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PRODUCTION OF ALCOHOL USING THE PULP OF *Parkia biglobosa*

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Abstract

This report explores the possibility of using the pulp of locust bean (*Parkia biglobosa*) to make wine in a large scale and in a form that compares favorably with imported commercially available wines. The pH of the pulp syrup was low (4.1) and could be preserved for six months without deterioration. The total titratable acidity, reducing sugar, crude protein and ascorbic acid contents of the pulp syrup were determined and compared with the locally available substrates used for making wine. However, reducing sugar content of pulp was low compared to that of other substrates and sucrose was added at 1% (w/v) to the pulp syrup for fermentation using *Saccharomyces cerevisiae*. The important characteristics of locust bean wine include alcoholic content, 12.40 %(v/v), pH 4.18, reducing sugar 1.1%, protein 1.0%, total titratable acidity, 0.44%. These values are comparable to those of cashew, orange, pineapple and "icheku" wines and also to values found for some commercially available wines.

Introduction

Considering the great economic importance of alcohol especially alcoholic beverages using local raw materials available in Nigeria, a number of fruits namely cashew (Macleod and Tracoins,1982), orange (Mitchell, 1982), pineapple (Felton, 1971) and *Dialium guineensis* (De and Ona, 1997) have been investigated for use in making wine. In Nigeria, locust bean (*Parkia biglobosa*) is abundantly available in Northern, eastern and western parts and the leaves and bark are used in remedies for treating guinea worm, skin infections and burns. The fermented seeds are used to prepare condiments but the yellow pulp of the fruits has no commercial demand though it has some applications in rural areas. It is interesting to see the suitability of this pulp, which is rich in carbohydrate as a substrate for making an alcoholic beverage.

Materials And Methods

Preservation

Drying completely under the sun preserved the pulp.

Preparation of pulp syrup for fermentation

About 500 g of the pulp was taken into a 5-liter flask and 2,500 ml of distilled water was added to it. The pulp and water were mixed vigorously to form syrup.

Quality assessment of pulp syrup

For **microbial analysis** of the syrup, one ml of syrup was transferred into 9 ml of distilled water and using this as a stock solution serial dilution of up to 10^{-3} were made and 1.0 ml of each dilution was plated in potato dextrose agar (PDA) and nutrient agar (NA) using the pour plate method and then the PDA plates were incubated at 28°C for 48 hours whereas NA plates were incubated at 37°C for 24 hours. The **pH** of the syrup was determined using a digital pH meter (Crison, micro-pH 2000). The total reducing sugar was determined by dinitrosalicylic (DNS) method adapted by Mandel (1974). The **sugar** content of the pulp syrup was determined by making dilution of 1 ml syrup to 2 ml with distilled water and to it 3 ml of DNS reagent was added. The tubes were shaken for 20 seconds and placed in

boiling water bath for 5 minutes. The tubes were allowed to cool at room temperature. The percentage transmittance was read at 550 nm with a water blank set to 100% transmittance. A standard curve was obtained using different concentrations of glucose. The sugar concentration of the sample was then obtained from the standard curve. For determination of **ascorbic acid,** 7.5 ml of syrup was added to 92.5 ml water and mixed vigorously. Five ml of this treated sample was taken in a boiling tube and 1.0 ml chloroform and 1.0 ml glacial acetic acid were added to it and was titrated against the dye 2,6 dichlorophenol indophenol contained in a burette.The *crude protein* was determined by Kjeldahl method using 5.0 ml of syrup. The total titratable acidity (TTA) was determined by the method of Heinzel and Truper (1989). Five ml of syrup was taken in a conical flask and 95 ml of water was added to it and the diluted sample was titrated against 0.1N sodium hydroxide solution.

Fermentation

About 2 liters of the pulp syrup was put in a sterile 5-liter flask fitted with a L-shaped tube passing through a rubber cork. To the syrup in the flask, about 20 gms of sucrose was added and mixed thoroughly. For the development of inoculum, about 8.0 gms of dry yeast, Saccharomyces cerevisiae (source: Vahine Professionnel, France) was added to another 200 ml pulp syrup containing 2.0 gms of sucrose and then incubated for 4 hours at 37°C. The inoculum was added aseptically to the contents of 5-liter flask and the pH was adjusted to 4.5. The fermentation was considered complete when gas bubbling stopped. The alcohol content was determined on 7th, 14th and 21st day of fermentation.

Qualitative assessment of fermented broth

The microbial analysis was done on 3rd, 7th, 14th and 21st day of fermentation following the procedure used for pulp syrup. For determination of alcohol content, 100 ml of fermented broth was filtered and 50 ml was taken for alcohol determination by fractional distillation method. The pH, total titrable acidity, reducing sugar and protein were determined following the procedures described for pulp syrup.

Results

Quality assessment of pulp syrup

No microbial growth was observed on PDA and NA plates for the pulp syrup. The pH of the pulp was 4.1. The values of reducing sugar, total titratable acidity, crude protein and ascorbic acid are listed in Table 1.

Fermentation

Gas bubbling in the fermentation flask was observed within 3 hours after inoculating the syrup with starter culture. Gas bubbling ceased completely on 21st day of fermentation.

Quality assessment of fermented broth

No contaminating microorganism was detected. The pHs of the broth on 7th, 14th and 21st day were 4.1, 4.15 and 4.18 respectively. The alcohols content on 14th and 21st day of fermentation were 5.3% and 12.40% respectively. No alcohol was detected on 7th day of fermentation. The values of total titratable acidity, reducing sugar, protein content, ascorbic acid and alcohol content of the broth on 21st day of fermentation are listed in Table 2.

Discussion

The value of reducing sugar (1.2%) in the extract is low compared to the 4- 12% obtained for banana, pineapple, orange, cashew and Dialium guineensis (Felton, 1971; Gallender, 1976 and De and Ona, 1997). The insufficient natural sugar content results in wine that is low in alcohol content, so sucrose (1%) was added to the pulp syrup.

The protein content of pulp syrup is comparable to those found for grape and cashew (Rose, 1977; Aderiye et al. 1991). Ascorbic acid value 7.04-mg/100 ml in syrup contrasts with the range of (34.56-97.11 mg/100 ml) reported for some other substrates (Temple et al., 1990). It was reported that ascorbic acid at a concentration of 1.0-1.5 mg/ml enhances enzymatic hydroxylation and accelerates glucose uptake as well as the excretion of ethanol in the fermenting must (Lehninger, 1975). The low pH value may be responsible for the sterility observed in syrup. The TTA level (0.045%) of the pulp syrup is comparable to reported TTA values for cashew, pineapple and orange juices (0.05 -1.5%). The alcohol content of the fermented broth is comparable with that of some commercially available wines such as Day country, Capel and Royal Banquette (7.9-14.6%). The protein content (1.1%) of the fermented pulp syrup was comparable to that of cashew wine. The ascorbic acid content of the fermented broth was not detectable. This may have resulted from the fact that the very low ascorbic acid of the syrup was used up by yeast for enzymatic hydroxylation. Increase in TTA in the product may come from organic acids like pyruvic, succinic and ascorbic acids produced during the fermentation (Rancine, 1965). Our results suggest that the pulp syrup may be used as a substrate for making an alcoholic beverage especially wine.

Table 1:
Chemical composition of pulp syrup

Components	Value
TTA (%)	0.045
Crude protein (%)	2.75
Reducing sugar (%)	1.20
Ascorbic acid (mg/100 ml)	7.04

Table 2:
Chemical Analysis of fermented broth

Parameter	Value
Protein (%)	1.00
Reducing sugar (%)	1.10
TTA (%)	0.44
Alcohol (% v/v)	12.40
Ascorbic acid nondetected (mg/100 ml)	

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