NCIM 3090. The culture was maintained on Potato Dextrose Agar slant. The culture were preserved at 4°C and used in all further study. In present investigation popular varieties of rice of Thanjavur and Vilupuram districts were selected for the study. CR 1009, ADT 36 and IR 20.

Preparation of the substrate

A known quantity of each of different varieties of rice were steep for 1 hour separately, cooked and mashed separately. Further 25 g of mashed substrate weighed separately and volume was made to 35 ml with distilled water for hydrolysis of fermentable sugars.

Optimization of enzymatic concentration

Commercial α -amylase (Diastase α -amylase) was prepared with buffer 10 mM CaCl_2 at 1 per cent concentration was prepared and added to the smashed substrate for saccharification.

Optimization of substrate

The prepared substrate was diluted with distilled water at 1:1 concentration (Substrate: distilled water). This was mixed with 1% of commercial α -amylase enzyme and kept for inoculation optimization of temperature. Pretreated substrate was kept for incubation at 60°C for maximum hydrolysis.

Optimization period for hydrolysis

The incubation period for hydrolysis of substrate for commercial α -amylase was 5 hours for maximum hydrolysis.

Production of wine by fermentation

The hydrolysate from the pretreatment was ameliorated by adding cane sugar and pH was adjusted to 3.5 by adding baking soda or Sodium bicarbonate. Activity of the natural flora of the rice was suppressed

by adding 200 ppm of Sodium sulphite and kept for 4-5 hours. The whole process was supplemented with di-ammonium hydrogen phosphate (0.5 g/l) as a source of Nitrogen and Phosphorus.

The sample was inoculated with *Saccharomyces cerevisiae* (previously prepared in 100 ml of rice variety must) at 5%. This rice was fermented anaerobically at $28 \pm 2^\circ\text{C}$ (21 days) it was filled through double folded clean muslin cloth, pasteurized and stored in glass bottle at 20 to 24°C. Then 100 ppm of Sodium benzoate was added. Then the clear wine was transferred into clean bottles and pasteurized at 65°C for 30 mins. The bottles were kept for maturation at low temperature (15-16°C).

Estimation of ethanol

The ethanol was estimated by colorimetric method as described by Caputi *et al.* (1968). 1 ml of representative samples from each treatment was transferred to 250 ml round bottom distillation flask connected to the condenser and was diluted with 30 ml distilled water. The sample was distilled at 74-75°C. The distilled sample was collected in 25 ml of 0.23 N $\text{K}_2\text{Cr}_2\text{O}_7$ reagent, which was kept at receiving end. The distillate containing alcohol was collected till total volume of 45 ml was obtained. Similarly standards (05-25 mg ethanol) were mixed with 25 ml of $\text{K}_2\text{Cr}_2\text{O}_7$ separately. The distillate of samples and standards were heated in water bath at 60°C for 20 minutes and cooled. The volume was made up to 50 ml with distilled water and the optical density was measured at 600 nm using spectrophotometer. The standard curve was plotted considering the concentration against absorbance.

Result and Discussion

The experimental result on selection of rice varieties, enzymatic pretreatment method for efficient hydrolysis, anaerobic fermentation with *Saccharomyces cerevisiae* for wine preparation was done. The chemical analysis of wine and organoleptic evaluation showed that efficient wine can be prepared using *Saccharomyces cerevisiae* at 5 per cent inoculum level with alcohol content.

In the present investigation, different rice varieties were studied for their starch and protein content. Significant difference was observed in starch and protein content between the different varieties. Previous work was done on rice by using different pretreatment methods. Among the different pretreatment method acid pretreatment, (Lancater *et al.*, 1954), Microbial pretreatment (Coronel *et al.*, 1981), using Bacterial culture of *Aspergillus oryzae*, *Aspergillus niger* and enzymatic

Table.1. Reducing Sugar, Starch and Protein content of different rice

S.No.	Rice varieties	Reducing sugar	Starch (mg/100 gm)	Protein (mg/100 gm)
1	CR 1009	5.11±0.02	309.5 ±0.12	189.61±0.11
2	ADT36	5.44±0.15	308.25±0.08	246.44±0.24
3	IR 20	5.56±0.08	309.5 ±0.13	163.38±1.02

pretreatment (especially Diastase α amylase) used for efficient hydrolysis of wine.

Among the different pre-treatments used for hydrolysis, commercial α -amylase pretreatment was selected for further hydrolysis and wine preparation from different rice varieties using *Saccharomyces cerevisiae* at 5 per cent inoculum level, since the pretreatment gave maximum reducing sugars as compared to other pre-treatments (acid, microbial and crude enzymatic pre-treatment). Maximum alcohol was obtained at an IR20 per cent (6.7%) followed by CR 1009 was (6.5%) while low alcohol content was obtained at ADT36 at (6.2%) percent.

The observations recorded on chemical analysis of wine prepared from different rice varieties clearly indicated that there was significant difference in the rice varieties with respect to pH. pH of the wines depends on the acid and sugar content of the wines. Similar work was done by Sanchez *et al.* (1988) and reported pH of wine prepared from different rice varieties which ranged from 4.65 to 5.0. Citric, malic and tartaric acids are important acids present in wine that measure the total titrable acidity of wine sample. Sanchez *et al.* (1988) used the characters viz., aroma, colour, flavour, astringency, acidity and general quality for organoleptic evaluation of wines from ten different rice varieties. Kundu *et al.* (1980) evaluated exotic grapes grown in Haryana for white table wines. Depending on the nutritional and organoleptic evaluation of rice wine from different varieties, the variety giving good quality wine with highest score was selected for further experiments. The saccharification rate was checked in enzymatic pretreatment in the selected rice variety. Optimized conditions were selected for enzymatic pretreatments. Pretreatment with commercial amylase showed highest release of reducing sugars of 63.11 mg per g. The effect of different pre-treatment on protein content was found to be non-significant.

Results obtained from enzymatic pre-treatment with commercial amylase was found effective and had potential to release the fermentable sugars. The potency of sugars and enzymes are biocatalysts having specific action and target oriented. In case of other enzymes there might be chances of nucleases which degrade the enzyme and there may be interference of other ions inhibiting the reaction.

Colour and brightness of the wine decreased significantly with increase in the inoculum level. Since, wine is a consumable product, it is evaluated

by a panel of judges by organoleptic procedures. Results showed that the organoleptic score increased with increase in the inoculum level. The sensory evaluation proved that the wine prepared by *Saccharomyces cerevisiae* was found superior than the wine prepared from native yeast.

Conclusion

From the present study, it can be concluded that the commercial amylase pretreatment giving maximum reducing sugars was suitable to screen different rice varieties for wine preparation using the *Saccharomyces cerevisiae*. The different rice varieties varied significantly with respect to starch and protein contents. Significant difference was observed between the wine samples of different rice varieties with respect to various parameters like protein, residual sugar and alcohol content. The present investigation clearly brought out that maximum hydrolysis of rice substrate can be achieved by pure amylase pretreatment at 1% concentration. The released reducing sugars can be successfully converted to alcohol using *Saccharomyces cerevisiae* at 5% inoculum level for producing good quality wine.

References

- Anonymous, 2003. Indian economic data research centre, Indian Agriculture p. 120. New Delhi.
- Caputi, A.J., Ueda, M. and Brown, T. 1968. Spectrophotometric determination of ethanol in wine. American Journal of Enology Viticulture 19: 160-165.
- Coronel, L. M., Velasquez, A. O. and Castillo, M. C. 1981. Some factors affecting the production of rice wine using an isolate of *Aspergillus oryzae*. Philippine Journal of Science 110(1/2): 1-9.
- Kundu, B. S., Bardiya, M. C., Daulta, B. S. and Tauro, P. 1980. Evaluation of exotic grapes grown in Haryana for white table wines. Journal of Food Science and Technology 17(5): 221-224.
- Lancaster, E. B., Moulton, K. J., Uhl, D. and Sohns, V. E. 1964. Modification of cereal flours with hydrochloric acid. Cereal Chemistry 41: 484-492.
- Park, K. I. and Mheen, T. I. 1977. Korean yakju and takju. In Park, K. I., Lee, L. H. Chang, C. H. Lee, S. R. and Kwon, T. W. Symposium on Indigenous Fermented Foods, p.122, Bangkok, Thailand.
- Sanchez, P.C., Juliano, B.O., Laude, V.T. and Perez, C.M. 1988. Nonwaxy rice for tapuy (rice wine) production. Cereal Chemistry 65(3): 240-243.
- Suresh, K., Kiransree, N. and Venkateswar Rao, L. 1999. Production of ethanol by raw starch hydrolysis and fermentation of damaged wheat and sorghum. Bioprocess Engineering 21: 165-168.