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Effect of Fermentation Period on Alcohol Content, pH, TSS and Titrable Acidity during Microbial Processing of Coconut Water for the Development of Coconut Wine

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ABSTRACT

Background: *Saccharomyces cerevisiae* is one of the important yeast found to be superior in the fermentation technology.

Methods: The present study was undertaken to study the periodical changes in TSS, pH, acidity and alcohol content during the fermentation process of wine preparation from coconut water. With different TSS levels (7, 9, 11 and 13°Brix) adjusted with sugar and 9°Brix was the optimized sugar level.

Result: TSS in wine was constantly reduced until fermentation was complete. Though the TSS reduced significantly during the first stage of fermentation, it continued to decline at a considerably slower rate until the fermentation was complete. It was observed that the TSS level does not affect the pH of coconut wine, but pH decreased with the increase in fermentation period because of increase in titrable acidity and alcohol. It was also noticed that the titratable acidity was found least at 6th day and maximum at 12th day, titratable acidity increased as fermentation progressed which was consistent with the fall in pH. The higher the TSS levels yields high titratable acidity during fermentation. Immediately after inoculation, the yeast count rises sharply on the first day of fermentation. It then tends to rise drastically till the third day, after which the yeast count is found to have declined until the end of fermentation. The coconut wine was prepared from coconut water added with sucrose as a source of sugar. *Saccharomyces cerevisiae (*MTCC 171) was used as reference strain and yeast isolate BRYI-1 produced the best results of alcohol content and sensory characteristics when compared to reference yeast strain.

Key words: Alcohol, Coconut wine, Fermentation, pH, Titratable acidity, TSS.

INTRODUCTION

Coconut is a fruit produced by coconut palm (*Cocos nucifera*) which belongs to the family of Arecaceae. Coconut is found in tropical regions generally within 22°N and S of the equator and most commonly near the sea coast (Polemer and Ronie, 2017).

Coconut water is a refreshing beverage consumed worldwide as it is nutritious and beneficial for health. There is increasing scientific evidence that supports the role of coconut water in health and medicinal applications. (Joshi *et al.,* 2013). Coconut water is naturally occurring, is very rich in potassium, contains sodium chloride, carbohydrate (Chavalittamrong *et al*., 1982) and is viewed as the hydrating beverage with antioxidant properties (Campbell *et al*., 2000).

The Karnataka state is blessed with ten agro-climatic regions suitable for growing coconut all round the year. The total area under coconut is 5.13 lakh ha in 2017-18 with production of about 6,773.05 million nuts and is the second largest in area, production and productivity (Anonymous, 2018).

An alcoholic beverage is a potable drink, which contains alcohol as an ingredient. The alcoholic beverages are broadly classified into three categories such as fermented beverages, distilled spirits and fortified wines. The fermented beverages are those which are produced from fruit juice or plant sap by natural fermentation. In India, wild dates (*Phoenix sylvester's*), coconut palm (*Cocos nucifera*), Palmyra (*Borassus flabellifer*) palms *etc*. are frequently used for the purpose of wine production. (Joshi *et al.,* 2013). The earliest winery is the 6100 years old in Armenia. The word "wine" comes from Proto-Germanic "Winam", an early borrowing from the latin Vinum, "vine" (Johnson, 1989).

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Coconut wine is light pale in colour has powerful aroma and sweet taste refreshing beverage. It is a fermented beverage, obtained from fermentation of coconut water with the inoculation of yeast (Polemer and Ronie, 2017). As garden fresh tender coconuts are devoid of wild yeasts and bacteria, wine made of it will be automatically pure and natural. The wine produced was healthy, hygienic and considered as nutritional beverage (Augustine, 2007). The wine production from coconut water and coconut milk gave good quality results for flavoring of coconut water wine with efficient alcohol percent, since the combination of those two products in the form of 'coconut wine' will serve healthy and refreshing drink (Sunil *et al*., 2013).

This beverage contains unique chemical composition of sugars, vitamins, minerals, amino acids and phytohormones and a rich source of electrolytes and natural salts, especially potassium and magnesium. It is low calorie and nearly fat-free, low in sugar as well as containing a little fiber to moderate absorption and is rich in cytokinins, or plant hormones, which have anti-aging, anti-cancer and anti-thrombolytic effects in humans (Idise and Okiemute, 2011).

Coconut water obtained from mature nuts, when harvested for the production of copra and coconut oil, is wasted on a large scale in several tropical countries (Brito *et al*., 2002). Large quantities of coconut water are produced in coconut industries, temples, which is unused; its nutritional value will just go waste and contribute to environmental pollution (Polemer and Ronie, 2017). This coconut water can be made use for the production of value added products like Nata-de-coco (Narayanaswamy 2010), tender coconut jam (Shahanas *et al*., 2019), coconut based chocolate (Divya and Baskaran, 2020), sweets (Singh *et al*., 2017), therapeutic drinks, refreshment drinks, vinegar, sparkling wine, coconut champagne, components for tissue culture media for plants (*e.g*., banana, orchids), fermentation media for enzyme production, growth regulator, biogas generation and fermented coconut water beverages (Chauhan *et al.,* 2014).

Local farmers and processors will be encouraged to gather more coconut to be utilized into a valuable product which is the coconut wine for commercialization and standardization (Neela and Prasad, 2013).

Keeping in view the above facts, the proposed study is aimed to develop quality fermented beverage from coconut water using yeast. The present study was conducted by isolating yeasts from different fruit sources and optimization and evaluation of beverage for the different fermentation factors with the following objectives.

MATERIALS AND METHODS

Microbial processing of coconut water for the development of coconut wine was carried out at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru during 2019. The following are the material and methods used to conduct the experiments.

Coconut water, sugar and rotten fruits were obtained from local markets and reference culture *Saccharomyces cerevisiae* from MTCC, rotten fruits and coconut water used.

The isolation of yeasts was carried out from different fruit sources as per method given by Zahra *et al*. (2011). Different fruits were collected from the markets of Sahakarnagar, Hebbal, Yelahanka and other places of Bangalore for isolation of yeasts. A slightly rotten portion of the fruits were inoculated to test tubes containing coconut water and were allowed to grow. The formation of turbidity and alcoholic smell after 7 to 8 days indicated the presence of yeast.

By using standard methods, the yeast isolates were purified and characterized for further research. After being stained with cotton blue, these isolated cultures were examined under a microscope. By using the yeast extract peptone dextrose agar (YEPDA) medium as well as the standard plate count technique, the yeast population in the samples was counted. The strain *Saccharomyces cerevisiae* MTCC 171 obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh was used as reference culture. Further, yeast identification was attempted by comparing their morphological traits to those of the reference yeast, Saccharomyces cerevisiae MTCC 171.

Colonies grown on surface of the YEPDA media of Yeasts were transferred to coconut water broths and incubated for 48 h to isolate different yeasts. The broth was again plated out on YEPDA medium by streak plate method and incubated for 48 h, from which more isolated colonies were obtained. These colonies were selected for further purification. The selected isolates were sub cultured by streaking on respective agar medium frequently until a pure colony growth of each isolate was obtained. Cells were observed under microscope. Yeasts were streaked on YEPDA slants and preserved at 4°C in refrigerator for further studies.

Characterization of yeasts was done by observing colony and cell morphology followed by biochemical tests *viz.* catalase activity, acid, gas production and Exopolysacchride production. The isolated yeast strains screened for maximum alcohol production using coconut water (Neela and Prasad, 2013).

Yeast culture *(Saccharomyces cerevisiae)* inocula that had been purified and authenticated was added in a loopful to a conical flask that contains 250 ml of coconut water. In order to promote growth, the inoculated flask was kept at 26-28°C overnight. For fermentation investigations, they were then utilized at 5% (v/v) Francisco *et al.* (2010), Sevda and Rodrigues (2011).

Experiment was set up to determine the variation of alcohol content, pH, TSS and titrable acidity at different days of fermentation. Coconut wine inoculated with yeast isolates BRYI-1, BRYI-2, JKYI and PIYI was compared with reference strain. Experiment was set up with 100ml coconut water with 9% sugar, 10% inoculum and incubated for 6, 8, 10 and 12 days. (Fig 1).

Particulars	Details
Design	Completely randomized design (CRD)
Replication	Three
Treatments	T_1=Coconut water + BRYI-1
	T_2=Coconut water + BRYI-2
	T_3=Coconut water + JKYI
	T_4=Coconut water + PIYI
	T_5= Coconut water + Ref. yeast

Based on the results of optimized parameters like sugar concentration, inoculum levels and incubation period the evaluation of fermentation efficiency of yeast isolates for preparation of coconut wine was taken up. The results of yeast isolates were compared with the reference strain. Parameters like pH, TSS, titrable acidity, alcohol % and sensory evaluation were considered.

Particulars	Details
Design	CRD
Replication	Three
Treatments	T_1=Coconut water + BRYI-1
	T_2=Coconut water + BRYI-2
	T_3=Coconut water + JKYI
	T_4=Coconut water + PIYI
	T_5= Coconut water + Ref. Yeast
	T_6= Coconut water (Control)

RESULTS AND DISCUSSION

The yeast isolates were isolated from different fruits and vegetable sources and were confirmed preliminarily based on alcoholic smell. Samples which gave excellent alcoholic smell produced more alcohol. Yeasts isolates BRYI, JKYI, PIYI, GRYI and WMYI showed good results with respect to alcoholic smell and alcohol percentage.

Sandeep (2015) isolated yeasts from fruits and different food samples and they were identified and characterized based on cell morphology. The potential yeasts for ethanol production were found out with the monitoring for alcohol smell and further followed with the measurement of ethanol concentration. Based on their alcohol production capacity best four isolates were selected for further studies *viz*. BRYI-1, BRYI-2, JKYI and PIYI. The growth of standard yeast and other isolates in fermented coconut water for various fermentation periods was examined using a spectrophotometer and OD readings at 600 nm. The cell density found increasing with the fermentation period drastically up to 10 days of fermentation, further the rate of cell density was found declining (Fig 2). These results are in support with works of Idise and Emmanuel (2011). Evaluation of different yeast isolates for fermentation efficiency of coconut wine with comparision to reference yeast are presented in (Table 1).

The alcohol percentage was observed increasing for 6th and 8th day of fermentation and decreased with increasing number of fermentation days (Fig 3). Maximum alcohol percentage was observed at 8th day of fermentation. These could be due to increased metabolic activities leading to exhaustion of available nutrients with the concomitant production of alcohol. These results are in agree with the reports of previous workers (Amerine and Kunkee, 1968), (Chaudary and Chincholkar, 2006). The pH declined at higher rate at 8th day of fermentation and it further reduced in the rest of the fermentation days at very slow rate (Fig 4). Sievers *et al*. (1995) obtained similar results, where the pH value declined from an initial value during room temperature incubation due to acid production. These results are also in agree with Blanc (1996). Brix values observed decreasing with the fermentation days (Fig 5). These results are in agree with Shuklajasha *et al*. (2005), Idise and Emmanuel (2011) showed reducing sugar content decreased from an initial value in must to wine. The titratable acidity was increased from must to finished wine. As a result of yeast metabolism, Blanc (1996) found that TSS of the cultures was higher at the start of the incubation period and rapidly declined with fermentation period. The results of titrable acidity during fermentation period was found increasing with the number of fermentation days (Fig 6), these results are in accordance (Rodriguez *et al*., 2012).

Fig 1: Experimental setup of coconut wine fermentation.

The titrable acidity was lowest on day six of fermentation and highest on day twelve. These results are in agree with Idise and Emmanuel (2011), Akubor *et al.* (2003) that the titratable acidity increased as fermentation progressed which was consistent with the fall in pH. The malolactic fermentability of wines produced from the must differs

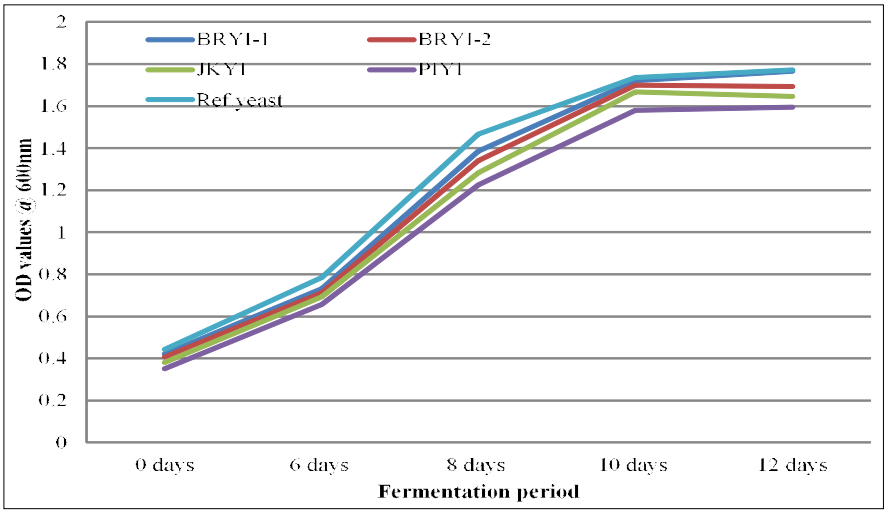

Fig 2: Yeast cell density during fermentation period.

Note: BRYI-1/BRYI-2 - Beetroot yeast isolate.

JKYI - Jackfruit yeast isolate.

PIYI - Pineapple yeast isolate.

Ref. yeast - *Saccharomyces cerevisiae* MTCC 171.

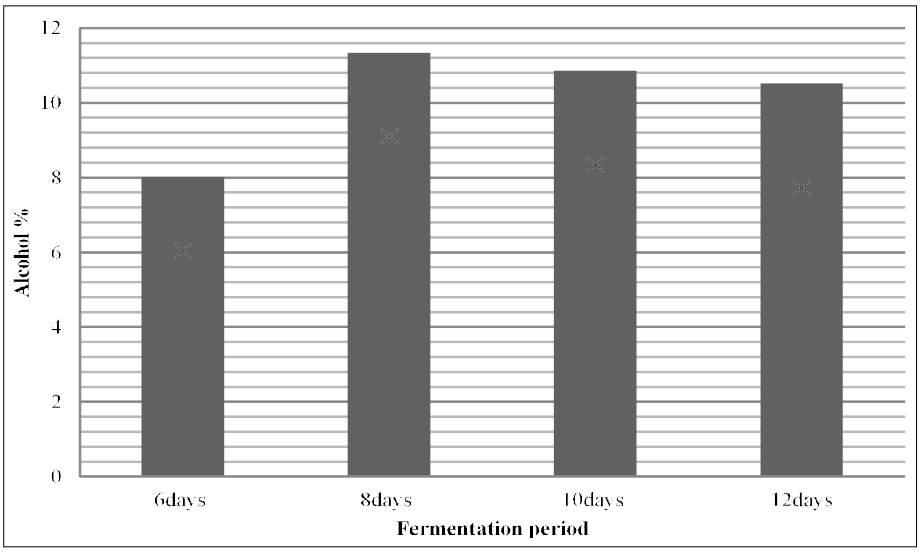

Fig 3: Effect of fermentation period on alcohol content in coconut wine.

Table 1: Evaluation of different yeast isolates for fermentation efficiency of coconut wine with comparision to reference yeast.

Treatments	pH	TSS (°Brix)	Titrable acidity	Alcohol (%)	Yeast counts ($\times 10^4$ cfu/ml)
BRYI-1	3.62^{e}	4.7^{f}	0.59^{a}	11.25^{a}	28.7
BRYI-2	3.83^{d}	5.0^{e}	0.55^{bc}	10.88^{b}	19.5
JKYI	3.72^{de}	5.4^{c}	0.53^{c}	10.56^{c}	17.8
PIYI	4.23^{b}	5.8^{b}	0.48^{d}	9.87^{d}	14.3
Ref. yeast	4.1^{c}	5.2^{d}	0.56^{b}	10.96^{b}	27.8
Control	4.95^{a}	7.2^{a}	0.40^{e}	2.20^{e}	7.6

Note: Initial pH: 5, initial TSS: 22 °Brix; Ref. yeast: *Saccharomyces cerevisiae*.

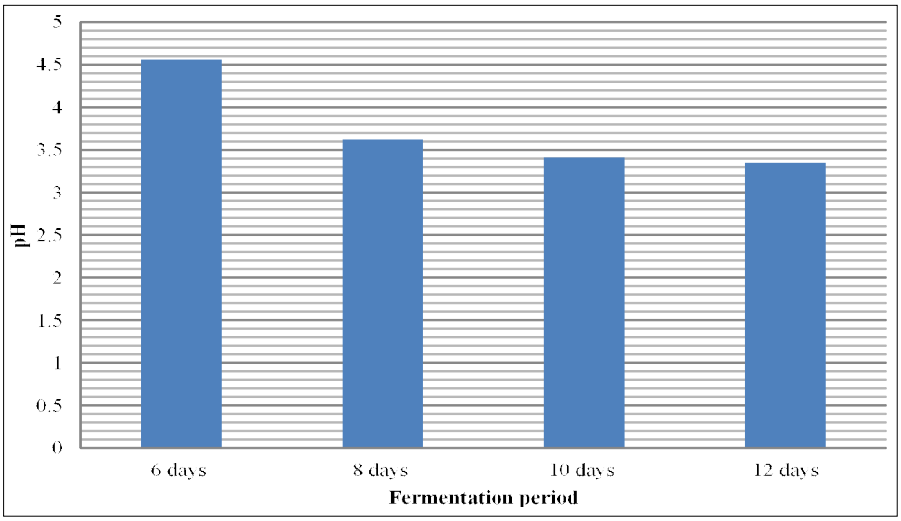

Fig 4: Effect of fermentation period on pH in coconut wine.

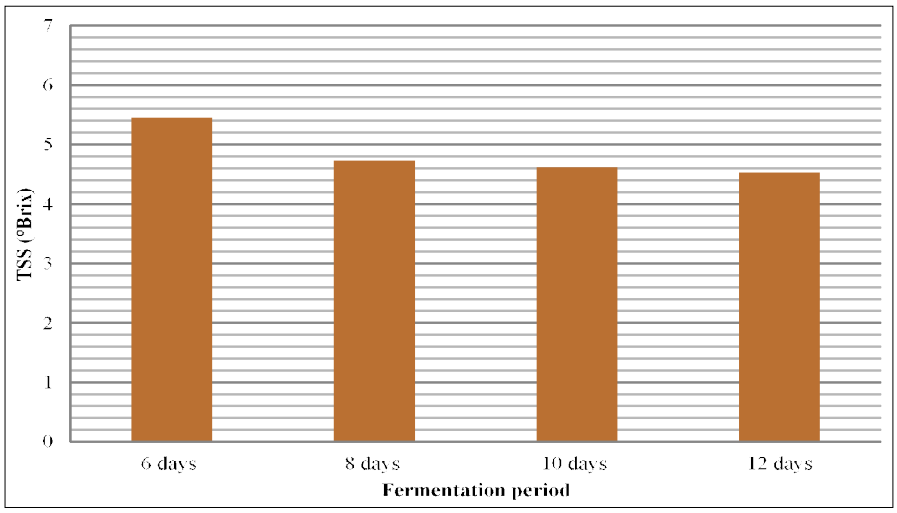

Fig 5: Effect of fermentation period on TSS in coconut wine.

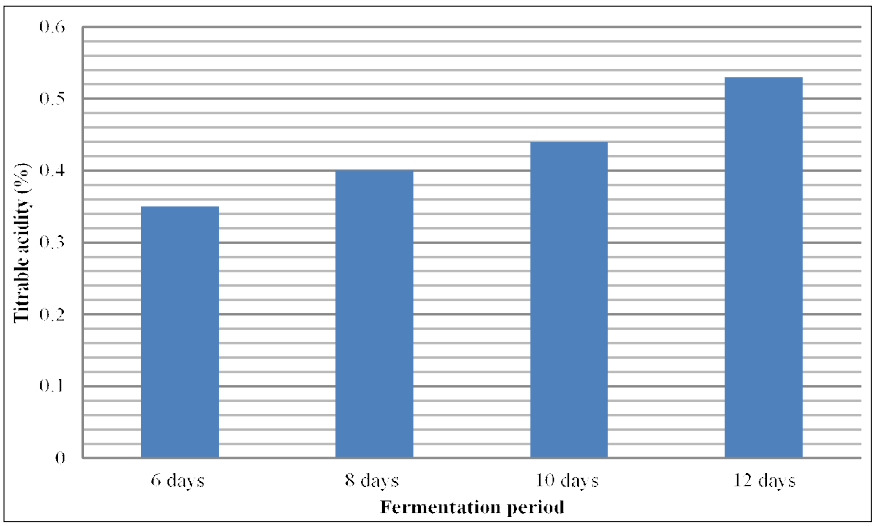

Fig 6: Effect of fermentation period on titrable acidity in coconut wine.

Fig 7: Coconut wine as influenced by yeast isolates.

Table 2: Effect of different yeast isolates on the sensory parameters of the coconut wine.

Treatments	Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Vinegar (2)	Total acidity (2)	Sweetness (1)	Body (1)	Flavour (2)	Astringency (2)	General quality (2)	Overall acceptability (20)
BRYI-1	1.8	1.8	1.9	1.6	1.5	1.6	0.7	0.8	1.8	1.7	1.9	17.1
BRYI-2	1.7	1.7	1.5	1.4	1.4	1.4	0.5	0.6	1.3	1.1	1.6	14.2
JKYI	1.5	1.5	1.1	1.2	1.0	1.4	0.6	0.5	1.2	1.3	1.4	12.7
PIYI	1.6	1.4	1.0	1.1	1.1	1.3	0.5	0.4	1.0	1.2	1.3	11.9
Ref. yeast	1.8	1.7	1.8	1.7	1.4	1.5	0.7	0.8	1.6	1.6	1.8	16.4
Control	0.5	0.4	0.5	0	0.5	0.4	0	0.2	0	0.4	0	2.9

according to yeast strain used in alcoholic fermentation, which has been attributed to varying amounts of amino acids and peptides produced by yeast. These amino acids and peptides being growth factors for lactic acid bacteria leading to increase in titrable acidity (Hua *et al.*, 2008).

The sensory values with respect to overall acceptability of the coconut wine fermented by the influence of yeast isolates were in the range between 11.9 to 17.1 out of 20 Swiegers *et al*. (2005), (Fig 7). But in terms of overall acceptability, BRYI-1 received the highest score. (17.1) followed by reference strain with the score (16.4), BRYI-2 (14.2), JKYI (12.7), PIYI scored the lowest (11.9). This showed that the coconut wine fermented by different yeast isolates is acceptable for consumption (Table 2) (Fig 7).

CONCLUSION

It was discovered that after 8 days of fermentation, the alcohol content was declining as a result of a drop in pH and an increase in titrable acidity. As fermentation progressed, the TSS concentration of the wine gradually decreased. Though the TSS reduced significantly during the first stage of fermentation, it continued to decline at a considerably slower rate until the fermentation was complete. The TSS level does not affect the pH of coconut wine, but pH decreased with the increase in fermentation period because of increase in titrable acidity and alcohol. The higher the TSS level, yields high titratable acidity during fermentation. Following inoculation, the yeast count rises quickly on the first day of fermentation and continues to rise alarmingly until the third day. Thereafter, the yeast count starts to decline until the fermentation is complete. Thus the effect of fermentation period on alcohol content, pH, TSS and titrable acidity are interconnected and varies according to the fermentation period of coconut wine.

Conflict of interest: None.

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