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Influence of aging on nutrient retention and organoleptic characteristics of wine developed from star fruit (*Averrhoa carambola* L.)

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Abstract

Wine is an alcoholic beverage normally prepared from fermented fruit juice. It is a popular drink enjoyed all over the world. The present investigation was carried out to standardize wine from star fruit using sugar and jaggery as a carbohydrate source for yeast and to study change in nutrients and sensory parameters during aging process. The results showed that the star fruit juice is most appropriate for the preparation of wine than its pulp. Star fruit wines were further analyzed for nutrients composition, microbial safety, and sensory characteristics during 6 months of aging under ambient temperature in a dark place. Initially the wine prepared using sugar had highest total soluble solids (TSS), total sugar; reducing and non-reducing sugars, ascorbic acid, and β-carotene content as compared to the wine prepared using jaggery. Mineral content did not vary in both wines. During aging, the nutrients and sensory parameters were decreased in both wines except taste scores. At the end of aging, the wine prepared using jaggery had better retention of TSS (92.34 %), total sugar (89.29 %), reducing sugar (93.31 %) and ascorbic acid (71.82 %) than wine prepared using sugar. The microbial study indicated that both wines were safe for consumption after six months of aging. Overall acceptability scores (8.95 and 8.90) were found reasonably similar in both the wines after 180 days of aging.

Keywords aging, alcohol, jaggery, nutrients, star fruit, sugar, wine

Introduction

Fruits and vegetables are vital components of a healthy diet. Almost every fruits offer great medicinal benefits through their abundant quantities of nutrients (carbohydrates, minerals, vitamins, and fiber), antioxidant potential and phytochemical properties (polyphenolics, flavonoids, carotenoids, tocopherols, ascorbic acid and glutathione) [1]. India is one of the hot spots for several tropical fruit tree species, most of them never cultivated commercially but appreciably associated with livelihood and household food security of many rural and tribal communities. Many tropical fruits are labeled as "Underutilized plant species" that are locally plentiful, but restricted in their plant geographical dispersion have superior use value [2].

As a result of the agricultural evaluation process, these underutilized fruit crops are now being globally considered as potential fruit crops especially due to their significant nutraceuticals values and improved market demands. Many underutilized fruits can significantly contribute to improve health and nutrition, livelihoods, household food security, ecological sustainability and also for curing diverse diseases and ailments [3].

Star fruit (*Averrhoa carambola* L.) popularly known as "Carambola" or "Five finger fruit" belongs to the family Oxalidaceae. It is an important underutilized fruit and an excellent source of nutritional and phytochemical components [4]. It is a juicy fruit with predominately golden yellow to green color in appearance with a succulent pulp. Star fruit is rich source of natural antioxidant, vitamin "C" and functional dietary fibers [5]. Traditionally, almost every part of star fruit plant can be used for therapy to certain ill-health symptoms. The tonic made from star fruit is also used to relieve eye afflictions and as a diuretic for kidney and bladder complaints in Brazil [6].

In Indian Ayurvedic medicine, the star fruits are used as an anti-helminth, anti-malarial, antipyretic, digestive tonic, febrifuge, antiscorbutic and antidote for poison [7]. Value-added products from star fruit include low calorie jam [8], cordials [9], minimally processed carambola [10], foam-mat dehydrated star fruit puree [11], Osmo-dehydrated star fruit slices [12 and 13] and star fruit wine [14].

Wines are healthy alcoholic beverages that have been seen as a natural remedy for illness since so many decades from early days. It is one of the functionally fermented foods and has many health benefits such as anti-ageing effects, destruction of cancer cells, prevention of stroke by keeping the arteries clean by polyphenols, improvement of lung function through antioxidants, reduction in coronary heart disease (CHD), development of healthier blood vessels in elderly people, reduction in ulcer-causing bacteria, decreasing ovarian cancer risk in women and making the bones stronger [15]. Many wines made from fruits are having medicinal value and such wines have extra health benefits [16]. Therefore, the purpose of this study was to develop a low and medium alcoholic wine from star fruit, and to study their nutrient retentions, microbial loads and sensory characteristics during aging process under ambient temperature in dark place.

Methodology

Selection of star fruit

Star fruits (Averrhoa carambola L.) were collected from Horticulture Research Station, Tamil Nadu Agricultural University, Thadiyankudisai, Kodaikanal, Tamil Nadu, India. The fruits were brought to the laboratory immediately after the harvest and were kept in refrigerated condition for further analysis.

Standardization of wine from star fruit

Alcohol strength, yield, and sensory characteristics were evaluated select two treatments for wine processing. The preliminary trials were made using different treatments as given in Table 1.

Table 1. Standardization of wine from star fruit

Treatments	Carbohydrate source	Alcohol (%)	Yield (ml)
Star fruit juice (500 ml)	Sugar	12.50	425
	Jaggery	5.25	420
Star fruit pulp (500 ml)	Sugar	11.75	238
	Jaggery	5.50	235

Based on the alcohol strength, yield and sensory characteristics, the star fruit juice was selected for further processing of low and medium alcoholic star fruit wine. The preparation of star fruit wines is given in Figure 1.

Extraction of juice from star fruit

Sound, healthy and fully ripened star-fruits were selected and washed thoroughly with running tap water. The fruits were cut into small pieces, seeds were separated manually and the fruits were crushed with the help of mixer and grinder. The juice was collected and strained through a double fold cheese cloth.

Preparation of must

The extracted juice was transferred into previously sterilized fermentation flasks and the total soluble solids (TSS) of the juice were adjusted to 24^{o} Brix by addition of sugar and jaggery. The pH was adjusted to 3.5

with the help of citric acid / potassium hydroxide, treated with 50 ppm of potassium metabisulphite (KMS), pasteurized (89 ±2°C for 20 minutes) and cooled.

Figure 1. Prepration of star fruit (*Averrhoa carambola* L.) wine

Fermentation

Wine yeast (10 ml kg^{-1} juice) was inoculated to the pasteurized star fruit juice to initiate the fermentation process, which was carried out at ambient temperature for 3 days under aerobic condition followed by the fermentation under the anaerobic condition for 14 days. The fermented star fruit juice was filtered through double-fold cheese cloth and pasteurized at 80°C for 4 minutes.

Clarification and corking

The pasteurized star fruit wines were clarified using 0.5 per cent pectinase (w/v) and left overnight without disturbance. Next day the clarified wines were drained carefully using the suction tube, filtered again through double-fold cheese cloth, poured into sterilized glass bottles (200 ml) and crown corked.

Pasteurization and aging

The crown corked wine bottles were pasteurized at 89 ± 2°C for 20 minutes in a water bath, cooled immediately and stored in dark place for aging. The changes in the nutrient composition were subjected to analysis at a monthly interval up to six months.

Physico-chemical and nutrient analysis

The pH was determined using digital pH meter (EUTECH Instruments, Mumbai, India) and the acidity was estimated by titrating against 0.1N NaOH solution using phenolphthalein as an indicator and the values were expressed as anhydrous citric acid (%). The TSS (° brix) was recorded using hand refractometer. Total, reducing and non-reducing sugars (g / 100 ml), ascorbic acid (mg / 100 ml) and β-carotene (µg / 100 ml) were also analyzed as per the standard procedures. The alcohol content of the samples was determined using the dichromate method as described by [17].

Mineral analysis

Calcium, magnesium, phosphorus and potassium were evaluated as per the standard methods [18]. Atomic absorption spectrometry (AAS) (ICE 3000 series, Systronics, India) was used for the determination of iron, zinc, and copper. The results were expressed on dry weight basis.

Microbial analysis

The fruit wines were subjected to microbial study initially and at the end of the aging of 6 months. The samples were weighed aseptically. Analysis for fungi, yeast, and bacteria was carried out for each sample. For fungi, commercially available Martins Rose Bengal Agar media (MRBA) were used. For determination of bacteria and yeast, nutrient agar media was used correspondingly.

Organoleptic evaluation

The wine samples were evaluated organoleptically using various quality attributes such as color, flavor, sweetness, taste and overall acceptability by a panel of ten semi-trained judges using 9 points hedonic scale at regular intervals of 30 days.

Statistical analysis

The data obtained in the present study were subjected to analysis of variance using Completely Randomized Design (CRD) as described by [19] to find out the impact of sugar and jaggery on the alcohol strength and retention of nutrients content in star fruit wines during aging process.

Results and Discussion

Chemical composition of star fruit wine

Physico-chemical and nutrient composition of wines were studied and the obtained results are reported in Table 2. TSS ranged from 9.40 ^{o}brix to 16.40 ^{o}brix in fruit wines developed using sugar and jaggery. Total sugar (17.37 g / 100 ml), reducing sugar (9.65 g / 100 ml), non-reducing sugar (8.20 g / 100 ml), ascorbic acid (57.26 mg / 100 ml) and β-carotene (73.85 µg / 100 ml) were obtained maximum in wine prepared using sugar. The percentage of alcohol content varied between 5.24 and 12.50. Both wines showed substantial amount of minerals such as calcium, phosphorus, potassium, magnesium, iron, zinc and copper.

Table 2. Chemical composition of star fruit wines

Chemical composition	Fruit wine (sugar)	Fruit wine (jaggery)
pH	3.26±0.02	3.46±0.06
Titratable acidity (%)	1.120±0.12	1.220±0.03
TSS (o brix)	16.40±0.68	9.40±0.16
Total sugar (g / 100 ml)	17.37±0.27	8.96±0.19
Reducing sugar (g / 100 ml)	9.65±0.03	6.43±0.02
Non - reducing sugar (g / 100 ml)	8.20±0.32	2.85±0.10
Ascorbic acid (mg / 100 ml)	57.26±1.22	52.10±0.92
β-carotene (µg / 100 ml)	73.50±1.74	27.86±0.40
Alcohol (%)	12.50±0.11	5.24±0.07
Calcium (mg / 100 ml)	7.42±0.05	7.47±0.02
Potassium (mg / 100 ml)	201.6±2.09	202.7±2.03
Phosphorous (mg / 100 ml)	17.93±0.31	17.65±0.23
Magnesium (mg / 100 ml)	15.42±0.26	15.47±0.20
Iron (mg / 100 ml)	1.47±0.04	1.49±0.04
Zinc (mg / 100 ml)	0.32±0.01	0.34±0.01
Copper (mg / 100 ml)	0.26±0.01	0.24±0.01

Table 3. Changes in pH, titratable acidity and TSS of star fruit wines during aging

Storage periods (Days)	pH		Titratable acidity (%)		TSS (o brix)	
	Fruit wine (sugar)	Fruit wine (jaggery)	Fruit wine (sugar)	Fruit wine (jaggery)	Fruit wine (sugar)	Fruit wine (jaggery)
Initial	3.26	3.46	1.120	1.220	16.40	9.40
30	3.26	3.46	1.126	1.227	16.40	9.40
60	3.06	3.24	1.131	1.233	16.00	9.10
90	3.02	3.20	1.135	1.236	15.64	8.94
120	2.99	3.17	1.139	1.241	15.30	8.80
150	2.97	3.14	1.144	1.248	15.02	8.74
180	2.95	3.09	1.149	1.253	14.74	8.68
Mean	3.07	3.25	1.135	1.237	15.64	9.01
CD at 0.0 5%	**T** - 0.019; **S** - 0.036; **Interaction** – NS		**T** - 0.008; **S** - 0.015; **Interaction** - NS		**T** - 0.081; **S** - 0.151; **Interaction** - 0.214	

Titratable acidity and pH

The changes in the titratable acidity and pH content of wines during aging in dark place are given in Table 3. Generally, the fresh star fruit is slightly acidic in nature due to the presence of high ascorbic acid (135.8 mg / 100 g) and titratable acidity (1.024 %). The titratable acidity and pH of freshly processed wines ranged from 1.120 to 1.220 per cent and from 3.26 to 3.46 respectively. During aging, a gradual decrease in pH and an increasing titratable acidity was observed in both wines. This might be due to oxidation of sugar molecules into organic acid which increased the titratable acidity and decreased pH content of the fruit wines during aging. The steady decrease in pH and increase in titratable acidity during aging were observed in wine prepared using jaggery, as the addition of jaggery produced dark brown color in the wine, which minimized the oxidation reaction during aging. Whereas the fruit wine prepared using sugar did not change the actual color of star fruit juice during wine processing, resulting from the considerable changes of pH and titratable acidity. Statistically, a highly significant difference among the wines and storage periods were observed. A similar view was also shared by [20] in sapota wine and [21] in guava wine..

Table 4. Changes in total, reducing and non-reducing sugars content of star fruit wines during aging

Storage periods (Days)	Total sugar (g / 100 ml)		Reducing sugar (g / 100 ml)		Non-reducing sugar (g / 100 ml)	
	Fruit wine (sugar)	Fruit wine (jaggery)	Fruit wine (sugar)	Fruit wine (jaggery)	Fruit wine (sugar)	Fruit wine (jaggery)
Initial	17.37	8.96	9.65	6.43	8.20	2.85
30	16.95	8.65	9.52	6.35	7.91	2.62
60	16.59	8.48	9.35	6.29	7.71	2.50
90	16.25	8.33	9.21	6.21	7.50	2.43
120	15.96	8.17	9.06	6.10	7.35	2.38
150	15.68	8.09	8.93	6.05	7.20	2.34
180	15.43	8.00	8.78	6.00	7.09	2.30
Mean	16.32	8.38	9.21	6.20	7.57	2.49
CD at 0.0 5%	**T** - 0.082; **S** - 0.154; **Interaction - 0.217**		**T** - 0.050; **S** - 0.093; **Interaction - 0.131**		**T** - 0.036; **S** - 0.067; **Interaction - 0.094**	

Total soluble solids (TSS)

Table 3 summarizes the changes in the mean TSS (o brix) content of star fruit wines during aging. During wine processing, the TSS content of star fruit decreased gradually and come down to 9.40 obrix in wine prepared using jaggery and 16.4 obrix in wine prepared using sugar. The difference in TSS was due to the utilization of sugar and jaggery as a carbohydrate source for growth and development of yeast during fermentation. The high TSS content while using sugar was due to the presence of high sucrose percentage and less impurity than jaggery. The TSS content of fruit wines was decreased with advancement in aging. The decreasing TSS content was lesser in wine prepared using jaggery. As discussed earlier, the addition of jaggery produced dark brown color in fruit wine which creates strong barrier on oxidation reaction against light and temperature. Whereas the wine prepared using sugar produced slight golden yellow color, which did not affect their oxidation reaction. It resulted in to slightly higher changes in TSS content in the wine prepared from sugar as compared to the wine prepared using jaggery during aging process. The decreasing trend in TSS content is in line with the findings of [20] who reported a decrease in TSS content of sapota wine during aging. Maragatham and Panneerselvam, [22] reported that the TSS content of papaya wine was decreased from 12.14 to 9.36 o brix with advancement in aging process.

Total, reducing and non-reducing sugars

The changes in the total, reducing and non-reducing sugar content of the star fruit wine during aging are given in Table 4. Due to the presence of highest sucrose percentage and less impurities in sugar, initially total (17.37 g / 100 ml), reducing sugar (9.65 g / 100 ml) and non-reducing (8.20 g / 100 ml) sugars content was two times more in wine prepared using sugar than that of the wine prepared using jaggery. During aging, a steady reduction in sugar content was observed in both wines. This might be due to slow mobility of sugar molecules within the wine and helped them being utilized in Maillard reaction and other degradative reaction such as formation of organic acid etc. The highest changes in total, reducing and non-reducing sugars content was observed in wine prepared using sugar which had reduced from 17.37 to 15.43 g / 100 ml for total sugar, 9.65 to 8.78 g / 100 ml for reducing sugar and 8.20 to 7.09 g / 100 ml for non-reducing sugar at the end of six months aging in dark place. The wine prepared using jaggery permitted less oxidation reaction against light and temperature due to dark brown color. Statistically, highly significant difference among the treatments, storage periods and their interaction effects were observed. The values obtained for total sugar, reducing sugar and non-reducing sugar showed much more synchronization with the results of an earlier study conducted in banana wine and [23] in Jamun wine.

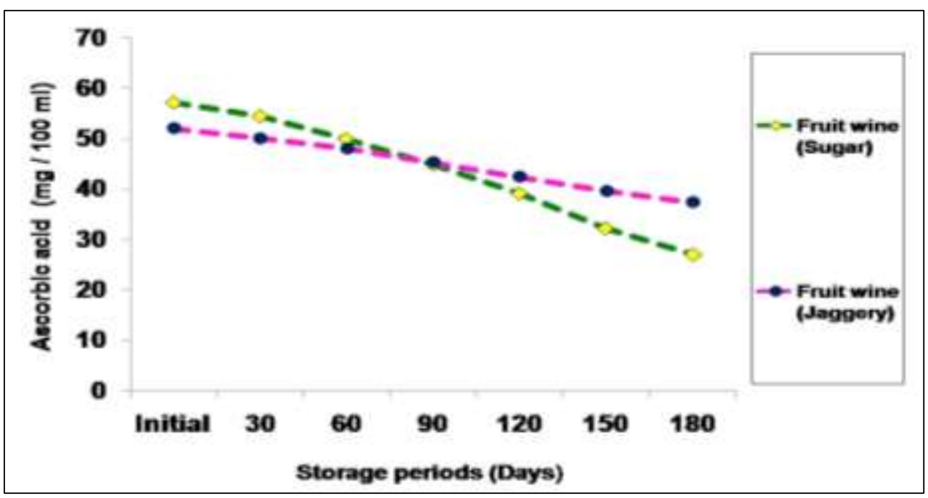

Figure 2. Changes in ascorbic acid content of star fruit wines during aging

Ascorbic acid and β-carotene

The freshly prepared fruit wine using sugar had slightly higher ascorbic acid (57.26 mg / 100 ml) content compared to the wine prepared using jaggery (Figure 2 and Table 5). Two and half times lower β-carotene content (27.86 µg / 100 ml) was found in wine prepared using jaggery. The chemical component of jaggery partially reduced the ascorbic acid and β-carotene content by accelerating Maillard reaction during wine processing. After bottling the wine, this Maillard reaction was stabilized and produced dark brown color, which minimized many oxidation reactions against light and temperature and also preserved more nutrients during aging process. However, at the end of aging, the notable loss of ascorbic acid (52.72 %) and β-carotene (27.40 %) content was observed in wine prepared using sugar. This may be due to the generation of high fructose from sucrose which might increase the rate of anaerobic degradation of ascorbic acid. Both the wines preserved significant level of ascorbic acid and β-carotene content during aging process. A similar trend in ascorbic acid and β-carotene reduction were reported by [20] in sapota wine, by [21] in guava wine and by [24] in Tendu fruit.

Table 5. Changes in β-carotene content of star fruit wines during aging

Storage periods (Days)	β-Carotene (µg / 100 ml)	
	Fruit wine (sugar)	Fruit wine (jaggery)
Initial	73.50	27.86
30	71.29	26.79
60	67.72	25.12
90	64.14	23.48
120	60.65	21.87
150	56.92	20.19
180	53.36	18.47
CD at 0.0 5%	**T** - 0.306; **S** - 0.573; **Interaction** - 0.810	

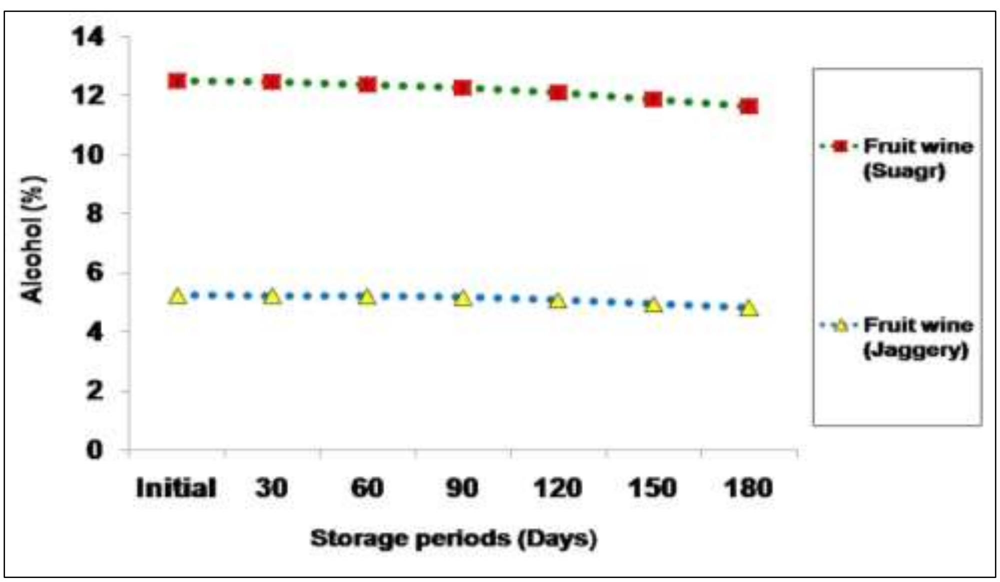

Figure 3. Changes in alcohol content of star fruit wines during aging

Alcohol content

At the initial stage, the alcohol content of both fruit wines ranged between 5.24 per cent and 12.50 per cent (Figure 3) respectively. The present study identified major discrepancy in alcohol content of the developed wines using sugar and jaggery in the identical anaerobic fermentations. This might be due to the impact and unequal distribution of carbohydrate sources from sugar and jaggery for the growth and development of yeast during fermentation. The less sucrose, impurities and other components of jaggery produced 5.24 per cent alcohol which was two and half times lower than the wine prepared using sugar. Moreover during fermentation, the yeast got of carbohydrate sources from sugar for their growth and development, as a result they produced highest percentage of alcohol (12.50 %). During aging process, the decreasing trend of alcohol content was observed in both wines. At the final stage of aging (180 days), the maximum decrease (12.50 to 11.64 %) in alcohol per cent was observed in wine prepared using sugar and minimum (5.24 to 4.84 %) in wine prepared using jaggery. These results, coupled with earlier studies conducted by [20] in sapota wine and by [14] in star gooseberry and carambola.

Mineral content

A significant amount of minerals such as calcium, phosphorus, potassium, magnesium, iron, zinc and copper was found in both wines (Table 6). Potassium was the major mineral element found in star fruit wines. It was ranged between 201.60 and 202.70 mg / 100 ml. Calcium ranged from 7.42 to 7.47 mg / 100 ml, phosphorous from 17.65 to 17.93 mg / 100 ml and magnesium from 15.42 to 15.47 mg / 100 ml. The ranges of variations for the concentrations of iron, zinc and copper were 1.47 - 1.49 mg, 0.32 - 0.34 mg and 0.26 to 0.24 mg / 100 ml, respectively. During the aging process, very negligible changes in mineral content were observed in both wines. At the end of six months aging, the corresponding values were decreased between 7.38 and 7.43 mg / 100 g for calcium, 17.60 and 17.88 mg / 100 g for phosphorous, 201.52 to 202.65 mg / 100 g for potassium, 15.37 to 15.43 mg / 100 g for magnesium, 1.44 to 1.46 mg / 100 g for iron, 0.28 to 0.31 mg / 100 g for zinc and 0.20 to 0.22 mg / 100 g for copper respectively.

Table 6. Changes in mineral content (mg / 100 ml DWB) of star fruit wines during aging

Mineral contents	Fruit wine (sugar)			Fruit wine (jaggery)		
	Initial	Final	Mean	Initial	Final	Mean
Calcium	7.42±0.05	7.38±0.03	7.40±0.04	7.47±0.02	7.43±0.02	7.45±0.02
Potassium	201.6±2.09	201.5±2.05	201.6±2.07	202.7±2.03	202.6±2.02	202.7±0.23
Phosphorous	17.93±0.31	17.88±0.28	17.91±0.30	17.60±0.23	17.60±0.22	17.63±2.03
Magnesium	15.42±0.26	15.37±0.22	15.40±0.24	15.47±0.20	15.43±0.20	15.45±0.20
Iron	1.47±0.04	1.44±0.02	1.46±0.03	1.49±0.04	1.46±0.02	1.48±0.03
Zinc	0.32±0.01	0.28±0.01	0.30±0.01	0.34±0.01	0.31±0.01	0.33±0.01
Copper	0.26±0.01	0.22±0.01	0.24±0.01	0.24±0.01	0.20±0.01`	0.22±0.01

Microbial load

The microbial load of the fruit wine was enumerated during the initial and final days of aging (Table 7). The microbial load of the wine prepared using sugar had 1.0 x 102 cfu 10ml-1 for bacteria and fungi and 2.0 x 103 cfu 10ml-1 for yeast. At the final stage of aging, the fungal growth was not observed whereas the bacteria and yeast were increased to 3.0 x 102 cfu 10ml-1 and 4.0 x 103 cfu 10ml-1 respectively. The same trend was also observed in wine prepared using jaggery except for fungi. Initially, the fungi was not found in the wine prepared using jaggery and this trend was maintained up to six months of aging. However, the bacterial (2.0 x 102 cfu 10ml-1) and yeast (3.0 x 103 cfu 10ml-1) counts were observed slightly higher as compared to the wine prepared using sugar which was increased to 3.0 x 102 cfu 10ml-1 for bacteria and 6.0 x 103 cfu 10ml-1 for yeast respectively. Kocher and Pooja [21] observed 6.2 x 106 cfu ml-1 total yeast count in guava fruit wine after one-month storage in ambient temperature.

Organoleptic Characteristics

Acceptability of star fruit wines by the consumers is highly dependent on its sensory attributes. The sensory characteristics (color, flavor, sweetness, taste and overall acceptability) of wines were evaluated by a panel of 10 semi-trained judges with 9 points hedonic scale and the values are expressed in Figure 4.

Initially, the sensory scores on color, flavor, sweetness, taste and overall acceptability of both wines were similar (9.0). At the end of 180 days of aging, the initial color score was retained in the wine prepared using jaggery. The panelist likely preferred the dark brown color of the wine than golden yellow color. Moreover, the addition of sugar did not change the actual flavor of star fruit

Table 7. Changes in the microbial load of star fruit wines during aging

Microbial load (cfu $10ml^{-1}$)	Storage periods	Fruit wine (sugar)	Fruit wine (jaggery)	Mean
Bacteria × 10^2	Initial	1.0±0.01	2.0±0.01	1.5±0.01
	Final	3.0±0.03	3.0±0.02	3.0±0.03
Fungi × 10^2	Initial	1.0±0.01	ND	1.0±0.01
	Final	1.0±0.01	ND	1.0±0.01
Yeast × 10^3	Initial	2.0±0.01	3.0±0.01	2.5±0.01
	Final	4.0±0.04	6.0±0.05	5.0±0.04

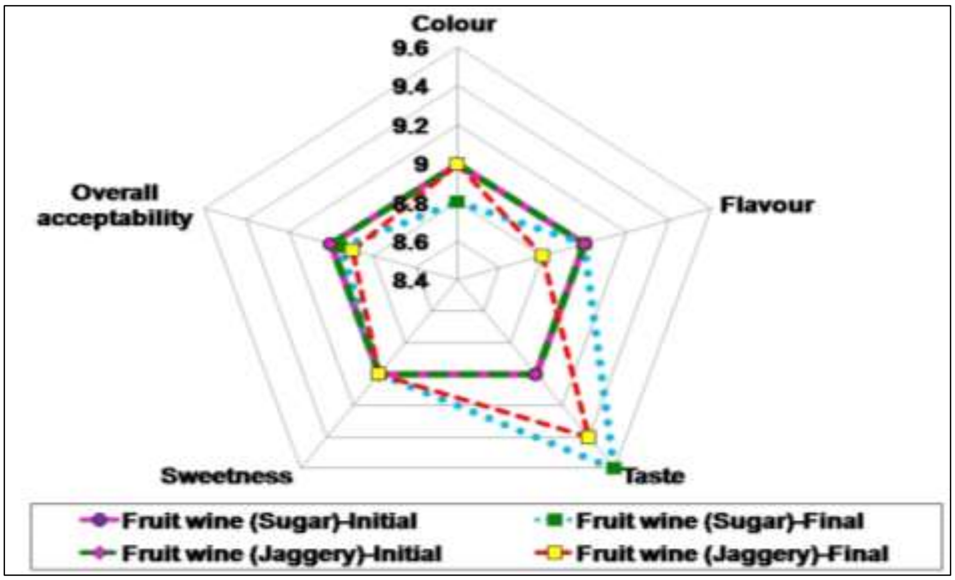

Figure 4. Sensory evaluation of star fruit wines during aging

juice during wine processing. The present study observed the increasing trend in taste scores with the advancement of aging process. At the final stage of aging, the maximum increase in taste score was found in the fruit wine prepared using sugar (9.0 to 9.6) and minimum in fruit wine prepared using jaggery (9.0 to 9.4). Comparatively both wines got similar overall acceptability score at the end of aging process. Similar results were reported by [20] in sapota and mango juice blended wine up to six months aging in ambient temperature and by [24] in Tendu fruit wine.

Conclusion

The optimized parameters viz., star fruit juice, 24 obrix total soluble solids, and 3.5 pH were identified as appropriate for the commercial production of wine from star fruit. The present study also determined that both sugar and jaggery are effective carbohydrate (energy) sources for the growth and development of yeast during the wine processing with better retention of nutrient and sensory parameters. The addition of jaggery produced dark brown color with slight jaggery taste and flavor in the fruit wine. Both the wines contained significant amount of minerals as well as below permissible limit of microbial loads after six months of aging in dark place at ambient temperature. Therefore, on the basis of the present study, jaggery is recommended as a carbohydrate source instead of sugar not only for the production of low alcoholic wine from star fruit juice, but also from other fruit juices in identical fermentation periods. Antioxidant potential, phytochemical properties, clinical and pathological target studies should be carried out to identify the health-promoting attributes of these wines in future.

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