



Physico-chemical and Sensory Properties of Wine Produced from Blends of Roselle Calyces Extract and Pineapple Juice

B. O. Idoko^{1*}, M. T. Ukeyima¹, G. M. Gberikon² and O. O. Olaleye³

¹Department of Food Science and Technology, Federal University of Agriculture, P.M.B. 2373,
Makurdi, Benue State, Nigeria.

²Department of Microbiology, Federal University of Agriculture, P.M.B. 2373,
Makurdi, Benue State, Nigeria.

³Nigerian Stored Products Research Institute, Km 3, Asa Dam Road, P.M.B. 1489, Ilorin,
Kwara State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author BOI managed the analyses of the study, performed statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Authors MTU and GMG designed the study, wrote the protocol and supervised the research. Author OOO managed the analyses in the laboratory. All authors read and approved the final manuscript.

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ABSTRACT

Wine was produced from must formulated by blending roselle calyces extract with pineapple juice at different ratios (v/v): A (100:0), B(90:10), C(80:20), D(70:30), E(60:40) and F(50:50). The must was pitched with *Saccharomyces cerevisiae*; primary and secondary fermentation lasted for five and three days, respectively, during which aliquot samples analyses of pH, titratable acidity, specific gravity, total soluble solids and alcohol were carried out daily using standard procedures. Wine was aged for 31 days. The pH of the must decreased during the period of fermentation with a range of 3.30 to 4.37. Specific gravity and total soluble solids were observed to reduce drastically as fermentation progressed. During the fermentation period, consistent increase

*Corresponding author: E-mail: idokobijo@gmail.com;

in titratable acidity and alcohol was observed with time. At the end of fermentation (8th day), alcohol content ranged from 10.19 to 12.23% with sample B and C having the highest values. Vitamin C analyses carried out on the wines had values ranging from 2.50 to 8.40g/100mL. Wines had volatile acid values range of 0.06 to 0.13g/100mL which was within acceptable limits. Sensory evaluation on a 9-point hedonic scale carried out on the aged wine indicated that all samples were accepted except sample F which had scores below average. Sample B was rated highest in terms of overall acceptability with value of 7.41±1.06. The result of this study showed that acceptable wines can be produced from blends of roselle hot water extract and pineapple juice.

Keywords: Wine; roselle calyces extracts; pineapple juice; physico-chemical; sensory properties.

1. INTRODUCTION

Wine is an alcoholic beverage produced principally from grapes or extracts of other fruits [1], through fermentation by yeasts producing significant quantity of ethyl alcohol depending upon the type of fruit wine. Different types of table wines such as dry, sweet and sparkling wines having alcoholic content of 8 to 15% by volume are popular throughout the world [2]. There are some soft fruits from both temperate and tropical regions whose pigment stability and flavour profiles match those of wine from grapes, but suffer from lack of intensive research and development to encourage commercial production.

As an alternative to full-strength wine, wines with a reduced alcohol content offer a number of potential social and health benefits for consumers. Wine has prominent health benefits in controlling cardiovascular diseases. Epidemiological studies from diverse populations have revealed that individuals who habitually consume moderate amounts of wine experience a 20 to 30% reduction in all causes of mortality particularly cardiovascular mortality, when compared with individuals who abstain or drink alcohol to excess [3].

Roselle (*Hibiscus sabdariffa*) is a member of the *Malvaceae* family and grows in the tropical and sub-tropical regions of the world [4]. It is one of the locally available vegetable that is still underutilized in Nigeria. It is an ideal crop for developing countries as it is relatively easy to grow and can be grown as part of multi cropping systems. 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 the red variety is used to produce 'karkade drink' in Sudan and 'Zobo' in Nigeria [5].

Non-alcoholic beverages have been made from a hot water extract of roselle calyx and usually

sweetened with sugar and may be flavored with ginger, pineapple, banana, vanilla and strawberry [6]. Roselle calyces extract has a characteristic red to red-brown colour. The red variety has been reported to be rich in anthocyanins and it contains a mixture of organic acids such as citric, malic and tartaric acids [7]. Since the hot water extracts of these calyces have a history of human consumption, producing wine from this extract or in combination with other tropical fruit juices may be feasible.

Pineapple (*Ananas comosus*) is a tropical and subtropical fruit grown in many countries in Africa. It belongs to the family *Bromeliaceae*. The pineapple is herbaceous perennial plant which grows to 1.0 to 1.5 m (3.3 to 4.9ft) tall, although sometimes it can be taller. In appearance, the plant has a short, stocky stem with tough, waxy leaves. It is consumed in many parts of the world as fresh and processed product. In recent years it has become one of the most demanded exotic fruits. The fresh pineapple juice is a popular product due to its pleasant aroma, flavor and numerous functional properties [8].

The pineapple juice satisfies the "5 a day" dietary requirement of fruits and vegetables set by many health agencies [9]. It has a high nutritive value and a rich source of vitamins A, B and C along with several minerals such as calcium, manganese, phosphorus and iron [10] as well as amino acids, various sugars and polyphenols [11]. It is considered as a functional drink due to its health-promoting properties and its anti-inflammatory, anti-atherosclerotic, anti-aging, and many other health promoting properties. The pH of pineapple juice is acidic which falls at 3.5 on the pH scale [12]. Pineapple juice has sugar of up to 22 to 25°Brix and can produce wine of about 12 to 13% alcohol, which can be preserved by pasteurization [11].

The aim of this work was to determine the physico-chemical and sensory properties of wine

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 pineapple fruits, baker's yeast, sugar, diammonium phosphate and potassium metabisulphite.

2.2 Raw Material Collection and Study Site

Dried roselle calyces (dark red cultivar), mature ripe pineapples, granulated sugar, baker's yeast (STK royal instant dry yeast) were purchased from modern market in Makurdi, Nigeria and stored under ambient conditions before processing. Diammonium phosphate, potassium metabisulphite were obtained from Chemistry Department, Benue State University, Nigeria. Raw materials obtained were taken to the Department of Chemistry, Benue State University Makurdi, Nigeria, where research was carried out under ambient conditions.

2.3 Sample Preparation

Dried roselle calyces were dry cleaned by hand picking. Impurities such as straws, dried grasses, etc., were removed. 10 g 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 mucilin clothes (0.8 mm). 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 0.8 mm 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 juice was blended according to the desired ratios (v/v) of 100:0; 90:10; 80:20; 70:30,

60:40, 50:50 designated as samples A-F, respectively.

Mixing of blends/must with water was done at 2:1 ratio. 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 (DAP) 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 Physico-chemical Analysis

2.4.1 Determination of specific gravity and alcoholic content

Specific gravity of the must during fermentation and after ageing of wine was determined by measuring 500 mL of the sample into a measuring cylinder at 20°C. A hydrometer was dipped into it to determine the specific gravity (with appropriate temperature correction factor). The percentage alcohol content was then calculated based on specific gravity chart according to AOAC [13].

2.4.2 Determination of total soluble solids

The total soluble solid (°Brix) during and after fermentation was measured in triplicate using handheld refractometer (Model: Erma inc. Tokyo Japan) at 20°C, according to AOAC [13].

2.4.3 Determination of pH

The pH was determined directly during and after fermentation using a pH meter standardized with buffer solution of 4.0 and 7.0, according to AOAC [13]. The glass electrode of pH meter was dipped in 30 mL of the sample at ambient temperature and allowed to stabilize for sometime before taking readings.

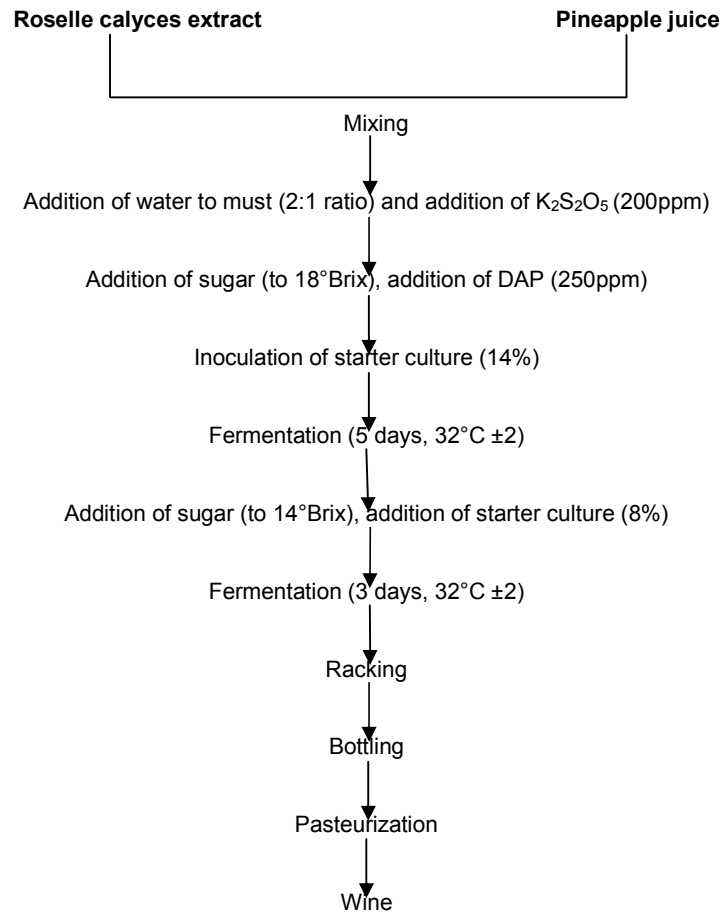


Fig. 1. Flow chat for production of roselle-pineapple wine

2.4.4 Determination of titratable acidity

The must and wine were separately mixed and 100ml aliquot of the individual sample solution was taken and titrated against 0.1 M NaOH using phenolphthalein solutions as indicator. Titratable acidity was calculated as percent tartaric acid, according to AOAC [13].

2.4.5 Determination of volatile acidity

The wine was thoroughly mixed and 100 mL aliquot of the sample solution was taken and titrated with 0.05 M NaOH using phenolphthalein solutions as indicator. Volatile acidity was calculated as percent acetic acid, according to AOAC [13].

2.5 Determination of Vitamin C Content

Ascorbic acid determination was carried out by iodine titration method as described by Ikewuchi et al. [14]. The iodine reagent was standardized

by titrating it against 5 mL of 1% ascorbic acid solution (to which 1 mL of starch indicator was added) until the appearance of the blue starch iodine color. Five milliliters of each sample was treated the same way. Iodine solution (0.005 mol/L) was prepared by dissolving 2 g of potassium iodide into a 100 mL beaker and adding 1.3 g of iodine and dissolving with distilled water. The solution was transferred into a 1L volumetric flask using distilled water making sure that the iodine was completely dissolved. The solution was made up to the 1L mark with distilled water.

The concentration of ascorbic acid in the samples was determined as follows:

$$\text{Concentration in the samples (g/100 mL)} = y/b$$

Where,

b = titre (mL) from the titration of the standard ascorbic acid solution;

y = titre (mL) from titration of the sample solution.

2.6 Sensory Analysis

Twenty semi-trained panelists made up of males and females who are familiar with wines tasting were selected from the Department of Chemistry, Benue State University. Panelists were provided with product information and requested to evaluate the various samples for colour, taste, aroma, mouthfeel and overall acceptability using a 9-point hedonic scale, where 1 correspond to “dislike extremely” and 9 correspond to “like extremely” as described by Iwe [15].

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 as described by Iwe [15].

3. RESULTS AND DISCUSSION

3.1 Changes in Physico-chemical Properties of Must during Fermentation

Fig. 2 shows the pH values during eight days fermentation of must. The pH values were observed to decrease gradually from day 0 to day 8 in all the samples. The wines showed low pH values (acidic range) throughout the period of fermentation. The decrease in pH towards acidity in all wine samples could be attributed to production and accumulation of organic acids during fermentation. This observation is similar to the report in other studies, where a pH range of 3.0 - 4.8 for passion, pineapple and watermelon wines [16,17] reported a steady decrease in pH during fermentation of mango juice by *Saccharomyces cerevisiae*.

A steady increase in acidity was observed in all samples (A – F) as there was a decrease in pH value during fermentation as presented in Fig. 3. The low pH of the must led to higher acid level [18]. At day 0, sample A had the lowest acidity of 0.23 and it decreased with increased addition of pineapple juice to the blend. There was no significant ($p > 0.05$) difference between sample E and F at the end of maturation. Organic acids such as lactic, acetic, formic together with phenolic compounds, carbon dioxide and esters are reported to contribute in lowering the pH and concomitantly increase the total acid content of the “must” during fermentation [19]. The total acidity of wine should fall within 0.5 and 1.0%

[16] and the result of the present study fell within this limit.

From Fig. 4, it was observed that there was a gradual decrease in the total soluble solids (°Brix) in all samples as fermentation progressed. The initial sugar content of the “must” ranged from 17.00 to 18.50. It was observed that sample F had the highest sugar content of 18.50 at day 0. This could be due to the fact that pineapple juice has higher sugar content when compared to roselle extract and so an increase in quantity of pineapple juice increased the sugar content of the blend. The decrease in sugar content during fermentation could be as a result of the activities of the fermenting yeast which utilized the sugar and produced alcohol. At day 5 the sugar content dropped and ranged from 3.00 to 6.00 for sample A to F. Addition of sugar to the must increased the sugar content and this provided the substrate (sugar) for the initiation of the secondary fermentation. At the end of secondary fermentation, samples were not significantly ($p > 0.05$) different from each other. The overall decrease in the sugar content during fermentation can be attributed to the breakdown of substrates by the fermenting organism [20].

Fig. 5 Presents the changes in alcoholic content during fermentation. There was an increase in alcohol content as fermentation progressed. Sample B and C had the highest alcoholic content of 12.23 and 12.24 respectively at the end of fermentation. An alcoholic content of 11.89 and 12.61% for wines produced from *Melodorum fruticosum* have been reported [21].

The specific gravity of the wines decreased ($p < 0.05$) significantly from day 0 to day 8 as presented in Fig. 6. Specific gravity is inversely related to alcoholic content. As the specific gravity of the wine decreased, its alcohol content increased. During fermentation, production of alcohol, acids and other ethyl compounds causes the must to become less dense thereby leading to the reduction or decrease in specific gravity. The decrease in specific gravity of the wines in this study compared favourably with results of [5] and [22].

3.2 Physico-chemical Properties of Wines

Table 1 shows the result of physico-chemical properties of the wine after fermentation. The pH values ranged from 3.32 to 3.56 from sample A-F, respectively. The pH increased with increase

in concentration of pineapple juice. However, all wines showed low pH and this could be attributed to the production and accumulation of organic acids during fermentation.

Titrateable acidity values ranged from 0.65 to 0.88 with sample F having the least value and sample A having the highest value. There exist a relationship between acidity and pH i.e a decrease in pH will mean an increase in acidity [18]. Organic acids such as lactic, acetic, formic together with phenolic compounds, carbon dioxide and esters are reported to contribute in lowering the pH and concomitantly increase the total acid content of the “must” during fermentation. A low pH and high acidity have been reported to give fermentation yeast comparative advantage in natural environments. Total soluble solids of samples ranged from 8.90 to 9.50° Brix. Samples were significantly different from each other. The variation in concentrations of total soluble solids could be as a result of the various concentrations of pineapple juice added. Sample B and C had the highest alcoholic content of 12.23 and 12.24% v/v, respectively. Alcoholic fermentation leads to the production of ethanol, esters, carbonyl compounds and hence

the presence of alcohol in the samples. Alcohol levels of 10 to 10.5% in wines from roselle and pawpaw have been recorded [5]. Specific gravity of wines ranged from 0.95 to 0.97. Results showed low values of specific gravity and samples varied significantly from each other.

The volatile acid content of the wines varied between 0.06 to 0.13 g/L with sample A having the highest volatile acid content and sample F having the lowest value. The volatile acid has a direct relationship with the total titrateable acidity. Volatile acid is a by-product of microbial metabolism and act as an indicator of spoilage. The volatile acid of the wine were lower as a result of the immobilised yeast used for fermentation (control fermentation) after the addition of SO₂. This reduced contamination by adhering microorganisms, most especially the acetic bacteria and wild yeast which could have caused spontaneous fermentation of the wine. This is an indication that the free yeast produces wine with higher volatile acid content than the immobilized yeast [23]. The average volatile acid value for red table wines was reported to be approximately 0.05 to 0.60 g/L [24] and the result of this study fell within this limit.

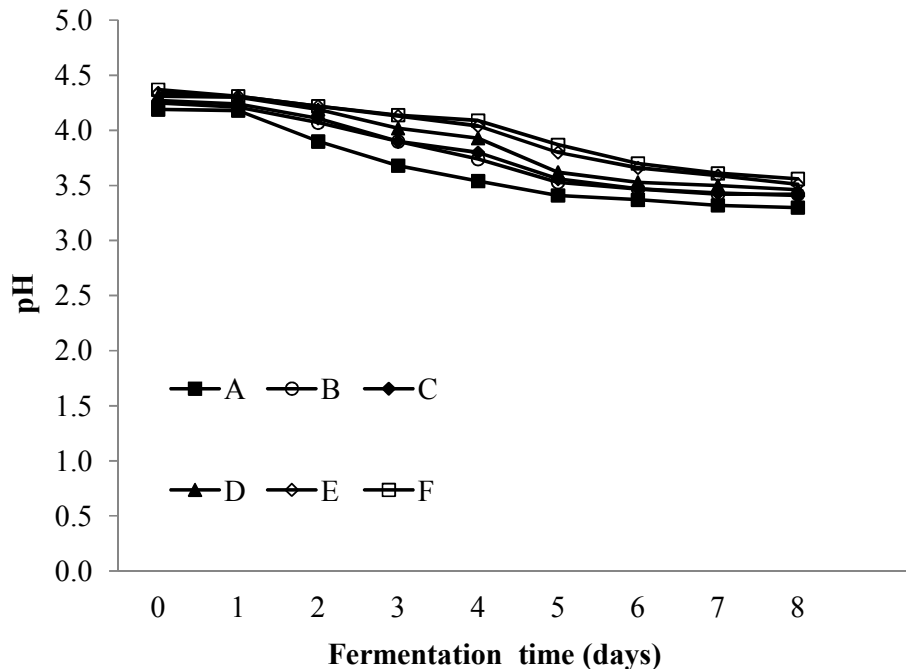


Fig. 2. Changes in pH during fermentation of the must
 Blending roselle calyces extract with pineapple juice at different ratios (v/v): A (100:0), B (90:10), C (80:20), D (70:30), E (60:40) and F (50:50)

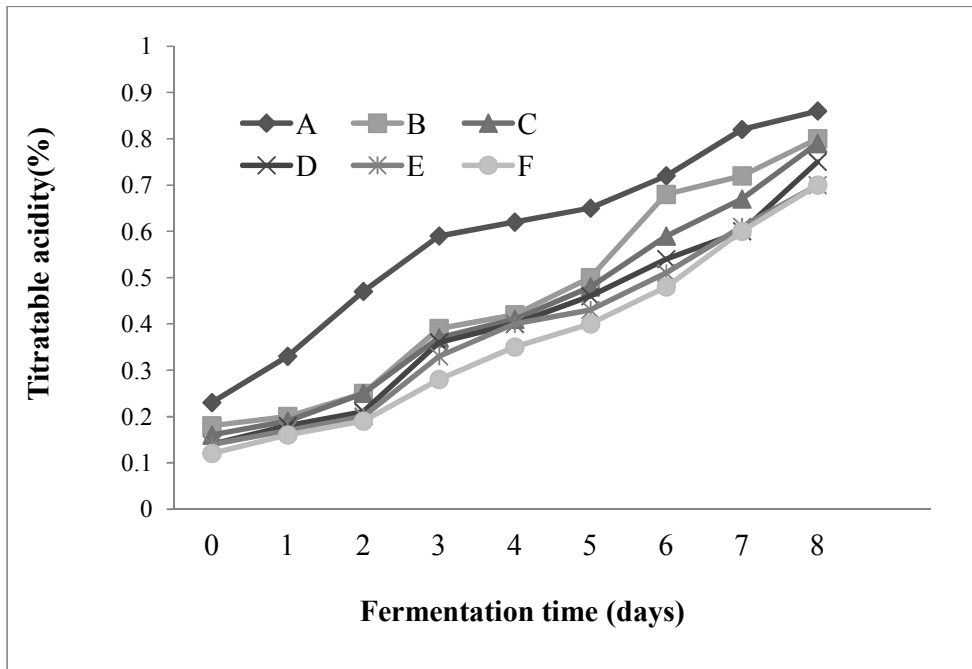


Fig. 3. Changes in titratable acidity during fermentation of the must
 Blending roselle calyces extract with pineapple juice at different ratios (v/v): A (100:0), B (90:10), C (80:20), D (70:30), E (60:40) and F (50:50)

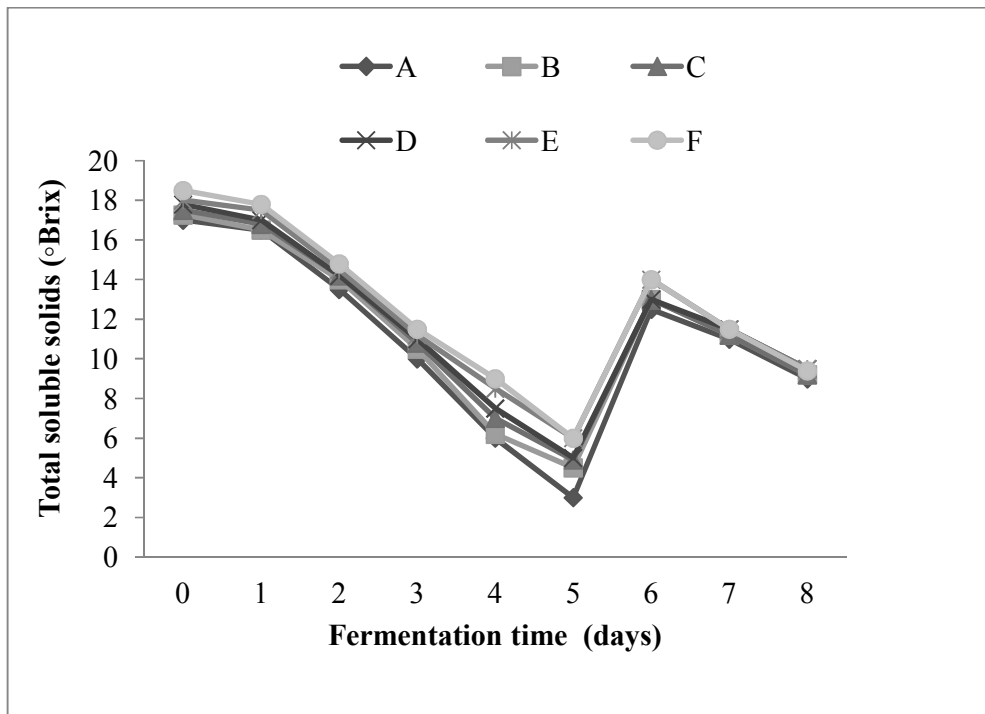


Fig. 4. Changes in total soluble solids during fermentation of the must
 Blending roselle calyces extract with pineapple juice at different ratios (v/v): A (100:0), B (90:10), C (80:20), D (70:30), E (60:40) and F (50:50)

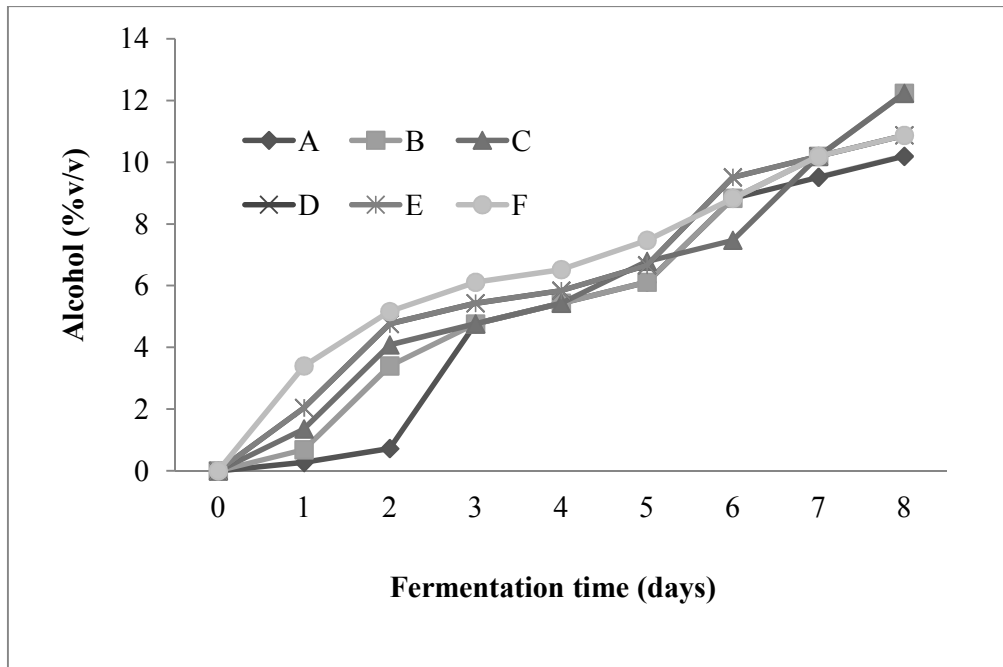


Fig. 5. Changes in alcohol during fermentation of the must
 Blending roselle calyces extract with pineapple juice at different ratios (v/v): A (100:0), B (90:10), C (80:20), D (70:30), E (60:40) and F (50:50)

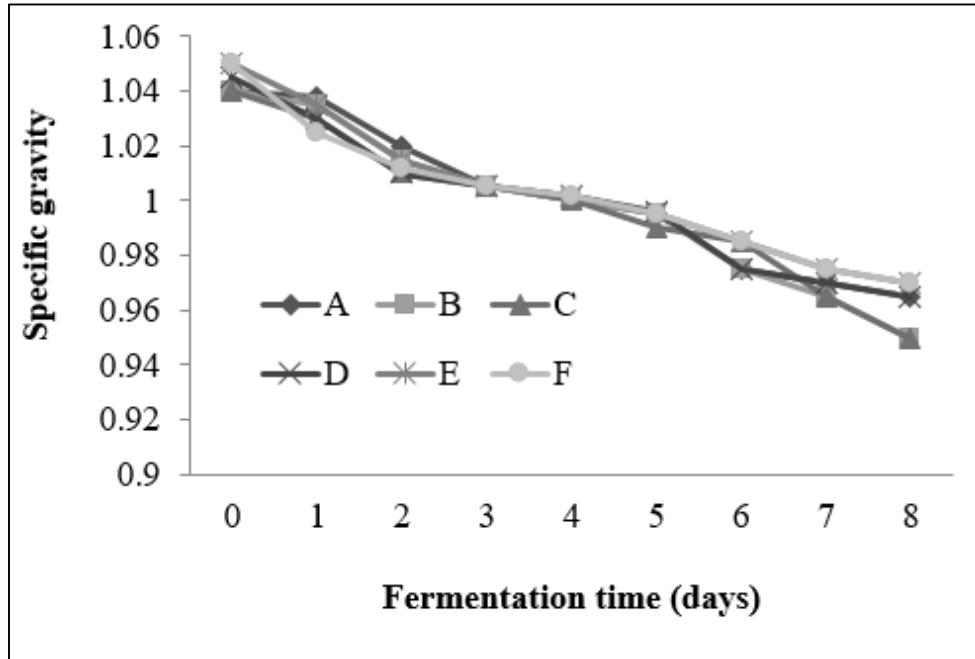


Fig. 6. Changes in specific gravity during fermentation of the must
 Blending roselle calyces extract with pineapple juice at different ratios (v/v): A (100:0), B (90:10), C (80:20), D (70:30), E (60:40) and F (50:50)

Table 1. Physico-chemical properties of wine

Samples	A	B	C	D	E	F
pH	3.32 ^a ±0.02	3.40 ^b ±0.00	3.40 ^b ±0.01	3.46 ^c ±0.01	3.52 ^d ±0.02	3.56 ^e ±0.01
TTA (%)	0.88 ^d ±0.02	0.80 ^c ±0.02	0.81 ^c ±0.02	0.74 ^b ±0.02	0.74 ^b ±0.02	0.65 ^a ±0.02
TSS (°Brix)	8.90 ^a ±0.20	9.20 ^{ab} ±0.20	9.18 ^{ab} ±0.02	9.50 ^b ±0.25	9.50 ^b ±0.25	9.30 ^{ab} ±0.30
Alcohol (%v/v)	10.20 ^a ±0.00	12.23 ^c ±0.00	12.24 ^c ±0.00	10.87 ^{ab} ±0.00	10.87 ^{ab} ±0.00	10.87 ^{ab} ±0.00
Specific gravity	0.97 ^b ±0.00	0.95 ^a ±0.00	0.95 ^a ±0.00	0.97 ^b ±0.00	0.97 ^c ±0.00	0.97 ^c ±0.00
TPC (gGAE/100mL)	2.92 ^a ±0.03	4.85 ^f ±0.02	4.81 ^e ±0.01	4.78 ^d ±0.03	4.76 ^c ±0.01	4.51 ^b ±0.04
Vitamin C (mg/100mL)	2.50 ^a ±0.00	2.70 ^{ab} ±0.00	3.00 ^b ±0.00	4.30 ^c ±0.00	5.70 ^d ±0.00	8.40 ^e ±0.00
Volatile Acidity (g/L)	0.13 ^e ±0.00	0.09 ^d ±0.00	0.08 ^c ±0.00	0.07 ^c ±0.00	0.06 ^b ±0.00	0.06 ^a ±0.00

Means on the same rows with different superscript are significantly different at ($p < 0.05$). A: 100% Roselle extract, B: 90% Roselle extract, 10% pineapple juice, C: 80% Roselle extract, 20% pineapple juice, D: 70% Roselle extract, 30% pineapple juice, E: 60% Roselle extract, 40% pineapple juice, F: 50% Roselle extract, 50% pineapple juice, TPC: Total Phenolic content, TTA: Total Titratable Acidity, TSS: Total Soluble Solid

Table 2. Sensory evaluation of wine

Samples	Colour	Aroma	Taste	Mouthfeel	Overall acceptability
A	7.35 ^{cd} ±1.05	6.70 ^c ±1.40	7.05 ^{cd} ±1.02	6.76 ^{bc} ±1.25	7.00 ^{bc} ±1.00
B	7.47 ^{cd} ±1.23	7.29 ^c ±1.21	7.29 ^d ±0.98	7.29 ^c ±1.10	7.41 ^c ±1.06
C	6.82 ^{bc} ±1.07	6.58 ^{bc} ±0.87	6.41 ^{bcd} ±1.17	6.64 ^{bc} ±0.78	6.70 ^{bc} ±0.84
D	6.64 ^{bc} ±1.32	6.47 ^{bc} ±1.41	6.29 ^{bc} ±1.35	6.23 ^b ±1.52	6.52 ^{bc} ±1.50
E	6.11 ^b ±1.57	5.70 ^{ab} ±1.49	5.64 ^{ab} ±1.57	5.82 ^{ab} ±1.38	6.11 ^b ±1.86
F	4.47 ^a ±1.54	5.17 ^a ±1.42	4.88 ^a ±1.57	5.11 ^a ±1.53	5.00 ^a ±1.11
REF	7.94 ^d ±0.65	7.11 ^c ±1.26	6.05 ^b ±1.51	6.41 ^{bc} ±1.66	7.29 ^c ±0.77

Means on the same columns with different superscript are significantly different at ($p < 0.05$). A: 100% Roselle extract, B: 90% Roselle extract, 10% pineapple juice, C: 80% Roselle extract, 20% pineapple juice, D: 70% Roselle extract, 30% pineapple juice, E: 60% Roselle extract, 40% pineapple juice, F: 50% Roselle extract, 50% pineapple juice, REF: Baron de valls

There was a significant ($P < 0.05$) difference in the total phenol content of wines. The total phenol contents of wines varied from 2.92 ± 0.03 to 4.85 ± 0.02 gGAE/100ml with sample B having the highest amount. Phenolic acids can scavenge free radicals and quench reactive oxygen species and therefore provide effective means of preventing and treating free radical-mediated diseases [25]. The phenolic contents in this study was higher than the phenol contents of other tropical fruits such as cashew wine (0.12 g/100 ml) [26], litchi wine (0.22 g/100 ml) [27]. Vitamin C content of the wines ranged from 2.50 to 8.40 mg/100mL for sample A-F with sample F having the highest value. Vitamin C, also known as ascorbic acid, is the body's primary water-soluble antioxidant, defending all aqueous areas of the body against free radicals that attack and damage normal cells [28]. Samples were significantly different from each other.

3.3 Sensory Evaluation of Wine

The result of the sensory evaluation of the wines is presented on Table 2. The colour of the reference wine was rated highest by the panelists and there was no significant ($p > 0.05$) difference when compared to samples A, B, C and D. The extraction of predominantly red pigments from the calyces of roselle is responsible for the bright red colour of the wine. Sample F had the least value of 4.47. It was observed that as the concentration of pineapple increased in the blend, the red colour became pale.

There was no significant ($p > 0.05$) difference in aroma between the reference wine and samples A, B, C and D. Sample E and F had the lowest scores for aroma.

In terms of mouthfeel, sample B was rated highest with the value of 7.29. Sample A, C and the reference wine can be compared to sample B. For overall acceptability, sample B was rated highest with value of 7.41. There was no significant ($p>0.05$) difference between sample B and the reference wine. Also samples A, C and D compared with the reference sample.

4. CONCLUSION

In this study, wine was produced from blends of roselle calyces extract and pineapple juice at different ratios. Results of physico-chemical analysis carried out on wine indicated that all samples had values which were within acceptable limits. However, sample B (90% roselle extract: 10% pineapple juice) was most preferred by panelist in terms of overall acceptability and therefore highly recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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