Studies on Fruit Wine Production from Mixed Fruits of Pomegranate (Punica granatum) and Sweet Oranges (Citrus sinensis) Using Palmwine Yeast (Saccharomyces Cerevisiae)

Mixed fruit wine was produced from fruits of Pomegranate (Punica granatum) and Sweet oranges (Citrus sinensis), using yeast (Saccharomyces cerevisiae) isolated from palm wine. The fresh palmwine was allowed to sediment and the sediment was inoculated onto Sabouraud dextrose agar (SDA) in duplicates. The plates were incubated at room temperature for 24 hours for the isolation of yeast strain. The must was mixed in the ratio of 2:1 for pomegranate and sweet oranges, respectively. The primary and secondary fermentation of the wine lasted for 7 and 14 days during which the pH, specific gravity, temperature, reducing sugar and alcohol content of the wine was observed. Specific gravity reduces drastically, the alcohol content increased and the reducing sugar level reduces. It was also observed that the titrable acidity was within the normal limit of 0.5% to 1.0%. The pH range is between 3.36 to 3.71. At the end of fermentation, the alcoholic content in the wine ranged from 0.252% to 7.492%. Specific gravity values gradually decreased from 0.961 to 0.034 throughout the fermentation period. The decrease was irrespective of the yeast strain and fruit used in the wine production. The temperature increased from 31.5°C from the first day of the fermentation to 33.9°C on the 14th day of the fermentation. This study shows that an acceptable and quality wine can be produced from the combination of pomegranates and sweet oranges with yeast isolated from palm wine.

Keywords: Alcohol, Fermentation, Orange wine, Pomegranate wine, Yeast

1. Introduction

Fruit 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.
Studies on Fruit Wine Production from Mixed Fruits of Pomegranate and Sweet Oranges

According to the European Union, Wine is legally defined as the fermented juice of grapes. Wine can be made from virtually many plants matters that can be fermented. Most fruits and berries have the potential to produce wine. Wine making involves the use of yeast to ferment the ‘Must’ of a chosen fruit or fruits for several days, depending on the objective of the wine maker [2].

Pomegranate (*Punica granatum L.*) is one of the ancient yet more sought-after fruit. The pomegranate, contrary to previous records citing that the pomegranate was considered native to the region of Iran and/or northern India [3], probably originated in northern Turkey, based on the fact that in the vicinity of the late-14th-century BCE Uluburun shipwreck near Kas, Turkey, pomegranate remains were found [4]. The pomegranate spread from Anatolia to Persia, Israel, India, China, Greece, Egypt, Tunisia, Spain, Indonesia, Mexico, South America, and, more recently, the United States. The pomegranate plant is a fruit-bearing, small tree that is highly branched but can grow up to 10 m tall and survive in extreme conditions [5]. The leaves have short stems and leathery surfaces; the flowers are flashy, from white to red in color [5].

Sweet oranges (*Citrus sinensis*) is the largest genus in the family Rutaceae and is the most traded horticultural product in the world [6]. Taxonomic identification is difficult because there are many spontaneous and commercial hybrids, but citrus can be generally classified into the following categories: Sweet oranges (most are *C. sinensis* but also includes blood and acidless oranges), mandarins (such as Satsuma (*C. unshiu*)), tangerines (*C. tangerina*, and *reticulata*), and clementines (*C. clementine*), sour/bitter oranges (such as Seville, *C. aurantium*), lemons (*C. limon*), limes (*C. aurantifolia* and *latifolia*), grapefruit (*C. paradisi*) and pummelos (*C. grandis*), hybrids (e.g., *tangelos, tangors, and limequats*), and citrons (*C. medica*), which has a rind that is used primarily for confectionary and is only commercially grown in limited areas [6].

Palm wine is the fermented sap of the tropical plant of the *palmae* family. It is produced and consumed in very large quantities in the south-eastern Nigeria [1]. It contains nutritionally important component including amino acids, proteins, Vitamins, and sugar. These make the wine a veritable medium for the growth of a consortium of microorganisms, where growth in turn, change the physiochemical conditions of the wine giving rise to competition and succession of organisms [7]. The yeast which is the main organism responsible for alcoholic fermentation usually belong to the genus *Saccharomyces*. Used palm wine isolates of *Saccharomyces cerevisiae* to produce artificial palm wine and beer, respectively, palm wine is tapped from the sap of *Elias species* and the sap of *Raphia species* which contains a heavy suspension of live yeast and bacteria. Most studies on palm wine have reported its potentials are source of yeast isolate for the fermentation industries [2]. The aim of this research work is to produce fruit wine from mixed fruits of Pomegranate and Sweet oranges using yeast isolated from palm wine.

2. Materials and Methods

2.1 Sample Collection

The Pomegranate fruits were sourced from a fruit store at Lagos State of Nigeria and was transported to Nnami Azikiwe University, Awka Anambra State while the sweet orange fruits were sourced from a local market in Nkwelle Ezunaka, Oyi LGA of Anambra State. The fruits
were identified at the Botany Department of Nnamdi Azikiwe University, Awka. Fresh palm wine samples were collected from tapped sources into sterile keg from Umuawulu in Awka south Local Government Area, Anambra state, Nigeria for the isolation of yeast and all samples were transported to Nnamdi Azikiwe University Microbiology Laboratory.

### 2.2 Isolation and Identification of Yeast from Palm Wine

The fresh palm wine (100ml) which was kept in a sterile conical flask was allowed to sediment and ferment, the sediment was inoculated onto Sabouraud Dextrose Agar (SDA) in duplicates. The plates were incubated at room temperature for 72 hours. Developing isolates were purified by repeated subculture techniques and slides of pure culture were prepared for microscopic observation and identification and the pure cultures were identified by their morphological characteristics [8].

### 2.3 Preparation of “mixed must” and Inoculum development

The Pomegranate and Sweet oranges were washed thoroughly with 0.1% sodium metabisulphite in water. The fruits were cut, manually deseeded, blended, and filtered to obtain the juice (must). Exactly 30ml of the juice (15 ml of Pomegranate juice, 15ml of Sweet Orange juice) were introduced into a clean sterile 250 ml conical flask and sterilized by autoclaving. Upon cooling, 4 loopful of the yeast culture isolated from the palm wine was used to inoculate the juice (must). It is incubated in a rotary shaker (attemperated Gallenkamp) for 48 hours. All these procedures took place under aseptic condition [9].

### 2.4 Fermentation of the Fruits Must

The Pomegranate and Sweet oranges was washed thoroughly with 0.1% sodium metabisulphite in water. The fruits were cut, manually deseeded, blended, and filtered to obtain the must. Aliquots of the extracted juice obtained are used for pH, temperature, temperature and reducing sugar analyses. The must was transferred into a sterile 3ltr glass fermenter, until it was ¾ filled. This was followed by the addition of g of sodium metabisulphite, 234.1g granulated sugar (for fortification),176.4g of 0.84% Ammonium sulphate, 25.2g of 0.12% potassium dihydrogen for yeast supplementation. The juice inoculated with yeast obtained by inoculum development and the set-up allowed to ferment for 14 days, with daily analysis of parameters such as pH, temperature, reducing sugar and titrable acidity [10].

### 2.5 Physiochemical analyses

#### 2.5.1 pH determination

Ten (10ml) of the “must” was introduced into a sterile beaker and the pH of the must determined using a digital pH meter (Model No: pH S-25). [11]
2.5.2 Determination of reducing sugar

The quantitative estimation of reducing sugar of the wine was determined using the method described by [12]. One of 3,5-Dintrosalicylic acid (DNS) was added to 1ml of supernatant of sample in a test tube and the mixture heated in boiling water for 10 minutes. The test tube was cooled rapidly in tap water and the volume adjusted to 12ml with distilled water. A blank containing 1ml of distilled water and 1ml of DNS was prepared. The optical density of the sample was read against the blank in the spectrophotometer or 540nm absorbance. The concentration of reducing sugar in the supernatant was estimated from the glucose standard curve. To supplement the sugar content of the “must”, granulated sugar which is from sugar cane was part of additives.

2.5.3 Determination of specific gravity

50 ml specific gravity bottle was thoroughly cleaned with distilled water dried in an oven for 50°C and allowed to cool [12]. The weight of the cooled dry bottle (W₁) was recorded. The dried bottle was filled with deionized water and surface of the bottle was cleaned with a cotton wool and weighed (W₂).

The bottle was empty and cleaned twice with 10ml of the “must”, thereafter the bottle was filled to the brim with the “must” and the bottle was filled with cotton wool and weighed (W₃). The specific gravity (S.G) was calculated

\[ S.G = \frac{W_3 - W_1}{W_2 - W_1} \times S \]

Where S= weight of volume of must (W₃-W₂)

\[ W = \text{weight of volume of water (W₂-W₁)} \]

2.5.4 Estimation of titratable acidity

This was determined by the methods described by [13]. Exactly 200ml of distilled water was introduced into a sterile 500ml conical flask and boiled. 1ml of 1% aqueous alcoholic phenolphthalein indicator solution was added. This was titrated with 0.1M NaOH solution to give a faint pink colour. 5ml of the “must” was pipetted and introduced into the boiling neutralized solution and titrated again to the end point using the same 0.1M NaOH solution.

The titratable acidity was expressed as tartaric acid and was calculated as thus;

\[ \text{Tartaric acid g/100ml} = \frac{V_1 x M x 75 x 100}{100 x V_2} \]

Where \( V_1 = \text{Volume of NaOH (Final reading – Initial reading)} \)

\( M = \text{Molarity of NaOH} \)

\( V_2 = \text{Volume of “must”} \)

2.5.5 Determination of Temperature

The temperature reading was taken about 50ml of the sample using a clean thermometer and measuring cylinder [12].
2.6 Determination of Alcoholic content

The Alcohol content of the fermented mixed juice was estimated using Dichromate Titration method [14]

3. Results and Discussion

Table 1 shows the cultural and biochemical characteristics of the isolate used for the fermentation. The results of the analysis carried out during the fermentation of the Must are presented on Table 2. Irrespective of the yeast strain, the pH of the Must was within the acidic range (3.36 – 3.71). There was a steady increase in the alcohol content as shown in Table 2. At the end of fermentation, the alcoholic content in the wine ranged from 0.252% to 7.492%

In the case of the specific gravity, the values gradually decreased from 0.961 to 0.034 throughout the fermentation period. The decrease was irrespective of the yeast strain and fruit used in the wine production.

The temperature increased from 31.5°C from the first day of the fermentation to 33.9°C on the 14th day of the fermentation. Figure 1 shows the changes in the pH of the Must during fermentation. Figure 2 shows the changes in Temperature while figure 3 shows the change in Alcoholic content.

**Table 1: Cultural and Biochemical characteristics of yeast isolated from palmwine**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Isolate</th>
<th>Gram stain</th>
<th>Colour</th>
<th>Shape</th>
<th>Margin</th>
<th>Sugar fermentation test</th>
<th>Probable Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>I</td>
<td>+</td>
<td>Creamy</td>
<td>Circular/Rod</td>
<td>Smooth</td>
<td>AG+ AG+ AG+ AG-</td>
<td><em>Saccharomyces cerevisiae</em></td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>Creamy</td>
<td>Circular budded</td>
<td>Smooth</td>
<td>AG+ AG+ AG+ AG+</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>Creamy</td>
<td>Circular budded</td>
<td>Smooth</td>
<td>AG+ AG+ AG+ AG-</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>+</td>
<td>Creamy</td>
<td>Circular budded</td>
<td>Smooth</td>
<td>AG- AG+ AG- AG-</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>Creamy</td>
<td>Circular non budded</td>
<td>Smooth</td>
<td>AG+ AG- AG+ AG-</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Physiochemical Parameters from the Fermentation

<table>
<thead>
<tr>
<th>Fermentation Days</th>
<th>Reducing sugar(mg/ml)</th>
<th>Specific gravity (Kg/m³)</th>
<th>Titratable acidity (w/v)</th>
<th>pH</th>
<th>Alcoholic Content (%v/v)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>0.961</td>
<td>1.03</td>
<td>3.36</td>
<td>0.252</td>
<td>31.5</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.811</td>
<td>1.13</td>
<td>3.41</td>
<td>0.301</td>
<td>31.7</td>
</tr>
<tr>
<td>3</td>
<td>0.16</td>
<td>0.630</td>
<td>1.19</td>
<td>3.45</td>
<td>0.391</td>
<td>32.3</td>
</tr>
<tr>
<td>4</td>
<td>0.11</td>
<td>0.501</td>
<td>1.27</td>
<td>3.49</td>
<td>0.496</td>
<td>32.5</td>
</tr>
<tr>
<td>5</td>
<td>0.08</td>
<td>0.395</td>
<td>1.35</td>
<td>3.53</td>
<td>0.632</td>
<td>32.9</td>
</tr>
<tr>
<td>6</td>
<td>0.05</td>
<td>0.225</td>
<td>1.45</td>
<td>3.59</td>
<td>1.12</td>
<td>33.2</td>
</tr>
<tr>
<td>7</td>
<td>0.03</td>
<td>0.103</td>
<td>1.48</td>
<td>3.66</td>
<td>2.46</td>
<td>33.6</td>
</tr>
<tr>
<td>14</td>
<td>0.01</td>
<td>0.034</td>
<td>1.53</td>
<td>3.71</td>
<td>7.49</td>
<td>33.9</td>
</tr>
</tbody>
</table>

Figure 1: Changes in pH over the fermentation period
Wine made from mixed fruits are rich sources of sugar, minerals, vitamins, organic and volatile compounds which act as good source of food for spoilage organisms as well as phenolic compounds which are present in fruits may be responsible for the enzymatic browning reactions which changes the colour and aroma of the wine [1]. The major problem associated with the use of tropical fruits in wine production is their low sugar content according to [15]. The fruits also contained reasonable amount of carbohydrate which gives an account of their high caloric value. The alcoholic content of the wine in this study ranges from 0.252% to 7.492%. The alcoholic content compares with the 10.46% reported by [1] during fermentation of mixed fruits of Pineapple (AnanasComosus) and Soursop (Annonamuricata L.). Alcoholic fermentation is known to lead to the production of esters, ethanol, carbonyl compounds, acids and acetyllys which affects the quality of the final product and associated with pleasant aromas. The concentration of these by-products can widely vary. [1] and [8] reported that the concentration of ethanol contributes to the whole characteristic quality and flavour of produced wine.

In the study of the specific gravity, it was observed that the specific gravity of the wine reduced drastically to 0.034. This was due to the type of yeast used in the production of the wine.
Saccharomyces cerevisiae isolated from palm wine has been reported to reduce specific gravity of fruit wines during fermentation according to [1][16][17]. In the study of the pH, the pH range of the wine is within 3.36 to 3.71. The decrease in pH towards acidity could be attributed to the production and accumulation of organic acids during fermentation. Generally, pH and acidity influence the taste of wines by imparting sour tastes to the end products according to [1].

4. Conclusion

This study showed that an acceptable wine can be produced from the fermentation of fruit juices from pomegranates and sweet oranges with yeast strains isolated from palm wine. The study also highlighted the efficiency of local yeast strains from palm wine in the alcohol fermentation of fruits. The wine produced from this work, when compared with the already existing wines in the market, competed favorably in terms of physiochemical, organoleptic properties and general acceptability.

Acknowledgement

The authors acknowledge the support of the Technologists at the Applied Microbiology and Brewing Laboratory of Nnamdi Azikiwe University Awka, Nigeria

Competing Interests

Authors have declared that no competing interests exist.

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[10] CC. Ezemba, Archibong E J. Comparative Studies of Wine Produced from Coconut (Cocos Nucifera) and Mango Fruit (Mangifera indica) using Yeast Isolated from Palm Wine International Journal of Research in Pharmacy and Biosciences. Vol 4, No. 8, 44-49, 2017, ISSN 2394-5885 (Print) & ISSN 2394-5893 (Online)


