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Effect of different concentrations of roselle on physico-chemical changes in pomegranate wine

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Abstract
Pomegranate (*Punica granatum* L.) fruits which were unmarketable were used for the preparation of wine. The effect of different concentrations of roselle on various physico-chemical properties of pomegranate wine was investigated. The wine prepared from pomegranate arils with 32ºB sugar syrup, 2.5 per cent rind powder and 0.4 % roselle was found best as compared to other treatments with higher TSS (11.45 ºB), ascorbic acid (81.55 mg/100 ml), antioxidants (342.43 mg AAE/100 ml), phenols (384.07 mg GAE/100ml), alcohol (10.69) during primary fermentation and at storage of 30, 60 and 90 days were found desirable with physico-chemical properties.

Keywords: Pomegranate wine, fermentation, TSS, alcohol, antioxidant activity

1. Introduction

Wine is one of the functional fermented beverages which have many health benefits. Commercially, wine is produced by the fermentation of yeast which involves the conversion of sugar to alcohol. The change in dietary habits, life style of people has increased the production and consumption of wine. At present, life style diseases such as obesity, diabetes, aging problems and cardio vascular problems have been on the rise. The antioxidants present in wine have a significant role in prevent the oxidative damage caused by free radicles. Studies have reported that certain types of wines reduce the risk of heart attacks and high cholesterol level (Katalinic *et al*., 2004) [6]. Also, fruit wines like cranberry, elderberry and blueberry are good source of flavonoids (Negi and Dey, 2009) [7]. Wine has been use as food and medicine since ages. The alcohol in wine stimulates gastric secretions and depresses nervous system. The excess consumption of wine however, causes severe depression in coordination of movements and loss of consciousness (Chavan, 2008) [4].

Fruit wine is an undistilled, low alcoholic beverage containing all the natural ingredients of the fruits like vitamins, amino acids, polyphenols, flavonoids, tannins, anthocyanins and minerals in it, which together makes it a nutritive health drink of high commercial value (Rana, A. and Singh H. P., 2013) [10].

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae is a native to the Iranian Plateau. Pomegranate has been extensively used as a source of traditional remedy for thousands of years. The rind of the fruit and the bark of the tree are used as a traditional remedy against diarrhea, dysentery and intestinal parasites. The seeds and juice are considered as a tonic for the heart and throat, and classified as a bitter-astringent (*pitta* or fire), and considered a healthful counter balance to a diet high in sweet-fatty (*kapha* or earth) components. The astringent qualities of the flower juice, rind and tree bark are considered valuable for a variety of purposes, such as stopping nose bleeds and gum bleeds, toning skin.

Roselle, also known as guinea or Indian sorrel is a member of the *Malvaceceae* family and grows in the tropic and sub-tropical regions of the world. Red roselle calyx has been reported to be rich in antioxidants and it contains a mixture of organic acids such as citric, malic and tartaric acids. These characteristics suggest that roselle calyx extract may be a suitable raw material for the production of colored wines.

2. Material and Methods

The present investigation on preparation of pomegranate wine was conducted at the Department of Post Harvest Technology, College of Horticulture, Bangalore, during the year, 2016-17. Pomegranate fruits which were unmarketable (cracked, bruised broken and cut) were obtained from the K.R. market, Bengaluru. All the chemicals used in this investigation were of analytical grade. Inoculum was prepared by using *Saccharomyces cerevisiae* var. *Ellipsoideus* strain (ZYMAFLORE FX10) which was used for entire experiment.

The culled (unmarketable) fruits bought from the market were washed and cleaned. The arils were separated from the fruits manually. Any infected arils were separated. The arils were treated with 0.2% sodium benzoate by dipping in the solution for one minute and drained immediately to remove the excess of solution.

For preparation of pomegranate rind powder to be used in the wine making, the rind was dried in dryer at 70° C for 8 hour; the dried rind was made to powder like flakes and used. For preparation of roselle concentrate to be used in the wine making, the dried calyx was subjected to water extraction 1: 10 ratio, later concentrated by rotary evaporator.

2.1 Preparation of pomegranate wine

Separated arils of one kilogram was weighed in a conical flask of two liters, sugar syrup of 32⁰B was prepared by using cane sugar. Pomegranate rind powder is prepared by drying rind in dryer at 65º C for 8 hours. One liter of sugar syrup of 32º B was transferred to conical flask. Immediately different concentrations of roselle of 0.1 %, 0.2 %, 0.3 % and 0.4 % and 10 ml of inoculum per Kg of arils were added to the must and the conical flask was cotton plugged. CO_2 discharging unit was fixed to flask allowing it for primary fermentation. After 7 days of primary fermentation transfer fermenting liquid into pre sterilized one litre bottles and the bottles were kept for secondary fermentation under low temperature (5-10º C). The flow chart of the entire procedure is shown in fig. 1.

The parameters like TSS, ascorbic acid, antioxidants, phenols, alcohol content were estimated by using standard procedures.

Select of mature, healthy fruits

⇓

Extract the arils and dip in Sodium benzoate (200ppm) solution

⇓

Drain out the extract solution

⇓

Prepare 2.5 % pomegranaterind powder and roselle concentration of
0.1, 0.2, 0.3, 0.4 %

⇓

Prepare sugar syrup of 32 ºB and was added to 2 litre conical flask containing1 kg of arils

⇓

Add 10 ml of inoculum

⇓

Close the conical flask with a cork having gas discharging unit

⇓

Allow for primary fermentation for about 21 days and then transfer the liquid to pre- sterilized bottles for ageing

⇓

Store at low temperature (10º C)

Fig 1: Flow chart of preparation of pomegranate wine

2.2 Treatment details

- T_1: Pomegranate aril + sugar syrup (32˚ B) + 2.5 % rind powder (control)
- T_2: Pomegranate aril + sugar syrup (32˚ B) + 2.5 % rind powder + 0.1 % roselle
- T_3: Pomegranate aril + sugar syrup (32˚ B) + 2.5 % rind powder + 0.2 % roselle
- T_4: Pomegranate aril + sugar syrup (32˚ B) + 2.5 % rind powder+ 0.3 % roselle
- T_5: Pomegranate aril + sugar syrup (32˚ B) + 2.5 % rind powder + 0.4 % roselle

2.3 Physico chemical parameters observed

Total soluble solids (TSS °B)

The content of total soluble solids (TSS) in the aonla blended beverage was determined with the help of digital hand refractometer and expressed as degree Brix (ºB). Care was taken that the prism of the refractometer was washed with distilled water and wiped dry before every reading.

Ascorbic acid (mg 100 g^{-1}) (AOAC, 2006)

Vitamin C content was determined by 2, 6-Dichlorophenol indophenol (DCPIP) method (AOAC, 967.21). One ml of juice sample was mixed thoroughly with 4 per cent oxalic acid solution and volume was made up to 25 ml and from that 5 ml was taken and volume made up to 25 ml. Vitamin C content present in the solution was estimated by titrating a 25 ml quantity of the extract against DCPIP. Vitamin C content was calculated as mg of ascorbic acid equivalents per 100 ml of juice using a standard curve of L-Ascorbiacid.

$$\text{Vitamin C (mg/100g)} = \frac{\text{titrevalue} \times \text{Std.value}(\mu g) \times \text{TotalVol.ofextract} \times 100}{\text{Assayvolume} \times \text{Wt.ofthesample}(g) \times 100}$$

Antioxidant activity (mg/100 g)

The antioxidant activity of the pomegranate wine was determined using Ferric Reducing Antioxidant Potential (FRAP)assay, which is based on the principle of the formation of *o*-phenanthroline-Fe($^{2+}$) complex and its disruption in the presence of chelating agents (Benzie *et al*., 1996) [3]. Pomegranate wine (0.2 g) was suspended in 15ml acidified methanol (1:99); 1.8ml of FRAP reagent was added to 0.2 ml aliquot and incubated for 40 min. Ascorbic acid standards (2-10 µg) were subjected to the same reactions. Absorbance was read at 593 nm in an UV-Visible spectrophotometer (PG Instruments, T80+, UK), and expressed in ascorbic acid equivalents in mg/100 g.

FRAP reagent: 10ml of Acetate buffer of 0.1 M pH 3.6 + 1ml of TPTZ (2, 4, 6-Tri (2-pyridyl)-s-triazine) + 1ml of $FeCl_3$

Phenols (Mg gallic acid equivalents 100 g^{-1})

Phenols react with the oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent and form a blue colored complex, molybdenum blue which is measured at 700nm.Five ml of sample was taken andvolume was made up to 50 ml. Take 0.5 ml of the extract in test tubes; add 0.2 ml of Folin-Ciocalteau's Phenol Reagent followed by 3.3 ml of distilled water and mix well. After 2 min, add 1ml of sodium carbonate solution and mix. Allow to stand at room temperature for 30 minutes and read the blue color in a spectrophotometer at 700nm. Prepare a standard curve for phenols using gallic acid (GA) as standard.

Alcohol (%)

The alcohol per cent of wine is obtained from OENOFOSS, a wine scan having FTIR laser technology. It is a pre calibrareted instrument, used to get the alcohol content of different wines, like finished wine, Must under Fermentation, Sweet wine, parameters.

Procedure: Take 5 ml of sample and dearrate it. Put this sample to wine scan (Oenofoss) and run for a minute, which provides instant result within two minutes.

2.3 Statistical analysis

CRD (Complete Randomized Design) was used for conducting the experiment and results were analyzed as per the guide lines suggested by Panse and Sukhatne (1978).

3. Results and Discussion

TSS (ºB)

The TSS content was found to decrease upon ageing of wine from the initial day of storage towards the end of storage period in pomegranate wine (Table 1). There was a significant difference in the TSS among treatments. TSS content found to be higher in the treatment T_5 (Pomegranate aril + sugar syrup (32˚ B) + 2.5 % rind powder + 0.4 % roselle) i.e., 11.45 as compare to control 9.32 (T_1: Pomegranate aril +sugar syrup (32˚ B) + 2.5% rind powder) this was mainly because of increased roselle concentration.

Table 1: Effect of different concentrations of roselle on Total soluble solids (TSS °B) during fermentation and storage of pomegranate wine. DAS - Days After Storage, T_1: Pomegranate aril +sugar syrup (32˚ B) + 2.5% rind powder (control), T_2: Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.1% roselle., T_3: Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.2% roselle., T_4: Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.3% roselle., T_5: Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.4% roselle.

Treatments	During fermentation				Storage period		
	1 DAY	3 DAY	5 DAY	7 DAY	30 DAS	60 DAS	90 DAS
T_1	26.56	20.13	15.65	11.43	9.72	9.45	9.32
T_2	27.61	22.45	17.02	12.55	10.5	9.62	9.56
T_3	28.09	23.98	17.56	13.97	11.02	10.53	9.89
T_4	28.57	24.81	18.09	14.78	11.76	11.37	10.89
T_5	29.45	26.03	19.52	15.63	12.32	12.01	11.45
S. Em. ±	0.09	0.07	0.07	0.13	0.07	0.09	0.06
CD @ 1%	0.65	0.49	0.47	0.87	0.51	0.65	0.45
Significance	**	**	**	**	**	**	**

Ascorbic Acid (mg 100 ml^{-1})

The decrease in the ascorbic acid content was observed in all the treatments of pomegranate wine during the storage period of 90 days (Table 2). The higher ascorbic acid content was observed in T_5 (Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.4%roselle) followed by T_4 (Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.3%roselle) this might be due to increased concentration of roselle. Whereas, the lowest ascorbic acid content was recorded in T_1 (Pomegranate aril +sugar syrup (32˚ B) + 2.5% rind powder -control). The decrease in ascorbic acid due to increase in the temperature and as the pH goes towards acidic it will depletes the ascorbic acid. Similar results obtained by Kalra and Tandon (1984) [5] were they reported decrease in TSS and ascorbic acid in storage of mango and guava juice. Vitamin C is directly correlated with antioxidant content during the storage period Vitamin C starts decreasing which is directly proportional to the antioxidant content of wine.

Table 2: Effect of different concentrations of roselle on Ascorbic acid content during fermentation and storage of pomegranate wine

Treatments	During fermentation				Storage period		
	1 DAY	3 DAY	5 DAY	7 DAY	30 DAS	60 DAS	90 DAS
T_1	86.80	95.90	99.05	83.30	78.75	72.45	69.65
T_2	88.55	99.40	105.00	85.75	81.20	74.55	72.10
T_3	92.05	103.25	106.75	89.25	87.50	77.35	72.45
T_4	93.10	105.70	107.80	90.30	88.90	78.40	79.10
T_5	95.90	108.50	110.95	92.75	91.35	83.30	81.55
S. Em. ±	0.66	0.71	0.85	0.85	0.71	0.60	1.20
CD @ 1%	4.32	4.67	5.55	5.55	4.63	3.95	7.83
Significance	**	**	**	**	**	**	**

Antioxidants (mg GAE 100 g^{-1})

In the present investigation the decreasing trend in the antioxidant content was observed during storage period (Table 3). The antioxidant content of 255.78 mg GAE 100 ml^{-1} was found highest with treatment T_5 (Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.4% roselle) has showed higher amount of anti-oxidant content of 342.43 followed by T_4 (Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.3% roselle) of 332.35 mg GAE 100 ml^{-1}. This was mainly because of increased concentration roselle. The antioxidant activity was increased with increased concentration of roselle extract in the blends as the roselle extract is known to be a good source of anthocyanins (Wong *et al*. 2002) [11]. Whereas, the lowest level of antioxidant of 276.77 mg GAE 100 ml^{-1}was observed in the treatment T_1 (Pomegranate aril +sugar syrup (32˚ B) + 2.5% rind powder -control).

Table 3: Effect of different concentrations of roselle on Anti-oxidants (mg AAE L^{-1}) during fermentation and storage of pomegranate wine.

Treatments	During fermentation				Storage period		
	1 DAY	3 DAY	5 DAY	7 DAY	30 DAS	60 DAS	90 DAS
T_1	120.98	190.32	268.97	301.08	292.42	287.06	276.77
T_2	142.76	210.98	290.43	332.7	323.56	309.34	304.68
T_3	159.05	235.89	320.55	348.34	339.8	332.12	320.52
T_4	167.85	264.74	349.78	352.47	341.75	336.89	329.04
T_5	189.54	278.83	367.42	378.91	365.37	357.92	342.43
S. Em. ±	2.03	2.26	2.03	2.42	3.06	4.69	5.08
CD @ 1%	13.24	14.77	13.24	15.82	19.93	30.59	33.14
Significance	**	**	**	**	**	**	**

Phenols (Mg gallic acid equivalents 100 g^{-1})

The decreasing trend in the phenolic content was observed during the storage time (Table 4). The highest phenolic content of 384.07 mg GAE 100 ml^{-1} was recorded highest in the treatment T_5 (Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.4% roselle). There was significant difference between the treatments with respect to phenols.

This might be due to releasing of higher amounts of phenols and tannins during fermentation. Similar results were reported by Wong *et al*. (2002) [11] that Total phenol and antioxidant activity (FRAP) content was increased with increased concentration of roselle extract in the blends as the roselle extract is known to be a good source of anthocyanins.

Table 4: Effect of different concentrations of roselle on phenolic content (mg 100ml^{-1}) during fermentation and storage of pomegranate wine.

Treatments	During fermentation				Storage period		
	1 DAY	3 DAY	5 DAY	7 DAY	30 DAS	60 DAS	90 DAS
T_1	150.23	189.59	259.32	330.21	303.24	282.12	263.58
T_2	175.56	198.23	314.78	352.27	328.25	302.21	293.6
T_3	201.42	267.37	352.19	387.93	356.76	379.45	375.62
T_4	234.78	298.36	375.84	412.32	372.23	381.94	378.28
T_5	259.01	320.65	389.85	423.87	394.5	386.63	384.07
S. Em. ±	4.67	9.20	7.02	14.43	1.52	1.55	0.37
CD @ 1%	30.47	59.96	45.77	93.99	9.96	10.13	2.42
Significance	**	**	**	**	**	**	**

Alcohol (%)

The increasing trend in the alcohol content was observed in storage period (Table 5). The alcohol content of 11.78 per cent was found to be significantly highest in the treatment T_5 (Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.4% roselle). The lowest alcohol percentage of 10.69 was observed in the treatment T_1 (Pomegranate aril +sugar syrup (32˚ B) + 2.5% rind powder - control). There was a gradual decrease in TSS with a corresponding increase in alcohol content. These changes became less pronounced after the 7th day of fermentation. The changes may be due to a gradual attenuation of the fermentation process due to decreased substrate concentration with a corresponding increase in alcohol concentration, which could adversely affect yeast activity. The roselle wine contained 10.8% (w/v) alcohol after aging. This value is comparable with that for lemon wine (10.1%) as reported by Alobo (2002) [1]. It is also comparable with that of wine produced by Okoro (2007) [8] from a blend of pawpaw and roselle extracts with a value of 10.5% (w/v).

Table 5: Effect of different concentrations of roselle on Alcohol (%) during fermentation and storage of pomegranate wine.

Treatments	During fermentation				Storage period		
	1 DAY	3 DAY	5 DAY	7 DAY	30 DAS	60 DAS	90 DAS
T_1	2.30	4.58	5.74	8.93	9.95	10.65	10.81
T_2	1.52	4.45	6.58	10.33	11.25	11.75	11.78
T_3	1.43	4.72	6.07	9.88	10.82	11.68	11.72
T_4	1.30	2.83	5.42	8.44	9.37	10.62	10.83
T_5	1.50	5.38	6.31	10.26	10.34	10.53	10.69
S. Em. ±	0.023	0.148	0.039	0.032	0.041	0.078	0.009
CD @ 1%	0.16	0.97	0.25	0.21	0.27	0.51	0.06
Significance	NS	**	**	**	**	**	**

4. Conclusion

The results of this experiment showed that the changes in physico chemical parameters of pomegranate wine viz. total soluble solids, ascorbic acid, antioxidants, phenols, alcohol content was found significant in all the treatments whereas, Treatment T_5 (Pomegranate aril + sugar syrup (32˚ B) + 2.5% rind powder + 0.4% roselle) has showed maximum retention of ascorbic acid, antioxidants compare to other treatments during storage.

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