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RESEARCH ARTICLE

Production, sensory and physicochemical evaluation of zobo and zobo-date wine from *Hibiscus sabdariffa* flower, pineapple, orange and lime juice using *Saccharomyces cerevisae*

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ABSTRACT

The need to harness the potency of underutilized local materials in wine production as well as extending the shelf life of the drink prompted this research study. Two equal concentrations of Zobo juice were produced from *Hibiscus sabdariffa*, pineapple, orange and lemon juice with same quantity of sugar and date syrup added to each before fermentation with *Saccharomyces cerevisae* to produce zobo wine and zobo-date wine respectively. Both Zobo wine and Zobo-Date wine produced significantly different ($p < 0.05$) physicochemical properties with pH 2.747 and 3.333, titratable Acidity; 0.4143 and 0.1220%, brix content; 6.1667 and 2.11%, Vitamin C; 12 mg/100ml and 20 mg/100ml, total dissolvable solids 6.52 Mg/L and 3.53 Mg/L, alcohol content; 9.18% and 6.56% respectively. The Zobo-Date wine however, tend to reduce microbial growth when compared to the Zobo wine as shown in the microbial count of both wines (9.703 ×106 and 1.2967 ×107 cfu/ml respectively). The sensory evaluation showed Zobo-Date wine to compete favorably with Zobo wine in terms of sensory attributes evaluated and overall acceptability showed no significant difference ($p > 0.05$). This study however showed Zobo-Date wine to impart more retention ability for vitamin C as well as reducing microbial growth. This established Zobo-Date wine as an excellent innovation in the production of wine using underutilized local materials and should be adopted for large scale production.

Keywords: Fermentation, *Hibiscus sabdariffa*, Physicochemical Evaluation, Zobo-Date wine

1. INTRODUCTION

Utilizing local and readily available raw materials in the production of foods and drinks is quite essential in boosting the economy of a nation as well as reducing production cost. Zobo (*Hibiscus sabdariffa*) is a common and extensively grown plant in Nigeria, particularly in the North-East and middle belt [1]. It produces a dark red flower which is considered as herb of economic importance which when processed into juice serve as a source of essential minerals and vitamins such as riboflavin, niacin, calcium and iron [2]. Zobo

drink is traditional refreshment and basically served chilled to consumers [3]. The purple-red juice are bubbled, sieved, sweetened with sugar, and flavoured with pineapple juice, ginger and lemon to give an improved drink. The significant reason for drinking this juice is, its immense potential and being great source of carbohydrate, vitamin C and protein [4]. Despite the inherent qualities embedded in this drink, Zobo drink has not been successfully produced at large commercial scale due to its short shelf life attributable to microbial activity. Microbial invasion is observed in this drink within twenty-four hours of production if not preserved either by refrigeration or controlled fermentation. This results into degradation of the nutrient components which in turn erodes the antioxidant property as well as other health benefits. The short shelf life of this potent nutritious drink aroused interest by researchers on possible ways of preserving this drink by fermentation using probiotic organisms such as *Saccharomyces cerevisae*, *Lactobacillus fermentum* among others to produce red wine that can impart additional nutrient on the drink as well as prolong shelf life.

Nigeria had spent about ₦171.56 million on wine importation. In 2016, Nigeria spent ₦9 billion on the importation of champagne (sparkling wine) alone [5]. The temperature restriction of grape to the temperate regions encourages such trend. However, high import duty on importation of wines has stimulated interest in the promotion of Nigerian indigenous drinks such as kunu and zobo even in the Presidential villa [6] and there was decline in the importation of wine by 24% [7].

Although fruit wines are generally produced from mango, pineapple, carrot and orange fruits, they are even prepared with lesser known tropical fruits like pawpaw [8]. These wines are priced lower than the imported white and red wines due to their poor colour. Generally, typical red wines have not been produced from tropical fruits because of their low content of extractable red pigments as red varietal grapes. Efforts have prior been made towards producing red wine by adding synthetic red colourants or dyes but their use and quantities are regulated by law to prevent toxicity in humans [9]. However, a tropical fruit with potential for producing red wines is the Roselle (Red or Indian Sorrel), *Hibiscus sabdariffa Var Sabdariffa*. The red variety of Roselle confers bright red juicy calyces.

Orange (*Citrus sinensis*) cultivation began about 7000 years ago and was believed to have originated from Southeast Asia, India and China. It was brought to Italy, Spain and Portugal in the fifteenth century. Prior to that, bitter oranges were cultivated for medicinal purposes. The introduction of sweet oranges in Europe has since encouraged worldwide commercial production and this was a turning point in the history of orange fruit.

Orange fruits are the most popular for consumers throughout the world due to their pleasant flavors and nutritional value. The fruit is commonly peeled and eaten fresh, or squeezed for its juice. The pulps, which are rich in soluble sugars, significant amounts of

vitamin C, pectin, fiber, natural antioxidants source and different organic acids, are mainly processed into juice.

Fruits and vegetables contain different components such as phytonutrients (including vitamins C and E, carotenoids, flavonoids) and phenolic components that significantly contribute to their antioxidant capacity. Oranges are eaten to allay fever and catarrh, the roasted pulp is prepared as a poultice for skin diseases while the fresh peel is rubbed on acne. An infusion of the immature fruit when taken relieves stomach and intestinal complaints. The flowers are employed medicinally by the Chinese people living in Malaya while Italy and France employ orange flower water (which is bitter and considered antispasmodic and sedative) as cologne. A decoction of the dried leaves and flowers is given in Italy as an antispasmodic, cardiac sedative, antiemetic, digestive and remedy for flatulence. The inner bark, macerated and infused in wine, is taken as a tonic and carminative.

Lime and lemon belong to the acid citrus fruit group. This group is characterized by an elliptical to round shaped fruit with high citric acid content. Limes differed from other fruits in the citrus family as they have both acid and sweet varieties. All citrus fruits have nearly the same structure; however, the elements that comprise these structures vary according to species and variety [10]. The external part of the rind consists of several morphologically different tissues called flavedo because of the presence of flavonoid compounds [11]. There are many juice vesicles within the carpels [12]. The number of carpels vary, with acid lime fruit normally containing around 8–11 segments [10].

Pineapple (*Ananas comosus*) fruit is mostly grown in countries which are situated in the tropical and sub-tropical regions. It is native to Central and South America. Nowadays, it is in several countries where the weather conditions are favorable. Pineapple belongs to the Bromeliaceous family and grows on the ground. Pineapple is considered the third most important tropical fruit produced in the world, after the banana and citric fruits. Total pineapple production worldwide is around 16 to 18 million tons [13] with Thailand, Brazil, India, Phillipines and China contributing to the total production. Pineapple is an important food which can be eaten fresh or in a processed form. It contains nutrients which are good for human health. Pineapple is composed mainly of water, carbohydrates, sugars, vitamins A and C and betacarotene and low amounts of protein, fat, ash and crude fibre. Pineapples contain antioxidants such as flavonoids, vitamin A and C. These antioxidants reduce the oxidative damage such as that caused by free radicals and chelating metals. It also has the enzyme complex protease (bromelain). Bromelain contains peroxidase, acid phosphate, several protease inhibitors and organically bound calcium [14]. Although most of the commercial pineapple produced worldwide is canned prior to consumption, the fresh fruit market is increasing.

Date Palm (*Phoenix dactylifera* L.) is known as the date palm [15]. The *Phoenix dactylifera* L. is a monocotyledonous woody perennial belonging to the Arecaceae family, which

comprises 3000 species and 200 genera [16]. Baliga *et al*., [17] stated that there are over 600 kinds of dates based on the shape and organoleptic properties. Date fruits are a significant component of the diet in the majority of the Arab countries with low cost.

Date palm is one of the most important trees in semi-arid and dry areas of the world and has become a part of the cultural life of the people in these regions. The tree is capable of providing a broad range of products and uses, and is considered a natural renewable resource. Date fruits have immense importance as a healthy food as well as a dessert fruit because they are rich in carbohydrates, vitamins and minerals thereby providing a wide range of essential nutrients [18].

Roselle (*H. sabdariffa*) popularly called zobo in Nigeria is also known as guinea or Indian sorrel. Zobo is a member of the malvaceceae family and grows in the tropic and sub-tropical regions of the world [19]. It is an erect branched subwoody 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 [20].

Thus we aim to analyse and produce zobo-date wine from *Hibiscus sabdariffa* flower, using *Saccharomyces cerevisae* as an alternative to expensive wine available and boost local economy.

2. METHODS AND MATERIALS

2.1. Sample Collection

The raw materials used for the production of wine were obtained from Eke Oko market, Orumba North Local Government Area, Anambra state. The materials acquired were zobo flowers, queen pineapple, sweet oranges, lime, date palm, sugar and baker's yeast (*Saccharomyces cerevisiae*). During the fermentation process, zobo flowers were used to produce wine and date syrup, pineapple juice and orange juice served as sweetener and carbon source for Zobo-Date wine while sugar was substituted for date syrup in Zobo wine.

2.2. Preparation of Materials for Juice Production and Fermentation

The pineapples, oranges and lime were washed twice with deionized water, peeled with separate sterile knife, blended/juice extracted aseptically. The date palm was sorted, washed twice with sterile water, deseeded, washed again, soaked for about 30 min in deionized water followed by blending, sieved and boiled for 20 min.

About 500 g of the sorted zobo leaves was weighed for each of the sample, washed with sterile water and boiled in 4 L of deionized water each for 30 min to aid juice extraction. The zobo suspension was filtered using the sterile muslin cloth, 100 ml each of the pineapple, orange and lime juice were added to each of the sample and 750 ml of date syrup was added to prepare the zobo-date sample while 750 g of sugar was added to zobo sample. Each mixture was inoculated with 50 g of bakers' yeast after which the bottle was sealed, corked and left to ferment anaerobically at room temperature for 14 days. Upon

completion of the 14 days fermentation, the juice was racked from its sediment and the racked wine was filtered, filled into prior strerilized bottles, corked and pasteurized at 70°C for 20 minutes, cooled and stored.

2.3. Physicochemical Analysis

The physicochemical parameters determined for all the wine sample with the pH, total titratable acidity, total dissolved solids, vitamin C content and alcohol content. All parameters were carried out in triplicates and the mean value was calculated.

2.3.1. pH Determination

The pH was assessed with the aid of pH meter. The pH electrode was calibrated with standard buffer solution then dipped in the wine samples until a steady reading was attained. The meter reading was recorded in triplicate then the average was calculated.

2.3.2. Total Titratable Acidity (TTA)

About 15 ml of the wine samples were each measured and 75 ml of distilled water was added followed by 3 drops of phenolphthalein indicator and then titrated against 0.1M NaOH solution, until the colour changed to pink. The value was recorded and used for calculating the total titratable acidity. Total Titratable Acidity was calculated by equation 1:

$$\text{TTA} = \frac{(\text{Volume of NaOH})}{(\text{Volume of Wine})} \times 0.75 \qquad \textit{eq (1)}$$

2.3.3. Total Dissolved Solids (TDS)

Exactly 50 cm^3 of the sample was measured and filtered using filter paper, then poured into sterile evaporating dish that had been prior cleaned, dried and pre-weighed. The content was then put in the oven at 105°C until the sample dried completely after which it was cooled inside a desiccator and weighed until the constant weight was recorded.

2.3.4. Alcohol Content Determination

The alcohol content was determined using the specific gravity method as described by Biri *et al* [21] by equation 2:

$$\text{Alcohol content by volume (\%)} = (\text{Original Gravity} - \text{Final Gravity}) \times 131.25 \qquad \textit{eq (2)}$$

2.4. Sensory Evaluation

Sensory evaluation was conducted according to a procedure described by Maragatham & Panneerselvam [22]. Zobo-Date and zobo wine were evaluated by a panel of 7 judges comprising of 5 male and 2 female who were very familiar with wine and aged between 25 to 30 years. The 7 panelists were selected based on interest, availability, familiarity with wine and health stability and were instructed to rinse their mouth with water after each tasting. Higher values indicated higher acceptance on a 7-hedonic scale with 7 as extremely liked and 1 as extremely disliked.

2.5. Total Viable Plate Count (TPC)

At the end of 14 days fermentation, microbiological culture was done on Potato Dextrose Agar to check for viable cells.

2.6. Statistical Analysis

Figure 1. Bottled and freshly prepared red zobo wine

Results were statistically analysed using Analysis of Variance (ANOVA) and means were separated by Least Significant Difference (LSD) procedure.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Properties and Microbial Count of Zobo Wine and Zobo-Date Wine

The zobo wine was red in color and was similar to commercial wines (figure 1). With the same quantity of date syrup serving as substitute for sugar in the wine samples, Table 1 showed a significant higher value ($p < 0.05$) in alcohol content, total titratable acidity (TTA), total dissolved solid (TDS), brix content and microbial load with lowered value in pH and vitamin C in Zobo wine compared to Zobo-Date wine after 14 days of fermentation. The higher TA value in Zobo wine can be attributed to the reduced pH of Zobo wine in comparison to the value obtained in Zobo-Date wine. Both TTA value of both wine samples however, considered them as high acid product because the pH values (2.747 and 3.333) are lesser than 4.6 which is a dividing line for high acid food and low acid food [23]. This corresponds to the finding of Adeleke and Abiodun [24]; Wong *et al.* [25] where it was claimed that high acid content is usually observed with Zobo drinks because Zobo is naturally acidic fruit endowed with organic substances with low pH (oxalate, tartarate, malate and succinic acid). Acidity is quite important in wine fermentation as it determines the quality of the wine and creates environment inhibiting potential spoilage microorganisms and supporting the growth of desirable one [26].

The higher alcohol content of Zobo wine compared to Zobo-Date wine as observed in Table 1 can be attributed to the concentration of the added nutritive sweeteners (table sugar and date syrup), other fermentable substrate (pineapple, orange and lime juice as well as *H. sabdariffa*), action of the fermenting yeast on the substrate and the sweeteners [27] and the conversion rate of the substrate to alcohol. However, a generally low alcohol content (9.18% and 6.56%) was recorded in this investigation for both wines when compared to the available wines in the market and this can be attributed to the reason reported in the findings of Yokotsuka *et al* [28] that *Hibiscus sabdariffa* contained small amounts of acetaldehyde, pyruvic acid and apha-ketone glutaric acid which normally react with SO_2 to form bisulphate complexes in fermentation, leading to the low amount of alcohol production at the end of fermentation.

The higher brix value obtained in Zobo wine as showed in Table 1 can be attributed to higher concentrated form of sucrose in table sugar used in zobo wine as might be less concentrated in date syrup used in Zobo-date

Table 1. Microbial Count and Physicochemical Properties of Zobo Wine and Zobo-Date Wine.

Samples/parameters	Zobo wine	Zobo-date wine
TTA	$0.4143^{a}\pm0.0015$	$0.1220^{b}\pm0.0017$
Ph	$2.7467^{b}\pm0.0680$	$3.3333^{a}\pm0.0351$
TDS (mg/l)	$6.5233^{a}\pm0.0873$	$3.5300^{b}\pm0.1081$
BRIX (%)	$6.1667^{a}\pm0.8621$	$2.1100^{b}\pm1.0493$
TPC (cfu/ml)	$1.2967\times10^{7b}\pm0.0208$	$9.7033\times10^{6a}\pm0.0550$
Vit c (mg/100ml)	$12.00^{b}\pm0.1321$	$20.00^{a}\pm0.0982$
Alcohol content (%)	$9.18^{a}\pm0.1212$	$6.56^{b}\pm-0.1805$

* Means with the same superscript in the same column are not significantly ($p < 0.05$) different.
TTA=total titratable acidity; TDS=total dissolvable solids; TPC=total plate count

Table 2. Sensory evaluation of Zobo Wine and Zobo-Date Wine

Samples/sensory attribute	Zobo wine	Zobo-date wine
Aroma	$4.40^{a}\pm0.17$	$4.20^{a}\pm0.07$
Taste	$5.00^{a}\pm0.13$	$4.40^{b}\pm0.08$
Clarity	$5.40^{a}\pm0.08$	$5.40^{a}\pm0.08$
Appearance	$6.20^{a}\pm0.11$	$6.20^{a}\pm0.07$
Alcohol content	$5.20^{a}\pm0.08$	$4.60^{b}\pm0.08$
Overall acceptability	$5.24^{a}\pm0.02$	$5.20^{a}\pm0.17$

* Means with the same superscript in the same column are not significantly ($p < 0.05$) different.

wine which has not been exhaustively utilized by the micro-organism for growth and conversion to alcohol and acids. This can also impart proportional increase in total dissolvable solids (TDS) obtained in Zobo wine when compared to Zobo-Date wine (table 1).

The higher vitamin C content obtained in Zobo-Date wine in comparison to Zobo wine can be attributed to the claim of Hossain [29] who reported that date fruits contained some considerable amount of vitamins (including vitamin C) along with minerals like calcium, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc.

As shown in Table 1, it is evident that the microbial load of the Zobo wine was higher compared to the Zobo-Date wine and this can be attributed to the high sugar content as indicated in the brix value of Zobo wine. This provides a better available substrate for the proliferation of microorganisms in the zobo wine compared to Zobo-Date wine. It is reported in the finding of Hossain [29] that a hot water treated date fruits contained reduced fructose, glucose and inverted sugar level as well as reduced nutrient level and in this investigation, the production of the date syrup used in Zobo-Date wine involved boiling of the blended date fruits for 20 min hence, reduction in the brix value of Zobo-Date wine and less available nutrient for the growth of microorganisms.

On a general note, it was observed statistically ($p < 0.05$) as shown in Table 2 that the sensory attributes of the Zobo-Date wine as adjudged by the panelists competed favourably with the Zobo wine in terms of aroma, clarity, appearance, alcohol content and overall acceptability.

Many investigations have been conducted for wine production using Zobo (*H. sabdariffa*). Okoro CE (2007) [30] produced red wine from

Roselle (*H. sabdariffa*) and Pawpaw (*Carica papaya*) using palm-wine yeast (*Saccharomyces cerevisiae*), Omole and Oranusi (2019) [31] produced wine from *Hibiscus sabdariffa* calyxes using probiotics starter cultures. Charles and Nwahia (2012) [32] produced wine from zobo flower juice using yeast (*Saccharomyces cerevisiae*) extracted from palm wine. Arubi *et al*, (2009) [33] produced colored wine from *Hibiscus sabdariffa* calyx extract possible wine production from Zobo drink will provide health benefits to its consumers due to abundance of phenolic compounds coupled with low alcohol content. There are reports on the use of the red variety of roselle catyx to produce 'Karkade drink' in Sudan and Zobo in Nigeria [34]. Red roselle Calyx has been reported to be rich in anthocyanins containing a mixture of organic acids such as citric malic and tartaric acids [35].

Clifford [36] reported that anthocyanins vary in colour from pinks to blue and violet. These characteristics suggest that roselle calyx extract may be a suitable raw material for the production of red wines.

4. CONCLUSION

The outcome of this study explicates that red wine can be produced from zobo-date and zobo, and if adopted for large scale production will boost Nigeria's economy by conserving revenues that would have been lost on wine importation. Job opportunities will also be availed for the teeming Nigeria youth thereby reducing the rate of unemployment in the country.

The wealth of benefits embedded in the production of wine from *Hibiscus sabdariffa* sweetened with date syrup, it is quite recommendable to utilize other probiotics like *Lactobacillus fermentum* in carrying out further studies on Zobo-Date wine production.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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