Fermentation of black plum (*Vitex doniana* Sweet) juice for production of wine

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Fermentation of black plum (*Vitex doniana* Sweet) juice for production of wine.

Abstract — **Introduction**. *V. doniana* Sweet fruit juice is sweet and has been fermented for many years in Nigeria using a traditional technology to produce wine. In this country, black plum is collected from wild trees in the natural ecosystem or from trees cultivated in isolated farms. Our study compared the chemical and sensory properties of wine produced from traditional or controlled fermentation of black plum juice. **Materials and methods**. Four types of experiments, two with spontaneous fermentation and two with controlled fermentation, were carried out, each with five replicates. The proximate composition, alcohol content and pH of the solutions obtained after spontaneous or controlled fermentation were determined. Moulds and yeasts isolated in the fermented juice were identified. **Results and discussion**. Wine obtained from controlled fermentation had 10.5% alcohol content while the spontaneous fermentation produced a wine of 5.0% alcohol content. The chemical analysis of juice produced from harvested ripe *Vitex doniana* fruit showed that it contained a soluble protein content of 75.62 $mg \cdot mL^{-1}$, pH of 4.5, total sugars of 95.10 $mg \cdot mL^{-1}$, titratable acidity of 0.33 $mg \cdot 100\ mL^{-1}$, and ascorbic acid of 5.2 $mg \cdot mL^{-1}$. The yeasts isolated from the wine were *Saccharomyces cerevisiae* and *S. coreanus*, and filamentous moulds were *Rhizopus stolonifer*, *Penicillium* sp. and *Aspergillus* sp. Single cell protein was produced from the fruit juice by cultivation in it of *Saccharomyces cerevisiae* isolated from the fruit. The yeast had a protein content of 6.8 $mg \cdot mL^{-1}$ when grown at 20 °C for 36 h at pH 5.8. **Conclusion**. Wine produced from *Vitex doniana* Sweet juice has quite high alcohol content and is palatable with good flavor. The stability of the juice for yeast growth is an advantage for the production of single cell protein.

Nigeria / *Vitex doniana* / fruits / postharvest technology / fermentation / wine yeast / *Saccharomyces cerevisiae*

Fermentation du jus de *Vitex doniana* Sweet pour la production de vin.

Résumé — **Introduction**. Le jus du fruit de *V. doniana* Sweet est doux et, au Nigeria, il est utilisé pour la production d'un vin produit à partir d'une fermentation effectuée selon des techniques traditionnelles. Dans le pays, ce fruit est récolté soit sur des arbres sauvages, soit sur des arbres plantés dans des fermes isolées. Notre étude a permis d'effectuer une évaluation chimique et sensorielle de ce vin en comparant deux types de fermentation : la fermentation traditionnelle et une technique de fermentation contrôlée du jus de *V. doniana*. **Matériel et méthodes**. Quatre types d'expériences, deux utilisant la fermentation traditionnelle et deux testant une fermentation contrôlée, ont été effectuées, chacune avec cinq répétitions. La composition, la teneur en alcool et le pH des solutions obtenues après les deux types de fermentation ont été déterminés. Les moisissures et levures isolées dans les milieux fermentés ont été identifiées. **Résultats et discussion**. Le vin obtenu à partir de la fermentation contrôlée a eu une teneur en alcool de 10,5 %, alors que le vin issu de fermentation traditionnelle a présenté un titrage en alcool de 5,0 %. L'analyse chimique du jus produit à partir de fruits mûrs de *V. doniana* a révélé une teneur en protéines soluble de 75,62 $mg \cdot mL^{-1}$, un pH de 4,5, une teneur en sucres totaux de 95,10 $mg \cdot mL^{-1}$, une acidité titrable de 0,33 $mg \cdot 100\ mL^{-1}$ et une teneur en acide ascorbique de 5,2 $mg \cdot mL^{-1}$. Les levures isolées dans le vin ont été *Saccharomyces cerevisiae* et *S. coreanus* et les champignons filamenteux identifiés ont été *Rhizopus stolonifer*, *Penicillium* sp. et *Aspergillus* sp. Développée à 20 °C pendant 36 h à un pH de 5.8, la levure a permis d'obtenir une teneur en protéines de 6,8 $mg \cdot mL^{-1}$. **Conclusion**. Le vin obtenu à partir du jus du fruit de *V. doniana* a une teneur en alcool assez élevée et il est agréable au goût avec une bonne saveur. La stabilité de ce jus pour la croissance de levures est un avantage pour la production de protéines unicellulaires.

Nigéria / *Vitex doniana* / fruits / technologie après récolte / fermentation / levure de vinification / *Saccharomyces cerevisiae*

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***Resumen Español**, p. 369*

1. Introduction

Vitex doniana Sweet (black plum, Verbenaceae), formerly known as *V. cunieata* [1], is a plant widely used by various communities in Nigeria for many purposes. In the Hausa language, it is called 'dinya', Fulanis call it 'galbihi'; in Yoruba, it is known as 'oriri' or 'orinla' and in Ibo's language it is called 'uchakokoro' or 'elili' [2, 3]. The fruit is sweet and has been fermented for many years in Nigeria, using traditional technology to produce wine for domestic consumption.

There are many reports on the production of local wines: production of palm wine from oil palm sap [4, 5]; pito (local wine in Nigeria) from fermented maize, sorghum or a mixture of both [6]; cocoa wine from cocoa seed [7]; carrot wine from carrot [8] and wine from cashew biomass [9], for example. The type and quality of wine produced depends on the initial fruit substrate, the fermenting microorganism and different cultural practices employed in their production.

Various studies have identified the yeasts involved in wine-making from the different substrates: *Saccharomyces cerevisiae, Schizosaccharomyces pombe*, *Candida tropicalis and Pichia* sp. [5] in oil palm wine; *Saccharomyces cerevisiae, Candida krusei*, *Pichia* sp. and *Kloechera* sp. [7, 8] from cocoa and carrot wines, and *Saccharomyces, Kloechera*, *Torulospora* and *Zygosaccharomyces* [10] from grapes.

With the growing emphasis on renewable resources in Nigeria, there is the need for exploitation of existing plants in currently under-utilized species. *Vitex doniana* represents one of our neglected, untapped forest resources [11]. In addition, the search for cheap sources of protein to enrich indigenous staple food, which is mainly starchy, means that microbial protein production has become important in Africa [8]. As a result, much attention is now being given to the production of single cell protein (SCP) in Nigeria.

This paper reports the chemical and sensory evaluation of wine produced from traditional and controlled fermentation of *Vitex doniana* and the ability of the juice to support the production of single cell protein.

2. Materials and methods

2.1. Collection of samples

Black plum ripe fruits were collected from the rain forest zone of Ojoto in South-Eastern Nigeria. Ripe fruits are known by their black color and softness. Collections were made from August to November, and samples were stored in the refrigerator at 4 °C until required for analysis.

2.2. Juice extraction

The skins of 250 fruits (close to 1.8 kg) were washed thoroughly with ethanol before being peeled off using sterile knives. The seeds of the peeled fruits were taken out and the fleshy tissue or pulp was diluted with 100 mL of water for easy maceration in a blender. The resulting solution, about 3 L of juice, was poured into a cloth bag and the juice squeezed out into a pot previously rinsed with 2% of potassium metabisulphite.

2.3. Fermentation of black plum and yeast propagation

Four types of fermentation with five replicates each were carried out: two different experiments with spontaneous and two other different experiments with controlled fermentation.

For the first experiment with spontaneous fermentation, 1 L of juice extracted was poured into a sterile 5-L aspirator bottle to stand for 36 h, after which the bottle was tightly corked and left to stand at room temperature for 10 d. The pH, temperature and alcohol content were determined on a daily basis. For the second experiment with spontaneous fermentation, an amelioration of the fruit juice with 150 $g \cdot L^{-1}$ commercial sucrose was carried out. Aeration was for 36 h. Anaerobic fermentation was for 10 d and sampling for yeast study was done at 2-d intervals. The temperature and pH were monitored on a daily basis. The yeast that predominated after this fermentation was isolated and identified. Adding 0.2 $g \cdot L^{-1}$ potassium metabisulphite before storage preserved the wine from fermentation.

The yeast species isolated during the experiments with spontaneous fermentation were incubated into pure culture and stored in slants. The best yeast strain for fermentation was determined by inoculating each of the yeasts isolated into 2% glucose/peptone water solutions in McCartney bottles containing Durham tubes, and observing their abilities to ferment glucose by the amount of gas bubbles which accumulated in the Durham tubes after (24 to 25) h.

For the experiments with controlled fermentation, 2 L of extracted juice were employed. They were pasteurized at 60 °C for 30 min. A propagated yeast with a packed cell volume of 500 mL was introduced into the pasteurized juice to give a final volume of 2.5 L. An amelioration was carried out by adding 150 $g \cdot L^{-1}$ of commercial sucrose and accessory nutrients including 0.55 $g \cdot L^{-1}$ ammonium sulphate and 0.8 g potassium dihydrogen phosphate (KH_2PO_4); 0.2 $g \cdot L^{-1}$ potassium metabisulphite was used as a stabilizer and antioxidant. These additions were made before adding the inoculum. Aeration was for 24 h after which the light cotton wool covering was replaced with a cork fitted with an air lock and thermometer, for anaerobic fermentation. The two experiments with controlled fermentation differed by dilution: one was with diluted juice, while in the other, no dilution was done. Each of the experiments used 2 L of juice. For all the fermentation, viable yeast cell counts, measurement of the pH, temperature and alcohol content of the fermentation medium were determined at 24-h intervals. At the end of the fermentation, the wine was run off into a clean bottle rinsed with 1% potassium metabisulphite.

2.4. Analytical procedures

The proximate composition, acidity, alcohol content and pH of the spontaneous and controlled fermentation of black plum were determined using AOAC methods [12]. Moulds were identified by means of the keys of Barnett and Hunter [13] and yeasts by the method of Lodder [14]. The bacterial species were identified with Bergey's Manual of Systematic Bacteriology [15].

The wine from controlled fermentation was assessed on the basis of dryness, sweetness, odor, color and flavor by a trained panel of seven adult men. The average scores of the panel were then used to determine the palatability of the wine. The significance between spontaneous and controlled fermentation was determined using the Student's *t* test for paired differences.

2.5. Extraction of protein from whole yeast cells

Yeast cells were fractionated before extraction of protein, and two methods were used. The amount of protein extracted by the use of the methods was measured by the biuret method [16].

– An autolysis of yeast cells was done by incubating the cells at pH 6.5 and at (45 to 50) °C for 24 h. After incubation, the protein content of the solution was determined [17].

– Five millimeters of distilled water were added to 2 g of yeast paste. The autolysis of the yeast cells was then achieved by the process of plasmolysis with the addition of 5 mL of 25% NaCl solution to the yeast sludge, which was allowed to stand for 24 h [18].

3. Results and discussion

3.1. Microbial components

The yeasts isolated from the two spontaneous fermentations were *Saccharomyces cerevisiae* and *S. coreanus*. We have already observed that *S. cerevisiae* is a major component of the yeast found in the ripe fruit of black plum [11]. The yeast count for the first spontaneous fermentation to which sugar was not added was 9.1×10^2 $cells \cdot mL^{-1}$ after 7 d. The second spontaneous fermentation in which there was addition of sugar had 2.4×10^2 $cell \cdot mL^{-1}$ after 9 d (*figure 1*). The moulds isolated from spontaneous fermentation were *Rhizopus stolonifer* and *Penicillium* sp. The population of the moulds in the first spontaneous fermentation was 1.4×10^2 colony forming $unit \cdot mL^{-1}$

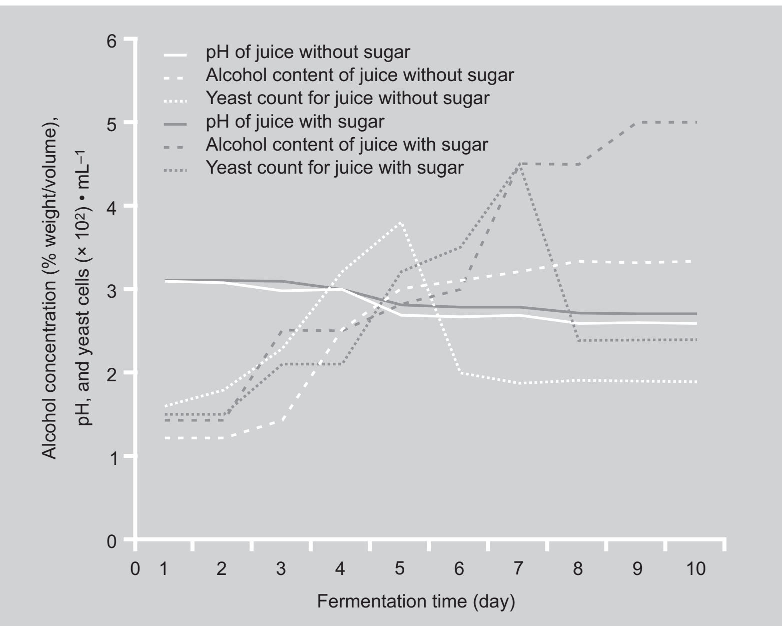
and the second spontaneous fermentation had 1.2×10^2 colony forming units·mL^{-1}.

3.2. Spontaneous fermentation

For the spontaneous fermentation without sugar, the pH decreased slowly (*figure 1*), which might be due to the microbial fermentation resulting in acid production. The alcohol content (3.30% after 10 d) of the product was low. There was a gradual increase in the yeast cell number up until 5 d, then a decrease until a constant count (1.9×10^2 cells·mL^{-1}), which was then maintained. That yeast count was low, probably because of insufficient nutrients required by the microorganism for growth.

For the spontaneous fermentation to which sugar was added, the pH showed a gradual decrease from 3.1 on the first day to 2.7 on the 10th day (*figure 1*). As the alcohol content increased, the acidity of the wine also increased, which led to a decrease in pH. This might be attributed to the fermentation of the juice by yeast, which utilized the sugar content. The yeast cell number increased gradually up until the fifth day, then it had a sudden increase before falling to a constant level of 2.4×10^2 cells·mL^{-1} (*figure 1*). The fifth day was the period during which fermentation was vigorous. As the alcohol content increased, it was most likely that some of the yeast could not tolerate such a high level. Aderiye and Mbadiwe [9] noticed a 78% reduction in the microbial population within 48 h of fermenting cashew pomace and that population was kept relatively low for the next 6 d. This pattern was attributable to a reduction in the fermentation sugar, enzyme hydrolysis and the increased acidity of the substrate due to microbial activity [9, 19].

Figure 1.
Changes in pH, alcohol content and yeast cell count with time of spontaneous fermentation according to addition or not of sugar to the fruit juice (*Vitex doniana* Sweet or black plum, Nigeria).



3.3. Controlled fermentation

The pH decreased with time of fermentation while alcohol content increased with this time (*figure 2*). The decrease in pH was probably as a result of some acids that might be produced by the yeast during metabolism [20]. After 10 d of fermentation, the alcohol content of the wine was 10.53% for undiluted juice and 7.60% for diluted juice. The alcohol content of the wine produced from controlled fermentation was high and this may be due to the initial sugar concentration and amelioration with ammonium sulphate, which supplied nutrients to the yeast starter. The yeast cell number increased most notably up until the seventh day, when it was highest; then it started to decrease to a constant number. Hung [21] reported a 22% reduction in mycelia growth of *Geotrichum candidum* at pH 4.3 and a reduction in excess of 93% at pH 4.0 after 24 h. At pH 2.7 or lower, there was no significant growth of the organism, in Sabouraud agar broth adjusted with citric acid, after incubation for 48 h at 30 °C.

The wine produced from controlled fermentation was chemically analyzed to determine its nutritional value (*table I*). The protein content of the fruit wine showed a decrease compared with the protein in the fruit juice. This was as a result of the utilization of the protein in the juice, along with other accessory nutrients, as fermentation proceeded. A decrease in the crude protein content of *Vitex doniana* fruit because of microbial degradation has been reported previously [3]. The total titratable acidity of the wine was higher than that of the fruit juice, probably because of the microbial activity [9].

3.4. Organoleptic assessment

The aggregate score of the seven-person panel showed that the wine was palatable. The flavor, color and dryness were rated good, while the sweetness and odor were fair.

The initial sugar concentration in the black plum juice had a major effect on the quantity of alcohol produced in controlled and spontaneous fermentations. From the organoleptic assessment, it could be deduced that wine from controlled fermentation was better than the wine from the ameliorated spontaneous fermentation. The wine produced from black plum (*V. doniana*) has a favorable balance between acids, alcohol and flavor, since it was palatable to taste.

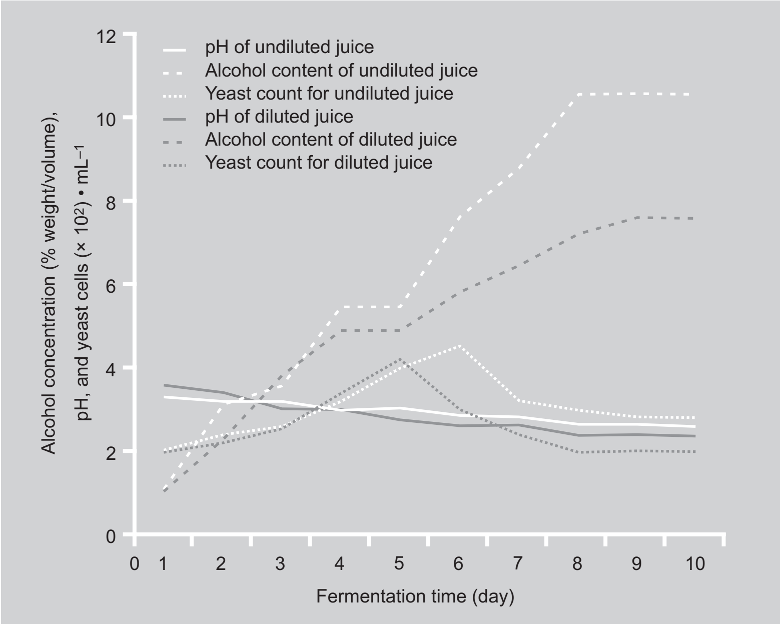


Figure 2.
Changes in pH, alcohol content and yeast cell count with time of controlled fermentation according to addition or not of sugar to the fruit juice (*Vitex doniana* Sweet or black plum, Nigeria).

3.5. Yeast propagation

Saccharomyces cerevisiae and *S. coreanus* were observed to be good for single cell protein. Yeasts found in *V. doniana* fruits have been graded into minor and major yeasts with *S. cerevisiae* included in the major components [11]. This might have been the reason for the dominance of *S. cerevisiae* in the spontaneous fermentation.

The fruit juice of black plum could be used as a medium for production of single cell protein (SCP) since it supported good growth of yeast. The increases observed in the yeast cell count of the juice left for 24 h supported this fact. One of the major advantages of single cell protein production is the flexibility in choosing not only the organism to be produced, but also the substrate to serve as the primary carbon source. The choice of a carbon source will depend on such factors as availability, purity, cost and lack of toxicity [22].

An adequate proportion of carbon source, nitrogen source and minerals are required for a profitable yield of single cell protein (SCP) in a substrate [23]. In addition to the carbon source, certain minerals and nitrogen are added to the aqueous medium, usually as ammonia or nitrates, for the production of single cell protein.

Between the two methods used for fractionating the yeast cells, the one which consisted of incubating the cells at (45 to

Table I.
Chemical composition of *Vitex doniana* Sweet (black plum) fruit juice and wine obtained from controlled fermentation (Nigeria).

Product analyzed	Crude protein ($mg \cdot mL^{-1}$)	Total sugar ($mg \cdot mL^{-1}$)	Titratable acidity ($mg \cdot 100mL^{-1}$)	Volatile acidity ($mg \cdot 100mL^{-1}$)	Alcohol content (%)	pH
Fruit juice	75.62	95.1	0.33	4.5	0.0	4.5
Wine	14.8	91.2	0.487	0.0571	10.5	2.7

50) °C for 24 h at pH 6.5 gave the best result. The protein content obtained was 6.8 mg·mL^{-1}. This method was economically cheaper than the second method adopted, which was done by disruption of the cell wall by growth in 25% sodium chloride for 24 h, and only produced protein concentrations of 6.2 mg·mL^{-1}.

4. Conclusion

Wine and single cell protein (SCP) were produced from black plum (*Vitex doniana* Sweet) juice. The alcohol content was quite high and the wine produced was palatable and had a good flavor. The stability of the juice for yeast growth is an advantage for the production of single cell protein and can serve as both the sole carbon and nutrient source. *Saccharomyces cerevisiae* and *S. coreanus* are good for production of single cell protein and they can serve as yeast starter for wine production.

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Fermentación del jugo de *Vitex doniana* Sweet para la producción de vino.

Resumen – Introducción. El jugo del fruto de *V. doniana* Sweet es dulce y, en Nigeria, se emplea para elaborar vino a partir de una fermentación realizada con técnicas tradicionales. En Nigeria, este fruto se recolecta en árboles silvestres o en árboles plantados en huertas aisladas. Nuestro estudio efectuó una evaluación química y sensorial de este vino comparando dos tipos de fermentación: la fermentación tradicional y una técnica de fermentación controlada del jugo de *V. doniana*. **Material y métodos**. Se efectuaron cuatro tipos de experiencia: dos con fermentación tradicional y dos con una fermentación controlada, cada una con cinco repeticiones. Se determinó la composición, contenido de alcohol y pH de las soluciones obtenidas tras los dos tipos de fermentación. Se identificaron los mohos y levaduras aislados en los medios fermentados. **Resultados y discusión**. El vino obtenido a partir de la fermentación controlada tenía un contenido de alcohol del 10,5%, mientras que el vino con fermentación tradicional presentó una graduación de alcohol del 5,0%. El análisis químico del jugo producido a partir de frutas maduras de *V. doniana* reveló un contenido de proteínas soluble de 75,62 $mg \cdot mL^{-1}$, un pH de 4,5, un contenido de azúcares totales de 95,10 $mg \cdot mL^{-1}$, una acidez valorable de 0,33 $mg \cdot 100\ mL^{-1}$ y un contenido de ácido ascórbico de 5,2 $mg \cdot mL^{-1}$. Las levaduras aisladas en el vino fueron *Saccharomyces cerevisiae* y *S. coreanus* y los hongos filamentosos identificados fueron *Rhizopus stolonifer*, *Penicillum* sp. y *Aspergillus* sp. Desarrollada a 20 °C durante 36 h con un pH de 5,8, la levadura permitió obtener un contenido de proteínas de 6,8 $mg \cdot mL^{-1}$. **Conclusión**. El vino obtenido a partir del jugo de fruta de *V. doniana* tiene un contenido de alcohol bastante elevado y es de gusto agradable y con buen sabor. La estabilidad de este jugo para el crecimiento de levaduras es una ventaja para la producción de proteínas unicelulares.

Nigeria / *Vitex doniana* / frutas / tecnología postcosecha / fermentación / levadura de vino / *Saccharomyces cerevisiae*