FERMENTATION STUDIES ON POMEGRANATE AND SWEET ORANGE BLENDED JUICE

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Abstract

The suitability of two therapeutic fruits, pomegranate (Punicagranatum L.) var. Bhagva and Sweet orange (Citrus sinensis L.)var. nucellar were studied for the first time for the production of quality wine using Saccharomyces Cerevisiae NCIM 3218.The juices were extracted using hydraulic press and analysed for the physicochemical properties. The Total Soluble Solids (TSS)of pomegranate, sweet orange and blend juice 70:30 (pomegranate: sweet orange) was adjusted to 23 ^{o}brix. The inoculum was added in pasteurized and blend juices, which was fermented at 25 °C for three weeks. The juices and fermentation products were analysed for TSS, acidity, presence of ascorbic acid, sugar content, total phenolic content, antioxidant capacity and total anthocyanin. In addition the alcohol profile, minerals and vitamin composition of fermented products were studied. The blend juice wine was found to be rich in nutritional profile with total phenolic content of 1673.7 mg/L GAE, antioxidant capacity 29.01 mg/L of AAE and 62.92 %inhibition by Ferric Reducing Antioxidant Potential (FRAP) and Dihydroxy Phenyl Picryl Hydrazine (DPPH) method respectively. The anthocyanin content was found to be 173.9mg/L of cyanidin. Mineral profile shows Potassium 214.5 mg/L, Sodium 33.94 mg/L and Iron 4.12mg/L respectively in the blended wine samples. The vitamin B spectrum reveals 1.6mg/100gof vitamin B2(riboflavin), 0.6mg/100g of vitamin B5(Pantothenic acid)in pomegranate wine,while 0.067mg/100g of B3(niacin),1.3mg/100g of vitamin B5(pantothenic acid) and 0.1 mg/100g vitamin B6 (Pyridoxine) in the blend juice wine sample respectively. The blend juice wine was found to be highly acceptable in terms of taste, flavour aroma and other nutritional parameters studied.

Keywords: Blend juice, phenolic content, antioxidant activity, Dihydroxy Phenyl Picryl Hydrazine method, Ferric Reducing Antioxidant Potential.

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1. INTRODUCTION

In recent studies, the researchers have focused on exploring the bioactive properties of fruits, which were manifested mainly due to phenolic compounds. For humans, phenolic compounds in plants are “real treasure” because of the nutritional, functional and therapeutic qualities (Bhardwaj and Pandey, 2011). Pomegranate and Sweet orange are well known for the medicinal properties. Since ancient time, these fruits were used as an alternative medicine in *Ayurveda* globally. The extensive research has been carried out on plant polyphenols. Pomegranate polyphenols exhibit not only anti-oxidant, anti-mutagenic, anti-inflammatory and anti-microbial potential but also prevent various types of non-communicable diseases (Wasillaet al., 2013). Sweet orange (*Citrus sinensis* (L.)) is commonly known as “*Mosambi*” in Indian subcontinent, is effective in the treatment of various diseases. The presence of unique flavonoid compounds not only act as anti-cancer, gastroesophageal reflux, anti-diabetic and anti-ulcer agents but also used for the treatment of urinary disorders, various skin diseases, as an immunity booster and in weight reduction (RajuLal et al. 2014).Presently, consumer demand for healthy beneficial functional foods and beverages has increased due to awareness regarding the effectiveness of functional foods in combating various kinds of chronic diseases. The pomegranate being a rich source of bioactive

compounds such as phenols, (ellagicacid, punicalgin, punicalin and flavonoids), anthocyanin, ascorbic acid, vitamins and minerals (potassium, calcium, phosphorous, magnesium, sodium) as well the complex polysaccharides and organic acids emphasised to be the outstanding functional food (Mirdehghan SH and Rahemi M. 2007).The other important medicinal fruit of the citrus family is sweet orange which is rich in phenols (hesperidin, niriginin, catachin, epicatachin, gallic acid, vanilic acid etc.). Sweet orange flavonoid also exhibit health related properties like anti-cancer, anti-viral effects, anti-inflammatory activities on capillary fragility and inhibition on human platelet aggregation (Marzouk, B., et. al. 2013). It is also a good source of ascorbic acid (vitamin C), potassium bio-flavonoid and folic acid (Syed et. al., 2011) because of all these health benefits it is selected for present blended juice wine study.

Epidemiological studies have introduced the higher consumption of fruit wines globally, as it reduced the risk of cardiovascular diseases, (Natella et. al., 2001). Now days, low calorie and health beneficial wine products are the consumer preference. Many people have made the wine consumption as a part of the daily routine because of its health benefits. Although, attempts were made to intensify the health benefits of pomegranate by development of fermented beverages, the pomegranate juice and its fermented products lacks characteristic flavor. For better consumer acceptance, marketability and to enrich the nutritional profile of pomegranate wine, the fermentation of juice blends of pomegranate and sweet lime was attempted in this study. The nutritional profiling of important components associated with radical scavenging properties of juices, blend of juice and their wines were studied.

2. MATERIALS AND METHODS

Fruit collection and juice extraction

The fully matured pomegranate (*Punica granatum* L.) fruits of var. Bhagwa grown in *Hastabahar* (particular season to get good quality mature fruits) were harvested in month of March2018 from the farm of ICAR-National Research Centre on Pomegranate Solapur,India.The sweet orange fruit(*Citrus Sinensis L.)*(Mosambi) var.nucellarwere harvested from farmer's field located in Aurangabad, India. Pomegranate and sweet orange fruits were transported on the same day to the laboratory and stored at 5°C until used for the experiment. Fruits were washed with chlorine water (200 ppm sodium hypochlorite) and cut into two equal halves. The juice was extracted in hydraulic press (Johnston make) from halved fruits. The juice was stored at 5^{o}C for 24 hours for sedimentation and filtered using muslin cloth. All the chemicals required for the experiments were purchased from the Hi media.

Inoculum preparation

The wine yeast strain *Saccharomyces cerevisiae* NCIM 3218 was collected from the National Collection of Industrial Microorganisms (NCIM), NCL, Pune India and maintained on malt extract, peptone, yeast extract, dextrose (MPYD) agar (malt extract 0.3%, peptone 0.5 %, yeast extract 0.3 % and dextrose 2% and agar 2%) slants at 4°C. Before fermentation yeast cells were activated by inoculating the slant culture on sterile MPYD agar medium and incubated at 25°C for 24 hours. The activated cells were transferred to the100 ml of pasteurized pomegranate, sweet lime and pomegranate: sweet lime (70: 30) juice blend respectively and incubated for 48 hours at 25°C until the cell density of $3x10^{6}$cfu/ml was achieved.

Fermentation of Juice

For pomegranate, sweet orange and pomegranate: sweet orange (70: 30) juices were blended separately and TSS was adjusted to 23^{o} brix by using sucrose. The juices were pasteurized at 80°C for 10 minutes. Juices and blended juice were added with 48 hours old 30% inoculum and incubated at 25°C until the TSS drop reached constant value. Fermented broths were added with 0.1% bentonite and filtered using Whatman filter paper no.4.The prepared wine samples were pasteurized at

50°C for 10 minutes. The pasteurized wine samples were bottled and kept for ageing at 5°C. The pomegranate, sweet lime, blend juice and the their wine samples were analysed for physicochemical parameters such as TSS, acidity, total sugar, and reducing sugar. Assessment of bioactive components (total phenolic content, FRAP antioxidant activity, DPPH free radical scavenging activity) was also performed. The vitamin content and elemental profiles of all wine samples were analysed by HPLC-DAD and AAS-MIS method respectively. Three significant alcohols in blend juice wine (methanol, ethanol and isopropanol) were analysed using GC-MS. All the experiments were performed in triplicate and mean values are presented.

Analytical Methods

Physicochemical and bioactive compound assessment

The total soluble solids (TSS) of juice samples were measured using digital pocket refractometer (Atago, Tokyo, Japan) values corrected to 20°C expressed as ° brix. Titrable acidity (TA) was measured by titration and is expressed as percent citric acid (AOAC 1984). Total and reducing sugar was measured using Lane and Eynon method as described by Ranganna (2000). Ascorbic acid in juices and wine sample was extracted by4% oxalic acid solution and determined quantitatively according to the standard method described in the (AOAC 2000) based on the reduction of 2,6 di-chlorophenol indophenol by L-ascorbic acid(Harris and Ray 1935).Total phenolic compounds were determined by Folin-Ciocalteu (FC) colorimetric method at 765 nm as described by Singleton and Rossi (1965).The results were expressed as mg of Gallic acid equivalent (GAE) per litre of the sample. Total anthocyanin contents were estimated by a pH-differential method using two buffer systems, 0.025 M potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5 using a spectrophotometric method at 510 and 700 nm wavelengths(Wrolstad et al., 2005). Anti-oxidant activity was determined by performing FRAP assay reported by (Benzie,I.F.F. and J.J. Strain 1996)with slight modifications. Free radical scavenging activity using DPPH assay was determined as like (Siddhurajuet al., 2002) and DPPH % inhibition was calculated.

Vitamin analysis

Vitamin analysis was performed only with wine sample. Diluted sample with water (1:1 proportion) was analysed by UHPLC-DAD. The analysis was performed by using a Dionex HPLC system with Chromeloen software. DionexLGP40 pump and AD 250 UV detector were employed along with a Thermo AS3500 auto sampler. Chromatographic separation was achieved by Hibar column Purospher, RP C_{18} (250 mm ×4.6 mm with internal diameter 5µm) column. The column temperature was maintained at 35º C, and the column elution was carried out by the gradient programming of the mobile phase with the flow rate 0.6 mL/ min; injection volume 20 µl, runtime 15 minutes). The wavelength used for the analysis was 232nm, 268nm, 214nm, 220nm, and 205nm for vitamin B2 (riboflavin), B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine). A gradient condition of a mobile phase composed water (A) and acetonitrile (B) was used for vitamin analysis.

Elemental analysis

Elemental analysis was performed for wine samples. The potassium, calcium, iron and sodium were analysed using atomic absorption spectrophotometer. The Agilent 4200 MP-AES system was used for analysis. For sample preparation, 0.1 ml of nitric acid was added to each sample (0.20 g) and volume was made to 10 ml using Milli-Q.

Alcohol profile

GC-MS analysis of blend juice wine sample was performed by using Agilent 7679A system coupled with quadrupole mass selective detector (electron impact ionization; positive mode) and operated at 70 eV. Helium gas was used as the carrier gas at a constant flow rate of 1.19ml/minute and injection volume of 1µl (split ratio 50:1).The oven temperature was initially held at 40 °C for 2.25 minutes and raised to 100°C at the rate of 20°C /minute.

With the holding time zero min. The equilibrium time was set to 0.5 minute the interface and quadrupole temperatures were 230°C and the total GC/MS running time was 5.25 minutes. The analysis was performed on Agilent J&W HP-5ms column and it was provided with (5% - Phenyl) - methyl polysiloxane material. The MS was operated in scan mode with a mass range of 30 to 250 a.m.u. The reference ion (m/z) and internal standards were used for identification and quantification of methanol, ethanol and isopropanol.

Sensory evaluation

The wine samples were Organoleptically evaluated by a twenty member panel of judges on nine point hedonic scale as per the standard methods (Ranganna2000)

Statistical analysis

Experimental data were analysed for analysis of variance (ANOVA) and differences between means were assessed by Duncan's new multiple range test at the significance defined $p \leq 0.001$using SAS 9.3 software. Sensory data were statistically analysed using Microsoft excel ANOVA ($p \leq 0.05$) at 95% confidence interval. The mean of each parameter was used for the graphical interpretation.

3. RESULTS AND DISCUSSION

The pomegranate juice has shown significantly higher TSS and total sugar as compared to sweet orange. Sweet orange juice shows high acidity (35.13%) than pomegranate juice. At the end of the fermentation process TSS was found to be reduced up to 12.4, 7.6 and 13.3°brix in pomegranate, sweet orange and blend juice wine respectively listed in Table 1. During fermentation TSS of blend juice wine, pomegranate and sweet orange wine decreased from 23 °brix to 13.4, 11.9 and 7.7 °brix respectively (Fig.1). The fermentation process completed at the end of the 20th day for pomegranate and blend juice wine but it took about 22 days for sweet orange wine. This signifies the utilization of sugar by yeast cells for the alcohol production with consequent reduction in TSS and also signifies that the yeast cells (*Saccharomyces cerevisiae*) supports in the sweet orange wine fermentation more effectively. The blending of sweet orange juice with pomegranate enhances the rate of fermentation.

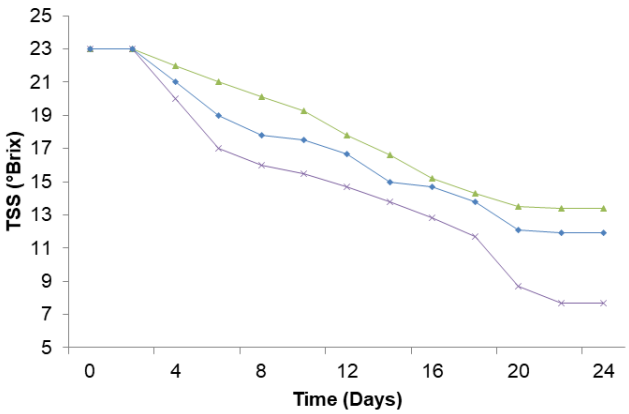

Fig. 1: Trend in TSS decrease recorded with three different wine samples

----- *Citrus wine;*
----- *Blend wine;*
----- *Pomegranate wine.*

Table 1: Physicochemical composition of juice and wine samples

Samples / Parameters	TSS (° brix)	Acidity (%)	Total sugar (%)	Reducing Sugar (%)
Pomegranate Juice	14.1^{a}	0.37^{d}	13.83^{a}	12.8^{a}
Sweet lime juice	10.4^{e}	0.50^{c}	10.13^{d}	9.2^{c}
Blend Juice	11.8^{d}	0.39 ^{d}	12.5^{c}	11.2^{b}
Pomegranate wine	12.4^{c}	0.68^{b}	13.83^{a}	12.8^{a}
Sweet lime wine	7.6^{f}	0.75^{a}	8.1^{e}	6.7^{d}
Blend wine	13.3^{b}	0.67^{b}	13.8^{a}	12.5^{a}

The values are mean of triplicates and mean with different letters are significantly different from each other at P≤0.05 according to Duncan's Multiple Range Test.

The acidity plays an important role in maintaining the organoleptic property of wine. The major acids which affect on sourness of wine are tartaric, malic, citric, lactic acids which are responsible for the astringency of wine (Sowalsky et al., 1998). The data presented in table 1 shows the remarkable difference in the acidity of pomegranate, sweet orange and blend juice. The blend juice was found to be more acidic than pomegranate juice. The acidity of juices and blend was found to be increased during fermentation process. This may be due to the development of non-volatile compounds during fermentation (Thoukis, G. et.al., 1965). The total sugar in blend wine was found to be 13.8% and reducing sugar was 12.5%. Sugar content varies according to the stage of ripening, climatic condition and analytical method used for estimation of the sugar content in fruits varies. The blend wine physicochemical composition was reflected or estimated at pH 3.5.

The obtained values of bioactive compound assessment are presented in Table 2, while fig. 2 reflects transition in the bioactive constituents for each juice (pomegranate, sweet orange, blend juice) before and after fermentation. The Phenolic compounds are main bioactive compounds in fruit juices and wine (Fukumoto et al., 2000).The phenolic content in pomegranate juice was 2546.6mg/100mL of GAE, which is in accordance with the findings of Janbi, A.H.A. and Al-Said, S.A., 2014.Phenolic compounds content in sweet orange was found to be 749.33mg/L of GAE which is almost 4 times lower than pomegranate juice. While the blend juice of 70:30 ratio of pomegranate and sweet orange was reported to have 2127.0 mg/L. After fermentation, the phenolic content were found to be1982.3 mg/L of GAE,568.0 mg/L of GAE and 1673.7mg/L of GAE respectively in pomegranate, sweet orange and blend juice wine. As per statistical analysis, the phenolic content in the blend juice wine was not significantly different from that of the pomegranate wine. The reduction in phenolic content in all wine samples than the respective juices were due to polymerization, condensation, hydrolysis, enzyme activity , interaction of antioxidants and yeast cell wall (Verdin H et al. 2003).Although fermentation stabilizes and preserves the bioactive components they get transformed during fermentation and remain unchanged after fermentation. Evaluation of phenolic content reflected pomegranate and blend juice wine are rich in phenolic compounds than available grape wine phenolic content. Which also indicate the noteworthy potential of blend juice wine as more health beneficial product than grape wine (Akalın, A. C et al. 2018).

Table 2: Assessment of bioactive components

	Total phenolic content (mg/LGAE)	FRAP antioxidant activity (mg/Lof AAE)	DPPH activity % Inhibition	Anthocyanin content (mg/L of cynidine)	Ascorbic acid (mg/100ml)
Pomegranate Juice	2546.66^{a}	31.79^{a}	73.44^{b}	26.23^{a}	14.16^{d}
Sweet lime juice	749.33^{d}	19.24^{e}	79.26^{a}	14.71^{e}	47.5^{a}
Blend Juice	2127.0^{b}	28.7^{f}	73.85^{b}	22.67^{b}	23.76^{c}
Pomegranate Wine	1982.3^{bc}	29.74^{b}	60.08^{d}	20.76^{c}	12.5^{d}
Sweet lime wine	568^{d}	19.240^{e}	77.51^{a}	10.86^{f}	37.5^{b}
Blend wine	1673.7^{c}	29.01^{c}	62.92^{c}	173.9^{d}	22.5^{c}

The values are mean of triplicates and mean with different letters are significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range Test.

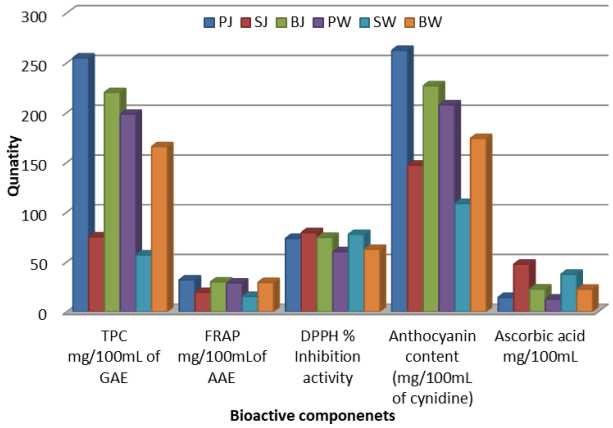

Fig. 2: Bioactive component in juices and wine samples

***PJ**: Pomegranate juice, **SJ**: Sweetlime juice, **BJ**: Blend juice, **PW**: Pomegranate wine, **SW**: sweetlime wine, **BW**: Blend wine*

Colour is the most important attribute used by consumers to select quality wine and its highly depend on the phenolic composition and anthocyanin content (Abe L.T et al. 2007).In the present study the pomegranate shows higher anthocyanin content of 26.23mg/L cyanidin which is lesser than the contents reported by Janbi, A.H.A. and Al-Said, S.A., 2014. The difference in similar fruit cyanidin content may be due to the changes in climate and the geographic region of cultivation. The anthocyanin content in sweet lime and blend juice was found 14.72 mg/L of cyanidin and 22.67 mg/L respectively. Blend juice anthocyanain content was detected significantly different (p<0.001) than pomegranate and sweet orange juice anthocyanain. Anthocyanin from pomegranate may useful in reducing blood pressure, preventing prostate cancer, colon cancer and arthritis and also helps in maintaining blood glucose level in the normal range (Raju Lal et al., 2014). After blend juice fermentation the anthocyanin content was found to be 17.39mg/L, which is significant reduction (5.3%). In pomegranate and sweet lime wine the anthocyanin content was also found significantly lowered, 20.76 and 10.86 mg/L respectively. Anthocyanin reduction because of fermentation is due to the reaction of anthocyanin with yeast cell wall, hydrolysis of the anthocyanin molecule to anthocynidine and glycosides or else yeast metabolic activities have generated polymerization of anthocyanin with aldehyde even the presence of oxygen, metal ion and light has adverse effect on anthocyanin content and induces brown polymer formation in wine (Gumienna, M.et al, 2016).

Ascorbic acid and polyphenols present in sweet orange are the chief nutritional components (Jayaprakasha G.K., et al., 1977**).** The present studies reveals the positive correlation of ferric ion reducing antioxidant activity with total phenolic content in pomegranate and blend juice wine. The FRAP activity in the blend juice wine was significantly higher 29.1mg/l of AAE than the blend juice (28.7mg/l of AAE). This may be due to fermentation more ferric reluctant formation was occurred and quantified as FRAP activity (Yadav N., et al 2018).In pomegranate juice antioxidant activity was 31.79 mg/L of AAE while in pomegranate wine it was 29.24 mg/L of AAE. Fermentation process reduces antioxidant activity significantly in pomegranate wine. In sweet orange there is no reduction in antioxidant activity (19.24 mg/L of AAE in juice and wine) because of the presence of higher ascorbic content (Gardner et al., 2002), which are contributory in antioxidant potential.

DPPH free radical scavenging activity, significant percentage inhibition reduction was observed as a fermentation effect in pomegranate and blend juice (73.44% to 60.08% and 73.85% to 62.92%) respectively, however in sweet orange wine statistical significant reduction in free radical scavenging activity was not reported even after fermentation (79.26 to77.52%).These studies also confirm the positive correlation between free radical scavenging activity and its phenolic content in pomegranate and blend wine. While in sweet orange juice and wine antioxidant activities attributed to phenolic and ascorbic content. Vitamin C was found to account for 65-100% of the antioxidant potential of beverages derived from citrus fruit (Gardner et al., 2000). These findings are in accordance

with the studies on antioxidant activity of guava fruit extract which also revealed the high correlation between the antioxidant activity and ascorbic acid content in vitamin C rich fruits (Thaipong, K., et al., 2006).The recorded value of antioxidant activity in the blend juice wine was higher compared to other fruit wine. (D Beer et al., 2003).

Ascorbic acid content in sweet lime juice was higher (23.54%) than pomegranate juice. Because of fermentation significant loss in ascorbic acid was detected in samples except blend juice. The blended effect of pomegranate to sweet orange enriched the wine with a significant amount of ascorbic acid. In present study pomegranate juice found to contain 3.5 times more ascorbic acid than sweet orange .The blend juice maximizes the ascorbic acid content to 23.76mg/100mLwhich was almost 1.5 times more than pomegranate juice. The reduction in pomegranate and sweet orange wine may be due to heat treatment and the fermentation process (Schvab M.D.C. et al.,2015), however said effect was not observed with blend juice its ascorbic acid remains unaffected even after fermentation. Higher ascorbic acid in blend wine not only make the wine ascorbic acid rich but also contribute to antioxidant enrichment. Ascorbic acid is more rapid and effective oxygen scavenger, hence may useful in prevention of blend juice wine from oxidation, despite of sensitive oxidisable wine components phenolic and flavour compounds (Cojocaru, G. A., and Antoce, A. O. et al., 2012).

Table 3 highlights on the content of major element and vitamins present in all wine samples. In pomegranate potassium is major element which is an electrolyte that regulate normal fluid balance in the human body. Deficiency leads to nerve irritability, cardiac and mental disorder, muscular weakness and paralysis. Sodium is also abundantly reported in pomegranate and blend wines. Sodium along with Chlorine (Cl) regulates the body fluid balance. Deficiency of sodium leads to body fluid loss and dehydration (Adotey et al., 2009).Significant amount of iron content was reported in pomegranate and blend wine, it regulates the synthesis of haemoglobin and normal functioning of central nervous system.Deficiency of iron leads to anaemic conditions (Adeyeye*et al*., 1999). Calcium was detected in high level in sweet orange wine, while very less in blend and pomegranate wine. Calcium is required for bone and dental tissue development, muscles contraction and nervous activity and blood clotting (Nandal U et al., 2013). Studies performed on vitamin assessment shows, higher vitamin B2 (riboflavin) in pomegranate (1.617mg/100 g) than sweet orange (0.17mg/100 g) while riboflavin content was not detected in blend wine. Riboflavin plays an important role in metabolism of carbohydrates. Highest riboflavin content in bananas and dates were 0.073 and 0.066 mg/100 g, respectively (USDA 2010). The blend wine reported with small amount of (0.067mg/100g) niacin while it is not detected in pomegranate wine and very less found in sweet lime wine (0.04mg/100g).

Table 3: Vitamin and mineral profile of blend wine

	Minerals (mg/L)				Vitamins (mg/100g)			
	Potassim	Iron	Calcium	Sodium	Vitamin B2 (Riboflavin)	Vitamin B3 (niacin)	Vitamin B5 (Pantothenic acid)	Vitamin B6 (Pyridoxine)
Pomegranate wine	230.0	6.05	0.02	38.25	1.617	ND	0.6	ND
Blend wine	214.50	4.12	2.70	33.94	ND	0.067	1.3	0.1
Sweet lime wine	166.68	1.65	9.41	8.46	0.17	0.04	ND	ND

All the values are mean of triplicates, ND: Not detected.

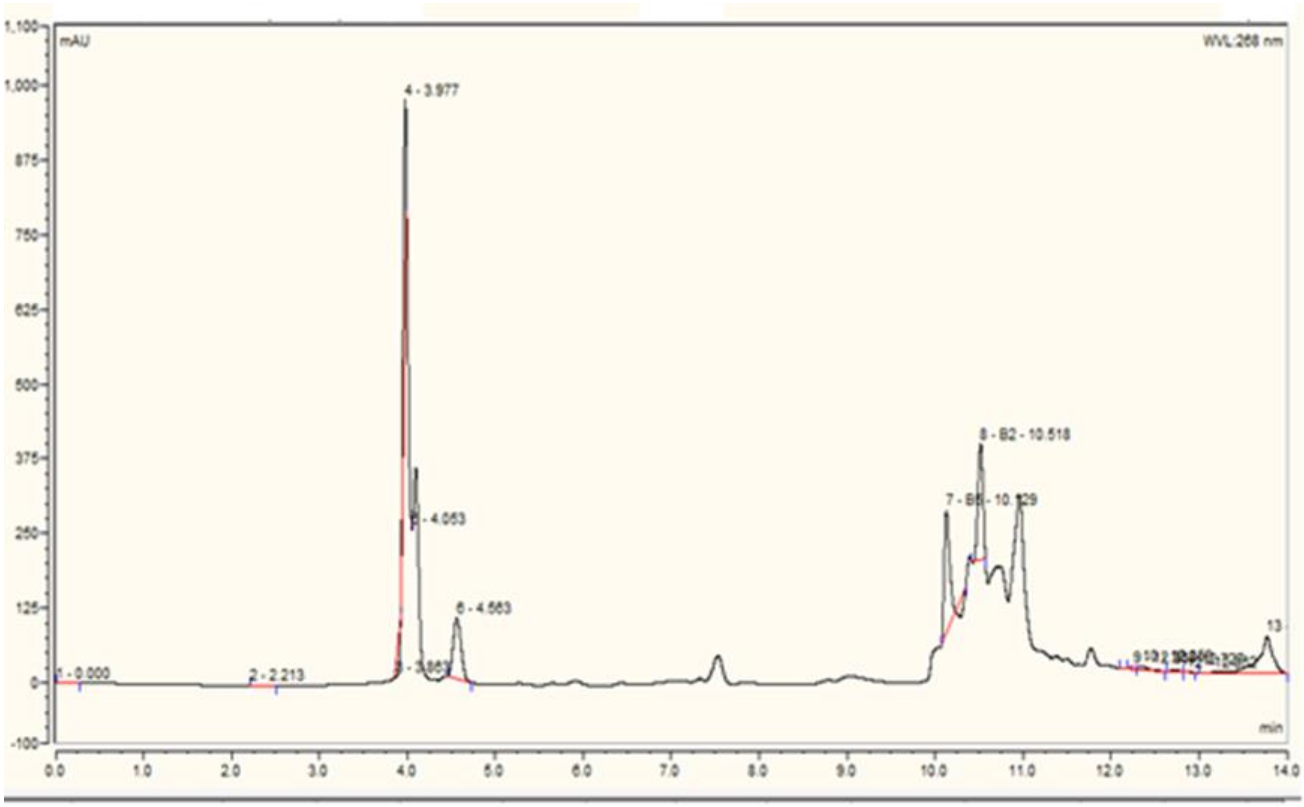

Fig. 3: Representative Chromatogram for sample blend wine showing Vitamin B3 at 268 nm wavelength

Fig. 3 is representative chromatograph showing the presence of vitamin B3 in blend wine at 268 nm wavelength. Pantothenic acid was found widely in all types of foods especially in nuts, vegetables, whole grains and various meats. (NIH, US department of health and Human services) However, in the present study prepared blend wine shows high value of pantothenic acid 1.3 mg/100g.The vitamin B6 is important in normal growth and development and homeostasis was found to be 0.1 mg/100g in blend wine, but not detected in pomegranate and sweet lime wine. Indeed various factors like climate, genotype, harvesting method maturity, storage conditions affects the vitamin composition (Elfalleh W.et al., 2011).Hence from the present investigation blend wine was found to contain good a amount of potassium sodium iron, vitamin B5 and B6.

Fig. 4 is a representative chromatogram of GC-MS analysis, which through the light on ethanol, methanol and isopropanol content in blend wine.

Ethanol content was detected 6.5%, 5.9% and 4% in pomegranate blend juice and sweet lime wine. While no methanol was detected in pomegranate wine and blend wine but 2.5% was found in sweet lime wine. The attempt to detect methanol was carried out because citrus fruit contains high amount of pectin which was converted into methanol during fermentation by a pectin methyl esterase enzyme (Ohimain, E.I. et al., 2016). Pectinase enzymes are present in fruits and even produced by yeast, bacteria, and fungi. The methanol production highly depends upon the type of yeast, quality of the fruits and maintenance of aseptic practices during the fermentation process. Isopropanol content in pomegranate, blend juice and sweet lime wine were found to be 1.1%, 0.9% and 2.5% respectively. Formation of higher alcohol depend upon the type of yeast, composition of juice fermentation condition and climate, even aeration during fermentation also leads to formation of higher alcohol. (B. C. Rankine et al., 1967).

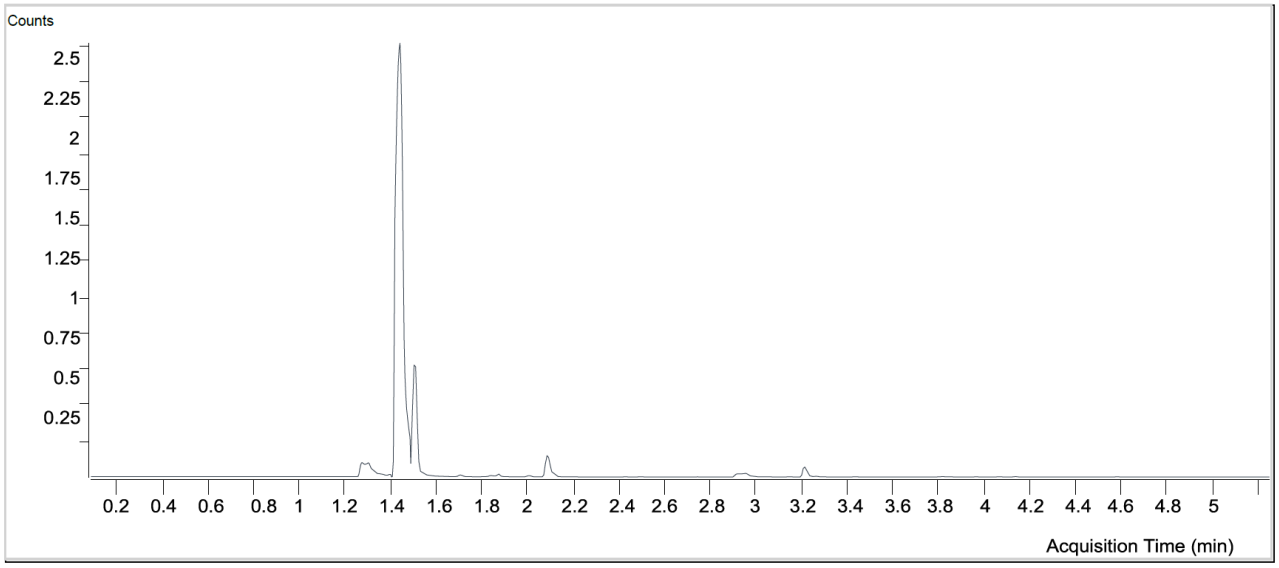

Fig. 4: Chromatogram showing the acquisition time for ethanol and isopropanol in blend wine

Higher alcohols and esters, produced during alcoholic fermentation, play an important role in the flavor of the wine, depending on the types of compounds and their concentrations. Higher alcohols positively affect the quality of wines when present in moderate quantities (Valero et al., 2002).

Sensory analysis

Sensory quality acceptance by consumer fosters the production distribution and marketing of any new type of beverage. The pomegranate sweet lime and blend juice wine were analysed for sensory analysis and scores are presented in fig 5, where pomegranate and blend juice wine were found good in all respect then sweet lime wine. However, blend juice wine was more acceptable in terms of flavour than pomegranate wine. The use of sweet orange in this study has enhance the flavour and aroma of wine significantly ($P<0.001$). The main contributory volatile compounds in fruits are esters, alcohols, aldehydes, ketones, lactonterpenoids and apocarotenoids (Wendakoon S. et al., 2006). Studies on volatile flavour compounds reported limonene, β-myrcene, linalool, hexanal, ethyl butanoate as a major contributory flavouring compounds of *Citrus sinensis* (Cuevas F, et al., 2007).Esters are also most significant to orange flavour. Ethylbutanoate followed by ethyl acetate, ethyl propionate, and methyl butanoate, in fruit juice mainly contribute in flavour (Qiao, Y, et al. 2008). Sweet orange flavour is probably the most widely recognized and accepted in the beverage industry. There was no significant difference in terms of colour, mouth feel, after taste parameters of blend juice wine and pomegranate wine. Sensory evaluation studies on blend wine shows good sensory acceptability especially in terms of flavour, aroma & taste than pomegranate and sweet orange wine.

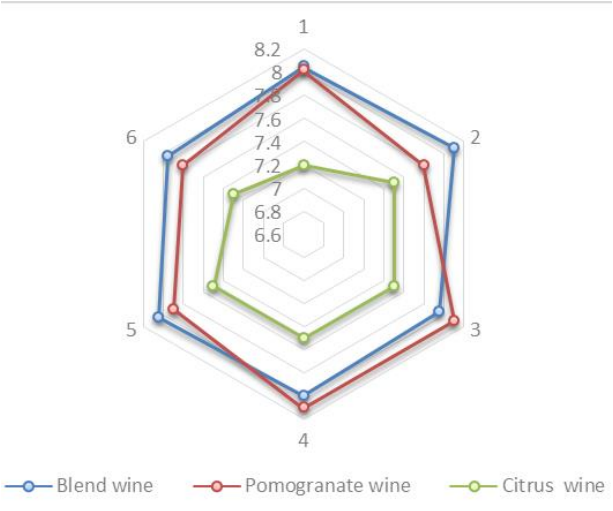

Fig. 5: Sensory evaluation of blend wine: 1-taste, 2- flavour, 3- colour, 4- mouth feel, 5-aroma & 6- after taste

4. CONCLUSION

Briefly to conclude, blend juice wine prepared from the extraction of the whole fruit of pomegranate and sweet orange was found better in terms of flavour and aroma. It was recorded with higher antioxidant activity, ascorbic acid content. The blend juice wine also found enriched with moderate amount of vitamin B and few important minerals. As per the obtained data, there is a significant loss of total phenolic content, total anthocyanin content and antioxidant activity, based on the technological treatments applied during fermentation. Novel technological treatments causing less adverse effect on wine quality need to be applied in further study. The investigation recommended the use of two therapeutic fruits for the preparation of quality blend wine. However, sensory properties are crucial in the choice of alcoholic beverages by the consumer; flavour is the most important aspect of quality. Furthermore employing state of art of analytical techniques for complete profiling of flavouring compounds would help to understand the optimization and fruit favour retention in blend wine for the benefit of both consumers and industry.

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