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Preliminary Investigation into the Use of Rosselle (*Hibiscus sabdariffa*) and Ugiri (*Irvingia gabonesis*) Fruits in Wine Production

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Abstract

Wine is an alcoholic beverage made from grapes, generally vitisvinefera, fermented without the addition of sugars, acids, enzymes, water, or other nutrients. Yeast consumes the sugar in the grapes and converts it to ethanol and carbon dioxide. Some fresh fruits, such as grape, orange, banana, apple, watermelon, cucumber and pineapple had been used for wine production. In this study, Ugiri (*Irvingia gabonesis*) and Roselle (*Hibiscus sabdariffa*) were used for table wine production. Fresh Ugiri fruit were processed and sieved with Muslin cloth. Dried Roselle were washed, soaked in hot water and sieved with muslin cloth. The Ugiri and Roselle juice were mixed to obtain the must. The must was inoculated with the pure culture of strain of *Saccharomyces cerevisiae* sourced from a stock culture isolated from fresh fermented palm wine. Sodium metabisulphite was also added into the must. The solution was allowed to ferment and during which some parameters such as pH, reducing sugar, specific gravity, titrable acidity and alcohol content determination were carefully carried out. The pH of wine ranged from 5.8 to 3.5, the specific gravity of the wine ranged from 0.63 to 0.40 kg/m. Alcohol content of the fermented wine ranges from 0.33 to 0.74 kg/100ml and the reducing sugar of the fruit wine is from 0.67 to 0.38. The fermentation of Ugiri and Rosell must using *Saccharomyces cerevisiae* led to the successful production of wine.

Keywords

Wine, Fermentation, (*Irvingia gabonesis*), *Sacharromyces cerevisiae* and *Hibiscus sabdariffa*

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1. Introduction

Wine is an alcoholic beverage typically made of fermented fruit juice [1]. Any fruit with good proportion of sugar may be used in producing wine and the resultant wine is normally named after the fruit. The type of wine to be produced dictates the fruit and strain of yeast to be involved [2]. Preservatives used in wine making include sulphurdioxide, potassium sorbate, sorbic acid and metabisulphides [3]. High concentration of these preservatives in wine, aside causing off odors, can induce lots of systemic disorderliness such as breathing problems in Asthmatic patients and gastrointestinal disturbances in allergic persons. The effects of bio accumulation of these chemicals could further compound these situations [1]. Due to their high fermentative capacity, the yeasts are essential in the production of alcoholic beverages. Among them, *Saccharomyces cerevisiae* is universally employed in processes for the production of alcoholic beverages, especially wines. The term wine is only used for the fermentation of grapes, however, many other fruits can be used in the same process [4].

Roselle *(Hibiscus sabdariffa L.)* belongs to the family *Malvaceae*, locally called “karkade”, is and important annual crop grown successfully in tropical and sub-tropical climates.

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The commercially important part of the plant is the fleshy calyx (sepals) surrounding the fruit (capsules). The whole plant can be used as beverage, or the dried calyces can be soaked in water to prepare a colorful cold drink, or may be boiled in water and taken as a hot drink. It also has some medicinal properties. The seeds contain 17.8–21% non-edible oil and 20% protein, and are sometimes used for animal feed. Roselle is a flexible plant with a number of uses. It is intercropped with crop staples such as sorghum and sesame, or planted along field margins. It requires little care. Its leaves, seeds, capsules and stems are used in traditional medicines.

In rural areas women are usually responsible for growing roselle. They add value to the crop by developing products for market. Mclean and Wilson and Menzel reported that *Hibiscus sabdariffa* is a tetraploid (2n = 4x = 72), whose chromosomes are related to the diploid (2n = 2x = 36) *Hibiscus cannabinus*. The two botanical types of roselle are Hibiscus Sabdariffa var. sabdariffa, grown for its fleshy, shiny-red csabdariffsystssabdariffa var. altissima grown for its phloem fiber. Despite its potential economic importance, karkadi has received little attention and there is a lack of information regarding its genetics, breeding and production, particularly under rain-fed conditions.

Ugiri (*Irvingia*. gabonensis) have a shelf life of less than 2 days if picked when ripe and not more than 10 days if harvested at the mature green stage due to high respiration rate, moisture loss and microbial attack [5, 6]. Poor storage conditions and handling, as well as pest attack, diseases and deterioration contribute to high losses of saleable fruit. Initially, [5] tried to lengthen the shelf life of mature green bush mangoes by refrigerating them at 12-15°C; a common method for delaying ripening. This resulted in chilling injuries such as pitting and black spots, which would reduce saleability considerably. They then trialed several different post harvest storage methods including dipping, wrapping and waxing the fruit, to see which was the most effective [6]. They found that the longest shelf life resulted from briefly submerging the fruits in a hot sulphite dip (commonly used in the food industry) and then wrapping them in PVC film. Hot water or other hot chemical dips used in conjunction with waxing or packaging in wrapped boxes were also effective combinations for enhancing shelf life at 22-35°C and 70-95% relative humidity, normal storage conditions in Africa.
Irvingiagabonensis fat, extracted from the kernels, has been stored for more than a decade with no adverse changes in it properties because it contains natural anti-oxidants that hinder oxidative decay [7]. The aim of this work is to investigate the use of roselle (*hibiscus sabdariffa*) and ugiri (*irvingiagabonesis*) in wine production.

2. Materials and Methods

2.1. Collection of Samples

Ten African bush mango (Ugiri) fruits and 100g of Roselle were purchased from Eke- Awka market, Awka, Anambra state. The samples were collected aseptically and packed in different sterile cellophane bags and transported to the laboratory of the Department of Applied Microbiology and Brewing, NnamdiAzikiwe University. Fresh palm wine was obtained from a Palm wine tapper in Orba village, Enugu state.

2.2. Isolation of Yeast

Fresh palm wine was obtained from Orba Village, Enugu, Enugu State and fermented for 24hours. The fresh palm wine was allowed to sediment and the sediment was inoculated onto Sabourauddextrose agar in duplicates. The plates were inoculated at room temperature for 72hours. Developing isolates were purified by repeated subculture technique according to [12].

2.3. Characterization and Identification of Yeast

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the colonies were gram stained for microscopic evaluation with reference to the manual of fungal Atlases.

2.4. Inoculum Development

The Roselle and Ugiri fruits were washed thoroughly with 0.1% sodium metabisulphide in water. The fruits were peeled and were sliced into chubs, blended and filtered to extract the must or juice. While 100g of Roselle were soak in 800ml of 80°c of boiled water and extracted the juice. About 400ml (200ml of Ugiri juice and 200ml of Roselle juice) of the filtered juice were introduced into a clean sterile 500ml conical flask and autoclaved to sterilize the juice. It was allowed to cool after sterilization and the yeast cell was inoculated into the plantain must and incubated in a rotary Shaker for 72hours.

2.5. Must Fermentation

The Ugiri and Roswell fruits were washed thoroughly with 0.1% sodium metabisulphite in water. The fruit was peeled and was sliced into cubes, blended and filtered to extract the must or juice. 1L of the filtered juice was introduced in a clean sterile into a sterile 4L fermenter. This was followed by the addition of 0.4g (per 400ml) sodium metabisulphite, 100g (per 400ml) of granulated sugar (for fortification), 29.4g of 0.84% (per 400ml) Ammonium sulphate, 4.2g of 1.012% (per 400ml) potassium dihydrogen for yeast supplementation. The juice was inoculated with yeast obtained by inoculums

development and the set up allowed to ferment for 18 days, With two days analysis parameters such as: PH, reducing sugar, titrable acidity, specific gravity and alcohol content

2.6. Parameters Evaluated

2.6.1. pH Determination

The pH meter was standardized with buffer solution. The buffer solution was prepared with pH buffer power of pH 4.00 at 25°C dissolved in distilled water. The electrode of the pH meter was immersed in a glass beaker containing the sample and the readings was obtained from the photo - detector of the pH. Meter.

2.6.2. Estimation of Reducing Sugar

This was determined using the method of miller (1959), [8]. 1ml of Dinitrosalicylic acid (DNS) was added to 1ml of must into a test tube and allowed to boil for ten minutes then allowed to cool. 2ml of DNS and 2ml of distilled water was put into another tube which serves as the blank. The optical density of the sample is read against the blank in a spectrophotometer at 540nm absorbance.

2.6.3. Alcohol Content

The refractometer method was used in determining the alcohol content. A clean dry applicator was used to place two drops of the sample on the prism of the refractometer and the value (Original gravity) of the refractive index taken. Also, after fermentation, two drops of the sample was applied on the prism of the refractometer and the value (Total gravity) was taken. The refractive index of the sample was gotten on two days interval.

2.6.4. Titrable Acidity

To 200mls of boiling distilled water in a 500ml Erlenmeyer flask was added, 1ml of a 1% phenolphthalein indicator. The solution was titrated with 0.1M sodium hydroxide solution to a faint but definite pink colour; 5mls of the sample was titrated to a pink color with the 0.1M NaOH, using 3 drops of 1% phenolphthalein as indicator. The titre was taken. This serves as the final titre. The titrable acidity was calculated with reference to tartaric acid [13].

The formulae is tartaric acid (g/100ml) = $\frac{V \times M - 75 \times 100}{100 \times V}$

Where V = volume of NaOH solution (final reading - initial reading)

M = Molarity of NaOH solution V

V = Volume of Must

2.6.5. Specific Gravity

50ml specific gravity bottle was thoroughly cleaned with distilled water, dried in an oven for 50°c and allowed to cool. The weight of the cooled dried bottle (W1) was recorded. The dried bottle was filled with deionized water and surface of the bottle was cleaned with cotton wool and weighed as (W2). The bottle was empty and cleaned twice with 10ml of the must therefore the bottle was filled to the brim with the must and the bottle cleaned with cotton wool and weighed as (W3). The specific gravity (S.G) was calculated using:

$$S.G = \frac{W_2 - W_1}{W_3 - W_1} = \frac{S}{W}$$

3. Results

The results of all the parameters evaluated are presented on the Table 1. The parameters evaluated are as follows; pH, Specific gravity, Temperature, reducing sugar and alcoholic content. The result of the pH of the fermenting wine shows a gradual decrease in pH as the fermentation time increases and it ranges from 5.8 on the 0.0 to 3.5 on 18th day. The table also shows that the result of the titrable acidity carried out increases from 0.33 on the 0.0 day to 0.74 on the 18th the day.

The result of the specific gravity is shown on table 1 as it decreases from 0.63 on the 0.0 day to 0.40 on the 18th day. The result shows that the reducing sugar reduces from 0.67 on the 0.0 day to 0.38 on the 18th day. The result shows that the alcoholic content of the fermented wine in table 1 increases from 0% on the 0.0 day to 6.5% on the 18th day.

Table 1. Results of the Parameters Evaluated.

Day	Specific gravity	Reducing sugar (g/1)	Titratable Acidity (g/100ml)	pH	Alcoholic content (%)
0	0.63	0.67	0.33	5.80	0.00
1	0.61	0.64	0.35	5.60	1.80
2	0.57	0.58	0.40	5.10	2.40
3	0.56	0.56	0.48	4.70	3.20
4	0.51	0.52	0.52	4.40	4.30
5	0.48	0.47	0.56	4.20	5.00
6	0.46	0.42	0.66	4.00	5.70
7	0.43	0.40	0.70	3.80	6.00
8	0.40	0.38	0.74	3.50	6.50

Note: Parameter determination were done after 2 days intervals.

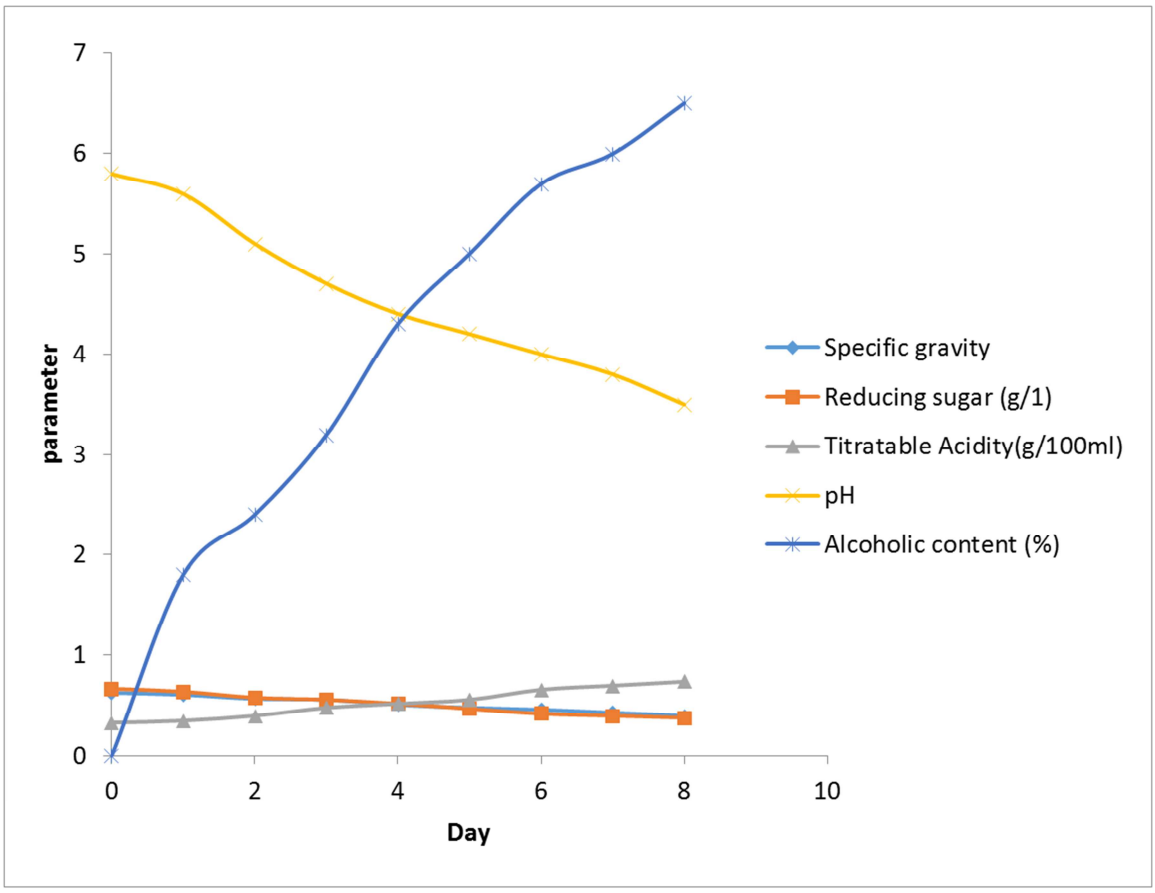

Figure 1. Graphical representation of Physicochemical parameters from the fermentation.

4. Discussion

Fermentation is a form of anaerobic respiration during which there is incomplete breakdown of sugar extracts which leads to the fermentation of alcohol, carbon dioxide, and the liberation of energy [9].

The sugar in the must derived from the Ugiri (*Irvingia gabonesis*) and roselle (*Hibiscus Sabdariffa*) and the granulated sugar used for fortification, were fermented by cultured yeast with the formation of ethanol [9]. The yeast used in the fermentation was stock yeast, saccromycescerevisiae isolated in the laboratory from fermented palm wine. The must was inoculated with high number of yeast cells. This was done to enhance the rate of fermentation and product formation. The estimation of the reducing sugar level showed that the fruits contained an appreciable amount of sugars. This made the fruits suitable for the production of wine. Generally, there was a decline in the specific gravity, pH and reducing sugar. The specific gravity dropped rapidly from 0.63 to 0.46. This was attributed to the uptake of sugars by the yeast for alcohol production [2, 15]. The specific gravity on the last day was 0.46; this was due to the fact that there were unfermentable sugars present that could not be fermented by the yeast. This was similar to the findings of [10, 14]. The pH was found to be decreasing. The pH of the fermenting must was 5.5 before fermentation. After fortification, the pH decreased from 5.5 to 4.0. This was as a result of production of carbondioxde during fermentation which dissolved in the wine as fermentation proceeded [2]. This corroborated the findings of [11] who found themaximum pH of fruit wines to be 3.5. The titrable acidity value increased steadily from 0.33 on the first day to 0.66 on the last day. The alcohol content at the end of the fermentation was 5.7%. There was a general increase in the alcohol content of the wine during the period of study.

5. Conclusion and Recommendation

The production of wine from roselle (*Hibiscus Sabdariffa*) and Ugiri (*Irvingiagabonensis*) using *Saccharomyces cerevisiae* was successfully carried out. The produced wine had good quality and taste. Ugiri and Roswell wine contained unique aroma, which differentiated it from other juice products. Roselle and Ugiricommercial wine production should be encouraged in the country because of it's unique taste.

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