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Isolation, identification and characterization of wine yeast from rotten papaya fruits for wine production

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ABSTRACT

Sixty four yeast strains isolated and identified from seven varieties of rotten papaya fruit were characterized using standard microbiological procedure. The ability of different yeast strain to produce ethanol was investicated such as Saccharomyces cerevisiae, Saccharomyces bayanus, Saccharomyces uvarum, Saccharomyces italicus, Saccharomyces pasteurianus, Schizosaccharomyces pombe and Zygosaccharomyces. Their ability for wine production were tested by using sugar and ethanol tolerance tests. The best biochemically active strain is Saccharomyces cerevisiae to produce wine from Co 2 papaya fruits. After fermentation for one month the highest (11.59%) alcohol concentration with corresponding residual sugar concentration of (1.87) were produced from Co 2 papaya fruits after fermentation with Saccharomyces cerevisiae. So, Saccharomyces cerevisiae was found to be the best yeast strain producing wine with the highest acceptable score of 4.8 from Co 2 papaya fruits. The study revealed the possibility of producing wine from our locally available fruits using simple, cheap, and adaptable technology with biochemically characterized yeast strains.

Key words: papaya fruits, yeast strains, fermentation, wine production.

INTRODUCTION

Wine and other alcoholic drinks are important in fulfilling social obligations such as marriage, christening, and burial ceremonies [17]. In cameroon, conference, rallies, marriage, as well as traditional and social gatherings are graced by a reception, and wine has become an integral part of it. Because many people have learned of its ability to prevent cardiovascular disease because of its high content of resveratrol [22]. Yeast is a group of fungi in which unicellular form is predominant. Most of the yeasts are represented in sub division *Ascomycotina* and

Basidiomycotina of the kingdom *Mycotina*. As a group of microorganisms yeasts have compolitan distribution. They have been isolated from natural substrates like leaves, flowers, sweet fruits, grains, fleshy fungi, exudates of trees, insect, dung and soil [20]. They play their role in the dynamics of biological and chemical turnover in soil, plants, animals and water [16]. There are about 100 genera and 700 species of yeast [9] of which only 5 genera and 7 species have been reported from Pakistan [10]. Rice *et al*., [14] confirmed that yeast causes spoilage in pineapple, while the species of Saccharomyces caused fermentation in damaged fruits and may also be a problem on ripe harvested fruits in the field. In this present study was carried out to isolate, identify and characterize the wine yeast from rotten papaya fruits and wine production from papaya fruits using yeast.

MATERIALS AND METHODS

Collection of sample:
Seven varieties of rotten papaya fruits collected from Tamil Nadu Agricultural University, Coimbatore.

Isolation of microorganism:
Seven varieties of rotten papaya fruits sample were taken and each variety of 1g was taken and diluted serially upto10^{-6} about 0.1ml of serially diluted sample was taken and done the spread plate technique by using Malt agar plate. The inoculated plates were incubated for 48hr at 30ºC.

Subculture technique:
The isolates of yeast species was subcultured on malt agar plates to check its purity and incubated at 30°C for 48hrs. Purified cultures were routinely maintained on malt extract slants and kept at -4ºC. The isolates were subjected to various physiological and biochemical tests, including sugar fermentation; assimilation of carbon and nitrogen compounds; urease testing; growth at 25, 30, 37 and 50ºC. Identification was based on an established scheme [7].

Processing of wine fermentation
Yeast such as *Saccharomyces cerevisiae, Saccharomyces bayanus, Saccharomyces uvarum, Saccharomyces italicus, Saccharomyces pasteurianus, Schizosaccharomyces pombe* and *Zygosaccharomyces* were isolated from rottened papaya fruits. 7 varieties viz. Co1, Co2, Co3, Co4, Co5, Co6 and Co7 were procured from coimbatore agricultural university. One kg each of sound, health and ripe fruits were selected and it was completely peeled off using knife. Then the pulp was macerated in mixie and pasteurized at 85 – 90ºC for 5minutes. After cooling the pulp required amount of cane sugar was added to adjust the final TSS to 24ºBrix. The isolated and confirmed yeasts used as starter cultures for fermentation were checked for biochemical activity by subjecting them to sugar and ethanol tolerance tests at different concentration as previously reported Gao and Fleet [5]. Yeast activity was suppressed by adding 100ppm of So_2 in the form of Potassium metabisulpate. The pulp was inoculated with yeast such as *Saccharomyces cerevisiae, Saccharomyces bayanus, Saccharomyces uvarum, Saccharomyces italicus, Saccharomyces pasteurianus, Schizosaccharomyces pombe* and *Zygosaccharomyces* (The ratio of 1:10). All the treatments were kept for primary fermentation at 24 to 26°C for 9 days with periodic aeration. After 9 days all the treatments were filtered through muslin cloth and the filterate was kept for secondary fermentation in plastic carbouys with air lock/water seal

to prevent the entry of external oxygen into the cans and for release the carbondioxide developed during fermentation. The secondary fermentation was carried out for a period of 2weeks at same temperature. After two weeks the evolution of CO_2 ceased and the wine was clarified by centrifugation at 5000 rpm. The sediment was discarded and the clear wine was filled into sterile bottles. The bottles were pasteurized at 50°C for 15minutes. The pasteurized bottles of wine were kept for aging at ambient temperature.

Method of analysis

According to the AOAC [2]the physico-chemical parameters of observations recorded were total soluble solids, total sugar, acidity, volatile acidity, pH, alcohol. The microbial count was observed by Ndip *et al* [11].

Organoleptic evaluation

The sensory evaluation was done using 8 judge panels after aging for 1 month. Observations were recorded for color, clarity, body & taste on a 5 point scale with 5 points for excellent quality & 1 point for bad quality.

RESULT AND DISCUSSION

Sixty four yeast strains isolated and identified from seven varieties of rotten papaya fruit were characterized using standard microbiological procedure. The ability of different yeast strain to produce ethanol was investicated such as *Saccharomyces cerevisiae, Saccharomyces bayanus, Saccharomyces uvarum, Saccharomyces italicus, Saccharomyces pasteurianus, Schizosaccharomyces pombe* and *Zygosaccharomyces*. Bhaskar Bhadra *et al* [3] reported the ascogenous yeast $YS16^T$ was isolated from a decaying papaya fruit. Yeasts of the genera *Rhodotorula*, *Cryptococcus*, *Sporobolomyces*, *Saccharomyces*, *Candida* and *Pichia*, amongst others, have been isolated from fresh and rotten fruits [3],[4],[13],[19]. These fruit-associated yeasts produce extracellular enzymes such as lipases, cutinases and pectinases and thus hasten the spoilage of fruits during storage and transportation [4]. A number of novel yeast strains have been isolated from both healthy and rotten fruits [3],[12],[13],[21]. Table 1 summarizes the percentage of sugars tolerated by the isolates. At 35% (w/v) sugar concentration, all isolates grew profusely. The percentage (v/v) of ethanol tolerated by the isolates was as shown in Table 2. All isolates grew well at 6% (v/v) ethanol, with *Saccharomyces cerevisiae* tolerating the highest concentration of 12% (v/v).

Analysis after fermentation

The TSS of must on the initial day of fermentation was 24 Brix. It kept on decreasing during fermentation and aging observed in all varieties. All treatment of juice (wine) with inoculum showed a gradual decrease in the acidity (volatile and non volatile) during fermentation. The decrease in the acidity during fermentation could be due to the utilization and production of carbon dioxide and alcohol by the yeast [8],[15]. The pH varied between 4.13 to 4.84 initially; subsequently this value is decrease in all the varieties indicating a decrease in acidity[8],[18]. The microbial population showed logerthemic increase during the primary fermentation subsequently there was decrease in its populations.

Table 1: Sugar tolerance of yeast isolates

Isolate	Sugar concentration (% [w/v])					
	35	40	45	50	55	60
Sa.c	+++	+++	+++	+	+	-
Sa.b	+++	++	+	-	-	-
Sa.u	+++	++	+	+	-	-
Sa.i	+++	++	++	-	-	-
Sa.p	+++	+++	++	+	-	-
Sc.p	+++	+++	+	-	-	-
Zyg	+++	+++	++	+	-	-

Sa.c = *Saccharomyces cerevisiae*, ***Sa.b*** = *Saccharomyces bayanus*,
Sa.u = *Saccharomyces uvarum*, ***Sa.i*** = *Saccharomyces italicus*,
Sa.p = *Saccharomyces pasteurianus*, ***Sc.p*** = *Schizosaccharomyces pombe*,
Zyg = *Zygosaccharomyces* sp.
+++, Crowded growth; ++, Moderate growth; +, Scandy growth; -, No growth.

Table 2: Ethanol tolerance of yeast isolates

Isolate	Ethanol concentration (% [w/v])					
	6	8	10	12	15	20
Sa.c	+++	+++	+++	++	-	-
Sa.b	+++	++	-	-	-	-
Sa.u	+++	++	+	+	-	-
Sa.i	+++	++	++	-	-	-
Sa.p	+++	+++	++	+	-	-
Sc.p	+++	+++	+	-	-	-
Zyg	+++	+++	++	+	-	-

Sa.c = *Saccharomyces cerevisiae, Sa.b = Saccharomyces bayanus,*
Sa.u = *Saccharomyces uvarum, Sa.i = Saccharomyces italicus,*
Sa.p = *Saccharomyces pasteurianus, Sc.p = Schizosaccharomyces pombe,*
Zyg = *Zygosaccharomyces sp.*
+++, Crowded growth; ++, Moderate growth; +, Scandy growth; -, No growth.

Table 3: **Physico chemical analysis of papaya wine from various papaya varieties using *Saccharomyces cerevisiae***

Types of wine (Papaya varieties + Organism)	TSS (%)	Titrable acidity (%)	Volatile acidity (%)	Total sugar (%)	Residual sugar	pH	Alcohol (%)	Sensory evaluation
Co1+ *Sa.c*	11.40	0.530	0.0070	0.560	7.64	3.70	7.34	3.0
Co2+ *Sa.c*	13.60	0.550	0.0060	0.534	1.87	3.72	11.59	4.8
Co3+ *Sa.c*	12.00	0.534	0.0054	0.573	5.43	3.44	8.34	4.3
Co4+ *Sa.c*	11.10	0.513	0.0053	0.549	7.22	3.40	7.43	3.2
Co5+ *Sa.c*	10.21	0.533	0.0073	0.600	6.78	3.45	8.44	3.5
Co6+ *Sa.c*	13.22	0.541	0.0110	0.650	6.34	3.70	8.11	3.5
Co7+ *Sa.c*	10.99	0.534	0.0070	0.610	6.56	3.49	8.77	3.4

Sa.c = *Saccharomyces cerevisiae*

This could be due to the fact that higher concentration of sugar substrates inhibited the growth and multiplication of yeast during secondary fermentation. With recorded to source of inoculums the pure culture had the leas number of CFU/ml (184 x 10^3). The alcohol content of papaya wine from all the varieties showed an increasing trend during fermentation. Maximum development of

alcohol was found in Co1 Papaya (7-9%), Co2 Papaya (11-12%), Co3 Papaya (8-10%), Co4 Papaya (7-9%), Co5 Papaya (8-9%), Co6 Papaya (8-9%) and Co7 Papaya (8-9%). Similar results were also observed by other workers [1],[8],[6]. The total sugar of papaya wine showed a decreasing trend during fermentation in all papaya varieties. This could be due to the utilization of sugar during alcohol production. The highest residual sugar (7.22[w/v]) was found in Co 4 papaya wine pitched with *Saccharomyces cerevisiae*. The sensory evaluation was done using 8 judges panel after aging for 1 month (Table 3).

CONCLUSION

Sixty four yeast strains isolated and identified from seven varieties of rotten papaya fruit were characterized using standard microbiological procedure. The ability of different yeast strain to produce ethanol was investicated such as *Saccharomyces cerevisiae, Saccharomyces bayanus, Saccharomyces uvarum, Saccharomyces italicus, Saccharomyces pasteurianus, Schizosaccharomyces pombe* and *Zygosaccharomyces*. Their ability for wine production was tested by using sugar and ethanol tolerance tests. The best biochemically active strain is *Saccharomyces cerevisiae* to produce wine from Co 2 papaya fruits. It can be concluded that all varieties of papaya are suitable for wine making It is important to screen a large number of varieties before attempting to produce the wine on large scale. In this study, Co2 papaya given more alcohol production using *Saccharomyces cerevisiae* compared with other varieties. Next to that, Co3 variety showed better results. Co5, Co6 and Co7 varieties showed optimum level alcohol production. Co1 and Co4 variety produced very low quantities of alcohol compared with all varieties.

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