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Jagadeesh U
Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, India

Narayanaswamy B
Professor, Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, India

Suvarna VC
Professor, Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, India

Corresponding Author:
Jagadeesh U
Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, India

Microbial processing of coconut water for the development of coconut wine

Jagadeesh U, Narayanaswamy B and Suvarna VC

Abstract
An investigation on "Microbial processing of coconut water for the development of coconut wine" was carried out at the Department of Agricultural Microbiology during 2018-19. Different isolates of yeast (BRYI-1, BRYI-2, JKYI and PIYI) were isolated from different fruit sources and were identified as *Saccharomyces sp*. Different fermentation factors (Sugar concentration, inoculum level and fermentation period) were optimized for the preparation of coconut wine using reference yeast (*Saccharomyces cerevisiae* MTCC 171) and coconut water. The results revealed that the sugar concentration of 9 %, inoculum level of 10 % and fermentation period of 8 days were found to be optimum for preparation of coconut wine. Different yeast isolates (BRYI-1, BRYI-2, JKYI and PIYI) were evaluated for their fermentation efficiency and quality improvement of coconut wine. The results revealed that the yeast isolate BRYI-1 found to be more efficient in alcohol production (11.25%), reduction in pH (3.62), TSS (4.7), with optimum titrable acidity (0.59 %) and with an overall acceptability of 17.10/20 followed by reference yeast which was found to be more efficient in alcohol production and with an overall acceptability of 16.40/20.

Keywords: Coconut water, fermentation¸ yeast, wine, alcohol

1. 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) [14].
Coconut water is a clear liquid found inside coconuts (fruits of the coconut palm). As the fruit matures, the coconut water is gradually replaced by the coconut meat and air. Coconut water (coconut liquid endosperm) is one of the world's most versatile natural products. This refreshing beverage is 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) [10].
The Karnataka state is blessed with ten agro-climatic regions suitable for growing coconut all round the year in almost all the districts of Karnataka. The total area under coconut is 5.13 lakh ha in 2017-18 and the production of coconut 6,773.05 million nuts and is the second largest in area, production and productivity. Karnataka produces about 25.5% of total production of coconuts in the country (Anon., 2018) [1].
Coconut water is naturally occurring, is very rich in potassium, contains sodium chloride, carbohydrate and is viewed as the hydrating beverage of choice in certain parts of the world (Chavalittamrong *et al*., 1982) [6]. Clinically, coconut water may be used as an oral rehydration aid to replace fluid loss from the gastrointestinal tract in patients suffering severe dehydration due to diarrhea. It has also been used intravenously with success. Although not linked specifically to hydration, coconut water has been reported to have antioxidant properties, which may aid in neutralizing reactive oxygen species production resulting from long duration exercise. (Campbell *et al*., 2000) [4]
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) [19].
Wine is an alcoholic drink prepared out of fermentation. Wine yeast responsible for fermentation consumes sugar and converts it into ethanol, carbon dioxide and heat. It is being

produced from thousands of years, where earlier traces of wine are from Georgia (6000 B. C) and Iran (5000 B. C). 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) [9]

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) [14]. 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) [2]. 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) [19].

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) [8].

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) [3]. 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) [14]. This coconut water can be made use for the production of value added products like Nata-de-coco (Narayanaswamy 2010) [11], 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) [5].

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) [12].

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.

2. Materials and Methods

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

2.1 Isolation and characterization of yeasts from different fruit and vegetable sources

The isolation of yeasts was carried out from different fruit sources as per method given by Zahra *et al*. (2011) [21]. 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.

The yeast isolates were purified and characterized for further studies by standard procedures. These pure cultures were observed under the microscope after staining with cotton blue. The population of yeasts were enumerated in samples by standard plate count method using Yeast Extract Peptone Dextrose Agar (YEPDA) medium. The strain *Saccharomyces cerevisiae* MTCC 171 obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh was used as reference culture.

2.2 Identification of yeast isolates

Identification of yeasts was attempted by studying their morphological characteristics by comparing with the reference yeast, S*accharomyces cerevisiae* MTCC 171.

2.3 Isolation and purification of Yeasts

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.

2.4 Characterization of Yeasts

Characterization of yeasts was done by observing colony and cell morphology followed by biochemical tests *viz.* catalase activity, acid, gas production and Exopolysacchride production.

2.5 Screening of yeasts for fermentation of coconut water to estimate alcohol content

The isolated yeast strains screened for maximum alcohol production using coconut water. The yeast strain inoculated into the coconut water and allowed for anaerobic alcohol fermentation for a week and then the alcohol per cent was estimated (Neela and Prasad, 2013) [12].

2.6 Preparation of yeast starter culture

Loopful of purified and authenticated inocula of yeast culture (*Saccharomyces cerevisiae*) was transferred to 250 ml conical flask containing 100 ml of coconut water. The inoculated flask was kept overnight at 26-28 °C for growth. Then they were used at 5 per cent (v/v) for fermentation studies.

2.7 Optimization of protocol

Optimization of protocol to develop a fermented product from coconut water was done. The parameters following fermentation were considered for optimization like sugar concentration, inoculum level and fermentation period. *Saccharomyce scerevisiae* MTCC 171 obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh was used as reference culture. Parameters like pH, TSS, titrable acidity, alcohol content and sensory evaluation were considered for optimization.

2.8 Optimization of sugar concentration for preparation of coconut wine using reference yeast

Coconut broth was prepared with different combinations of sugar (7, 9, 11, 13%) which were added to 100 ml coconut water and inoculated with 5 % inoculum of reference yeast starter and incubated at room temperature and observations were taken on 7th day of fermentation. The experimental setup is shown in Fig.1.

Sl. No.	Particulars	Details
1	Design	Completely randomized design
2	Replication	Four
3	Treatments	T_1 =7% sugar + Ref. Yeast
		T_2=9% sugar + Ref. Yeast
		T_3=11% sugar + Ref. Yeast
		T_4=13%sugar + Ref. Yeast

Note: Ref. Yeast (Reference yeast = *Saccharomyces cerevisiae* MTCC 171)

2.9 Optimization of inoculum level for preparation of coconut wine using reference yeast

Coconut broth was prepared with 9% sugar concentration and different inoculum levels (6, 8, 10, 12%) of reference yeast starter culture were added to the 100 ml coconut water of 9% sugar concentration and incubated at room temperature and observations were taken on 7th day of fermentation.

Sl. No.	Particulars	Details
1	Design	Completely randomized design
2	Replication	Four
3	Treatments	T_1 =6% inoculum + Ref. Yeast
		T_2=8% inoculum + Ref. Yeast
		T_3=10% inoculum + Ref. Yeast
		T_4=12%inoculum + Ref. Yeast

Note: Ref. Yeast (Reference yeast = *Saccharomyces cerevisiae* MTCC 171)

2.10 Optimization of fermentation period for preparation of coconut wine using reference yeast

Coconut broth of 100 ml was prepared with 9% sugar concentration and inoculated with 10% inoculum of (Reference yeast culture). This was incubated at room temperature and observations were taken on 6^{th}, 8^{th}, 10^{th} and 12^{th}days of fermentation.

Sl. No.	Particulars	Details
1	Design	Completely randomized design
2	Replication	Four
3	Treatments	T_1 =6 days + Ref. Yeast
		T_2=8 days + Ref. Yeast
		T_3=10 days + Ref. Yeast
		T_4=12 days + Ref. Yeast

Note: Ref. Yeast (Reference yeast = *Saccharomyces cerevisiae* MTCC 171)

2.11 Evaluation of different yeast isolates for fermentation efficiency of coconut wine in comparison to Reference yeast

Based on the results of 2nd objective, the 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.

Sl. No.	Particulars	Details
1	Design	Completely randomized design
2	Replication	Three
3	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)

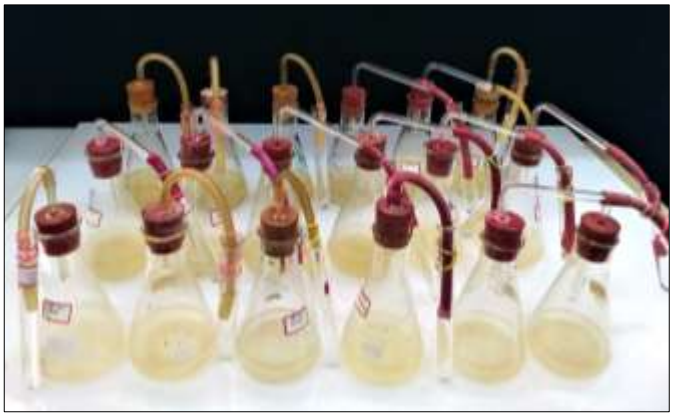

Fig 1: Experimental setup for optimization studies

2.12 Biochemical analysis

To assess the bio-chemical composition of coconut wine, different treatments were analysed for their pH, TSS, titrable acidity, alcohol and sensory evaluation.

3. Results

The yeast isolates showed characteristics creamy white, circular, raised, slimy and spreading colonies which were similar to reference yeast strain on YEPDA medium. Yeast cells were stained with cotton blue for morphological observations and budding was normal phenomenon.

The results revealed that all yeast isolates including reference yeast strain was positive for catalase activity and they were able to produce gas on fermentation of carbohydrates but unable to produce acid and were positive for exopolysacchride test indicating that isolates had resemblance to the characteristics of *Saccharomyces* spp.

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 (Table 1). The population of yeast isolates which showed good results in terms of alcohol production from different fruit and vegetable sources screened are presented in Table 2.

Table 1: Screening for efficient alcohol producing yeast isolate

Sl. No.	Yeast isolates	Source	Intensity of alcoholic smell	Alcohol (%)
1	GRYI	Grapes	+ + + +	6.43
2	CIYI	Citrus	+ + +	3.26
3	JKYI*	Jackfruit	+ + + + +	7.45

4	PIYI*	Pine apple	+ + + + +	7.21
5	APYI	Apple	+ + + +	4.69
6	WMYI	Watermelon	+ + + +	5.23
7	MMYI	Muskmelon	+ + +	4.86
8	BRYI*	Beetroots	+ + + + +	7.85
9	POYI	Pomegranate	+ + + +	4.98
10	CKYI	Sapota	+ + +	3.67

Note: * Selected isolates for the study
Scale of Intensity of alcoholic smell
+ + + + + Excellent
+ + + + Good
+ + + Fair
+ + Average
+ Poor

Table 2: Yeast isolates population (cfu/g) isolated from different fruit and vegetable sources

Yeast isolate	Source	Yeast ($x10^2$cfu/g)
BRYI-1	Beetroot	42.3
BRYI-2	Beetroot	40.1
JKYI	Jack fruit	36.4
PIYI	Pine apple	34.6
Reference yeast	MTCC	38.8

Note: Each value is mean of three replications; cfu: Colony forming unit

3.1 Optimization of sugar concentration for the preparation of coconut wine using reference yeast

Effect of different sugar concentrations on the biochemical parameters like pH, TSS, titrable acidity and alcohol percent of the coconut wine are presented in the Table 3. The coconut wine was prepared using 7 %, 9 %, 11 % and 13 % of sugar concentration and with coconut water as substrate with an initial pH of 5. The results revealed that after 7 days of fermentation the TSS varied from 6.2 to 5.2 between treatments. The coconut water fermentation with the sugar concentration of 9 % had optimum pH (4.3), TSS (5.2 ºBrix) and titrable acidity (0.72 %) with an alcohol content (8.08 %) compared to 7 %, 11 % and 13 %.

Table 3: Optimization of sugar concentration for preparation of coconut wine using reference yeast

Yeast	Sugar (%)	pH	TSS (ºBrix)	Titrable acidity (%)	Alcohol (%)
Reference yeast	7	4.5^b	5.6^c	0.60^c	7.82^b
	9	4.3^c	5.2^d	0.72^a	8.08^a
	11	4.6^b	6.0^b	0.64^b	7.61^c
	13	4.8^a	6.2^a	0.56^d	7.18^d

Note: initial pH: 5, initial TSS 7%: 20ºBrix; 9%:22ºBrix; 11%:26ºBrix; 13%: 29ºBrix; Reference yeast: *Saccharomyces cerevisiae*

3.2 Optimization of inoculum level for preparation of coconut wine using reference yeast

Effect of different inoculum levels on the biochemical parameters like pH, TSS, titrable acidity and alcohol % of the coconut wine are presented in the Table 4. The coconut wine was prepared using 9% of sugar concentration and with inoculum levels of 6 %, 8 %, 10 %, 12 % with an initial pH of 5. The results revealed that after 7 days of fermentation the TSS varied from 5.0 to 6.0 between treatments. The coconut water fermentation with the inoculum level of 10 % had optimum pH (4.5), TSS (5.0 ºBrix) and titrable acidity (0.68%) with an alcohol content (9.22 %) compared to 6 %, 8 % and 12%.

Table 4: Optimization of inoculum level for preparation of coconut wine using reference yeast

Yeast	Inoculum level (%)	pH	TSS (ºBrix)	Titrable acidity (%)	Alcohol (%)
Reference yeast	6	5.2^a	6.0^a	0.58^d	7.18^d
	8	5.0^b	5.5^b	0.65^b	8.08^c
	10	4.5^d	5.0^d	0.68^a	9.22^a
	12	4.8^c	5.2^c	6.30^c	8.73^b

Note: initial pH: 5, initial TSS: 20 ºBrix; Reference yeast: *Saccharomyces cerevisiae*

3.3 Optimization of fermentation period for preparation of coconut wine using reference yeast

Fermentation period plays an important role in an efficient and complete fermentation for development of a beverage. Thus, optimization of fermentation period was carried out with four levels *viz*., 6, 8, 10 and 12 days.

The results revealed that as the duration of fermentation increased pH and TSS decreased because of increase in titrable acidity and alcohol. After 6 days of fermentation pH reduced to 5.2, TSS to 5.4 ºBrix with 6.1 % alcohol and 0.52 % titrable acidity. After 8 days of fermentation pH reduced to 4.8 with a fall of TSS to 5.2 ºBrix, raise in alcohol content of 10.55 % with 0.46 % titrable acidity. Further, reduction of pH to 4.5 was observed at 10 days of fermentation with an alcohol content of 7.77 % due to increase of titrable acidity to 0.55 %. Finally, after 12 days of fermentation pH was reduced to 4.33, TSS to 4.8 ºBrix, alcohol to 6.56% with an increase in titrable acidity to 0.57 %. Coconut wine with 8 days of fermentation had the best sensory characteristics (Table 5).

Table 5: Optimization of fermentation period for preparation of coconut wine using reference yeast

Yeast	Fermentation period (days)	pH	TSS (ºBrix)	Titrable acidity (%)	Alcohol (%)
Reference yeast	6	5.2^a	5.4^a	0.52^c	6.10^d
	8	4.8^b	5.2^b	0.46^d	10.55^a
	10	4.5^c	5.0^c	0.55^b	7.77^b
	12	4.3^d	4.8^d	0.57^a	6.56^c

Note: initial pH: 5, initial TSS: 20 ºBrix; Reference yeast: *Saccharomyces cerevisiae*

3.4 Evaluation of different yeast isolates and reference yeast for fermentation efficiency of coconut wine

The sugar concentration of 9%, inoculum level of 10% and fermentation period of 8 days were used as standards. The fermentation was carried out for 8 days and the set up was filtered, pasteurised and bottled. These were subjected to various analyses. Treatment-6 without inoculation of microorganisms was taken in to consideration as control.

The results obtained due to the fermentation are presented in (Table 6). Yeast isolate BRYI-1 showed maximum alcohol production of about 11.25 %, followed by reference strain which produced 10.96 %, BRYI-2 with 10.88 %, JKYI with 10.56 % and PIYI with 9.87%.

Table 6: Evaluation of different yeast isolates for fermentation efficiency of coconut wine with comparision to reference yeast

Treatments	pH	TSS (ºBrix)	Titrable acidity	Alcohol (%)	Yeast counts (x10^{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 ºBrix; Ref. yeast: *Saccharomyces cerevisiae*;

3.5 Sensory evaluation of coconut wine (mean average of five judges)

The developed coconut wine from coconut water by the influence of different yeast isolates are shown in (Fig. 2). It was evaluated by 5 semi trained panel members with 20point hedonic scales (Polemer and Ronie, 2017) [14] was taken in to consideration. Which was mainly based on the appearance, color, aroma, flavor and acceptability. Also supported by Swiegers *et al*. (2005) who worked on wine aroma and flavor. Data recorded on organoleptic evaluation is presented in Table 7.

Table 7: 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

Fig 2: Coconut wine as influenced by yeast isolates

4. Discussion

Sandeep (2015) [16] 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 results with sugar concentration of 9 % was found to be optimum for coconut water fermentation with having the best sensory characteristics (Table 11). It was noted that sugar concentration of 11 % and 13 % yielded 7.61 % and 7.18 % of alcohol respectively. The alcohol % of sugar concentration 7 % and 9 % were 7.82 % and 8.08 % respectively which are on par with each other. The results were in agreement with the studies conducted by Sankpal and Kulkarni (2002) [17], Neela and Prasad (2013) [12] who reported that termination of the fermentation is after 90 % of sugar is utilized, where the ethanol production increased significantly and TSS could be responsible for higher alcohol content.

The results with inoculum level of 10% was found to be optimum for coconut water fermentation with having the best sensory characteristics (Table 13). It was noted that inoculum levels of 6 %, 8 % and 12 % yielded 7.18 %, 8.08 % and 8.73 of alcohol respectively. Any further increase in the inoculum level did not affect alcohol production. Therefore, this size of inoculum was adopted in subsequent experiments. The results were in agreement with the studies conducted by Sevda and Rodrigues (2011) [18] who reported that experiments with higher inoculum size rapidly reached the completion of fermentation and at the later stage of fermentation decrease in TSS was slower and with more alcohol percentage. These results are alo in agree with Francisco (2010) [7], Neelakandan and Usharani (2009) [13] and Neela and Prasad (2013) [12].

These results are supported by Idise and Okiemute (2011) [8] and also by Rodriguez *et al.* (2012) [15] who found that 6 days of fermentation was enough to produce volatile compounds with flavour notes desirables in cocoa out of 2, 4, 6, and 8 fermentation days. The concentrations of some undesirable compounds occurring after 8 days of fermentation suggest over-fermentation, therefore it was not necessary to extend this process for long periods.

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. However, BRYI-1 had the highest score with respect to overall acceptability (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.

5. Conclusion

Yeasts were isolated from different fruit sources and identified based on morphological and biochemical characteristics. Efficient isolates were screened for microbial processing of coconut water. Four best isolates of yeast *viz*., BRYI-1, BRYI-2, JKYI and PIYI were selected.

The optimization of coconut water for the preparation of coconut wine was carried out and biochemical characters were analyzed.

Sugar concentration of 9 % was optimized for the fermentation of coconut water by using reference yeast culture, based on increase in alcohol, reduction of pH, TSS and sensory evaluation. It was noticed that as the sugar concentration increased, alcohol content increased too but with high concentrations of sugar, there was less breakdown of sugar and there was no increase in alcohol content. Inoculum concentration of 10 % was optimized for which high alcohol percentage was obtained with low TSS, pH and titrable acidity. Alcohol content was found to increase along with fermentation time and after 8 days of fermentation alcohol was found to be decreasing due to decrease in pH and increase in titrable acidity. Hence, 8 days of fermentation was optimized for fermentation by using reference yeast culture based on alcohol content and sensory evaluation.

The evaluation of different yeast isolates for fermentation efficiency and quality improvement of coconut wine was carried out. It was noticed that the yeast isolate BRYI-1 produced the best results of alcohol content and sensory characteristics when compared to reference yeast strain. Vitamin B_6 content was found increased slightly in BRYI-1 after fermentation which was present in small amounts in coconut water.

From the study it can be concluded that microbial processing of coconut water using yeast turns coconut water into alcoholic beverage which is light in colour with strong aroma and rich taste.

6. References

1. Anonymous. Coconut development board (Government of India) 2018, www.coconutboard.nic.in
2. Augustine SP. Wine produced using tender coconut and product 2007, Patent US2007/ 017897 A1.
3. Brito N, Navickiene S, Polese L, Jardim G, Abakerli RB, Ribeiro ML. Determination of pesticide residues in coconut water by liquid–liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high performance liquid chromatography with ultraviolet detection, J Chromatogr. A 2002;957(2):201-209.
4. Campbell D, Thomas T, Falck T, Tutuo N, Clem K. The intravenous use of coconut water. American J Emerg. Med 2000;18(1):108-11.
5. Chauhan OP, Archana BS, Ashasingh PS, Raju, Bawa AS. A refreshing beverage from mature coconut water blended with lemon juice. J Food Sci. Technol 2014;51(11):3355-3361.
6. Chavalittamrong B, Pidatcha P, Thavisri U. Electrolytes, sugar, calories, osmolarity and pH of beverages and coconut water. Southeast Asian J Trop. Med. Public Health 1982;13(3):427-31.
7. Francisco C, Karina M, Laura F, Eduardo B, Eduardo D. Effect of *Saccharomyces cerevisiae* inoculum size on wine fermentation aroma compounds and its relation with assimilable nitrogen content. Int. J Food Microbiol 2010;143(2):81-85.
8. Idise, Okiemute E. Studies on wine production from coconut (*Cocos nucifera*). J Brew. Distilling 2011;2(5):69-74.
9. Johnson H. Vintage: The Story of Wine. S and S 1989, 11-6.
10. Joshi S, Vishnu J, Gaikwad S, Patil S, Ashiya Y. Comparitive study of production of wine from milk and water of *Cocos nucifera* L. Int. J Adv. Biotechnol. Res 2013;4(2):274-279.
11. Narayanaswamy B. Utilization of coconut water from coconut industry for production of nata-de-coco: a microbiologically processed food product. Environ. Ecol 2010;28(3):1577-1579.
12. Neela S, Prasad NB. Production of fermented coconut water beverages. Assoc. J Food Ag-Ind 2013;6(5):281-289.
13. Neelakandan T, Usharani G. Optimization and production of bioethanol from cashew apple juice using immobilized yeast cells by *Saccharomyces cerevisiae*. American Eurasian. J Sci. Res 2009;4(2):85-88.
14. Polemer MC, Ronie FM. Development of young coconut (*Cocos nucifera*) wine. Asia Pacific J Res 2017;5(2):89-93.
15. Rodriguez J, Orozco IA, Jaramillo E, Flores E. Effect of fermentation time and drying temperature on volatile compounds in cocoa. Food. Chem 2012;132(1):277-288.
16. Sandeep T, Rajani S, Anjali T, Arjun S, Yuvraj K. Isolation of yeast from soil and different food samples and its characterization based on fermentation. Nepal. J Biotechnol 2015;3(1):29-34.
17. Sankpal K, Kulkarni D. Optimization of fermentation conditions for gluconic acid production using *Aspergillus niger* immobilized on cellulose microfibrils. Process Biochem 2002;37:1343-1350.
18. Sevda S, Rodrigues L. Fermentative Behavior of *Saccharomyces* Strains During Guava (*Psidium Guajava* L.) Must fermentation and optimization of guava wine production. Food. Process. Technol 2011;2(4):2-9.
19. Sunil S, Joshi Vishu, Gaikwad J, Sandip S, Patil, Ashiya Y. Makandar Comparative study of production of wine from milk and water of *Cocos nucifera* L. Int. J Adv. Biotechnol. Res 2013;4(2):274-279.
20. Swigers J, Bartowsky E, Henschke P, Pretorius. Yeast and bacterial modulation of wine aroma and flavour. Aust. J Grape Wine Res 2005;11:139-173.
21. Zahra P, Mohammad R, Gholam RG. Isolation and characterization of cellulose - producing bacteria from local samples of Iran. Int. J Microbiol. Res 2011;2:240-242.