Research paper

Preparation and evaluation of wine from tendu (*Diospyros melanoxylon* L) fruits with antioxidants

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Abstract

Wine was prepared from tendu (*Diospyros melanoxylon* L.) fruits and evaluated. Must from tendu fruits was made by raising total soluble solids (TSS) to 20° B, 0.1% (NH₄) SO₄ as nitrogen source and fermenting it using 2% *Saccharomyces cerevisiae* var. *ellipsoideus* into wine. The wine had the following proximate compositions: TSS, 2°B; total sugar of 3.78g/100ml; titratable acidity, 1.32g tartaric acid/ 100ml; pH, 3.12; total phenolics, 0.95g/100ml; β -carotene, 8µg/100ml; ascorbic acid, 1.52mg/ 100ml; lactic acid, 0.39mg/100ml, methanol, 3.5% (v/v) and ethanol,6.8% (v/v). The tendu wine had a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of 52% at a dose of 250µg/ ml. Principal Component Analysis(PCA) reduced the 10 original analytical and proximate variables (TSS, total sugar, TA, pH, phenol, β -carotene, ascorbic acid, lactic acid, ethanol and DPPH scavenging activity) into three independent components that accounted for 83.42% of the total variations. Sensory evaluation was carried out by 16 trained panelists on various attributes such as taste, aroma, flavour, colour/ appearance and after-taste. The analysis showed that the taste of tendu wine was liked strongly by the panelists and there was no significant difference between the two replicate sets (p<0.05) in most sensory parameters.

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Keywords: Carotene, ethanol, phenol, Saccharomyces cerevisiae, tendu, wine, antioxidant.

Tropical countries possess a wide diversity of fruits with many possibilities of commercial exploitation; some of them are considered exotic and rare. Tendu (Diospyros melanoxylon L.) is an evergreen tropical tree with height up to 10-15 ft, belonging to family Ebenaceae. It is a native and endemic tree of India and widely found in the peninsular plains and lower hills especially in the dry deciduous forests of central, western and northern India (Stewart and Brandis, 1992). Tendu fruits ripen during the month of May-June. Fruit is ovoid or globose yellow to light orange when ripe, 1-23 across, supported by the flat spreading calyx-lobes with undulating edges. Pulp is yellow, glutinous, soft, sweet and slightly astringent. These fruits are rich in sugars, proteins, fibre and vitamin C. Further, the presence of several benevolent phytochemicals such as β-carotene, terpenoids, flovanoids, saponin and tannins in the fruit adds advantage to its nutritive value (Maridass, 2010).

Several tropical and sub-tropical fruits like jamun (Chowdhury and Ray, 2007), litchi (Kumar *et al.*, 2008), cashew apple (Mohanty *et al.*, 2006), mango, jackfruit, banana (Muniz *et al.*, 2008) have been shown to be suitable for fermentation into wine. However, fermentation of tendu fruits into wine has not been studied yet. This article deals with the studies related to the preparation of wine from tendu fruit by fermenting with the wine yeast, and evaluation of the wine with reference to nutrients, proximate composition and sensory attributes.

Materials and Methods

Tendu fruits

Fully ripened and undamaged tendu fruits were collected from the local market of Baripada, Odisha during the month of May 2009 (Day temperature $38\pm2^{\circ}$ C, night temperature $30\pm2^{\circ}$ C). The fruits were brought fresh to the laboratory of North Odisha University for further processing.

Wine yeast

The wine yeast, *S. cerevisiae* var. *ellipsoideus* was maintained on Potato Dextrose Agar (PDA) slants at 4° C in a refrigerator. The wine yeast was obtained from the Microbiological Laboratory of Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar. The yeast strain was provided by Dr. E. Suresh, principal scientist (Microbiology), Division of Postharvest Technology, Indian Institute of Horticultural Research, Bangalore, India. This yeast culture has earlier been employed for wine making from different fruits and other substrates (Kumar *et al.*, 2008; Ray *et al.*, 2012; Panda *et al.*, 2012).

Fermentation process

Tendu fruits weighing approximately one kg were sliced into two halves by a knife and the pulp was scooped out using the thumb finger. The seeds as well as the fibres were manually removed from the pulp. The pulp was then crushed with water 1:1 (w/v ratio) and the juice was extracted using a sterilized cheese cotton cloth. Approximately 400 ml of juice was extracted from one kg of pulp. The juice (must) filtered through cotton cloth had 14º B and was treated with potassium metabisulphite (KMS) (100 µg/ml) to inhibit the growth of undesirable microorganisms such as acetic acid bacteria, wild yeast and moulds. Then cane sugar and tartaric acid were added into the juice (amelioration) to attain total soluble solids (TSS) of 20^o B and pH of 5.1, respectively. The must was inoculated with 24 h old starter culture of S. cerevisiae (2%, vD v), and (NH₄)₂SO₄ at 0.1% concentration was added as a nitrogen source. Fermentation was carried out at ambient temperature for 7 days. Three replicates were maintained for conducting this experiment.

Preparation of starter culture

One hundred grams of good-quality sweet grapes (var. Bangalore blue) was selected and washed under running tap water. The grapes were mashed in a mixer-cum-grinder and the juice was extracted using a juice squeezer. The volume of the juice (ûltered through muslin cheese cloth) was measured, and an equal volume of water was added. It was boiled for 10 min on a hot plate and was cooled at room temperature $(28\pm2^{\circ}C)$. After cooling, the grape juice was inoculated with *S. cerevisiae* culture from the stock culture under aseptic conditions and was incubated at 30° C for 24 h to prepare the starter culture.

Racking and bottling

After fermentation, ûrst racking was carried out at an ambient

temperature. Racking of the tendu wine was carried out when TSS reached around $2-3^{\circ}$ B. This process was repeated three times at 20-days interval to discard the deposited residues at the bottom. Bentonite (0.04%) was added before the final racking to remove the last remaining residues. After final racking, food grade KMS (100µg/ ml) was added as a preservative before bottling. The bottles were filled full with wine, corked and sealed with bee wax. The flow chart for making wine from tendu fruits is shown in Figure 1.

Bio-chemical analysis

The biochemical composition of tendu fruit pulp was determined as described here. Weight of pulp was determined by an electronic balance. Moisture was determined by vacuum oven, and values were expressed as g/100g (CTCRI, 2000). Total sugar, ascorbic acid, total phenolic content and β -carotene content were quantified as described by Mahadevan and Sridhar (1998) and were expressed as g/100g. The pH of the must /wine was measured by using a pH meter. Ash content of the fruit was determined by the muffle furnace methods (CTCRI, 2000).Mineral contents of the fruit pulp were determined by a 3400 atomic absorption spectrophotometer (PerkinElmer, Fremont, CA, USA) (Emmel *et al.*, 1977).

The biochemical constituent (total soluble solids, total sugar, titratable acidity, pH, phenol, lactic acid, β -carotene, methanol and ethanol) of the tendu must and wine was determined by standard methods (Amerine and Ough, 1980). DPPH (2,2-diphenyl-1picryl hydrazyl) scavenging activity of the must and wine was determined by spectrophotometric method (Marxen *et al.*, 2007).

Microbiological analysis

Microbiological analysis of the wine was also performed. Triplicate samples from fermentation medium as well as from finished wine were analyzed for enumeration of microorganisms SMS by plate count methods. Two hundred and fifty ml of wine sample (in triplicates for enumeration of bacteria, fungus and yeasts) was filtered by using a filtered disc of 0.45μ m and a suction pump. The discs were plated on nutrient agar, potato dextrose agar and wort agar plates for enumeration of bacteria, fungus and yeasts, respectively (Dubey and Maheswari, 2004).

Sensory evaluation

Sensory attributes of tendu wine such as (taste, aroma, flavour, colour and after- taste) were evaluated using a 9-point Hedonic scale (where 1 = dislike extremely and 9 = like extremely)by 16 panelists (8 men and 8 women, aged 20-35 years) selected from staff and faculty members of North Odisha University, Baripada, who were familiar with wine consumption. Samples were served in clean transparent glasses (tumblers), which

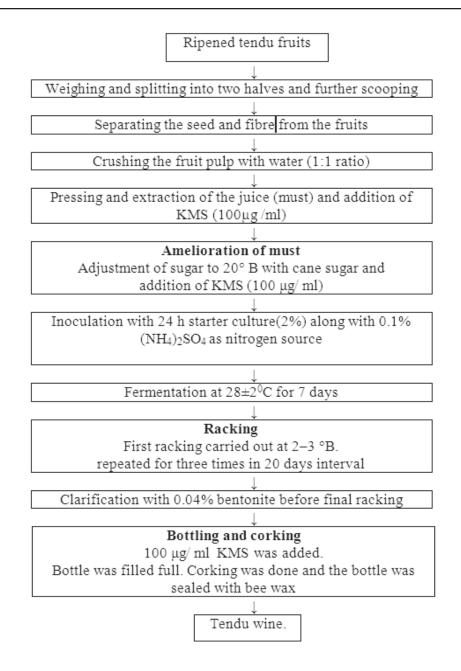


Figure 1: A flow chart for making tendu wine

session.

had been labeled with 3-digit random numbers. Questionnaires and water for mouth ringing between each tasting were provided. Prior to evaluation, a session was held to familiarize the panelists with the product. The panelists were asked to read through the questionnaires and the meaning of each attribute (taste, aroma, flavour, colour/appearance and after taste) was explained to the panelists to avoid any misinterpretation (Kilcast and Subramanian, 2000). Tasters were not allowed to discuss their scores during the evaluations

The tendu wine produced was presented to the trained panel for sensory analysis. Another set of tendu wine sample was evaluated as a second replication the following day. The sensory evalution data were presented as the means of the panelist's score. A standard test as used to test for the statistical significance of the difference observed between scores of the two sets was carried out (Cass, 1980).

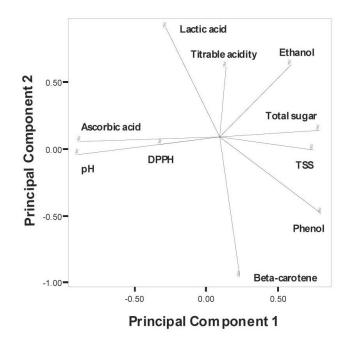


Figure 2: Graphical representation of principal components (PC1 and PC2) of analytical variables

Statistical methods

Multivariate statistical methods (correlation analysis and PCA) were applied for the evaluation of the results of the proximate composition and consumer's acceptability. PCA was applied to group the related variables into smaller components for the interpretation of their relationship. All analyses were performed using statistical software SPSS (SPSS Software for Windows release 17.0 SPSS Inc., Chicago, IL, USA).

Results and Discussion

Fermentation changes

The biochemical composition of the tendu fruit pulp is presented in Table 1. The titratable acidity (g tartaric acid/ 100ml) increased from 0.85 in the must to 1.32 in the finished wine prepared from the tendu fruits (Table 2). Lactic acid (LA) content was negligible (0.01mg/100ml) in the must and it increased to 0.39mg/100ml in the wine in the course of fermentation. The increase in LA was mostly because of malolactic fermentation, which is very common under tropical conditions in which sugar is partly converted to malic and lactic acid, (Liu, 2003; Saigal and Ray, 2007). The increase in TA and LA content was concomitant with the decrease in pH i.e. from 5.1 in must to 3.12 in wine The acidic characteristic of the tendu wine was comparable to that of other tropical fruit wines such as cashew apple (pH, 2.92; TA, 1.21g tartaric acid/ 100ml) and jamun (pH, 3.3; TA, 1.11g tartaric acid/100ml) wine. The decrease in pH was probably due to accumulation of organic acids such as lactic acid during fermentation and it minimizes the influence of spoilage bacteria (Liu, 2003). As excepted total sugar content decreased from 24.55 g/100ml in must to 3.78 g/100ml in wine. Similarly, the TSS decreased from 20°B in must to 2°B in wine. The decrease in total sugar content and TSS from must to wine was indicative of the consumption of the sugar sources by the wine yeast to produce ethanol and a part of it was converted to other metabolites such as methanol and lactic acid. Ascorbic acid is an essential water-soluble vitamin and is well known for its nutritional importance. Ascorbic acid (Vitamin C) content of the must was 2mg/g and it was reduced to 1.52mg/g in the wine. Due to the redox potential of ascorbic acid, it facilitates intestinal absorption of iron and functions as a cellular antioxidant alone and coupled to the antioxidant activity of vitamin E. Therefore, adequate intake of vitamin C from foods and/or supplements is vital for

Table 1: Chemical composition of tendu pulp

Parameters	Pulp (g/100g)	Juice (g/100ml)
Weight	72.0±2.7	ND
Moisture	68.8±2.3	ND
Total sugar	28.6±3.0	22.1±1.7
Ascorbic acid (VitC)	2.8±0.56	1.78 ± 0.6
Phenols	1.72 ± 0.64	1.36 ± 0.55
β-carotene	22.0±1.0	18.0±0.27
Ash	0.25 ± 0.04	0.23 ± 0.05

ND: Not determined

Parameters		
	Must	Wine
TSS(°Brix)	20±0.01	2.0 ± 0.00
Total sugar(g/100ml)	24.55±0.95	3.78±0.13
Titrable acidity		
(g/tartaric acid/100ml)	0.85±0.16	1.32 ± 0.52
pH	5.10±0.28	3.12±0.12
Phenols (g/100ml)	1.00±0.32	0.95 ± 0.45
Ascorbic acid (Vit -C) (mg/g)	2.00 ± 0.05	1.52 ± 0.22
Lactic acid(mg/100ml)	0.01 ± 0.00	0.39 ± 0.05
â-carotene (mg/100ml)	18.00 ± 2.1	8.00 ± 0.00
Methanol(%)	ND	3.5 ± 0.00
Ethanol(%)	ND	6.8±0.32
DPPH(%)	72.5 ± 2.80	52.0±2.15

Table 2: Composition of Tendu must and	d wine
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normal functioning of the human body (Phillips et al., 2010). However, the phenolic content varied slightly between the tendu must and wine (1g/100ml in must; 0.95g/100ml in wine). Phenolics are ubiquitous secondary metabolites in plants. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, anti-mutagenic, anti-carcinogenic as well as ability to modify gene expression (Marinova et al., 2005). Polyphenols can act as free radical-scavengers quenching hydroxyl radicals (OH) or superoxide anion radicals (O_{2}) (Sichel et al., 1991). Further, lower pH (3.1) of the wine is highly favourable for the stability of the polyphenols as they are known to auto-oxidize with increase in pH (Mochizuki et al., 2002). β -carotene content fell from 18µg/100ml in the must to $8 \mu g/100 ml$ in the wine. The degradation of \hat{a} - carotene was because of the physical stresses occurred during wine making such as grinding of the fruit pulp, filtration of the fruit juice, absorption by addition of bentonite, etc (Saigal and Ray, 2007). β - carotene possesses pro-vitamin A activity and is a powerful anti-oxidant, thus being able to scavenge reactive chemical species such as singlet oxygen, triplet photochemical sensitizers, and free radicals that would otherwise damage DNA inside cells and could trigger cancer - inducing mutations, and adversely affect specific immune functions (Weissenberg et al., 1997). The human body can convert provitamin-A carotenoids into the active form of vitamin-A (Faber et al., 2002). DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable synthetic compound which has been commonly used for the determination of antioxidant capacity of food. DPPH scavenging activity of the tendu wine was 52% at a dose of 250µg/ml. The high antioxidant activity of wine may be due to the redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides. Compared to the fruit must it is lesser than that of the must (72%) at the same dose. From the present study it may be interpreted that the DPPH scavenging activity

of the tendu wine was attributed from the cumulative contribution of higher concentration of poly-phenols, ascorbic acid and β - carotene pigments present in the tendu fruit. Tendu wine obtained from our study was slightly yellowish (in appearance) beverage with alcohol (ethanol) content of 6.8% (v/v). Low ethanol concentration (6-8%) was also reported for other tropical fruit wines such as mango wine (Reddy and Reddy, 2005), jamun fruit wine (Chowdhury and Ray, 2007), which is common for table wine (Saigal and Ray, 2007). Further, methanol concentration was 3.5%, which was quite low as reported for other fruit wines (Reddy and Reddy, 2005; Saigal and Ray, 2007). However, there is need to reduce the amount of methanol in the wine.

The biochemical composition of the tendu wine corroborated with the biochemical parameters of other tropical fruit wines. Akubor et al. (2003) reported the following biochemical composition of banana wine: pH, 3.3; TA, 0.85g tartaric acid 100mL^{-1} ; ascorbic acid, $1.4 \text{mg} 100 \text{ml}^{-1}$ and alcohol, 5% (v/v). Jamun (Syzgium cumini L.) fruits were successfully fermented to produce wine by using the yeast strain S. cerevisiae. The wine was acidic in taste [titratable acidity $(1.11 \pm 0.07 \text{ g tartaric})$ acid100 ml⁻¹)], with high tannin $(1.7 \pm 0.15 \text{ mg}100 \text{ml}^{-1})$ and low alcohol (6%) concentration (Chowdhury and Ray, 2007). Other tropical fruit wines with similar compositions have been reported from carambola (Averrhoa carambola L.), papaya (Carica papaya L.), mango (Mangifera indica L.), black mulberry (Morus nigra L.), water melon (Cucumis melo L.), yellow mombin (Spondis purpureal L.), custard apple (Annona squamosa L.) and manaba (Hancornia speciosa Gomes) (Muniz et al., 2008).

Microbiological analysis

Microbial studies of the fermentation medium have not shown the presence of any bacteria, fungi or any microorganisms except S. cerevisiae. This was because of the addition of KMS at a dose of 100µg/ml which suppressed the other microorganisms except S. cerevisiae. (Winter et al., 2011). As the fermentation tends towards completion the cells of S. cerevisiae moved towards death phase because of the depletion of nutrients (sugars) with concomitant increase in CO₂ and ethanol. S. cerevisiae also produces other metabolites like medium chain fatty acids, octanoic and decanoic acids and other lethal factors which interfere with the plasma membrane integrity of other microorganisms within the same environment (Mill et al., 2008). The finished wine after clarification with bentonite hardly contains any significant number of S. cerevisiae cells. Bentonite added to the wine formed precipitate with dead yeast cells, fine sediments and other proteins and settle down at the bottom. Enumeration of yeast cells in the wine by wort agar plates showed the presence of 4 CFU (colony forming units)/250ml of wine which was negligible. No other microorganisms were detected in the wine samples which ensured its sterility.

Sensory evaluation

Tendu wine obtained was slightly acidic [lactic acid, 0.39(g/100ml), titratable acidity, 0.85(g/100ml)], which together with tannin imparted characteristic exotic flavour of the tendu wine. Sensory evaluation analysis (Table 3) shows that tendu wine having strong exotic taste was "liked extremely" by the panelists and there was no significant difference between the two sets (p<0.05) in most sensory parameters.

Table 3: Sensory evaluation of the tendu wine*

Attributes**	Tendu wine
Taste	8.0
Aroma	5.5
Flavour	7.0
Colour/appearance	e 4.5
After taste	6.7

n=32, * values are means of the panelist scores;

**1=dislike extremely; 2= dislike strongly; 3= dislike moderately; 4=dislike slightly 5= neither like or dislike; 6= like slightly; 7= like moderately; 8= like strongly; 9= like extremely

Statistical evaluation of proximate data

Using PCA, the ten original proximate and analytical variables were reduced to three principal components (PC1-PC3), which had eigen values larger than one and retained for rotation (Hair *et al.*, 1998). PC1, PC2 and PC3 accounted for 31.92%, 27.39% and 24.11%, respectively and the total variations (83.42%) are presented in Table 4. Graphical representation of principal components (PC1 and PC2) of analytical variables is given in Figure 2.

To assist interpretation of dimensions, the factor pattern was rotated using the varimax method. Based on the guidelines provided by Stevens (1992), an attribute was correlated to load heavily on a given component if the factor loading was greater than 0.72. A total of ten proximate attributes loaded

Table 4: Results of principal component analysis of biochemical data of Tendu fruit wine

Biochemical variable	PC1	PC2	PC3
TSS	0.743	-0.007	0.349
Total sugar	0.789	0.139	0.065
Titrable acidity	0.140	0.609	0.682
рН	-0.895	-0.046	0.014
Phenol	0.800	-0.485	-0.228
Ascorbic acid	-0.879	0.052	0.155
Lactic acid	-0.284	0.900	0.279
Ethanol	0.590	0.622	-0.214
â-carotene	0.233	-0.955	0.116
DPPH	-0.312	0.035	0.897
Eigen value	3.891	2.439	2.013
Variance explained (%)	31.921	27.398	24.111
6			

Extraction method: principal component analysis.

Rotation method: varimax with Kaiser normalisation (Eigen value >1).

heavily on three dimensions. Five analytical variables, *i.e.* TSS (+), total sugar (+), pH (-), phenol (+) and ascorbic acid (-) were loaded on PC1 indicating a strong correlation among these attributes. Substantial factor loadings of â-carotene (-) and lactic acid (+) were loaded on PC2. DPPH (+) was loaded on PC3.

There are several studies where PCA was applied for the evaluation of wine. PCA was used for the study of relationship among proximate variables of litchi wine. PCA reduced the eight original proximate variables to two independent components which accounted for 100% of the variations (Kumar *et al.*, 2008). Similarly, PCA reduced the 11 original analytical and proximate variables of herbal sweet potato wine to four independent components, which accounted for 74.53% variations (PC1, 24.18%; PC2, 20.18%; PC3, 15.41%; PC4, 14.76%) (Panda *et al.*, 2012).

Conclusion

In the present study, we have evaluated bio-chemical and sensory attributes of tendu wine which was well acceptable among the consumers. Tendu fruits are seasonally available in South Asian countries like India in sufficient quantities during the summer months (May-June). The shelf-life period of the fruit is very less (<1 week). This study provides an avenue and scope to ferment these fruits into value-added products such as wine to preserve its nutrients, minerals, aroma and taste to the consumers round the year.

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