Full Length Research Paper

Production and quality attributes of vegetable wine from *Hibiscus sabdariffa* Linn.

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Juice was extracted from dark red cultivar of Roselle calyx (*Hibiscus sabdariffa*) using water at a temperature of 100°C for 20 min in an automatic steam-jacket kettle. The juice was treated with 250 ppm. of sodium metabisulphite and sucrose was added to raise the sugar content of the juice. Pure wine yeast strain (*Saccharomyces cervisiae*) was inoculated into the juice to initiate fermentation. The resultant wine (9.06% v/v alcohol) was left to age for four months to allow for the development of the characteristic flavor and bouquet associated with wine. During fermentation, physico-chemical analysis of the wines indicated decrease in specific gravity, soluble solids, pH and colour intensity. Sensory evaluation of the aged roselle wine in terms of colour, flavor, taste and overall acceptability showed no significant difference compared to commercial wine samples. From the present study, it was observed that acceptable red table wine of good quality can be produced from Roselle calyx and its industrial potential should be exploited.

Key words: Red wine, alcohol, color, Hibiscus sabdariffa.

INTRODUCTION

Fruits and vegetables are consumed fresh and largely used in food industry for the production of canned fruit, jam, candy and concentrated juice. Fruits and vegetables could also be used in wine production. Some fruit juices are fortified with sugar to enable the yeasts produce enough alcohol (Duarte et al., 2010). In recent times, there has been an increase in demand for alcoholic beverages. Likewise, there has also been an increase in the demand for the use of home grown fruits and vegetables as raw materials in the manufacturing sector in Nigeria. This has led to the use of several locally grown tropical fruits and vegetables for this purpose. Among arrays of such indigenous tropical fruits are Kolanut (Cola acuminata), Cocoa (Theobroma cacao L.), African star apple (Chrysophyllum albidium) and Pawpaw (Carica papaya). The use of home grown fruits and vegetables

for wine production would enhance efficient utilization of these abundant natural resources and would invariably increase the nation's Gross Domestic Product (GDP).

One of the locally available vegetables that is still under-utilized in Nigeria is Roselle (Hibiscus sabdariffa) popularly called 'Zobo' in the Northern part of Nigeria (Omemu et al., 2006). The plant belongs to the family, Malvaceae. Non-alcoholic beverages have been made from a hot water extract of H. sabdariffa calyx. The nonalcoholic beverage produced from this extract is usually sweetened with sugar and may be flavored with flavorings such as ginger, pineapple, banana, vanilla and strawberry (Omemu et al., 2006). H. sabdariffa calyx extract has a characteristic red to red-brown colour. The pigment of the extract, anthocyanins possesses medicinal values (Haji and Haji, 1999). Wang et al. (1997) reported anti-oxidative capacity of anthocyanins via absorption free radicals in the living system. Anthocyanins is also effective as anti-inflammatory, antihepatoxic, antibacterial, antiviral antithrombic and antiallergenic (Mazza, 2000). In view of the medicinal

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values of anthocyanins, production of red wine from *H.* sabdariffa will perhaps serve dual purpose to man.

Harnessing the potential of this under-utilized vegetable in Nigeria justifies the present study, aimed at the assessment of physico-chemical and sensory qualities of Roselle calyx as a possible raw material substitute for wine production in Nigeria.

MATERIALS AND METHODS

Dried Roselle calyx (Dark red cultivar) was purchased at an open market in Ibadan, while active dried wine yeast, *Saccharomyces cerevisiae* (Na33 / *S. bayanus* EC1118 (mixture, 80/20)) were also obtained from Lallemand Inc. Canada.

Preliminary operations such as cleaning, sorting were carried out to remove extraneous materials from the Roselle calyx. Aqueous extract of Roselle calyx was obtained at a temperature of 100°C for 20 min in a steam jacketed kettle. The dilution factor was 15 g of Roselle calyx per 1 L of hot water. The juice was filtered from the calyx and 250 ppm of sulphur (IV) oxide in the form of sodium metabisulphite was added to the juice to inhibit the growth of bacteria and wild yeast. 200 g of sucrose was added to the juice to adjust the soluble solids from 4 to 18 °Brix. This was followed by the addition of ammonium sulphate (0.67 g/L) and citric acid (0.3 g/L) to serve as yeast nutrient. The juice was then poured into an aspirator (fermenting vessel) and dried wine yeast (S. cerevisiae) was pitched into the juice at $27 \pm 2^{\circ}C$ (room temperature). It was covered for about 15 to 20 min to allow for the yeast population to build up. The fermenting vessel was covered with a safety lock which has 200 ppm of sodium metabisulphite at the lid of the lock to control oxidation.

Racking was done at room temperature immediately after the evolution of gases terminated; the yeast lees were removed from the fermenting must to prevent further fermentation. Second racking was done with the introduction of bentonite slurry to aid racking and clarification. The second racking lasted for 12 days. This was followed by filtration and the filtered wine was bottled aseptically. Bottled wine was aged for five months prior to sensory evaluation.

Physicochemical analyses of the Roselle wine

Routine physiochemical analyses were carried out in triplicates using standard methods during fermentation (Majdak et al., 2002; Ough and Amerine, 1988; Martínez et al., 2001). The colour of Roselle wine was measured with a Unicam UV-VIS (UV 2) spectrophotometer in 10 mm cells at 420 nm. The pH of Roselle wine was measured using electrode pH meter (Model IQ 150, IQ Scientific Instruments, Inc., San Diego, California). Soluble solids (SS) of Roselle wine was determined using a hand refractometer (Leica Model Atago E Type series, Leica Inc., NY), the total titratable acidity (TTA) was determined using Official method of analysis (AOAC 926.12, 1990), TTA was expressed as percentage citric acid after titrating 10 ml of wine with 0.1 N NaOH with phenolphthalein as an indicator. In addition, total acidity, ash content and volatile acidity of the Roselle wine was determined using the method of Ough and Amerine (1988). Finally, percentage alcohol in the Roselle wine (v/v) was measured by the specific gravity adopting Official method of analysis (AOAC 11. 005, 1990).

Microbiological analysis

10 ml of Roselle wine was homogenized in 90 ml sterile peptone water (pH = 7.0) to obtain a 1:10 dilution. Further 10-fold dilutions

were prepared from this and 0.1 ml each of appropriate dilutions was plated using the pour plate method (Harrigan and McCance, 1976). Enumeration of the total viable aerobic bacteria counts were carried out using Plate Count Agar (PCA) (Oxoid CM325, Hampshire, UK) and de Mann Rogossa and Sharpe (MRS) agar (Oxoid CM 361) while Sabouraud Dextrose Agar (SDA, Oxoid CN 41) was used for fungal counts. SDA plates were incubated at 25°C for 72 h for fungi while PCA and MRS plates for bacteria were incubated at 30°C for 48 to 72 h. One set each of MRS and PCA plates were incubated under anaerobic conditions, stimulated, using a CO₂ gas generating kit (Oxoid, Hampshire, UK).

Sensory evaluation

A bottle of red wine was purchased from a supermarket in Ibadan, Nigeria; this was used as reference sample of a preference test between commercial wine and the Roselle wine prepared. A panel of twenty judges was drawn from the University of Ibadan community for the sensory evaluation. The panelists were familiar with all the quality attributes of a good wine. The reference sample wine (Redman - 2004 Coonawarra) and the Roselle wine produced were refrigerated at 11°C for 24 h prior to sensory evaluation. Each panelist received the wine sample in a random presentation order, a glass of water for rinsing and crackers for consumption between samples. Each panelist was served 30 ml of chilled wine in transparent glasses, which were coded with random three-digit numbers and served prior to sensory evaluation (Jackson, 2002). Coded samples were assessed organoleptically using a 9-point hedonic scale, where 9 correspond to "like extremely" and 1 corresponds to "dislike extremely".

Statistical analysis

Physico-chemical analysis of Roselle wine was carried out using one way analysis of variance (ANOVA) using SPSS 17.0 at α = 0.05, while sensory analysis data were analyzed using T test.

RESULTS AND DISCUSSION

Changes in physico-chemical qualities of the Roselle wine during fermentation are reported in Table 1. During fermentation, the specific gravity, and pH decreased with days of fermentation. This may be linked to utilization of soluble solids by yeast cells during fermentation. The sharp decline in specific gravity and soluble solids of the fermenting Roselle wine indicates high rate of fermentation (that is, high rate of sugar utilization by the yeast cells). Fermentation process occurred in two stages; the primary stage, characterized by a high rate of fermentation, and a secondary stage which showed a slowdown of the fermentation rate as a result of the effect of alcohol concentration on yeast cells. The high rate of fermentation in the primary stage is due to the presence of yeast nutrients at the beginning of fermentation which was gradually used up as fermentation progressed. This is in agreement with previous work (Amerine et al., 1980).

The reduction in soluble solids from 18 to 3.4 °Brix correspondingly results in the production of alcohol. The reduction in soluble solids of the must from 18.1 to 4.8 °Brix at day 12 of fermentation shows efficiency of the

Days	Colour (abs. @ 420 nm)	рН	Soluble solids (°Brix)	Specific gravity	Alcohol (% vol.)	TTA (g/L)
1	0.5964 ± 0.23	3.78 ± 0.02	18.1 ± 0.03	1.049 ± 0.01		0.52 ± 0.02
2	0.5901 ± 0.06	3.76 ± 0.07	15.0 ± 0.00	1.044 ± 0.02	1.3 ± 0.22	0.54 ± 0.25
3	0.5866 ± 0.01	3.70 ± 0.19	13.2 ± 0.07	1.030 ± 0.01	2.1 ± 0.14	0.57 ± 0.01
4	0.5504 ± 0.03	3.66 ± 0.23	11.0 ± 0.13	1.022 ± 0.01	3.3 ± 0.11	0.61 ± 0.07
5	0.5093 ± 0.02	3.60 ± 0.12	10.1 ± 0.05	1.018 ±0.01	4.5 ± 0.02	0.64 ± 0.01
6	0.4789 ± 0.27	3.51 ± 0.05	9.0 ± 0.01	1.001 ± 0.00	5.8 ± 0.08	0.64 ± 0.02
7	0.4552 ± 0.14	3.45 ± 0.00	8.5 ± 0.02	0.999 ± 0.00	6.1 ± 0.06	0.66 ± 0.04
8	0.4451 ± 0.07	3.40 ± 0.04	8.0 ± 0.22	0.991 ± 0.01	6.9 ± 0.01	0.67 ± 0.01
9	0.4348 ± 0.03	3.33 ± 0.08	7.5 ± 0.09	0.989 ± 0.00	7.5 ± 0.0	0.68 ± 0.17
10	0.4106 ± 0.18	3.26 ± 0.01	6.8 ± 0.03	0.981 ± 0.01	8.1 ± 0.03	0.69 ± 0.05
11	0.4069 ± 0.07	3.18 ± 0.02	5.2 ± 0.05	0.979 ± 0.02	8.8 ± 0.02	0.71 ± 0.21
12	0.3902 ± 0.12	3.09 ± 0.04	4.8 ± 0.01	0.975 ± 0.01	9.6 ± 0.03	0.73 ± 0.03

Table 1. Changes in physico-chemical attributes during fermentation.

Table 2. Physicochemical composition of Roselle wine.

Analysis	Roselle wine
Colour	0.3902 ± 0.12
Specific gravity (20°C)	0.975 ± 0.01
Alcohol (% v/v)	9.6 ± 0.03
рН	3.09 ± 0.04
Volatile acidity ^a (g/L)	0.33 ± 0.02
Total titratable acidity ^b (g/L)	0.73 ± 0.03
Ash content	3.06 ± 0.11
Reducing sugars	4.22 ± 0.39
Total solids (°Brix)	4.8 ± 0.01

^a expressed as acetic acid; ^b expressed as citric acid.

yeast. The high yield of alcohol is attributed to the breakdown of soluble solids in the must to alcohol. This is also responsible for gradual decrease in pH and increase in TTA of the must during fermentation. TTA of the wine increased from 0.52 to 0.73 g/L of citric acid. The generally low TTA of the wine is a reflection of initial low pH of the Roselle calyx extract (Bolade et al., 2009).

The mean count of bacteria in fermenting must was 2 logcfu/ml. Generally, counts of bacteria were too low to be of any significance. Coliforms and other members of the enterobacteriaceae were below detectable levels in the must, possibly due to the low pH of the product. Similarly, count of spore was also 2 logcfu/ml. Variations in counts of bacteria and aerobic spores within fermenting must are significant with coefficient of variation (γ) of 60.29%. Yeast is the most dominant microorganisms in the fermenting must. Mean counts was 6 logcfu/ml. Yeast counts showed significant variation during fermentation due to its increase in population.

The physicochemical composition of freshly prepared Roselle wine obtained is reported in Table 2. The pH,

volatile acidity, total acidity, reducing sugars and ash content were in agreement with previous studies reported by Revilla and González-Sanjose (2001) and Kelebek et al. (2007). The colour of Roselle wine produced had 0.3902 absorbance unit at 420 nm which has a positive correlation with the low pH of the wine. The alcohol content of the wine was in conformity with European Commission (EC) Wine Regulations as reported by Kirk and sawyer (1991). The volatile acidity of the wine is 0.33 g of acetic acid per 100 ml of wine; therefore, the safety of the wine for consumption is assured. The pH of the wine has direct relationship with titratable acidity (that is. the higher the titratable acidity, the lower the pH and vice versa). Consequently, the wine would maintain good shelf stability (Ough and Amerine, 1988). The soluble solids content and gravity of the wine also falls within EC Wine Regulations, reported by Kirk and Sawyer (1991). The soluble solid content of the wine suggests that the quantity of reducing sugars present in the wine is responsible for low sweetness. Hence, the Roselle wine produce can be classified as dry wine for its residual sugar content is < 7.5 g/L.

Attributes	Redman wine	Roselle wine
Colour	7.15 ± 0.39 ^a	7.05 ± 0.34^{a}
Clarity	7.45 ± 0.21^{b}	7.40 ± 0.34^{b}
Taste and aroma	$6.10 \pm 0.48^{\circ}$	7.45 ± 0.21^{d}
Flavour	6.80 ± 0.41^{e}	7.21 ± 0.23^{e}
Overall acceptability	7.05 ± 0.33^{f}	7.15 ± 0.21^{f}

Table 3. Sensory attributes of the Roselle Wine.

Wine attributes having same superscript in not significantly different at (P < 0.05).

From the sensory analysis result shown in Table 3, Roselle wine was not significantly different (P < 0.05) from reference wine sample in terms of colour, clarity, flavor and overall acceptability. However, the taste of Roselle wine was significantly different from that of reference wine sample. Roselle wine is rated higher than reference wine sample in all attributes except in clarity. Correlation test was also conducted for the sensory attributes, there were significant correlation (P < 0.01) between colour and clarity and overall acceptability.

Conclusion

There are a number of underutilized fruits and vegetables in the tropics which can be exploited for wine production purposes. The wine produced from *H. sabdariffa* has been found to be acceptable, as well as meeting all the standards required by a good wine in terms of color, flavour, taste, aroma and overall acceptability for a bouquet wines. The high acidity gives it an edge in terms of storability and its resistance to microbial spoilage. *H. sabdariffa* is readily available and it is cheap, thus, it can be a good substrate for wine industry. The use of the vegetable for wine can be exploited commercially as this would lead to conservation of foreign exchange. Equally, more studies are needed that would compare quality of the *H. sabdariffa* wine aged for a long period of time with commercial wines from other sources.

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