

Assessment of Diverse Substrates for Quality Wines Production

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ABSTRACT

The present study was conducted to assess a variety of substrates for quality wines production. Vegetable (black carrots), fruit (Chikoo), mixed fruit juice waste and herb (rose petals) with grapes were evaluated for their nutritional status and potential as a substrate for wine production. Black carrots juice (22°B), Chikoo juice (20°B), mixed fruit juice (21°B) and grape juice with rose petals (22°B) were fermented using *S. cerevisiae* MTCC 11815 and 0.2% (w/v) DAHP supplementation that yielded 13.1, 9.7, 13.4 and 12.3 (% v/v) ethanol in 5, 3, 4 and 11 days, respectively. All prepared wines showed an ample amount of total phenolics and ascorbic acid. Black carrot and grape juice rose wine also contained a high amount of anthocyanins that add to antioxidant their antioxidant value. Rose wine and black carrot wine possessed *in vitro* antioxidant potential and total free radical scavenging activities with EC₅₀ value 88.9 and 76.0% whereas mixed fruit wine and Chikoo wine have 50.1 and 30.0% activities. Concentrations of rose, black carrot, mixed fruit and Chikoo wine needed for 50% inhibition were 53.01, 63.4, 100.0 and 179.7 μM and their AEAC values were 0.32, 0.27, 0.17 and 0.09 μM, respectively. Hence, all these substrates can serve as a suitable alternative to grapes to produce quality wines containing adequate amount of nutrients and antioxidants.

Keywords

Antioxidants, Black carrots, Chikoo, Mixed fruit juice, Rose, Wine.

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Introduction

The talent to produce appetizing effervescent beverage and wine by alcoholic fermentation of natural fruit juices is a revelation of inherent ingenuity of man. Fruit wines are fermented alcoholic beverages made from a variety of base ingredients (other than grapes) such as fruits, vegetables, flowers, and herbs; that add additional flavors. Most fruits and berries have the potential to produce wine. Few fruits other than grapes have the balanced quantities of sugar, acid, tannin, nutritive salts for yeast feeding and water to naturally produce a stable, drinkable wine, so most country wines are adjusted in one or more respects for fermentation of local fruits

to produce their wines (Okunowo *et al.*, 2005).

There are plenty of tropical fruits and vegetables in India which includes guava, watermelon, pineapple, plum, papaya, orange, black carrots, muskmelon, watermelon etc that are rich in nutrients. These fruits are highly perishable, and prone to bacterial and fungal contamination as a result they fail to reach the market due to spoilage, mechanical damage and over ripeness (Ihekoroye and Ngoddy, 1985). Besides, these fruits are difficult to keep for considerable length of time; hence the ripe fruits are utilized either

as fresh or processed into juice and specialty products (Oyeleke and Olaniyan, 2007). High rate wastage of these fruits and vegetables especially at their peak of production season necessitates the need for alternative preservation and post-harvest technologies towards an enhanced utilization of these fruits.

Fruit juices are fermented to produce wine, an undistilled nutritional alcoholic beverage. Using species of *Saccharomyces cerevisiae* which converts the sugar in the fruit juices into alcohol and organic acids, that later react to form aldehydes, esters and other chemical compounds which also help to preserve the wine (Fleet 2003; Duarte *et al.*, 2010; Isitua and Ibeh, 2010). Home-made wine production has been practiced with various fruits such as apple, pear and strawberry, cherries, oranges, watermelon, guava, etc. Yeasts from other sources such as palm wine has also been used (Ayogu, 1999) in the production of fruit wine. Evaluating different fruits, vegetables and herbs for wine production is always being a new area of research in terms of wine diversification. The production of wines from common fruits and vegetables can also help reduce the level of post-harvest losses besides increasing diversity of wines in India (Okoro 2007; Aloba and Offonry, 2009).

The present study evaluated the fruit (Chikoo), vegetable (black carrot), herb (rose) and mixed fruit juice waste for production of quality wines.

Materials and Methods

Procurement of culture

Pure culture of *Saccharomyces cerevisiae* MTCC 11815 was collected from Department of Microbiology, PAU, Ludhiana, India and sub-cultured on Glucose yeast extract (GYE) agar medium and stored in refrigerator at 4°C.

Inoculum preparation

The inoculum was prepared by inoculating the slant culture in 100ml of sterile GYE broth taken in 250ml capacity flask and cultured on a rotary shaker (100rpm) for 24 hours.

Seed culture preparation

From the prepared inoculum of *Saccharomyces cerevisiae* 11815, 5% (v/v) was added to the required amount of respective juices (5% v/v) and kept on a rotary shaker (100rpm) for 24 hours at 28±2⁰ C for incubation for seed culture preparation.

Extraction of juice

Black carrots (10 kg), Chikoo (2kg), mixed fruit juice (2.5 kg) and grapes (10kg) were taken, washed, peeled (except for fruit juice) and juice was extracted that yielded 5L of black carrots and grape juice and 1L of each Chikoo and mixed fruit juice from respective substrates. Physiocochemical analysis of juices were carried out to estimate TSS, pH, acidity, total sugars (Dubois *et al.*, 1956), reducing sugars (Miller, 1959), ascorbic acid (AOVC, 1996), anthocyanins (Zoecklein, 2005), total phenols (Malik and Singh, 1991). Sugar was chaptalized to adjust final Total Soluble Solids (TSS) of all juices to 20-22° Brix for ethanolic fermentation.

Potassium metabisulphite (KMS) was added @ 100mg/L to all juices to avoid growth of wild yeast. Following pasteurization of juices at 60°C-65°C for 10 minutes, cooled juices were inoculated with their respective seed cultures of wine yeast *Saccharomyces cerevisiae* 11815 @ 5% (v/v) and Diammonium hydrogen orthophosphate (DAHP) was supplemented @ 0.2% (w/v) as a source of nitrogen and phosphorus for yeast

growth. Fermentations were carried out under controlled temperature conditions of $28 \pm 2^\circ\text{C}$ till °Brix falls to zero. Periodic samples of different ongoing fermentations were taken and analysed for °Brix (By hydrometer), pH (pH meter; Hanna HI96107), acidity (Amerine and Roessler, 1976) and ethanol content (Caputi *et al.*, 1968).

Total antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to determine free radical scavenging activity of wines according to the method of Sanchez-Moreno *et al.*, (1999). EC_{50} value is the effective concentration of the sample showing 50% inhibition of free radical generation (Shimamura *et al.*, 2014).

The percent inhibition was calculated as following:

$$\text{Percent inhibition} = \frac{\text{O.D (Control)} - \text{O.D (Test sample)}}{\text{O.D (Control)}} \times 100$$

Ascorbic acid equivalent antioxidant activity (AEAC) was calculated as:

$$\text{AEAC} = \frac{EC_{50} \text{ of ascorbic acid } (\mu\text{M})}{EC_{50} \text{ of sample } (\mu\text{M})}$$

The higher AEAC value means the higher free radical scavenging activity.

Results and Discussion

Physicochemical analysis and ethanolic fermentation of various juices

Black carrots wine

Black carrots juice showed the initial TSS content of 4.7°B with 3.6% total sugars and 3.2% reducing sugars (Table 1). Juice was

chaptalized to raise the sugar level to 22°B before inoculation with *S. cerevisiae* seed culture. During the fermentation period of 5 days, pH drops to 4.4 from 5.5, acidity drops to 0.25% with non-significant decrease in ascorbic acid and anthocyanins content. While total phenolics content increased from 96.8 to 106.8 (mg/100ml) due to production of various secondary metabolites by yeast.

Final wine had 13.1% (v/v) ethanol content with fermentation efficiency of 93.0% (Table 2). In a similar study, Kocher *et al.*, (2016) reported the fermentation of black carrots juice using *S. cerevisiae* 5% (v/v) of starter inoculum culture and incubation at 25°C that lead to an ethanol content of 10.8% (v/v) with fermentation efficiency of 94.7% and an ethanol yield of 0.48 g/g of sugar.

Chikoo wine

Chikoo juice fermentation took 3 days to descend the fermentable sugars to 0°B from 20°B chaptalized by sugar from initial 8.9°B (Table 1 and 2). During the fermentation pH decreased from 5.4 to 4.6, acidity decreased to 0.24% and final ethanol content was 9.7% (v/v) produced with fermentation efficiency of 75.7%.

Mixed fruit wine

Mixed Fruit juice had 4.3°B that was increased to 22°B . Fermentation of mixed fruit juice yielded 13.4% (v/v) ethanol in 4 days with the fermentation efficiency of 95.1%. pH decreased to 4.6 from 5.8, acidity decreased to 0.25% during the process (Table 1). Gavimath *et al.*, (2012) also evaluated the different fruits (papaya, banana, orange and lime fruits) Comparative analysis of wine prepared from them revealed that banana fruits yield good quantity and quality alcohol (15.49%) when compare papaya orange and lime.

Table.1 Physicochemical analysis of black carrot, chikoo, mixed fruit juice and grape rose juice

Parameter	Black carrot	Chikoo	Mixed fruit juice	Grape Rose wine
TSS	4.7	8.9	4.3	16.8
Total sugars	3.6	8.3	4.0	16.3
Reducing sugars	3.2	7.9	3.2	15.6
pH	5.5	5.4	5.8	4.3
Acidity (%w/v)	0.46	0.38	0.41	0.59
Total phenols (mg/100ml)	96.8	76.8	70.1	232.6
Ascorbic acid(mg/100ml)	3.62	26.3	86.9	587.1
Anthocyanins (mg/100ml)	179.1	1.88	4.80	38.5

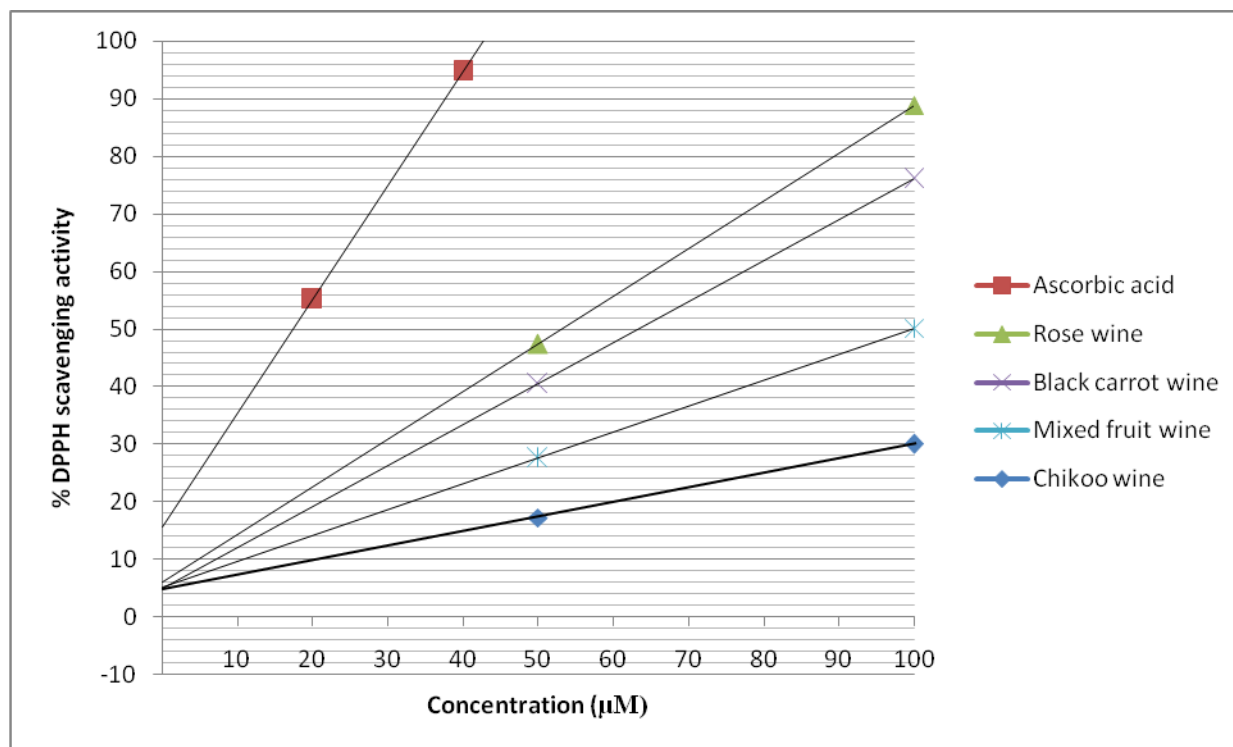
Table.2 Ethanolic fermentation of Black carrots, chikoo, mixed fruit juice and grape rose juice and comparative wine profile

Wine	Black carrot	Chikoo	Mixed fruit juice	Grape rose wine
Fermentation scale (L)	5L	1L	1.5L	5L
Initial TSS (°B)	22°B	20°B	22°B	21°B
Final TSS (°B)	0°B	0°B	0°B	0°B
pH	4.4	4.6	4.6	4.5
Acidity (%w/v)	0.25	0.24	0.25	0.26
Alcohol (%v/v)	13.1	9.7	13.4	12.3
Days to complete fermentation	5	3	4	7
Fermentation efficiency (%)	93.0%	75.7%	95.1%	91.5%
Total phenols (mg/100ml)	106.8	85.9	74.8	276.7
Ascorbic acid (mg/100ml)	3.06	24.9	84.8	564.2
Anthocyanins (mg/100ml)	178.1	1.37	4.82	37.9

Table.3 DPPH scavenging activity in respect of concentrations of black carrot, chikoo, mixed fruit and grape rose wine

Wine	% DPPH inhibition	EC ₅₀ Value	AEAC (µM)
Black carrot	76.0	63.4	0.27
Chikoo	30.0	179.7	0.09
Mixed fruit	50.1	100.0	0.17
Grape rose	88.9	53.01	0.32

Fig.1 Percent DPPH scavenging activity of rose, black carrot, mixed fruit and chikoo wine with ascorbic acid as positive control for calculating EC₅₀ values ($y=Ax+B$)



Grape rose wine

Grape juice (16.8°B) was supplemented with rose petals and physicochemically analysed. Rose petals known to increase the phenolics, flavonoids and anthocyanin content in the grape juice that add a characteristic dry flavor to the resulting wine. Phenolic content (232.6 mg/100ml), ascorbic acid (587.1 mg/100ml) and anthocyanins (38.5mg/100ml) was found in ample amount in the juice after addition of rose petals. Grape rose juice (22°B) fermentation completed in 7 days with production of wine with ethanol content 12.3% (v/v) with fermentation efficiency of 91.5% (Table 1 and 2). With a non-significant decrease in anthocyanins and ascorbic acid, phenolics showed a significant increase during the fermentation process. Earlier, Kumar *et al.*, (2017) reported the ethanolic fermentation of grape and guava juice at 50L scale by inoculating freshly prepared 24 h old

inoculum of *S. cerevisiae* MTCC 11815 (in jaggary solution @ 150 g/l) @ 6% (v/v) and 9% (v/v), respectively followed by incubation at 28±2°C (Pooja *et al.*, 2014).

Total antioxidant activity of wines

Antioxidant activity was in terms of % inhibition of DPPH was determined for wines produced. DPPH scavenging activity (Table 3) for black carrot, Chikoo, mixed fruit juice and grape rose wines were tested for determination of EC₅₀ values (Fig. 1) and AEAC values. Results revealed that concentrations of rose, black carrot, mixed fruit and Chikoo wine needed for 50% inhibition were 53.01, 63.4, 100.0 and 179.7 µM and their AEAC values were 0.32, 0.27, 0.17 and 0.09 µM, respectively. Further, the concentration of ascorbic acid taken as positive control needed for 50% inhibition was 17.2 µM. Earlier, grape juice has been

shown to possess DPPH activity in the range of 8.23 ± 0.17 , 2.51 ± 0.03 and 8.24 ± 0.19 mM in homemade, commercial and organic juice respectively (Burin *et al.*, 2010). Fruit wines reported to contain significantly higher total polyphenol content and hence possess greater antioxidant capacity compared to distilled beverages (Pinsirodom *et al.*, 2010).

Wines produced from different fruits i.e. rose, black carrot, mixed fruit and Chikoo having 12.3, 13.1, 13.4 and 9.7 (%v/v) ethanol, respectively. Grape rose wine and black carrot wine reported to have high ability to scavenge free radicals (88.9 and 76.0%, respectively) due to excess anthocyanins content. Whereas mixed fruit wine and Chikoo wine have low but significant 50.1 and 30.0% free radical scavenging activity. Hence, all evaluated substrates (black carrots, Chikoo, mixed fruit juice and grape juice with rose petals) wines can act as a nutritional source for people providing an ample amount of antioxidants and other nutrients.

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