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CANE WINE BLENDED WITH POMEGRANATE FOR VALUE ADDITION

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ABSTRACT:

Food processing imparts value addition. Antioxidant *resveratrol* in grapes are readily bioavilable when they are processed and converted into wine and wine is one of the most popular beverages of the world. Wine preparation could be an alternative method for post harvest management. Earlier wine is prepared from grapes, but grapes are costly and seasonal. Sugarcane (*Saccharum officinarum* L.) which contain very high amount of sugar, cheaper and easy available throughout the year when blended and fermented with antioxidant rich Pomegranate juice (*Punica granatum* L.) yields a high alcohol percentage and desirable colour, and maximizing the bioavailability of antioxidant flavonoids. It inhibits oxidized LDL uptake and Cholesterol (Parashar, 2012)[1]. The study will help in managing the post harvest overripe fruits from rotting and value added wine production.

KEY WORDS: *Value Addition, Acidity, Reducing Sugar, Sensory Evaluation.*

INTRODUCTION:

Wine production occurs in Georgia around 6,000 BC (Mark, 1996)[2]. Earlier, grapes where the main raw material used for wine preparation. Less attention is given to other fruits and vegetables as a source for wine making. The sweet characteristics of the sugarcane juice was exploited for wine production (Espinoza et al., 2005)[3]. Wine from sugarcane is traditionally prepared by Philippines (Sanchez, 1981)[4]. Most of the winery in the country or abroad at domestic as well as commercial level uses grapes or other fruit juices but idea of blending seems to be left out. Acknowledging the high contents of sugar in cane and the presence of high percentage of active antioxidant flavonoids in pomegranate, and increase bioavailability of these active components after processing; the present research has been designed using high flavonoid containing pomegranate, blending in sugarcane wine. The blending is expected to increase the efficiency in terms of health benefit and desirability.

MATERIAL AND METHODS:

Fresh sugarcane juice and pomegranate were collected from the local market (Mohanpur, Nadia, West Bengal, India). Extraction of juice from pomegranate was carried out following: washing, peeling, removing of juicy seeds and crushing in fruit juice extractor. The juices are then filtered through a strainer. The total soluble solid (TSS) of cane juice was made to 45^0Brix by adding sugar. Cane juice of 45^0Brix was blended in different concentrations (30%, 40%, 50% and 60%) with the juice of pomegranate (70%, 60%, 50% and 40% respectively) and final volumes were made up to 250 ml for each concentration. All blended substrates were adjusted to pH 4.5 by adding citric acid. The substrates were inoculated individually with "Y_4" yeast strain isolated from palm juice (an unknown strain collected from Department of Plant Pathology, B.C.K.V., Nadia, Mohanpur, India). Starter inoculums (1.2×10^8 cells per ml) at the rate of 10% of the substrate were inoculated and kept in the incubation chamber at temperature (26±2) ^{0}C for fermentation. After maturation of fermentation i.e., when the fermenting substrate show same ^{0}Brix consecutively for (2-3) days, it was subjected to analysis (fresh wine analysis) and the result was compared with those analysed after 6 months of storage (storage wine analysis). The analysis involves TSS using pocket refractrometer (expressed in terms of ^{0}brix.), pH by pH meter (Anon., 1995)[5], acidity as percentage citric acid by A.O.A.C. method (A.O.A.C., 1995)[6], reducing sugar by copper reduction method expressed as percentage (Ranganna, 2000)[7] and alcohol percentage by HPLC. The wine sample to be analysed was first distilled. The high-pressure liquid chromatography apparatus (HPLC) was equipped with a quaternary pump, a manual injection valve and C-18 Column. Maintaining the temperature of the column at 25^0C during analysis, HPLC grade water, mobile phase is injected at the flow rate 1ml/min. Then, 20 µl of distilled wine samples were injected for the analysis of ethanol and the peaks were recorded with the corresponding time in minutes (retention time) (Sun *et al.*, 2003)[8]. Sensory evaluation for colour, flavour and overall acceptability (OA) were carried out by 5 point hedonic scale (Ranganna, 2000)[9] (table 3). Statistical analysis of the observed data was carried out in CRD (Completely

Randomized Design, Single Factor) following standard analysis method by IBM SPSS STATISTICS 19 software. The whole processes were carried out in laboratory with the annual average room temperature of (5-25) °C and Relative Humidity of (40 – 85) % in 2011-12 (Fig. 1).

RESULTS AND DISCUSSION:

Changes in the quality of different percentage of pomegranate blended cane wine were compared, and the changes in the quality after 7 days fermentation were compared with the 6 months storage (Table 1).

Total soluble solids

As against the reported optimum of ethanol percentage (11%) of unblended 'CW' at TSS 24 ^{0}Brix, pH 5.5 and fermentation temperature at 26 ^{0}C by Tzeng *et al*, 2009[10], a higher optimum of ethanol percentage of 12.5% (after 7 days fermentation) and 12.7% (after 6 months storage) were obtained from pomegranate blended cane wine (Table No. 1).

Cane juice blended with 30%, 40%, 50% and 60% pomegranate (i.e. cane juice percentage being 70%, 60% and 50% and 40% respectively), and fermented at pH 4.5, at (26±2) ^{0} C for 7 days, shows corresponding lowering of TSS respectively. Subtracting the final from the initial TSS value gives the amount of actual TSS utilized during fermentation, and the amount utilized were respectively higher with increasing pomegranate % (up to 50%) in the blending, i.e. TSS (19.4 ^{0}Brix) from 30%, TSS (21.8 ^{0}Brix) from 40% and TSS (23.8.0 ^{0}Brix) from 50% blending. The utilized TSS value reduces to 21.0 ^{0}Brix at 40% cane juice blending. The TSS (U) value increases with the decrease in CJ% and the corresponding increase of POMJ%, while the alcohol percentage obtained corresponds to the increasing TSS (U) in the blended wine. The highest alcohol percentage was obtained at 50% blending, both in the fresh fermentation / 7 days fermentation (12.5 %) and after 6 months storage (12.7%). During storage the percentage of ethanol increases from the fresh wine at all the percentages of blended can wine.

Therefore, it can be concluded that if pomegranate is blended in cane, during wine production and stored, the percentage of ethanol production will be highest at initial TSS value of 31 ^{0}Brix, achieved at 50% blending (Table No. 1).

pH

During fermentation of the blended juice the pH was set to 4.25, to initiate fermentation, but, varies during the process and the optimum fermentation occurs at pH 3.4.

Acidity

The Titrable acidity (TA) value decreases with the higher ethanol percentage, which may be due to basic nature of alcohol. With the increase in alcohol production during storage TA value also decreases correspondingly from their fresh wine TA values.

Reducing sugar

Total reducing sugar (TRS) values decreases in fresh wine with the increasing ethanol percentage. The value further decreases after 6 months storage. It may be due to TRS of fresh wine further converting to alcohol during storage making further increase in the alcohol percentage.

Percentage of Alcohol (% alcohol)

The alcohol percentage gradually increases with the increase in POM blending up to 50%, and highest (12.5%) in 50% POM blended CW. The increase in alcohol % corresponds to the amount of TSS(U). At the highest TSS(U) value, alcohol production was highest. After 6 months of storage the alcohol percentage further increases from their fresh value, so the highest alcohol obtain after storage was 12.7%, from the POM: CW ratio (1:1) blending. This may be due to slow fermentation by the yeast isolates during storage period. The higher TSS concentration and lower ethanol yield after fermentation and before storage could be due to death of yeast cells because of high osmotic concentration, nutritional limitation or accumulation of some metabolites (Phisalaphong et al., 2006)[11].

Sensory Evaluation

The flavour of alcoholic beverages is due to numerous volatile and non-volatile compounds which confer the typical taste and odour of the beverage. The volatile compounds of wine perceived by the olfactory system are greatly dependent on the concentration of ethanol (Rothe and Schrodter, 1996)[12] Cane 40%: pomegranate 60% blending produces the most flavoured (3.8 hedonic scale) (Figure 2). The colour of cane: pomegranate blending was much acceptable by the panel of experts and acceptability hedonic scale value increases with the increase in pomegranate percentage up to 50% blending. Among the samples, the higher pomegranate percentage i.e. 50% cane: pomegranate blending scores highest overall acceptability (3.8). It may be due to flavonoids of pomegranate imparting the colour increases with its 50% blending concentration and beyond it the colour was too intense to be preferred.

In this experiment fresh starter culture was inoculated @ 10% of substrate with cell number of 1-1.6$\times 10^8$ /ml. Normally starter culture could be used at lower percent than 10, but to start immediate fermentation and avoid any contaminant growth (from unpasteurized materials), 10% fresh starter culture was used during the experiment.

Antioxidant property

Punica granatum L., posses antioxidant property in terms of Trolox Equivalent Antioxidant Capacity (TEAC) average values of (18-20) TEAC/100g, (Adel A. Kader, *et al.*, 2000)[13] much higher than that of Green Tea 3.8 TEAC/100g and grapes of 2.29 ± 0.41 mmol L^{-1} to 5.74 ± 2.38 mmol L^{-1} (Sensoy, R.I.G., 2002)[14] (table 2).

Storage

After 6 months of storage there was a slight decrease in TSS, TRS and TA but increase in the alcohol %. These variations in values may probably be due to further utilisation of the remaining sugar by the residual yeast, converting the sugar to ethanol and CO_2. The increased in acidity after storage may probably be due to oxidation of ethanol and formation of acetic acid.

The increase in ethanol production during storage was more in the blended wine having higher percentage of cane contrary to the fresh wine. Therefore, it can be concluded that, in pomegranate blended cane wine production, storage helps in the production of higher percentage of ethanol and is directly proportional to the amount of TSS present; hence, fixing of optimum TSS value will be erroneous for the storage wine.

CONCLUSION:

Cane juice blended with pomegranate juice at different concentrations (30%, 40%, 50% and 60%) and subjected to fermentation by the yeast isolate, Y_4 at pH 4.5 and incubated at (26±2) ^{0}C for 7 days and further storage for 6 months show highest alcoholic fermentation of 12.5% ethanol yield at TSS value of 31.0 ^{0}Brix before storage and a higher 12.7% ethanol with TSS(S) 6.8 ^{0}Brix after storage. This shows that in the freshly prepared wine of 50% pomegranate: 50% cane blending, fermented under similar pH (4.5) and temperature (26±2) °C, the alcohol% production was much higher than the optimum of unblended cane wine (11.0 °Brix) obtained by Tzeng *et al*. (2009)[15], and a much higher percentage of 12.7% was obtained after 6 months storage. Storage helps in higher ethanol percentage formation than the fresh wine. Increasing of cane percentage in blended wine increases the %alcohol during storage contrary to the fresh wine.

The antioxidant capacity of pomegranate juice (18-20 TEAC) is much higher than other fruit juices like grapes (2.29 – 2.38 TEAC) and Green tea (8.3 TEAC) (Table No. 2). Which could be due to the presence of six different types of anthocyanins viz., delphinidin 3-glucoside and 3, 5-diglucoside, cyanidin 3-glucoside and 3, 5-diglucoside, and pelargonidin 3-glucoside and 3,5-diglucoside developed at different developmental stages (Melgarejo P., *et al.*,2000)[16]. Anthocyanin content of grape is more than ten times higher than pomegranate, yet pomegranate is reported to be 4-5 times higher antioxidant activity (Table No. 2). The polyphenolic flavonoids (tannin, anthocyanins and ellagic acids) of pomegranate is also claim to be the reason of being more potent antioxidant against oxidation of LDL cholesterol better than grapes, green tea, blue berry etc. Pomegranate juice increases paraoxonase activity (inhibiting lipid peroxidation or breaking the oxidised lipids), thus act as second line of defence against oxidative stress (M. Arvind, 2004)[17].

Therefore, it can be concluded that pomegranate blending in cane juice produces higher quality wine, if fermentation is followed by storage. Ethanol% production is also directly proportional to the amount of TSS present for the blended and stored wine and optimum TSS value cannot be fixed or predicted as that of the fresh wine and unblended wine. Decrease in TSS, TRS and TA during storage was for further conversion to alcohol in pomegranate blended cane wine. The colour of the blended wine was much acceptable at 50% blending, with overall acceptability of 3.8 under 5 point hedonic scales, suggesting pomegranate as one of the best candidate for blending in 'CW' preparation. The present study provides the room for further research on the characterization and bioactivity of the bioactive antioxidant and flavouring compound compounds present in the blended wine and also creates the idea of value addition of wine by fruit blending.

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Table 1: Changes in quality of different percentage of pomegranate blended cane wine before and after storage

POM J (%)	CJ (%)	AFTER 7 DAYS FERMENTATION							AFTER 6 MONTH STORAGE			
		TSS(I) in ^{0}Brix	TSS(F) in ^{0}Brix	TSS(U) in ^{0}Brix	pH(F)	% ol	TA	TRS	TSS(S) in ^{0}Brix	%ol(S)	TA(S)	TRS(S)
30	70	35.4	16.0	19.4	3.4	10.8	0.47	13.97	15.5	11.1	0.92	12
40	60	33.0	11.2	21.8	3.4	12.2	0.40	13.07	10.8	12.4	0.94	9.1
50	50	31.0	7.2	23.8	3.4	12.5	0.35	12.2	6.8	12.7	0.95	8.6
60	40	30.0	9.0	21.0	3.4	11.0	0.32	11.4	8.7	11.2	0.54	7.8
SEm(±)		32.35 ±1.73	10.85 ±3.29	21.5 ±1.58	3.4 ±00	11.9 ±0.78	0.38 ±0.06	12.64 ±0.96	10.45 ±3.24	9.48 ±2.47	0.84 ±0.17	9.37 ±1.58
CV		5.35	30.35	0.74	00	6.60	14.48	7.59	31.01	0.26	0.21	16.91

Abbreviations:

(TSS(I): (Initial)Total Soluble Sugar, TSS(F): (Final) Total Soluble Sugar, TSS(U): (Utilized) Total Soluble Sugar, TA: Titrable Acidity, S: Storage, POMJ: Pomegranate Juice, CJ: Cane Juice, Initial pH 4.5, SEm: Standard Error of the Means, Co-efficient of Dispersion)

Table 2: Comparative pigments and antioxidant property of Pomegranate, Grapes and Green tea

Material	β- carotene (µ g/100g)	Anthocyanin (mg/kg fresh wt. ± SD)	Antioxidant property (TEAC ± SD)	References
Grapes	6.6 ± 1.4	500 to 4990	2.29 ± 0.41 mmol L^{-1} to 5.74 ± 2.38 mmol L^{-1}	*Josef Balik *et al.*, 2013 Sensoy, R.I.G., 2012, Fish, W.W. et al., 2002
Pomegranate	40	50-267*	18-20 TEAC	*F. Hernández *et al.*,, 1999 Adel A. Kade, *et al.*, 2000
Green Tea	12000-29000*	†Catechins (3,942mg/100g) (−)-Epigallocatechin gallate (flavonoid)	8.3 TEAC	†S. Bhagwat, 2003. * http://greentealovers.com/greenteahealthcatechin.htm Adel A. Kade*etal.*, 2000

Abbreviations: **(TEAC): Trolox Equivalent Antioxidant Capacity, TE: Trolox Equivalent**

Table 3. Rating of wine by 5 point hedonic scale (Ranganna, 2000)

Scale	Rating
1	Dislike very much
2	Dislike moderately
3	Neither like nor dislike
4	Like moderately
5	Like very much