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PRODUCTION AND EVALUATION OF SOME BIOCHEMICAL INDICES IN FERMENTED FRUIT PULP OF *P. Clappertoniana* (Locust bean) USING *Saccharomyces cerevisiae*

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ABSTRACT
The pulp of African locust bean fruit *Parkia clappertoniana* was utilized for production of table wine using *Saccharomyces cerevisiae* on must samples with total sugar 8.5^{o} brix, specific Gravity 1.047, pH 3.7 and fermented for 20 days at 28^{0}C. The produced wine had specific gravity (S.G) 1.03, total sugar 7.6^{o} brix, titratable acidity 0.57 at pH 3.2 and maximum alcoholic yield 9.4% (v/v) after 20 days of primary and secondary fermentations. The results of parameters in the wine sample compared favorably with some selected imported wines, and confirm the feasibility of wine production on small scale for cottage industries using the abundant and yet fully utilized pulp of *P. Clappertoniana* hitherto washed away in the process of locust bean condiment*Iru, Dawadawa, Ogiri* production in the tropics.

KEYWORDS: S. *cerevisiae,* fermentation, lag phase, flocculation, and pasteurization.

INTRODUCTION

Wines constitute products made by alcoholic fermentation of fruits or fruit juices, cereals, honey by direct conversion of their sugars; glucose, fructose and/or saccharose through enzyme-induced chemical alteration processes to ethanol and carbon (IV) oxide (Benda, 1959). Wines are regarded as classy alcoholic drink because of the expenses involved in procuring them for use based on their being mostly imported using foreign exchange as their local production on commercial scale is just emerging. Although wines are generally produced from carbohydrates materials, the nature of such materials used and the way they are processed largely determine the final characteristics of the wine (Amerine and Joslyn, 1970). Among the factors that may affect wine compositions are the changes to which wine is exposed during fermentation and ageing, temperature conditions and pH effects. These factors also determine the final aroma, fermentation duration and characteristics, degree of wine infections, sugar and alcoholic contents of wine. (Armerine *et al.*, 1980). Wines as alcoholic beverages are generally cherished on accounts of their flavour, stimulating effects and the pharmacological actions of some ingredients they contain (Amerine *et al.,* 1980).Fortified wines are additionally known to serve as energy, vitamins and mineral sources. The common wine yeast, *Saccharomyces cerevisiae* is known to thrive well in a medium containing utilizable energy source, carbon, nitrogen and inorganic salts and also shows enhanced tolerance to alcohol (Zoecklein *et al,* 1990). The monosaccharide sugars serve as the normal and perhaps the preferred medium for yeast cell growth in addition to a variety of other carbon sources like laevulose and dextrose (Amerine and Joslyn, 1970). However, under very high sugar contents of about 60 – 70%, most wine yeasts would not ferment sugar due to osmotic effect (Reed, 1995). Previous works on tropical fruits utilization for wine productions had been documented. Mango wine 8.0 – 10% (v/v) , Pawpaw juice 12.62% (v/v) and Cocoa juice wine 9.5 – 11.6% (v/v) alcohol content had been produced and charactersed. (Obisanya *et al*., 1987 Obayanju *et al*., 1992). One primary reason responsible for research interests of utilizing the locally sourced fruit pulp of *P. clappertoniana* in fruit wine production was the favorability of the middle belt climate of Nigeria to the growth and thriving of locust bean plant and the abundance of the raw material for use in the area of study. The second interest being the presently low utilization of the nutritious yellow fruit pulp of the plant containing 19% reducing sugars, 9% non – reducing sugars and 36% of other forms of carbohydrates (Oyenuga, 1968). The pulp is at best presently fed on by animals and used as sweeteners among the local populace. This study was aimed at exploiting the high levels of fermentable sugars in the fruit pulp for fruit wine production using the common wine yeast *Saccharomyces cerevisiae* to induce its fermentation, and thereafter evaluate the selected parameters in the wine samples.

MATERIALS

Collection of samples of the pulp of *P. clappertoniana* was done in Kutchi – Woro and Doko Villages in the suburbs of Bida, Niger State – Nigeria. The sun-dried pulp (2 days) was oven-dried (to about 5% moisture content) and used for the work. Brewer's yeast *Saccharomyces cerevisiae* already activated and ready for direct inoculation (obtained from the Quality Control Laboratory of the Production Unit of Nigerian Breweries Plc, Ibadan – Nigeria) was used on the must samples.

The experiment was carried out in the biochemistry laboratory of the Science Laboratory Technology

Department, Federal Polytechnic, Bida Niger State. Nigeria.

METHODOLOGY

The oven-dried *P. clappertoniana* fruit pulp (150g) was extracted using 1500 ml hot water to obtain the must and transferred into a 2000 ml capacity Erlenmeyer flask, pasteurized at 62.5^0C for 30 min and stored overnight. Inoculation of the sterile must sample was done with propagated yeast suspension in the ratio of 50ml of the yeast to 1500ml of must. Proper agitation was done and the inoculated must left at 28^0C under laboratory condition. Samples were periodically (at five day intervals) analyzed for sugar levels, acidity, specific gravity and alcohol content using standard methods.

At the end of primary fermentation, when the yeast cells had flocculated and a decrease in carbon (IV) oxide evolution noticed, first racking was carried out. Secondary fermentation was accomplished by re-inoculation with 20% of yeast cells from active fermentation. Samples were, as usual, collected over time as in primary fermentation and stipulated parameters determined before second racking. Decanted wine sample was subjected to fining using a mixture of 1% gelatin and 1% solution of tannic acid. The wine so treated was incubated for ten days at 4^0C. This was followed by pasteurization of the wine sample at 62.5^0C for 30 min in a thermo stated water-bath.

Determination of Acidity

The must and wine samples (50ml) were collected and the pH determined using (Jenway model 3070 portable automatic temperature compensation) digital pH meter. The PH value was read on the digital scale.

Determination of Specific Gravity

Must and wine samples (100ml) were collected into measuring cylinders and the specific gravity determined using a Brix hydrometer. The hydrometer was immersed and the specific gravity read off the scale.

Total Soluble Sugars Determination

The must and wine samples (1drop each) was placed on the receptor of automated digital bench refractometer and the sugar content directly read on the digital scale.

Quantitative Estimation of Reducing Sugars

The method of Plummer (1971) was used in the estimation and it involved the use of Benedict's quantitative reagent in titrimetric analysis.

Determination of Alcohol Levels

Wine sample (50ml) was analyzed for its alcohol yield using calibrated Peter Stevenson's wine hydrometer from where the alcohol % was read off the scale.

Statistical analyses

The data obtained from the above treatments were analyzed for variance using Duncan Multiple Range Test(DMRT) to locate differences among the sample means at 5% levels of significance and presented in the result table(1)

RESULTS

The results obtained for must and wine samples were presented in Table 1 while the Table 2 compares the wine characteristics as presented. There was an initial lag phase of five days before resumption of active fermentation followed by a progressive increase in alcohol yield. The peak of fermentation on day 14 was followed by a progressive decline in alcohol yield. Fermentation was carried out over a period of 20 days at the ambient laboratory mean temperature of 28^oC.The sugar levels and the specific gravity showed gradual decrease over the period of fermentation followed by an increase in alcohol yield; a reflection the efficiency of breakdown of fermentable sugar in the must. The low PH variation was indicative of the ability of the wine yeast to survive and produce alcohol at acceptable yield under the condition. The survival of the yeast cells even at 9.4 % alcohol content of the medium supports the resistance in yeast to moderate alcohol contents.

TABLE 1: Table of Wine Characteristics

Fermentation duration(days)	Total sugar (% brix)	Titratable acidity (%)	pH	Specific gravity(SG)	Temperature(^{o}C)	Alcohol content % (v/v)
1	8.5±0.2	0.50±0.1	3.7±0.01	1.047±0.2	27.8±0.01	0.1±0.01
5	8.5±0.2	0.53±0.1	3.6±0.03	1.057±0.02	29.5±0.01	5.8±0.03
1	7.8±0.4	0.55±0.2	3.6±0.2	1.035±0.4	28.2±0.02	8.0±0.01
15	7.6±0.2	0.57±0.3	3.5±0.01	1.032±0.4	28.0±0.03	8.6±0.04
20	7.6±0.1	0.57±0.1	3.3±0.03	1.031±0.3	27.3±0.01	9.4±0.02
Mean values ($\bar{x}$)	8.0	0.54	3.54	1.04	28.2	6.4
Standard deviation S.D (δ)	0.464	0.030	0.158	0.011	0.820	3.58
Standard Error (S.E)	0.197	0.013	0.071	0.005	0.367	1.601

TABLE 2. Comparison of selected wine Characteristics

wine type	balling(^{0}brix)	total acidity(g/100ml)	alcohol(%v/v)
APPLE	4.6	0.411	12.8
CHERRY	6.8	0.534	12.3
BLACKBERRY	8.2	0.890	12.2
RASBERRY	8.8	0.903	12.0
STANDARD WINE	10-15	0.1-0.5	10.2-14.2
*PARKIA FRUIT WINE	7.6	0.57	9.4

Source:(Yang and Weigand 1949(Obayanju and Ademokoya,1992)

DISCUSSION

The fermentation characteristics of *Parkiaclappertoniana* fruit wine monitored by CO_2 evolution showed a lag phase of about fourteen hours preceding the active fermentation and alcohol production. The lag phase marked the adaptation period of yeast cells to the must medium prior to their active growth and sugar metabolism. Active fermentation therefore effectively commenced after the lag phase and peaked on day 14 (Table1). The reduced progress of fermentation after day 14 could possibly be attributed to depleted sugar levels, decreased yeast cells population, or the likely inhibitory effects of high alcohol contents on yeast cell metabolism. The fermentation of the fruit pulp of *Parkia clappertoniana* produced wine that had comparable characteristics to wines hitherto produced from other tropical fruits like pawpaw, mango, cashew and orange, among others in terms of total acidity, residual sugar content and alcoholic yield. The *P. clappertoniana* fruit wine produced by the adopted procedures had maximum total sugar content 7.6% brix, titratable acidity 0.57%, pH 3.3, specific gravity 1.031 at 27.3 and maximum alcohol content 9.4%. It had comparable attributes with imported and other locally produced wines in terms of colour, taste and acceptability (Table 2). The comparatively lower alcohol yield for *Parkia clappertoniana* fruit wine may however, arise from the environmental production conditions of low pH (high acidity) and high temperature; factors known to affect metabolic activities, fermentation efficiency and life span of micro–organisms involved in biodegradation of the carbohydrates to alcohol and carbon (iv) oxide (Amerine *et al.,* 1980).This value however, still falls within the acceptable levels for wine production activities.

CONCLUSION

From the present level of success recorded in the utilization of fruit pulp of *Parkia clappertoniana* as raw material for fruit wine production, commercial scale production of the fruit wine is feasible in this agro-ecology and may save the nation some foreign exchange presently expended on wine importations. The raw materials are cheap, available and easy to store all year round to guarantee unhindered production off-season.

REFERENCES

Amerine, M. A. and Joslyn, M. A. (1970) Table Wines: The Technology of theirproduction, 2nd edition, University of California Press, USA.

Amerine, M. A., Kunkee, R. E., Ough, C. S., Singleton, V. L. and Webb, A. D. (1980) Red Wine Productions. The Technology of Wine Making.4th Edition. Avi Pub. Co Inc. USA.359-380

Bassir, O. (1968) Some Nigerian Wines *West Africa Journal of Biological and Applied Chemistry* 10(2):42 – 45.

Benda, I. (1959) Wine and Brandy, 293. Prescott and Dunn's Industrial Microbiology, 4th edition, USA.

Duncan, D.B. (1955) Multiple range and multiple F-tests. Biometrics.11 1-42.

Plummer, D. I. (1971) An Introduction to Practical Biochemistry, Tata McGraw Hill Publishing Co., New Delhi, India.

Obayanju, S. V. and Ademokoya, A. A. (1992) Utilization of Pawpaw Juice for Wine Making.*Journal of Technical Education* 10; 15-20.

Obisanya, M. O, Aina, J. O. and Oguntimehin, G. B. (1987) Production of Wine from Mango Juice using *S. cerevisiae* and *Schizo-saccharomyces. Journal of Applied Bacteriology* 63(3): 191 – 196.

Oyenuga, V. A. (1968) Nigerian Foods and Feeding Stuffs.Their Chemistry and Nutritive Values. 3rd Edition, Ibadan University Press, Ibadan.

Reed, G. (1959) Production of yeasts, Prescott and Dunn Industrial Microbiology, 4th Ed USA. p593.

Yang, H. Y. and Weigand, E. H. (1949) Production of fruitwines in the Pacific North- West. *Fruits Prod Journal* (29) 8-12, 27-29.

Zoeklein, B., W., Fulgelsang, K. C., Cump, B.H. and Nurry, F. S. (1990) Production and Wine Analysis 1st Ed.Avi Publication Co Inc Westport, Connecticut P 71-243.