Development of wine from pectinase treated Palmyrah fruit pulp by using toddy yeast

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Abstract - Borassus flabellifer L (Palmyrah palm) is widely distributed in North - East part of Sri Lanka. Among the palmyrah products palmyrah fruit pulp is rich in sugar, provitamin A, vitamin C and lycopene which could be used for the development of wine. Fermentation of fruit pulp was carried out by using yeast which was isolated from palmyrah toddy and selected based on the alcohol tolerance and cell mass in fruit pulp. Palmyrah fruit pulp contains 6.7% of pectin. To improve the clarification of wine, pectinase treatment was carried out at different concentration, temperature and incubation time and the selected optimum condition was 1%, 40 oC and an hour respectively. Fermentation medium were prepared by using optimized amount of brix (20o) and pH (4.5) with pectinase treated and untreated palmyrah fruit pulp. Then selected yeast strain (P7) was inoculated into the pasteurized pulp and allowed to ferment. Changes in alcohol content, total soluble solids, pH and titratable acidity were determined for 5 days. After 14 days, volatile acidity, total residue on evaporation and methanol content were determined. Pectinase treated wine gave the highest alcohol content (9.6 ± 0.06 v/v %) compared to untreated wine (6.8 ± 0.12 v/v %). According to the analysis of residue on evaporation, pectinase treated (3.94 ± 0.115g/100ml) wine gave fewer residues on compared to untreated wine (9.29 ± 0.035g/100ml). Pectinase treated wine is free of methanol but the untreated wine contains methanol. The pectinase treated wine was selected as the best based on high alcohol content, absence of methanol, less residue on evaporation. Therefore this study revealed that best wine could be produced by using pectinase treated palmyrah fruit pulp with optimum fermentation condition.

Keywords - Palmyrah, Pectinase treated wine, Toddy, Yeast isolate.

Introduction

Borassus flabellifer L, belongs to family Arecaceae, commonly known as Palmyrah palm is a native of tropical Africa. It is widespread in the arid tropic country like India, Sri Lanka and South East Asia, East Africa and South America[1][2].

The Palmyrah fruit pulp contains a considerable amount of soluble sugars. Therefore, it can be considered as a potential source of raw material for other industries or for the development of new products through value addition. Pulp has many potential uses apart from traditional products. These include its use in jams and cordials and as a source of pro-vitamin A, pectin and potable alcohol [2]. Nearly 50% of the Palmyrah fruits are underutilized. Most are thrown or used for animal feeding because its uses are limited mainly due to the presence of a bitter compound called flabelliferins and lack of interest in converting this pulp into consumer attractive value-added products [2].

Fruit wines are undistilled alcoholic beverages usually made from grapes or other fruits. Wines made from fruits are often named after the fruits. According to the Turkish Food Codex (2008) the alcohol content of the wine should be minimum 9%.

Development of Palmyrah fruit pulp wine is an alcoholic beverage which optimizes the underutilized fruit pulp into viable industrial product in Sri Lanka.

The yeast strain used during fermentation can have a great influence on the ultimate quality and quantity of the final product. So, the selection of the yeast strain is the crucial step for an expected and assured good quality wine[3].So first, best yeast strain for the wine production was found out.

As Palmyrah fruit pulp contains 6.7% of pectin, after degradation, methyl groups associated with pectin are released as methanol which is poisonous. Thus, removal of pectin before wine making is necessary [2][4]. So, optimum conditions needed for pectinase treatment such as enzyme concentration, incubation temperature and time was found out.

Materials

Same types of fresh Palmyrah fruits were collected from Navatkuli area in Jaffna. The pulp was extracted manually using water in the ratio (v/v) of 1:1.Palmyrah Toddy was collected from Karanavai area in Jaffna

Methodology

Isolation of yeast

Yeast from toddy was isolated using the streak plate method. Then it was activated using YEPDA medium. Isolated yeast was identified using light microscope.

Selection of best strain

Ten yeast strains were isolated and inoculated into 6%, 8%, and 8.5% alcohol medium and also inoculated into 25% Palmyrah fruit pulp. After 4 days test tubes were shaken well. Then the absorbance was recorded at a wavelength of 595 nm using UV Spectrophotometer.

Pectinase treatment

Fruit pulp was treated with PEC600 purchased from Sunson Industry Group Co LTD, China. Concentration, incubation time, and incubation temperature were taken as the variables to treat the Palmyrah fruit pulp. To determine the optimum conditions, total soluble solids, titrable acidity were measured. Finally TSS/TA value was analysed through general full factorial design in Minitab 17 statistical package.

Selection of optimum Fermentation condition

The pH was adjusted to 3.5, 4.0 and 4.5 using citric acid. And brix was adjusted to 80,100,120,150,200 using sugar. Then alcohol was measured on 2nd day and 4th day.

Comparison of selected yeast strain with baker's yeast

At optimum fermentation condition selected yeast strain and bakers' yeast were inoculated. Alcohol content was compared for 7 days.

Development of wine

Selected best yeast strain was activated using YEPDA medium. Then optimum conditions (pH and Brix) were set. Then using the results of statistical analysis, Pectinase treatment was done at selected optimum conditions. (Normal wine was prepared without pectinase treatment). Pulp was pasteurized in 850 for 30 minutes. Then it was cooled to room temperature. After that the juice was filtered using muslin cloth. Then activated yeast strain was inoculated into the fruit pulp and allowed to ferment.

Analysis of wine

Alcohol content (Ebulliometer), pH (pH meter), Total soluble solids (Refractometer), Titrable acidity (AOAC 962.12) were measured for 5 days after the inoculation of yeast. After 14 days volatile acidity, residues on evaporation (Food safety and standards authority of India, 2015) and Methanol content (GC-MS) were measured.

Results and Discussion

Selection of best strain

The shape of the cells was found as oval, circular and all the isolates had budding cells similar to yeast. Quality of wine depends upon the growth and activity of yeast and growth differs with genera and species of yeast strains. Efficient yeast strain can produce maximum cell biomass, metabolize different carbon sources and produce higher alcohol. Efficiency of yeast strains which were isolated from the Palmyrah toddy was measured on the basis of growth in Palmyrah fruit pulp and ethanol tolerance in alcohol medium. According to the Fig. 1, Based on high alcohol tolerance and high growth in Palmyrah fruit pulp, yeast strain P7 was selected as the best and it was followed by P4 and P9 .

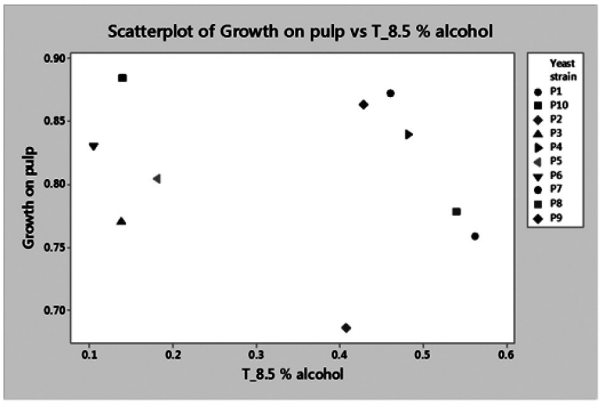

Fig.1: Effect of 8.5% alcohol content on growth of yeast strains

Pectinase treatment

Commercial pectinases are used in wine making process to improve juice yields, and also to improve clarification and filterability. According to the recent studies conducted in the physicochemical analysis of grape juice samples after the treatment of pectinase enzyme, the TSS/TA was considered as the factor representing the balance between the acid and sweet taste of grape juice [5].

According to the results of ANOVA 1% pectinase enzyme, 40 0C incubation temperature, 1 hour incubation time and 2 % pectinase enzyme, 40 0C incubation temperature, 1 hour incubation time were determined as the best two levels of treatment combinations, respectively have a highest TSS/TA ratio value in Palmyrah fruit juice.

Selection of optimum Fermentation condition

Juice sometimes fails to possess the desired acidity and pH favourable for the fermentation. So it must be adjusted before fermentation. According to Fig.2, pH 4.5 is considered as optimum pH for alcohol production. The pH of raw Palmyrah juice is in the range of 4.5-5.0.So if needed pH can be adjusted to 4.5.

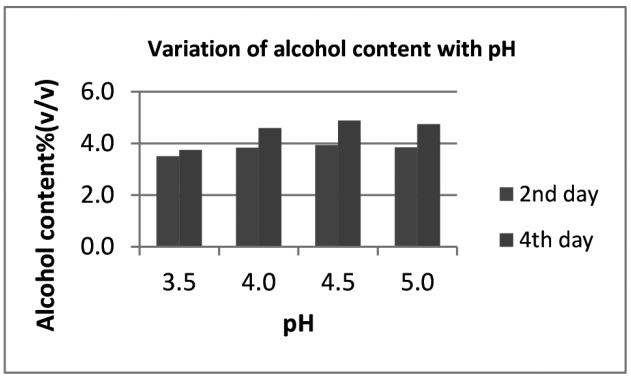

Fig. 2: Variation of alcohol content with pH

Comparison of selected yeast strain with baker's yeast

Amount of alcohol produced depends on the type of yeast strain used as well. According to the Fig. 3, selected palm yeast produce high amount of alcohol rapidly compared

to baker's yeast. Maximum alcohol content 7 %(v/v) was obtained within 3 days when using selected palm yeast under the fermentation conditions of brix 200 and pH 4.5.

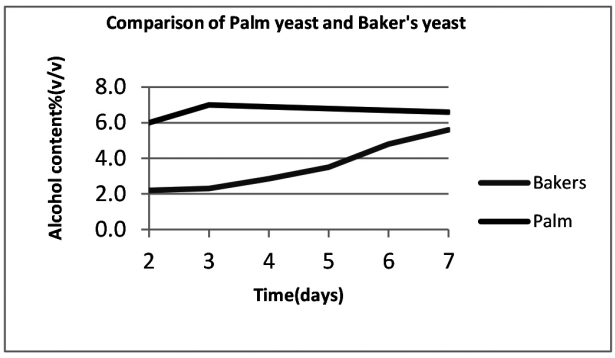

Fig. 3: Comparison of Palm yeast with Baker's yeast

Analysis of wine

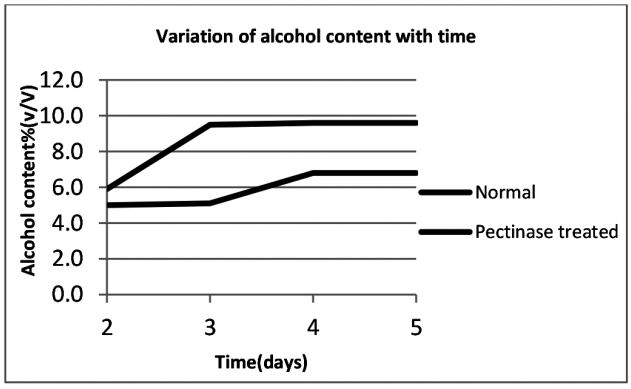

Fig. 4: Variation of alcohol content of wine with time

According to the Fig.4, highest alcohol content of 6.8 ± 0.12% was got for the normal wine on 4th day after the inoculation of yeast. But for the pectinase treated wine highest alcohol of 9.6 ± 0.06% was got on 4th day after the inoculation of yeast.

But pH of normal wine decrease from 3.58 to 3.31 in 5days. And the pH of pectinase treated wine decrease from 3.30 to 3.11 in 5days. The decrease of pH pectinase treated sample with normal wine can be justified because of the formation of galacturonic acid during the pectinase treatment has an effect in reducing the pH.

For the normal wine sample titrable acidity increased from 0.29 to 0.38 in 5 days. But for the Pectinase treated wine it was increased from 0.48 to 0.53.

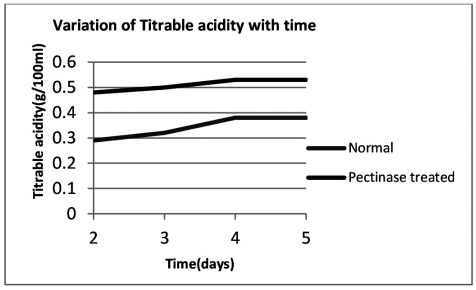

Fig. 5: Variation of Titrable acidity with time

For the normal wine volatile acidity was got as 0.11 ± 0.006. And for pectinase treated wine it was got as 0.14 ± 0.010. Volatile Acidity is caused by a type of bacterial spoilage which produces large amounts acetic acid (vinegar) which is a serious wine fault. The legal limit of VA in finished wines is 1.2 gram/liter in whites, 1.4 g/l in reds, and 1.2 g/l in dessert wines.

Total residue on evaporation represents the sum of both dissolved and suspended (including colloidal) material in a sample. Normal wine (9.29 ± 0.035g/100ml) has more residues on evaporation compared to pectinase treated wine (3.94 ± 0.115g/100ml).This was because of the addition of this enzyme lowers viscosity and causes cloud particles to aggregate into larger a unit, which settles as sediment makes the filtration and clarification of wine easier.

According to Fig.6, Methanol was detected in normal wine using GCMS. But not detected for pectinase treated wine. This prove that, use of pectinase enzyme reduce the release of methanol as a result of hydrolysis of pectin by the pectinase.

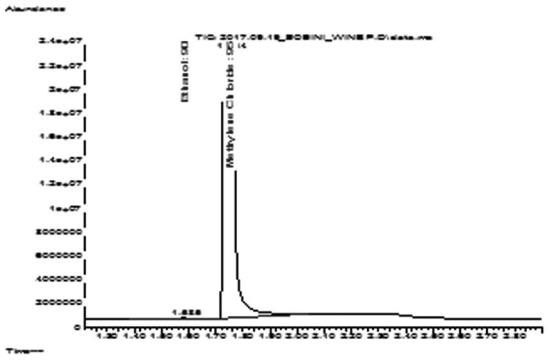

Fig. 6: GCMS result for normal wine

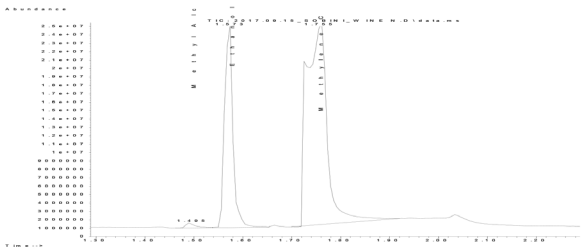

Fig.7: GCMS result for pectinase treated wine

Conclusion

The pectinase treated wine was selected as the best based on high alcohol content, absence of methanol, less residue on evaporation. Therefore this study revealed that best wine could be produced by using pectinase treated palmyrah fruit pulp with optimum fermentation condition of brix (20o), pH (4.5) and yeast which was isolated from palmyrah toddy.

Recommendations

To specifically identify the best isolated yeast strain from toddy, Molecular and genetic characterization can be done using PCR method. Further analysis on wine can be done to find out other volatile compounds in wine using GCMS method

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