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Production and Quality Analysis of Blended Fruit Wine from Apple, Orange and Black Grapes

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ABSTRACT

Combination of different fruits viz. apples, oranges and black grapes enriched the mixed fruit wine with ample health benefits. In the present work, for fermentation of the fruits, must was inoculated with *Saccharomyces cerevisiae*. Wine must with different brix (20 and 22) were prepared and matured by cold clarification. Clarified wine was evaluated for specific gravity, physiochemical properties, sensory and non-sensory factors. The different blends were having colour ranging from orange to dark red. They were slightly acidic, dry to sweet with alcohol content ranging from 11.5%-13.25%. Organoleptic analysis indicated that all the blends of mixed fruit wine were acceptable in terms of taste and quality. No major differences in biochemical aspects of the different wines were found. According to sensory evaluation, the blend MF 2.4 had maximum overall acceptability of 8.6, maximum antioxidant activity of 96% and also good alcoholic content of 12.25%. Therefore, MF 2.4 was found to be the best among the 8 wine blends. Storage of the wine further enhanced the acceptability of wine.

Key words: Mixed fruit wine, Fermentation, Apple, Orange, Black grapes

Wine is among the most discernible and high valuable yields from fruits. A good number of fruits and berries possess the potential for production of this alcoholic infusion. It is generated from variety of fruits like guava, watermelon banana, cucumber, Paw-Paw, mango, pineapple, apple, pear, strawberry, cherries, plum, oranges etc., [1] by fermentative action of microorganisms either instinctively or seeding with a selective strain (*Saccharomyces species*) to obtain a particular class of wine [2]. The fabrication of wines from common fruits could assist in reducing high-rate wear and tear of these fruits particularly at their peak of production [3-4]. *Saccharomyces cerevisiae* converts the saccharides present in the fruit must alcohol and organic acids. After a period of time they chemically changes to aldehydes, esters and other chemicals that aids wine preservation [5-6]. Because wines are fruit-based beverages, they therefore contain nutrients similar to those present in the original fruits. Nutritive value is further augmented due to release of amino acids and other healthy nutrients during fermentation process.

Apple, orange and grapes are locally available popular fruits that offer multiple health benefits. Apple (*Mallus domestica*) contains copious minerals, vitamins, and fibers. Due to presence of polyphenolic compounds, it exhibits antioxidant activity [7]. Apple wine production was first

initiated in the 1970s and 1980s [8]. Previous investigations have records of “ice apple wine” produced using *Saccharomyces cerevisiae* [9]. It has a glucose tolerance trait in addition to quality uniqueness of apple fruit wines along with medicinal herbs [10]. Sweet oranges (*Citrus sinensis*) contain high content of water (87%), saccharides, organic acidic compounds, minerals, vitamin C and minor content of flavonoids, lipids, carotenoids, volatile compounds and proteins [11]. They are vastly perishable and vulnerable to microbial contamination. They fall short to arrive at the marketplace due to mechanical injury, spoilage, and over ripeness [12]. Therefore, they are selected for production of white wine [13]. Grapes (*Vitis vinifera*) are highly enriched in antioxidants (resveratrol, catechin, epicatechin and proanthocyanidins. Resveratrol is main component of grape skin. Proanthocyanidins are residing merely in the seeds. Recent research finding has revealed that resveratrol and proanthocyanidin are the main components present in grapes and wines accountable for cardioprotection [14]. Grapes are typically favored for commercial production of wines [15] because of their fermentation route that occurs exclusive of sugars, acids, enzymes, or other nutrients [16].

Wine produced by blending of these three fruits is expected to have cumulative health benefits. Thus, the objective of this study is to manufacture blended wine from fruits like apple, orange and black grapes and to analyze physiochemical, biochemical sensory and non-sensory properties of the prepared blended wines.

MATERIALS AND METHODS

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The project was carried out in the Department of Biotechnology, Government College, Hisar (Fig 1).

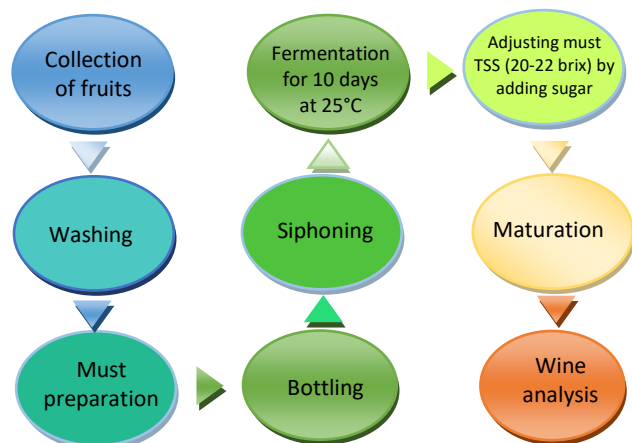


Fig 1 Outline of mixed fruit wine preparation

Substrate: The fresh and ripened apple, black grapes and oranges were collected from local market Hisar and used for wine preparation. The pulp and juice were extracted and stored separately in refrigerated state till use.

Reagents: Reagents consumed in the laboratory preparation were of analytical grade and procured from Himedia Labs and E. Merck, India.

Yeast and its activation: *Saccharomyces cerevisiae* was obtained commercially and activated by keeping it for 10-15 minutes in hot water containing sugar in dark place.

Table 1 Naming and coding of flask

Item	Code	Item	Code
Apple juice	AJ	Flask 1	MF 1
Orange juice	OJ	Flask 2	MF 2
Grape pulp	GP	Flask 3	MF 3
Mixed fruit	MF	Flask 4	MF 4

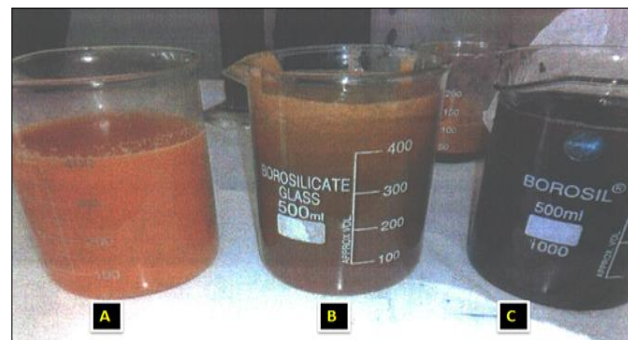


Fig 2 (A) Orange Must (B) Apple Must (C) Black grapes Must

Preparation of fruit must

Must was prepared using cold clarification method. After washing fruits, they were crushed and sieved. Their must was collected separately. Four combinations of juices having different concentration were added to four different flasks. They were labeled properly (Fig 2, Table 1). Fruit concentration was determined (Table 2).

Table 2 Concentration of juice in different combinations

Flask No.	Juice concentration (%)		
	AJ	OJ	GP
MF1	33.33	33.33	33.33
MF2	50	25	25
MF3	25	50	25
MF4	25	25	50

Estimation of Sugar and its adjustment

Sugar content was measured using hydrometer. Initial brix was found to be 14 in MF1, 14.25 in MF2, 14.5 in MF3 and 17.5 in MF4. Each combination was then divided into 2 subsets (Set 1 and Set 2 having four flasks each) (Table 3). Potassium bisulphite was added to these 8 flasks to avoid contamination. Fermentation was done at 25°C in BOD incubator. The flasks were kept in the incubator till the fall in the Brix (Fig 3-4).

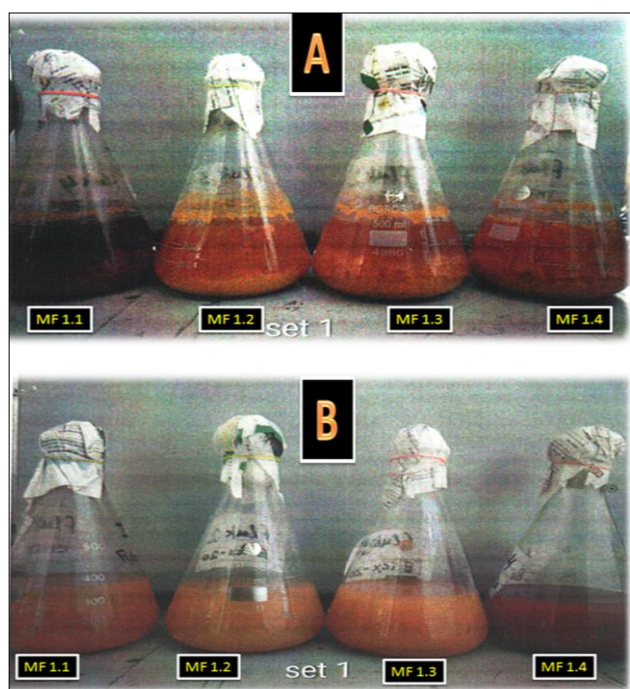


Fig 3 Mixed Fruit Wine Set 1
(A) Before Fermentation (B) After Fermentation

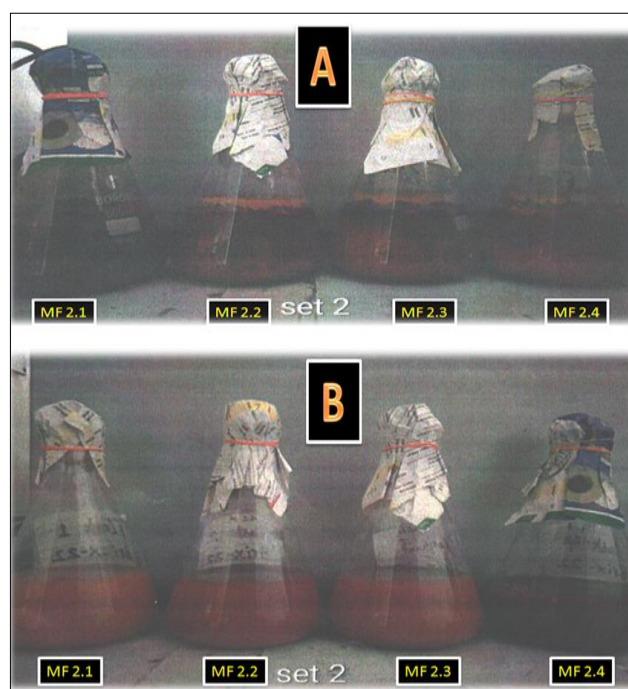


Fig 4 Mixed Fruit Wine Set 2
(A) Before Fermentation (B) After Fermentation

Table 3 Mixed juice Must concentration, initial Brix and Final Brix of Set 1 and Set 2

Set 1	AJ% : OJ% : GP%	Initial Brix	Final Brix	Set 2	AJ% : OJ% : GP%	Initial Brix	Final Brix
MF1.1	33.33 : 33.33 : 33.33	14	20	MF2.1	33.33 : 33.33 : 33.33	14	22
MF1.2	50 : 25 : 25	13.5	20	MF2.2	50 : 25 : 25	15	22
MF1.3	25 : 50 : 25	15	20	MF2.3	25 : 50 : 25	14	22
MF1.4	25 : 25 : 50	13	20	MF2.4	25 : 25 : 50	22	22

Siphoning

After fermentation filtration of juices was done 3-4 times using muslin cloth at regular interval (3-4 days) till the must became clear. Brix of the must was checked until it reached zero (indicates complete fermentation of sugar in the sample).

Table 4 Chemical composition of apple, orange and grapes

Constituents	Average Range		
	Apple	Orange	Grapes
Energy (KJ)	229	460	586
Water (%)	85.3	87	80
Total Sugar (%)	11.8	12	8
• Sucrose	2.4	2	2
• Fructose	6.0	0.7	3
• Glucose	2.2	0.7	1.4
Organic Acids (mg/100g)			
• Citric acid	16	910-1690	640
• Malic acid	550	-	1280
• Ascorbic acid	283.3	53.2	45
• Oleic acid	20	-	-
Total phenol (mg/l)	230.4	167.5	115
Total anthocyanin (mg/l)	46.8	-	-
Protein (%)	2.74	1	0.8
Titration Acidity (%)	-	0.00020	-
Potassium	-	181	-
Calcium	-	40	-
Tannin	-	-	164
Phytic acid	-	-	1320

Bottling

Different samples of wine were bottled separately and labeled properly (Fig 5). They were then refrigerated. Blended juice was used for wine making as per Somesh *et al.* [17] with some modifications.



Fig 5 Different blends of mixed fruit wine

Wine maturation: Wine maturation was done for two months in refrigerator. Biochemical changes were recorded at different time intervals. The wine was analyzed for different parameters during storage.

Specific gravity: Specific gravity (SG) was determined by measuring 50ml of sample into measuring cylinder at 20°C and then dipping hydrometer into it. The percentage alcohol content (%ABV), calories (energy), residual sugar (RS/ brix),

apparent fermentation velocity and attenuation (%App. Attn) were then calculated by specific gravity chart (American Society for Brewing Chemists:

$$\text{Percent alcohol by volume (\%ABV)} = \{(\text{Initial SG} - \text{Final SG}) / 7.36\} \times 100$$

$$\text{Residual sugar (\%RS)} = \text{brix} = 231.3 \{1 - (1 / \text{Final SG})\}$$

$$\text{Apparent attenuation (\%App. Attn)} = \{(\text{Initial SG} - \text{Final SG}) / (\text{Initial SG} - 1)\} \times 100$$

$$\text{Apparent fermentation degree (\%AFD)} = \{(\text{Initial SG} - \text{Final SG}) / \text{Initial SG}\} \times 100$$

$$\text{Fermentative capacity (\%Vc)} = \text{Initial RS} - \text{Final RS}$$

$$\text{Fermentative velocity} = \{ \text{alcoholic content (\%)} \} / \% \text{Vc}$$

Fermentation capacity and apparent fermentation both the parameters measure the quantity and rate of sugar conversion to alcohol.

Physiochemical analysis of wine

Estimation of pH: pH was determined using pH meter, Rangana [18].

Titration acidity: It was estimated by titrating known quantity of sample (10 ml) against standardized 0.2 NaOH using few drop of 1% phenolphthalein solution as indicator to achieve pink color end point (that persist for 15 sec) Patharkar *et al.* [19].

$$\text{Acidity (\%)} = \{ \text{volume of 0.2 NaOH used} \times 0.2 \times 75 \times 100 \} / \{ \text{sample taken (10ml)} \times 1000 \}$$

Determination of free SO₂: Iodine (0.005 ml) was filled in a burette. In a beaker 10ml of sample was taken. To it 2ml of starch indicator solution, 1ml of H₂SO₄ and little amount of NaHCO₃ was added. Titration was done using iodine solution until bluish color persisted for few seconds. Amount of Sulphur dioxide is calculated using following equation:

$$\text{SO}_2 \text{ (mg/l)} = \{ M_1 \times N \times 32 \times 1000 \} / M_2$$

Where, M₁ and M₂ are volume of iodine used and volume of sample used, respectively; N is the normality of Iodine.

Estimation of Vitamin C: 25ml of standard Vitamin C solution was added into a flask. After that 10 drops of 1% starch solution was incorporated in the flask. In burette iodine solution was added (initial volume was noted) and titration was done until bluish black color persisted for 20 sec. Final volume of iodine in burette was measured (Patharkar *et al.* [19].

$$\text{Amount of vitamin C} = (\text{final volume} - \text{initial volume}) \text{ of Iodine solution}$$

Determination of % ethanol: Preparation of K₂Cr₂O₇ solution was done. For this, 325 ml H₂SO₄ was added to 200ml of distilled water. It was then mixed and boiled. 33.768g of K₂Cr₂O₇ was added and volume was made up to 1L. Simultaneously, standard solution was prepared by pouring different concentration of 1ml of ethanol water solution into 6 test tubes with 25ml of K₂Cr₂O₇. All the test tubes were mixed except 6th (blank). 1ml of alcoholic sample was added and diluted to 30ml. distillation was carried out and

distillate was collected in flask having 25ml of $K_2Cr_2O_7$. It was then heated to 60°C (15-20 min) and then cooled and diluted to 50 ml. Absorbance was recorded at 600nm. Amount of ethanol in each sample was determined using standard curve of ethanol (Patharkar et al. [19]).

Estimation of reducing sugar: 3,5 dinitrosalicylic (DNS) acid is a reagent that is reduced by reducing sugars. 0.1g of glucose was added to 100ml distilled water. It was then diluted and different combination of glucose and water were added to 10 test tubes. To it added 3ml of DNS was added. It was kept at water bath for 20 min. absorbance was noted at 600nm using spectrophotometer. 30µl of sample, 970 µl of water and 3ml of DNS reagent was added in the test tube. The mixture is kept on hot water bath for 20 min and absorbance is noted at 600nm.

Determination of antioxidant activity: Stock solutions of wine were made by diluting 5ml of wine with 10ml of 13.5% neutral alcohol. Diluted samples of 5, 10, 15, 20 ml. 25 µl were mixed with DPPH (1,1 diphenyl 2 picrylhydrazyl-2900 µl of 0.03 mg/ml solution). Adequate amount of methanol (CH_3OH) was added progressively to each sample to obtain a final volume of 3 ml. After 30 min the UV spectroscopic absorbance was measured at wavelength 517nm (visible range). High extent of absorbance (517nm) indicated low free radical scavenging activity. The quantity of wine (in diluted solutions) required to reduce the initial DPPH concentration by 50% collectively with the quantity of aromatic phenol (mg/l) were used to determine LC_{50} value.

$$\% \text{ Antioxidant activity} = \{(A_0 - A_1)/A_0\} \times 100$$

Where A_0 is negative control (100µl methanol+2900 µl DPPH= 0.77); A_1 is absorbance recorded. Ascorbic acid

(organic) was taken as standard that has 98.7% total antioxidant capacity.

Sensory and non-sensory evaluation of wine

It was done by students, teachers and staff of Govt. College Hisar, Haryana, India. Selected parameters for sensory evaluation included smell, taste, colour, mouth feel and overall acceptance. Non sensory factors that were studied in the current research included colour, relative sweetness, alcohol content, effervescence and acidity/alkalinity.

RESULTS AND DISCUSSION

The investigational results originated from the present study are presented in this paper. The research was concentrated on the objective to investigate the production of mixed fruit wine using apple, orange and grapes. Production of wine was conducted in the lab in batch reactor set up. Process monitoring and final analysis was then done.

Preparation of wine

Must preparation: Apple and oranges were washed, and juice was extracted. For grapes its pulp was used. The fruit Must should have high sugar and low acidic content that should be adjusted if required. In case fruit has low content of natural sugar, it could be incorporated to must to speed up fermentation [20]. If acidic fruits are used, sugar level was maintained (sugar content of pulp/juice was less than the optimum amount required for wine formation) [20]. Grape must was adjusted to 22 brix with cane sugar [21]. Orange juice was adjusted to 23 brix TSS with sucrose and glucose [22]. Mixed fruit wine Set 1 and Set 2 had 20 and 22 brix, respectively (Table 5).

Table 5 Brix, pH and temperature of mixed fruit wine

Name	Set 1				Set 2			
	MF1.1	MF1.2	MF1.3	MF1.4	MF2.1	MF2.2	MF2.3	MF2.4
pH	3.80	3.89	3.91	3.93	3.90	3.88	4.02	4.09
Brix	20				22			
Composition of Must	Mixed juice				Mixed juice			
Temperature	25°C				25°C			
Brix after 10 days	0				0			

Fermentation: The majority of organic fruit wine production is done using *S. cerevisiae* strains because it ensures rapid and reliable fermentation. Furthermore, it reduces the risk of wedged fermentation and microbial contagion [23]. Prepared must was inoculated with yeast (1g/l). An optimum inoculum level during wine production was recorded to be 4-6% [24]. Increase in inoculum levels showed decrease in fermentation rate with time [25]. Thus, inoculums concentration of 10.0%(v/v) was found to be optimum for fermentation, which also prevents contamination of fermenting media [25]. pH and acidity were also maintained to further avoid microbial

contamination. Less acidic fruits need to be acidified before wine preparation [22]. Fermentation of orange was conducted by [19] at pH 4.5. Finally, mashers were incubated for 5 days (25°C). Previous studies have suggested optimum temperature range between 20-28°C [26] for fermentation. Generally, time for completion of fermentation varies (2-3 weeks) for different fruits and it also depends on the fermentation conditions. In the current work, fermentation of our wine was completed in 10 days. After fermentation wine was filtered through muslin cloth and racked for a period of 1 month. The wine obtained was thus clarified and analyzed.

Table 6 Enological properties of wine produced by *Saccromyces cerevisiae*

Name	Set 1				Set 2			
	MF1.1	MF1.2	MF1.3	MF1.4	MF2.1	MF2.2	MF2.3	MF2.4
Initial specific gravity	1.0830				1.0920			
Final specific gravity	1.00				1.00			
Residual sugar	0				0			
Apparent fermentation (%AFD)	7.663				8.424			
Fermentative capacity (%Vc)	14	13.5	15	13	14	15	14	22
Fermentative velocity	0.910	0.851	0.866	1.019	0.946	0.883	0.928	0.556

Specific gravity

The specific gravity, residual sugar (RS), apparent fermentation velocity are shown in (Table 6). A phenomenal correlation exists between fermentative capacity and apparent fermentation degree of blended wine. Both the parameters measure the quantity and rate of sugar utilization. Fermentative velocity measures the rate of sugar conversion to alcohol. In the present investigation, all blends were found to have similar Fermentative velocity.

Apparent attenuation measures percentage sugar conversion rate. In present study, it was found to be 100% in all wine blends. No trend was observed in the final specific gravities of the wines after fermentation. Residual sugar was negligible in all wine blends.

Physiochemical analysis of wine

It was done using different methods as discussed earlier and results are presented in (Table 7). Titrable acidity of 0.97 of MF1.3 was maximum among all subsets of wine. Overall

ethanol content was also higher (13.25) when compared to previous wine studies [27]. Vitamin C content was found to be in range of 0.025-0.075g/l. Free Sulphur varied from 40-112, which indicates efficient bactericidal and fungicidal activity of wine. Molecular Sulphur SO₂ released during wine formation, as a by-product of fermentation, inhibits acetic as well as lactic bacteria. Moreover, it allows longer storage of wine. The antioxidant property of fruit is directly related to the presence of efficient free radical scavengers (Vitamin C and phenolic compounds) present in it. Several researchers have published total antioxidant capacity (TAC) of various fruits, vegetables and cereals [28]. Oranges have greater TAC among various fruits (2-11 folds > than apple, grapes etc.) [29]. Antioxidant activity varied from 41–91% in our research. Antioxidant capacity of Set 2 revealed good TAC of Mixed fruit wine when compared to standard. In the present study ascorbic acid served as standard compound [30]. Thus, our mixed fruit wine exhibited significant physiochemical properties that are characteristic of a classic wine.

Table 7 Physiochemical Properties of Blended Wine

Name	Set 1				Set 2			
	MF1.1	MF1.2	MF1.3	MF1.4	MF2.1	MF2.2	MF2.3	MF2.4
Titration acidity	0.90	0.90	0.97	0.71	0.72	0.60	0.67	0.60
Vitamin C (g/l)	0.025	0.025	0.031	0.035	0.034	0.037	0.075	0.069
Ethanol (%)	12.75	11.50	13.00	13.25	13.25	13.25	13.00	12.25
Antioxidant activity (%)	41.5	54.5	49.3	81.8	54.5	84.4	80.5	96.0
Free sulphur (mg/l)	48	56	48	48	40	56	112	48
pH	3.80	3.89	3.91	3.93	3.90	3.88	4.02	4.09
Reducing sugar (%)	0.0025	0.0004	0.0001	0.0001	0.0052	0.0062	0.0010	0.0003
Antioxidant activity (%)	41.5	54.5	49.3	81.8	54.5	84.4	80.5	96

Table 8 Sensory and non-sensory evaluation of mixed fruit wine

Name	Sensory factors							
	Set 1				Set 2			
	MF1.1	MF1.2	MF1.3	MF1.4	MF2.1	MF2.2	MF2.3	MF2.4
Aroma	8	8	7.5	8	6.5	7.5	8.5	8.5
Taste	6.5	7	6.5	8	6	7.5	6.5	8.5
Mouth Feel	7	7	6	8.5	6.5	7.5	7.5	8.5
Overall Acceptance	7	7.3	6.5	8.25	6.25	7.6	7.6	8.6
Appearance	6.5	7.5	6	8.5	6	8	8	9
Non-sensory factors								
Colour	Red	Orange	Orange	Dark red	Orange	Red	Red	Dark red
Relative Sweetness	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Sweet
Alcohol content	Natural							
Effervescence	Still							
Acidity/Alkalinity	Acidic							

Sensory and Non sensory evaluation of wine: Colour, taste and aroma of wine are very complex and depend on number of factors such as cultivator, agricultural conditions and vinification practices [23]. The selected parameters for sensory and non-sensory evaluation are depicted in (Table 8). For sensory evaluation results were obtained according to 9-point Hedonic scale [14]. The different blends were having colour ranging from orange to dark red. They were slightly acidic, dry to sweet with alcohol content ranging from 11.5%-13.25%. Organoleptic analysis indicated that all the blends of mixed fruit wine were acceptable in terms of taste and quality. No major differences in biochemical aspects of the different wines were found. According to sensory evaluation, the blend MF 2.4 had the maximum overall acceptability of 8.6, maximum antioxidant activity of 96%. Therefore, MF 2.4 was found to the best among the 8 wine blends.

CONCLUSION

Vast majority of fruits could be blends for production of mixed fruit wine. The blended wine obtained in the present work met with all the requirements as essential for a classic wine in terms of aroma, colour, taste, flavour etc. It was very well accepted during sensory evaluation. Because of acidic nature mixed fruit wine was itself protected from microbial contamination. This further enhanced its storage. Hence, aforesaid research exhibited amalgamation potential of apples, oranges and grapes among themselves. They could thus act as substrate for producing superior quality wine in terms of its constituents and nutritional value. Moreover, usage of these seasonal fruits could minimize their spoilage. In nutshell, this single beverage will be highly efficient in imparting lots of health benefits.

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