Original Research Article

Antioxidant, Mineral and Microbiological Properties of Wine from Blends of Roselle Calyces Extract and Pineapple Juice

Abstract

Wine was produced from must formulated by mixing roselle calyces hot water extract with pineapple juice at ratios of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50 for A, B, C, D, E and F respectively. Must were pitched with *Saccharomyces cerevisiae*, fermented at room temperature for 8 days followed by racking, pasteurization and ageing at room temperature for 31 days. The antioxidant capacity, mineral content and microbiological analyses were carried out on the musts and wine samples using standard procedures. There was a significant ($p<0.05$) difference between must and wines as fermentation was observed to increase its antioxidant capacity (2, 2-diphenyl -1- picrylhydrazylradical scavenging activity, ferric reducing antioxidant properties, trolox equivalent antioxidant properties and hydroxyl radical scavenging activity). Fermentation decreased the mineral contents (manganese, zinc and magnesium) of wines except for sodium that increased significantly. Values ranged from 3.81 – 7.77 mg/L for Manganese, 162.38 – 166.66 mg/L for sodium, 2.71 – 4.71 mg/L for zinc and 35.45 – 40.67 mg/L for magnesium. Microbial count was done at different stages of production and there was no detectable growth on cultured wine samples. The result of this study showed that wines of nutritionally high quality can be produced from blends of roselle hot water extract and pineapple juice.

Keywords: Wine; roselle calyces extracts; pineapple juice; antioxidant; minerals.

1. INTRODUCTION

Tropical countries possess a wide diversity of fruits and vegetables with many possibilities of commercial exploitation. Fruits and vegetables are consumed fresh and largely used in food industry for the production of canned fruits, jam, candy, concentrates and some are also used in wine production [1]. Traditionally, wine is produced from the juice of grapes.

Wine is any alcoholic beverage produced from juices of variety of fruits by fermentative action of microorganisms either spontaneously or seeding with a particular strain mainly of yeast species. Wine is one of the most recognizable high value-added products from fruits [2]. Fruit wines are undistilled alcoholic beverages usually made from grapes or other fruits. These fruits undergo a period of fermentation and ageing. They usually have an alcohol content ranging between 5-13% [3]. Wines made from fruits are often named after the fruits. Since fruit based wines are fermented and undistilled product, they contain most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to release of amino acids and other nutrients from yeast during fermentation [3].

Roselle (*Hibiscus sabdariffa)* is a member of Malvaceae family and is a known medicinal plant with a worldwide fame [4]. Roselle calyces are edible and the red variety is used to produce 'karkade drink' in Sudan and 'Zobo' in Nigeria [5]. Roselle is also called "red sorrel". It is mainly cultivated for its calyx, which is of three types: green, red and dark red. The red calyces are the most used and they are characterized by their concentration of anthocyanin. Anthocyanins are water-soluble compounds that provide color to plant tissues (leaves, stems, roots, flowers and fruits) ranging from red, purple to blue according to the environmental conditions, pH and their structural composition [6]. Regarding to human consumption, the high intake of foods rich in anthocyanins offers potential health beneficial effects on various disorders associated with cancer, aging diseases, obesity, neurological diseases, inflammation, diabetes as well as bacterial infections [7, 8]. Those health benefits are mainly associated with their antioxidant effects, which clearly are influenced by the molecular mechanism related to the expression and modulation of key genes [9].
Pineapple (*Ananas comosus*) is a tropical and subtropical fruit belonging to the *Bromeliaceae* family which composes of many flowers whose individual fruitlets fuse together around a central core. Each fruitlet can be identified by an "eye," the rough spiny marking on the pineapple's surface [10]. Pineapples have exceptional juiciness and a vibrant tropical flavor that balances the tastes of sweet and tart. They are second only to bananas as America's favorite tropical fruit [11].Pineapples contain vitamin A, B-complex vitamins, vitamins C, E, K, essential amino acids and minerals such as calcium, iron, magnesium, phosphorous, potassium, zinc, copper, manganese and selenium [12]. They are especially high in potassium and vitamin C and provide the body with powerful antioxidant activity. Pineapple also contains 12-15% sugars of which two-third is in the form of sucrose and the rest are glucose and fructose.
The aim of this work was to determine the antioxidant capacity, mineral content and microbiological properties of musts and wines produced from blends of roselle calyces extract and pineapple juice.

2. MATERIALS AND METHODS

2.1 Materials

Materials used include dried roselle calyces, mature ripe pineapples, baker's yeast (STK royal instant dry yeast), sugar, diammonium phosphate and potassium metabisulphite.

2.2 Raw Material Collection and Study Site

Dried roselle calyces, mature ripe pineapples, granulated sugar, baker's yeast were purchased from Modern market Makurdi, Nigeria, they were stored under ambient conditions before processing. Diammonium phosphate, potassium metabisulphite were obtained from Chemistry Department, Benue State University Makurdi. Raw materials obtained were taken to the Department of Chemistry, Benue State University where samples were produced under ambient conditions. The mineral content and microbiological counts of samples were analyzed in Chemistry and Biology Departments respectively in Benue State University Makurdi, while the antioxidant capacity was analyzed in National root crop research institute Umudike, Nigeria.

2.3 Sample Preparation

Dried roselle calyces were dry cleaned by hand picking of impurities such as straws, dried grasses. Ten grams of the calyces was weighed using an electronic weighing scale (Adam AE 437544). Weighed samples were washed with cold water to remove dust and other adhering impurities. Cleaned calyces were transferred into stainless steel pots where water (80 mL) was added for heating. It was allowed to boil for 20 minutes after which samples were filtered using muslin clothes (0.8mm). Rinsing was done twice by adding 10 mL of cold water at each time to the supernatant. The filtrate and two rinse water extracts (100 mL) were mixed to give 10% w/v of roselle calyces hot water extract. The extract was allowed to cool to room temperature.

Mature fresh pineapples were washed using clean flowing tap water and peeled using stainless steel knives. The peeled pineapples were sliced and cored. It was then reduced into smaller sizes and blended using electronic blender (Eurosonic type). It was strained through 0.8mm sieve to obtain juice. The juice was pasteurized at 80°C for 30 seconds after which it was allowed to cool. Roselle calyces extract and pineapple juices were blended according to the desired ratios of 100:0; 90:10; 80:20; 70:30, 60:40, 50:50 designated as samples A-F respectively.

Mixing of blends with water was done at ratio 2:1. This is called amelioration and it was done to raise the pH from a range of 3.0-3.3 to a range of 4.1- 4.4. Granulated sugar was also added to each of the blends to raise the brix level from 7.50-8.00 to 17.00-18.50. Also, 200ppm of potassium metabisulphite was added to each blend. This was done to kill all wild yeast that may be present in the samples. The roselle and pineapple blend known as the must was covered and allowed to rest for 24 hours and 250ppm diammonium phosphate was added to the rested must before pitching or inoculation with 14% of activated *Saccharomyces cerevisiae*. The must was allowed to ferment for 5 days - this is called the primary fermentation. Sugar was again added to raise the sugar content to 14 ◦Brix and 8% of activated *Saccharomyces cerevisiae* was added to the must to begin the secondary fermentation. Fermentation was allowed to continue for 72 hours after which it was stopped and wines were racked, bottled and pasteurized. Wines were allowed to age for 31 days.

2.4 Determination of Antioxidant Capacity

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant property (FRAP), hydroxyl radical scavenging activity (HRSA),trolox equivalent antioxidant capacity (TEAC) were carried out according to methodsdescribed by [13, 14, 15, 16] respectively.

2.5 Determination of Mineral contents

Mineral elements such as sodium, manganese, magnesium, potassium and zinc were determined as described by AOAC [17]. Fifty milliliters of each sample were digested with a mixture of nitric acid and perchloric acid in the ratio of 15:5 (v/v) on hot plates. After complete digestion, samples were cooled to room temperature and filtered using filter paper. The volume was made up to 50ml using distilled water. Samples were then analyzed using Atomic Absorption Spectrophotometer (PG990).

2.6Microbiological Analysis

The microbiological analysis was carried out according to a method described by [18]. Nutrient agar was used for enumeration of bacteria while potato dextrose agar was used for fungi count. A well homogenized sample was serially diluted with 0.1% peptone water up to 10^{-4}. One ml aliquot from a suitable dilution was transferred aseptically into sterile petri dishes. To each plate about 15ml of melted and cooled media was added. The inoculum was evenly mixed with media by rotating the plates which was then allowed to solidify. The inverted plate was incubated for 24hours at 37◦C. The total bacterial count (CFU/mL) and total fungal count was determined using a colony counter [18].

2.7 Statistical Analysis

Data were obtained in triplicate and subjected to statistical analysis using one-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test and judged significantly different at 95% confidence level ($p<0.05$) [19].

3. RESULTS AND DISCUSSION

3.1 Antioxidant capacity of musts and wines from roselle calyces extract and pineapple juice blends

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Fruits and vegetables contain a wide range of flavonoids and other phenolic compounds which possess antioxidant activity [20].

Fig.1 show 2,2 diphenyl -1- picrylhydrazyl (DPPH) scavenging activity of musts and wines from blends of roselle calyces extract and pineapple juice. The results show an increase in wine samples as compared to the musts samples. This indicates that fermentation may have increased the radical scavenging power of the wine samples. In general, there was significant difference in variation of antioxidants in each sample. Jagtap *et al.* [21] reported that jackfruit wine showed high radical scavenging capacity of 69%and this can be compared with the result from this study which had values ranging from 72.15 to 79.44%.

The Ferric reducing antioxidant capacity (FRAP) of musts and wines from blends of roselle calyces extract and pineapple juice is shown in Fig. 2. The musts had higher concentration when compared to the wine samples except in sample A where the wine had a higher value. The least FRAP value was recorded in sample A (musts) (26.52%) while the highest value was found in wine sample F (51.44%). For the fresh samples, the FRAP increased as the quantity of pineapple juice increased in the blends. The depletion in the FRAP values in the wine may be associated with the Fe^{2+}/Fe^{3+} acting as catalysts in the fermentation process. There was observable difference in the values for all samples.

Fig. 3 shows the trolox equivalent antioxidant capacity (TEAC) of musts and wines from roselle calyces extract and pineapple juice blends. The result indicates that wine samples had higher values for TEAC when compared with the musts. For the musts, the TEAC increased as the ratio per volume of pineapple increased. A similar trend was observed for the wines with the least value for TEAC recorded in must sample A (3.72%) and maximum value in wine sample F (7.47%). It is possible that pineapple contains essential components which tend to increase the effect as seen from the observed trend.

Fig. 4 shows hydroxyl radical scavenging activity (HRSA) of musts and wines from blends of roselle calyces extract and pineapple juice. From the results, the wine samples showed higher values than the musts. The must samples recorded lower inhibition ranging from 0.52 to 4.01% for A – F respectively. Wine samples had values which ranged from 3.72 to 7.47% for A – F respectively. The trend suggests that an increase in pineapple increased the hydroxyl radical scavenging activity while fermentation further increased this activity.

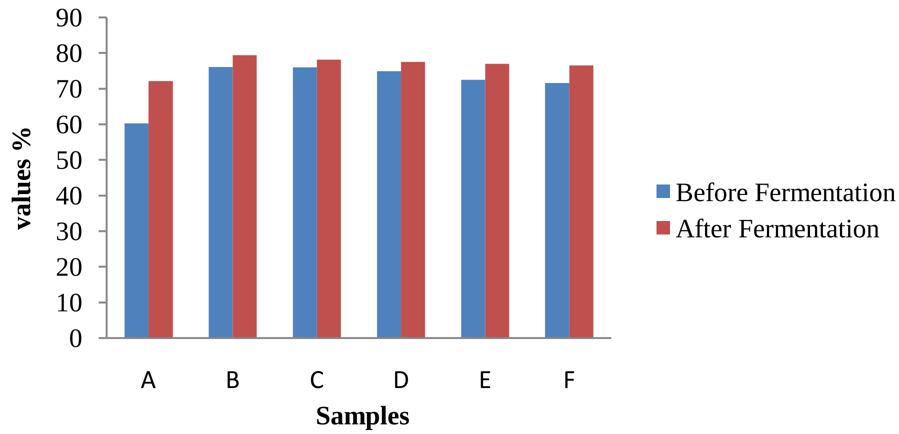

Fig 1: DPPH radical scavenging activity of musts and wines produced from roselle extract and pineapple juice blends

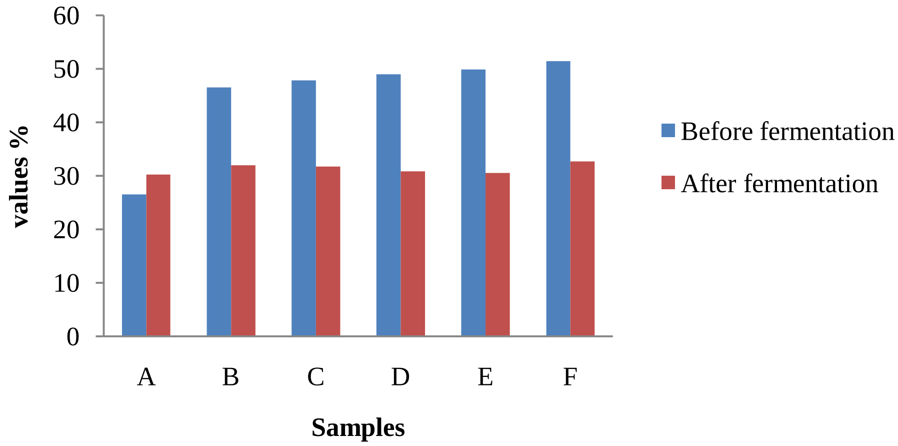

Fig 2: Ferric Reducing Antioxidant Power of samples of roselle extract and pineapple juice blends

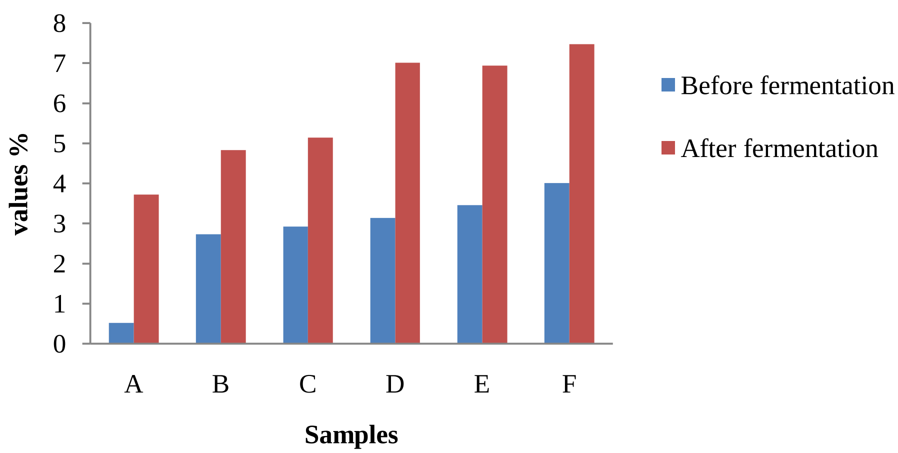

Fig 3: Trolox Equiva lent Antioxidant Capacity of musts and wines from roselle extract and pineapple juice blends

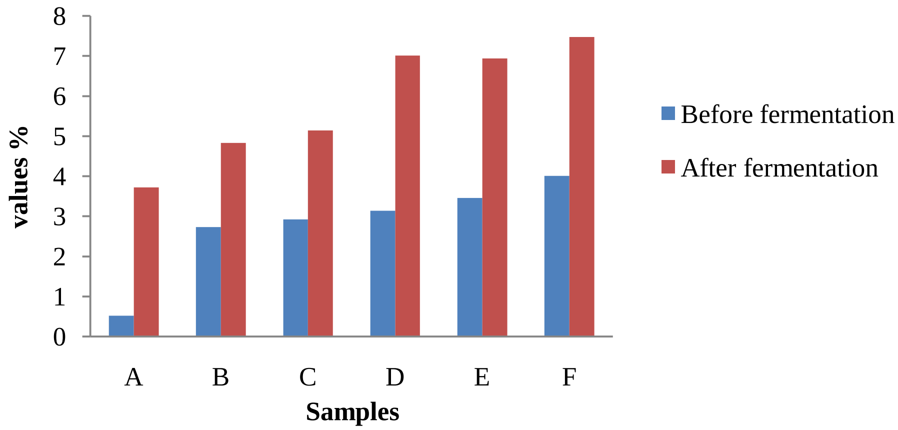

Fig 4: Hydroxyl Radical Scavenging Activity of musts and wines from roselle extract and pineapple juice blends

3.2 Selected mineral composition of musts and wines from blends of roselle calyces extract and pineapple juice

Table 1 and 2 present the results of selected minerals (mg/L) of musts and wines from blends of roselle extract and pineapple juice. Juice contains many minerals that affect fermentation and subsequent reactions. The source of minerals in wine could be from the raw materials used, the processing of the juice to wine, bottling, and storage [22]. Minerals contribute to particular characteristics in wine such as colour, flavour and aroma.The manganese content was found to decrease with addition of pineapple juice. In the musts, values ranged from 7.55 to 12.85 mg/L while manganese values of the wines decreased with values ranging from 3.81 to 7.77 mg/L, the value of manganese was found to decrease ($p<0.05$) significantly among the samples.

Sodium content in the musts decreased with increased concentration of pineapple juice with sample A having the highest value of 118.81 mg/L. The sodium content increased in the final wines with sample A (Fermented 100% roselle extract) having the highest value of 166.6 mg/L. The increase in sodium content in the wines could be attributed to the presence of sodium metabisulphite which was added to the wine to stop fermentation. Sodium can be added to wine during sulfur dioxide additions when SO_2 is added in the form of sodium metabisulphite ($Na_2S_20_5$) [23].

Zinc content in the musts was highest in sample D with value of 5.69 and least in sample F with value of 2.95 mg/L. There was a general decrease in zinc content after fermentation. The decrease in zinc could be attributed to the fact that yeast uses zinc during fermentation as its nutrient for growth and multiplication [24]. Samples were significantly different from each other at $p<0.05$.

Magnesium content decreased in the wines. However, there was no significant ($p>0.05$) difference for wine samples B, C, D and E. The general decrease in the mineral content during fermentation is due to precipitation. Clarification of wine may also lead to a decrease in concentration of heavy metals [24].

Table 1: Selected mineral composition of musts from blends of roselle calyces extract and pineapple juice (mg/L)

Sample	Mn	Na	Zn	Mg
A	12.85^{e}±0.00	118.81^{cd}±0.00	4.64^{cd}±0.00	42.87^{c}±0.00
B	11.72^{d}±0.00	97.54^{b}±0.00	4.43^{c}±0.00	42.79^{c}±0.00
C	11.59^{d}±0.00	108.47^{c}±0.00	4.26^{c}±0.00	42.67^{c}±0.00
D	10.65^{cd}±0.00	104.75^{bc}±0.00	5.69^{e}±0.00	42.55^{c}±0.00
E	9.54^{b}±0.00	94.53^{b}±0.00	3.94^{b}±0.00	41.97^{b}±0.00
F	7.55^{a}±0.00	83.18^{a}±0.00	2.95^{a}±0.00	40.69^{a}±0.00

Values are means± standard deviation for triplicate determinations. Means on the same columns with different superscript are significantly different at ($p<0.05$)

RE:PJ A (100:0); B(90:10); C(80:20), D(70:30); E(60:40), F(50:50)

Table 2: Selected mineral composition of wine from blends of roselle calyces extract and pineapple juice (mg/L)

Sample	Mn	Na	Zn	Mg
A	3.81^{a}±0.00	166.66^{ab}±0.00	2.71^{a}±0.00	35.45^{a}±0.00
B	7.77^{c}±0.00	165.59^{ab}±0.00	5.61^{e}±0.00	40.67^{c}±0.00
C	7.66^{c}±0.00	164.56^{a}±0.00	4.12^{c}±0.00	40.54^{c}±0.00
D	7.43^{c}±0.00	163.74^{a}±0.00	3.82^{b}±0.00	40.41^{c}±0.00
E	7.09^{c}±0.00	163.54^{a}±0.00	3.86^{b}±0.00	40.31^{c}±0.00
F	5.25^{b}±0.00	162.38^{a}±0.00	4.71^{cd}±0.00	38.88^{b}±0.00

Values are means± standard deviation for triplicate determinations. Means on the same columns with different superscript are significantly different at ($p<0.05$)

RE:PJ A (100:0); B(90:10); C(80:20), D(70:30); E(60:40), F(50:50)

3.3 Microbial counts of musts and wines from blends of roselle calyces extract and pineapple juice

Total bacterial count and total fungal counts are presented on Table 3 and 4 respectively. The freshly prepared unfermented must had high microbial load ranging from 7.20×10^{4} to 3.43×10^{5} CFU/mL and 6.30×10^{4} to 4.37×10^{5}CFU/mL for total bacterial count and total fungi count respectively. After fermentation, sieving, addition of metabisulphite, bottling and pasteurization, there was no detectable microbial growth on cultured wine samples. This suggested that the wines did not contain living or wild yeast cells or any contaminating microorganisms, this could be as a result of pasteurization that was carried out and addition of metabisulphites, thereby making the wines safe for consumption. Addition of sulphite inhibits bacteria and fungi growth [25]. Bacteria are part of the natural microbial ecosystem of wine and play an important role in winemaking. They contribute to the wines flavor and aroma. On the other hand, they can cause wine spoilage thereby reducing its quality [25].

Table 3: Total Bacterial Counts of Musts and Wines from blends of Roselle calyces extract and Pineapple Juice

SAMPLE	Must (TBC) x10^{5} CFU/mL	Wine (TBC) x10^{2} CFU/mL
A	1.10±0.10^{b}	ND

B	0.72±0.03[a]	ND
C	3.43±0.15[d]	ND
D	3.17±0.06[c]	ND
E	3.07±0.12[c]	ND
F	0.86±0.06[a]	ND

Values are means of triplicate determinations ± standard deviation
Means on the same columns with different superscript are significantly different at ($p<0.05$)
RE:PJ A (100:0); B(90:10); C(80:20), D(70:30); E(60:40), F(50:50)
ND: Not detectable

Table 4: Total Fungal Counts of Musts and Wines from blends of Roselle calyces extract and Pineapple Juice

SAMPLE	Must (TFC) $x10^5$CFU/mL	Wine (TFC) $x10^2$CFU/Ml
A	4.37±0.12[e]	ND
B	3.90±0.10[d]	ND
C	0.63±0.12[a]	ND
D	3.43±0.15[c]	ND
E	3.13±0.06[b]	ND
F	3.03±0.06[b]	ND

Values are means of triplicate determinations ± standard deviation
Means on the same columns with different superscript are significantly different at ($p<0.05$)
RE:PJ A (100:0); B(90:10); C(80:20), D(70:30); E(60:40), F(50:50)
ND: Not detectable

4. CONCLUSION

This study established that wine of good quality could be produced from roselle calyces extract and pineapple juice. The antioxidant properties showed improved characteristics in the wine compared to the must. Also, the selected minerals analyzed had contents which are within the acceptable limits with sodium having the highest values of 162.38 - 166.66 mg/L and zinc having the least value of 2.71 – 4.71 mg/ L. Microbial counts also showed that the wines are safe for consumption. From the results, therefore, it is evident that nutritious wine of high quality can be produced from roselle calyces extract and pineapple juice

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