Characteristics of Coloured Wine Produced from Roselle (*Hibiscus sabdariffa*) Calyx Extract

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ABSTRACT

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Wine was produced from roselle (*Hibiscus sabdariffa*) calyx extract using strains of *Saccharomyces cerevisiae* and subjected to physico-chemical analyses using standard analytical methods. Imported commercial red wine was used as the reference for sensory evaluation. The results showed that ameliorated roselle calyx extract (must) had 4.21% protein, 0.69% titratable acidity, and 21°Brix total soluble solids. The roselle calyx wine had a pH value of 3.43, a titratable acidity of 0.75%, and an alcohol content of 10.8% (w/v), which were all within values for grape wine. Total anthocyanin (TACY) content and total colour density (TCD) of the wine was 22.26 abs/mL and 25.20 abs/mL, respectively. The sensory properties of the roselle wine showed no significant difference with those of the imported red wine and it appears that roselle calyx could be used to produce acceptable coloured wine.

Key words: Anthocyanins, coloured wine, physico-chemical properties, roselle calyx, sensory properties.

INTRODUCTION

Non-availability of grapes, the fruit of choice for wine production in the tropics has necessitated the use of alternative fruits in the production of wine in Nigeria and other tropical countries[1,2,16,17]. Tropical wines are subjectively perceived as inferior in quality on the basis of flavour, aroma, bouquet, and colour[3]. These attributes are the outcome of the synergistic effect of flavour compounds, alcohol content, organic acid profile and the content of extractable pigments in wines, and they determine the acceptability of the wines. The major problems associated with the use of tropical fruits in wine production include their low sugar content, high acidity[2] and the presence of an array of microorganisms other than wine yeast (*Saccharomyces cerevisiae* var *ellipsoideus*). In addition, tropical red wines have not been produced from tropical fruits because of their low content of extractable red pigments, unlike those in red varietal grapes[19]. Modification of the processes for the production of grape wines and application of such modification to tropical fruits could lead to the production of good quality wines. These include pasteurization, amelioration of the fruit juices with sugar, addition of water and reduction of acidity. In addition, sulphur dioxide, ambient temperature fermentation and diammonium phosphate have been recommended and used in the production of acceptable tropical wines[1,6,11,21]. Addition of synthetic colourants or dyes to tropical wines appears to be an alternative in the production of coloured tropical wines. However, the use of synthetic colourants and azo dyes in foods and drinks is restricted, as they may be toxic to humans after prolonged exposure to such dyes[14].

Roselle, also known as guinea or Indian sorrel is a member of the *Malvaceceae* family and grows in the tropic and sub-tropical regions of the world[5,8,10]. It is an erect, branched sub-woody annual shrub that bears alternate leaves and flowers that are borne with large leafy calyces. Roselle calyces are edible and have been used in the preparation of 'cacody tea' and fermented drinks in Egypt[9,15]. There are reports on the use of the red variety of roselle calyx to produce 'karkade drink' in Sudan and 'Zobo' in Nigeria[18,25]. Red roselle calyx has been reported to be rich in anthocyanins and it contains a mixture of organic acids such as citric, malic and tartaric acids[18,25]. Karppa et al.[14] reported that anthocyanins vary in colour from pink to blue and violet. These characteristics suggest that roselle calyx extract may be a suitable raw material for the production of coloured wines. The objective of this study was to evaluate the quality attributes of wine produced from roselle calyx extract.

MATERIALS AND METHODS

Dry mature roselle calyces used in this study were obtained from a local market in Idah, Nigeria. Dry yeast (*Saccharomyces cerevisiae*) (Royal Instant, Danboli, China) was collected from the pilot processing plant of the Department of Food Science and Technology, Federal Polytechnic, Idah, Nigeria.

Preparation of roselle must

The roselle calyces were manually sorted and washed in cold water to remove dirt. About 100 g of the clean calyces were boiled in batches in 500 mL of water for 10 min. They were strained using sterile muslin cloth and the residual calyces were further re-extracted by heating with 500 mL of water. The filtrates were pooled to obtain a 1:10 roselle to water extract, which was then ameliorated, using sucrose sugar to raise the sugar level to 21°Brix. In addition, potassium metabisulphite calculated to 150 ppm and diammonium phosphate (0.3 g/L) were added to stabilize the extract and provide a source of nitrogen, respec-

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Table I. Physico-chemical properties of roselle extract, must and wine. Values are means ± SD of triplicate determinations.

Parameter	Extract	Must	Legal standards for wines*	Roselle wine % (w/v)**
Total soluble solids (°Brix)	2.00 ± 0.02	16.00 ± 0.02	4.00[a]	4.90 ± 0.02[b]
Titratable acidity (%, as tartaric)	0.65 ± 0.06	0.69 ± 0.14	0.69–0.90[a]	0.75 ± 0.03[a]
Volatile acidity (%)	0.04 ± 0.12	0.0 ± 20.15	0.14[a]	0.13 ± 0.03[a]
Fixed acidity (%)	0.61 ± 0.01	0.67 ± 0.03	0.40[a]	0.62 ± 0.03[a]
pH	3.78 ± 0.03	3.72 ± 0.04	3.60[a]	3.43 ± 0.04[a]
Specific gravity	1.0017 ± 0.04	1.0688 ± 0.01	0.9726[a]	0.9806 ± 0.02[b]
Ash (%)	0.70 ± 0.05	0.68 ± 0.02		0.43 ± 0.04
Total anthocyanins (abs/mL)	28.43 ± 0.04	28.30 ± 0.10		22.65 ± 0.08
Total colour density (abs/mL)	30.65 ± 0.15	30.14 ± 0.14		25.20 ± 0.14
Crude protein (%)	2.63 ± 0.23	4.21 ± 0.26		1.75 ± 0.09
Sulphur dioxide (%)	0.00	13.40 ± 0.12		10.50 ± 0.06
Alcohol (% [w/v])	0.00	0.00	4.00–14.00[a]	10.80 ± 0.08[b]
Ascorbic acid (mg/100 mL)	1.12 ± 0.06	1.06 ± 0.14		0.6 ± 90.13
Colour (visual)	Deep red	Deep red		Brilliant rose

* Amerine et al.[2]
** Aged for 30 days after fermentation. Values with the same letters attached are not significantly different (p>0.05).

tively[1]. The treated roselle extract (must) was pasteurized at 60°C for 30 min and then kept (0°C) until used.

Preparation of yeast starter

Ten grams of dry yeast was mixed with 300 mL of roselle must, which had been preheated to 37°C. The must was sweetened to 10°Brix with sucrose to provide the yeast with food. The mixture of must and yeast was held in a propagation flask at 30 ± 2°C for 48 h. The methylene blue staining procedure (according to IOB[13]) was used to determine the viability of the yeast. The yeast culture was 96% viable and had an initial cell count of 5.6×10^7 CFU/mL.

Fermentation of roselle calyx must

The standard must (4.5 mL) was poured into a 5 L sterile plastic aspirator jar and seeded aseptically with 135 mL of the 48 h yeast culture to give a pitching rate of 3% (w/v). The jar was fitted with an air lock containing 150 ppm sulphur dioxide. The must was subjected to primary fermentation at 25 ± 2°C for 14 days to produce wine. The young wine was racked into a sterile aspirator jar fitted with air lock and then allowed to undergo secondary fermentation for a further 7 days at the same temperature. The racked wine was fined using 10 mL of 0.5% (w/v) bentonite and aged at 10 ± 2°C for 30 days. It was filtered using a sterile muslin cloth that was folded four times. The wine was bottled and pasteurized at 60°C for 30 min.

Analytical methods

The roselle extract, must and the fermenting wine were analyzed physico-chemically. The amount of total soluble solids (TSS) was determined according to the AOAC[4] method using an Abbe 60 refractometer (Bellingham and Stanley standard model, England) that was set at 20°C. Titratable acidity (TA), volatile and fixed acidities (VA and FA, respectively), pH (Pye Unicam pH meter, Model 290 MK 2 England), specific gravity and alcohol were determined by the methods of the AOAC[4], as were crude protein (N × 6.25), ascorbic acid and sulphur dioxide. A spectrophotometric method was used to estimate the total anthocyanin concentration (TACY), expressed as absorbance per mL, according to the method of Fuleki and Francis[7]. Total colour density (TCD) was determined by the method of Somers[24]. Absorbance was measured at 420 nm, 520 nm and 700 nm on a spectrophotometer (Spectronic 20, Bausch & Lomb, Belgium) using a 10 mm cell.

Sensory evaluation

The roselle calyx wine was evaluated for colour, clarity, flavour, aroma, taste and over all acceptability by a 20-member panel composed of male and female subjects who were familiar with wine. The assessment was conducted under fluorescent illumination inside isolated booths within an air-conditioned sensory laboratory. A commercial red wine (Baron de Valls, manufactured by Baron de Valls, Spain) was used as a reference (control) for the assessment. The assessment was carried out using a 9-point hedonic scale (where 9 = like extremely and 1 = dislike extremely)[12]. Samples were served in coded transparent drinking glasses. The tasters were instructed to rinse their mouth with water after each evaluation.

Statistical analysis

Triplicate samples were used for the physico-chemical evaluation, and means and standard deviations (SD) are reported. The sensory data generated were submitted to the student's t-test and differences were discriminated at the 5% level of significance[22].

RESULTS AND DISCUSSION

Table I shows the physico-chemical properties of roselle calyx extract, must and wine. The roselle calyx extract had low amounts of sugars (TSS, 2°Brix) and crude protein (2.63%), which necessitated its amelioration to raise the levels of sugars and protein to 21°Brix and 4.21%, respectively for adequate fermentation by the yeast. The titratable acidity, volatile and fixed acidities, pH, ash, total anthocyanin, total colour density and ascorbic acid values of the roselle extract did not change appreciably upon amelioration. The pH value of 3.72 for the roselle must falls within the recommended optimal pH level of 3.5–4.0 for wine fermentation[2,21]. Dissolution of potassium metabisulphite in the roselle calyx must resulted in the formation of bisulphite ions. The pH value decreased to 3.43 after fermentation and aging of the wine. The low pH and sulphite are desirable, as they in-

Table II. Changes in some physico-chemical properties during fermentation of roselle calyx wine.[a]

Days	TSS[b] (°B)	pH	TA[c] (%)	VA[d] (%)	FA[e] (%)	Alcohol (% [w/v])
0	21.00 ± 0.02	3.72 ± 0.01	0.6 ± 90.06	0.02 ± 0.04	0.67 ± 0.05	0.00
1	19.40 ± 0.12	3.68 ± 0.13	0.6 ± 80.08	0.03 ± 0.12	0.65 ± 0.06	1.20 ± 0.15
2	17.80 ± 0.11	3.67 ± 0.09	0.66 ± 0.05	0.03 ± 0.06	0.63 ± 0.07	2.20 ± 0.11
3	15.50 ± 0.05	3.66 ± 0.16	0.64 ± 0.12	0.04 ± 0.03	0.60 ± 0.05	3.00 ± 0.06
4	13.10 ± 0.08	3.65 ± 0.14	0.62 ± 0.14	0.05 ± 0.15	0.57 ± 0.10	4.30 ± 0.08
5	12.20 ± 0.09	3.62 ± 0.04	0.63 ± 0.08	0.07 ± 0.16	0.57 ± 0.07	5.80 ± 0.12
6	11.60 ± 0.13	3.61 ± 0.17	0.65 ± 0.12	0.07 ± 0.06	0.58 ± 0.03	6.30 ± 0.16
7	10.10 ± 0.06	3.58 ± 0.15	0.67 ± 0.14	0.08 ± 0.03	0.59 ± 0.05	7.50 ± 0.09
8	8.60 ± 0.13	3.56 ± 0.12	0.67 ± 0.10	0.10 ± 0.11	0.57 ± 0.09	8.60 ± 0.02
9	8.10 ± 0.06	3.55 ± 0.08	0.68 ± 0.14	0.12 ± 0.08	0.56 ± 0.12	8.80 ± 0.06
10	7.60 ± 0.11	3.53 ± 0.16	0.69 ± 0.11	0.13 ± 0.06	0.65 ± 0.04	9.00 ± 0.04
11	7.20 ± 0.07	3.50 ± 0.05	0.71 ± 0.04	0.13 ± 0.13	0.58 ± 0.04	9.10 ± 0.12
12	6.80 ± 0.10	3.47 ± 0.09	0.72 ± 0.06	0.14 ± 0.12	0.58 ± 0.08	9.20 ± 0.08
13	5.70 ± 0.08	3.45 ± 0.05	0.72 ± 0.13	0.14 ± 0.05	0.58 ± 0.06	9.20 ± 0.06
14	5.60 ± 0.14	3.44 ± 0.16	0.73 ± 0.05	0.15 ± 0.06	0.58 ± 0.04	9.20 ± 0.04

[a] Values are means ± SD of triplicate determinations.
[b] Total soluble solids.
[c] Titratable acidity.
[d] Volatile acidity.
[e] Fixed acidity.

Table III. Comparative mean sensory scores of roselle and commercial wine samples.*

	Sensory attributes					
Sample	Colour	Clarity	Flavour	Aroma	Taste	Overall acceptability
Roselle	7.6[a]	7.0[a]	6.9[a]	7.2[a]	6.4[a]	7.0[a]
Baron de Valls	7.3[a]	7.3[a]	6.4[a]	7.8[a]	7.0[a]	7.1[a]

* Mean values with the same letters attached are not significantly different (p>0.05). Higher values indicate greater preference on a 9-point hedonic scale.

hibit the activity of undesirable microorganisms that may be present in the wine.

The titratable acidity of the roselle extract and must were comparable to that of bitter lemon must, which was 0.60%[1] and consistent with 0.65% for roselle water extract as reported by Udom et al.[25]

The TACY of the roselle extract reduced by 20.4% as a result of the vinification procedure employed in this study. The change in TCD of the fermenting must followed the same pattern as indicated by a reduction from 30.6 to 25.2 abs/mL (17.6%) after fermentation and aging of the wine. Although these decreases were low, a change in colour of the extract from deep red to a brilliant rose for the wine was visually evident. A combination of factors, which may include browning and anthocyanin loss as a result of oxidation probably contributed to the variations in the TACY and TCD values. The roselle wine contained 10.8% (w/v) alcohol after aging. This value is comparable with that for lemon wine (10.1%) as reported by Alobo[1]. It is also comparable with that of wine produced by Okoro[19] from a blend of pawpaw and roselle extracts with a value of 10.5% (w/v). The alcohol content of the roselle wine falls within the legal standard specification for wines[2]. The ascorbic acid content of the roselle extract was reduced by 35.7% during the conversion of the extract to wine. This may be due to heat destruction and oxidation. The acid may have served as an oxygen scavenger to protect the wine against oxidative changes.

Table II data shows the physico-chemical changes during the fermentation of roselle wine. There was a gradual decrease in TSS with a corresponding increase in alcohol content. These changes became less pronounced after the 10th day of fermentation. The changes may be due to a gradual attenuation of the fermentation process due to decreased substrate concentration with a corresponding increase in alcohol concentration, which could adversely affect yeast activity. The reduction of the soluble solids from 21°Brix to 5.6°Brix and the resultant formation of 9.20% (w/v) alcohol by the 14th day of fermentation suggest the efficiency and suitability of the yeast for use in the fermentation of roselle wine. Earlier reports[1,23] showed that yeast, which ferment sugars rapidly and tolerate a high level of alcohol, could be useful in wine production. The value for the volatile acidity of the roselle must increased, while that for the fixed acidity decreased with increasing period of fermentation. Peynauld and Domereq[20] reported the disappearance of malic acid during alcoholic fermentation and attributed this to its reduction to ethanol.

The results of the sensory evaluation of the roselle wine are presented in Table III. There were no significant differences (p>0.05) in colour, clarity, flavour, aroma, taste and over all acceptability between the roselle wine and a reference imported red wine. The roselle wine received higher scores for colour and clarity than the reference sample. Based on physico-chemical properties and panel responses, it appears that roselle calyx produced an acceptable rose wine. We are currently examining the effect of treatments on the variation and stability of the colour of roselle wine.

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