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Growth of *Raphia farinifera* and *Elaeis guineensis* Wine Yeast on Cassava (*Manihot esculenta*) and Poultry Manure Media

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Abstract
The aim of this research was to determine the suitability of cassava (Manihot esculenta) and poultry manure (locally available resources) as substrates for the production of raffia and palm wine yeasts. Yeast growth was estimated as a measure of their medium's optical density at 600 nm ($OD_{600\ nm}$) after 36 h of growth. The various media used were formed from different combinations of cassava flour and poultry manure. The results obtained showed that the growth of the palm wine (Elaeis guineensis Jacq.) yeast in the locally formulated media 3:2, 4:1, 5:0 [poultry manure (PM)(g)/ cassava flour (CS)(g)], and yeast extract-peptone dextrose medium (YPD) was 2.101, 2.830, 3.131 and 2.057 respectively. The $OD_{600\ nm}$ measurement of the growth of the raffia wine (Raphia farinifera) yeast was 2.912, 2.859, 4.000, and 2.110 respectively in the same media above. This shows that formulations 3:2, 4:1, and 5:0 [PM (g)/CS (g)] are highly suitable for the growth of palm wine and raffia wine yeasts when compared with their growth in the standard medium (YPD). It was also an indication that they contain adequate amounts of reducing sugar and protein which served as sources of carbon and nitrogen respectively, for the growth of the two yeasts (palm wine and raffia wine yeasts). The results of this study indicate that cassava and poultry manure are suitable substrates for yeast production.

Keyword: African oil palm (*Elaeis guineensis* Jacq.), raffia palm (*Raphia farinifera*), yeast (*Saccharomyces cerevisiae*), growth media, poultry manure, cassava (*Manihot esculenta*) flour.

Introduction
Raffia wine is an attractive milky sap obtained from raffia palm (*R. farinifera*) while palm wine is that obtained from the palm tree (Arecaceae or Palmae). Leuconostoc and Lactobaccilus bacteria are present in the early stages of fermentation of raffia wine and palm wine, while *Saccharomyces cerevisiae* is mainly responsible for alcoholic fermentation[1]. *Saccharomyces cerevisiae* is perhaps the most useful yeast owing to its use since ancient times in baking and brewing[2]. The industrial production and commercial use of yeast started at the end of the 19^{th} century after their identification and isolation by Pasteur[3]. During commercial production, yeast is grown under carefully controlled conditions on a sugar containing media typically composed of beet cane molasses. Under ideal growth conditions, a yeast cell reproduces every two to three hours. Studies show that organic nitrogen sources, such as yeast extracts support rapid growth and high cell yields of microorganisms because they contain amino acids and peptides, water soluble vitamins and carbohydrates[4]. The basic carbon and energy source for yeast culture are sugars[5]. Starch cannot be used because yeast does not contain the appropriate enzymes to hydrolyze this substrate to fermentable sugars. Beets and cane molasses are commonly used as raw material because the sugars present in molasses, a mixture of sucrose, fructose and glucose, are readily fermentable. In addition to simple sugar, yeast (*S. cerevisiae*) also requires certain minerals, vitamins and salts for growth. The number of yeast cells increases about five to eight-fold during fermentation[6].

Cassava (*Manihot esculenta*) also known as manioc, tapioca or yucca, is one of the most important food crops in the humid tropics, being particularly suited to conditions of low nutrients' availability and is able to survive drought[7]. The major harvested organ of the cassava plant is the tuber, which is actually a swollen root commonly called cassava. Cassava is a source of calories for both human and animal feed[8]. The nutrient reserve of cassava is made up of starch which is consumed as food and used in industrial processes[9], although cassava leaves are sometimes consumed. The acid-catalyzed hydrolysis of starch is a complex heterogenous reaction. The molecular mechanism of acid catalyzed hydrolysis of starch involves cleavage of glucan bonds, mainly on the α (1,4) bond of amylase and α (1,6) bonds of amylopectin to produce several thousands of glucose residues. The reaction is regulated by both acid concentration and temperature and the physical state of starch[10]. The treatment of starch as a mixture of several glucans with concentrated H_2SO_4 show a reaction rate, two orders of magnitude higher than that of untreated starch about the same magnitude. The major hydrolytic product of

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cassava starch, glucose (a reducing sugar), can serve as a suitable carbon source for the production of *S. cerevisiae*.

Millions of kilograms of nitrogen collected and disposed off each day in animal manure and municipal waste represent a valuable reservoir of nitrogen potentially for conversion to protein for livestock and poultry feeds as well as other valuable products[11]. However, the so called waste materials are now coming under close scrutiny because of increased demand for plant proteins for food[12,13,14]. Various studies have been carried out on the utilization of poultry manure on various applications[15,16]. This study estimated the biochemical parameters as indices of growth of *Raphia farinifera* and *Elaeis guineensis* Jacq wine yeast on media formulated from cassava and poultry manure.

Materials and Methods

Materials

Cassava tubers were purchased from Otorho-Agbon main market, Delta state, Nigeria. The tubers were peeled and thoroughly washed (to reduce cyanide content). They were then sliced into small pieces, dried under the sun, after which it was ground to flour and passed through a sieve of 0.25 mm before it was stored at room temperature ready for use. While poultry manure was purchased in a poultry farm at the University town of Abraka, Delta state, Nigeria. It was separated from saw-dust and other debris and then dried under the sun and mashed to fine particles using mortar and pestle after which it was passed through a sieve of 0.25 mm. This study was carried out in the Biochemistry Department of the Delta State University, Abraka – Nigeria and African Research Laboratory, Otorho-Agbon - Nigeria, between 4th – ***25th*** October, 2010.

Methods

Sulphuric acid (0.5%, w/v) was used to hydrolyze 20% (w/v) of cassava flour and 20% (w/v) of poultry manure, respectively, and heated to dryness. A mixture of the acid-hydrolyzed poultry manure (g) and cassava flour (g) (in powdered form) in various ratios of 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0, respectively were used to formulate local growth media. These formulated media, alongside with YPD (as control), were then inoculated with raffia wine and palm wine yeasts (*S. cerevisiae*) and left to grow for 36 h at 30°C. Assays were carried out in triplicate and expressed as mg reducing sugar/mg of sample or as mg protein/ml of sample and YPD. The total content of reducing sugar was determined[17], while protein was determined by the method of Gornal et al[18]. The pH values of media were determined before and 36 h after inoculation using the Extect pH meter. The growth of the raffia wine and palm wine yeasts was estimated as a measure of their medium's optical density at 600 nm ($OD_{600\ nm}$).

Statistical analysis

The results were expressed in mean ± SD. The one way analysis of variance (ANOVA) was used for the evaluation of statistical significance.

RESULTS

Total reducing sugar contents

From the results in Table 1, growth media formulation 0:5 [poultry manure (g)/ cassava flour (g)] yielded 0.349 ± 0.011 mg reducing sugar/ml of the medium, while formulations 1:4, 2:3, 3:2, 4:1 and 5:0 [poultry manure (g)/ cassava flour (g)] yielded 4.935 ± 0.003, 4.737 ± 0.02, 4.567 ± 0.381, 4.961 ± 0.055 and 5.248 ± 0.549 mg/ml of total reducing sugar, respectively, before inoculation with the raffia wine and palm wine yeasts. Among the growth media formulated for the growth of raffia wine yeast, the lowest residual concentration of reducing sugar (0.031 ± 0.002 mg/ml) was found in the 0:5 (PM:CS) medium after the duration of growth, followed by 2:3 (PM:CS) medium (0.263 ± 0.011 mg/ml). Similarly, among the growth media formulated for the growth of palm wine yeast, the lowest residual concentration of reducing sugar (0.085 ± 0.004 mg/ml) was found in the 0:5 (PM:CS) medium after 36 h of growth, which was also followed by the 2:3 (PM:CS) medium (0.358 ± 0.012 mg/ml).

Total soluble protein contents

The results of the total protein content obtained from the acid-hydrolyzed poultry manure are as shown in Table 2. While formulation 0:5 [poultry manure (g)/ cassava flour (g)] yielded 0.651 ± 0.101 mg protein/ml of the medium, formulations 1:4, 2:3, 3:2, 4:1 and 5:0 [poultry manure (g)/ cassava flour (g)] yielded 4.000 ± 0.225, 8.450 ± 0.656, 10.683 ± 0.483, 12.376 ± 0.208 and 14.462 ± 0.597 mg/ml of total protein, respectively.

pH values of media

Table 3 shows the pH values of the different formations and YPD, a standard yeast growth medium. The formulated growth media were neutral (pH 7) before inoculation with the respective yeast cells from raffia and palm wines. However, after a growth period of 36 h, the media in which raffia yeast cells were grown turned alkaline with a pH range of 8.8 -10.7 (Table 3). Similarly, the media containing grown palm wine yeast cells also turned slightly alkaline with a pH range of 8.5 – 9.7 with the exception of formulation 0:5 (PM:CS) that had a pH of 7.7. The YPD media used as standard for both raffia and palm wine yeast cells had pH of 7.4 and 5.5, respectively.

Growth of raffia wine and palm wine yeasts

Growth of yeast cells (*S. cerevisiae*) was observed in all the cassava flour/poultry manure locally formulated media (Table 4). The growth of both raffia and palm wine yeast cells in some of the local media (3:2, 4:1, and

5:0) was comparable with their respective growth in YDP media. While the growth of the palm wine yeast in the locally formulated media 3:2, 4:1, 5:0 [PM(g)/CS(g)], and YPD was 2.101, 2.830, 3.131 and 2.057, respectively, that of the raffia wine yeast was 2.912, 2.859, 4.000, and 2.110, respectively.

Results and Discussion

This experimental design seeks to explore the use of cassava flour as a source of reducing sugar (carbon) and poultry manure as a source of nitrogen in the production of palm wine and raffia wine yeasts culture media[19]. This is due to the enormous presence of starch in cassava (which can easily be broken down to yield simple sugars) and also the relative high availability of cassava plants and tubers and poultry waste or droppings in nature[20,21]. Acid hydrolysis of cassava flour was used for the breakdown of the complex polysaccharide because of the advantages it has over saccharification by enzymes. The processes involved in acid hydrolysis of cassava flour are fast, cheap, high yielding and not affected by contamination[22].

In order to decipher the extent of suitability of the locally formulated acid-hydrolyzed cassava flour/poultry manure media for the growth of African oil palm (*Elaeis guineensis* Jacq.) wine yeast and raffia palm (*Raphia farinifera*) wine yeast as compared to the standard yeast culture medium (YPD), the amounts of reducing sugar, protein, and pH values of the locally formulated media and YPD were determined before and after inoculation of the media (Tables 1, 2, 3), followed by estimation of the growth of the two yeasts, raffia wine and palm wine yeasts (OD_{600nm}).

The results obtained in this study (Table 1) showed that the hydrolyzed cassava flour/poultry manure gave a high yield of reducing sugar which could serve as a good and cheap carbon source for the growth of palm wine and raffia wine yeasts production. This is in agreement with the findings of an earlier study which observed that the hydrolytic product of cassava starch – glucose (a reducing sugar), serves as a suitable substrate in providing carbon source for the growth of yeast (*S. cerevisiae*)[10]. The results confirm the work of Osumah and Tonukari[11] that showed that, yeast biomass (at $OD_{600\ nm}$) increases with increasing amount of acid hydrolyzed cassava as carbon source.

Similarly, the results of estimated amounts of total soluble protein (Table 2) in the locally formulated yeast growth media also showed that the local media have good amounts of protein which could serve as sources of nitrogen for microbial growth. The relatively high protein content of the media is believed to have emanated from the hydrolyzed poultry manure present in the combination. This again suggests that yeast growth culture can be enhanced with the use of poultry manure to serve as an alternative to peptone, which is commonly used as nitrogen source for the growth of yeast.

Table 1. Reducing sugar content of locally formulated media before and 36 h after inoculation with raffia wine and palm wine yeasts.

Formulated medium [(PM (g):CS (g)]	Reducing sugar (mg/ml) before inoculation	Reducing sugar (mg/ml) 36 h after inoculation with raffia wine yeast	Reducing sugar (mg/ml) 36 h after inoculation with palm wine yeast
0:5	0.349 ± 0.011	0.031 ± 0.002[a,c]	0.085 ± 0.004[a,c]
1:4	4.935 ± 0.003	1.668 ± 0.007[a,c]	0.462 ± 0.054[a,c]
2:3	4.737 ± 0.002	0.263 ± 0.011[a,c]	0.358 ± 0.012[a,c]
3:2	4.567 ± 0.381	0.445 ± 0.002[a,c]	1.180 ± 0.064[a,c]
4:1	4.961 ± 0.055	0.424 ± 0.003[a,c]	2.850 ± 0.008[a,c]
5:0	5.248 ± 0.549	1.051 ± 0.007[a,c]	5.496 ± 0.253[a,c]
YPD	5.005 ± 0.003	0.539 ± 0.002[a]	3.190 ± 0.555[a]

Results are expressed as mg/ml of sample mean ± standard deviation of mean. PM represents acid-hydrolyzed poultry manure (g); CS, acid-hydrolyzed cassava flour (g).
[a]Values of reducing sugar content that are significantly different ($p< 0.05$) from the corresponding values before inoculation.
[b]Values of reducing sugar content that are not significantly different ($p> 0.05$) from the corresponding values before inoculation.
[c]Values of reducing sugar content that are significantly different ($p < 0.05$) from YPD values.
[d]Values of reducing sugar content that are not significantly different ($p> 0.05$) from YPD values.

Table 2. Concentration of protein (mg/ml) in local media inoculated with palm wine and raffia wine yeasts.

Formulated medium [(PM (g):CS (g)]	Protein content (mg/ml) before inoculation	Protein content (mg/ml) after inoculation with palm wine yeast	Protein content (mg/ml) after inoculation with raffia wine yeast
0:5	0.651 ± 0.101	0.438 ± 0.104[a,c]	0.073 ± 0.032[a,c]
1:4	4.000 ± 0.225	2.049 ± 0.138[a,c]	2.680 ± 0.270[a,c]
2:3	8.450 ± 0.656	2.704 ± 0.902[a,c]	2.049 ± 0.122[a,c]
3:2	10.683 ± 0.483	2.532 ± 0.888[a,c]	3.327 ± 0.241[a,c]
4:1	12.376 ± 0.208	4.274 ± 0.071[a,d]	3.835 ± 0.122[a,c]
5:0	14.462 ± 0.597	4.563 ± 0.003[a,c]	4.199 ± 0.045[a,c]

YPD	2.229 ± 0.032	4.08 ± 0.041[a]	4.056 ± 0.055[a]

Results are expressed as mg/ml of sample mean ± standard deviation of mean. PM represents acid-hydrolyzed poultry manure (g); CS, acid-hydrolyzed cassava flour (g).
[a]Values of protein content that are significantly different ($p < 0.05$) from the corresponding values before inoculation.
[b]Values of protein content that are not significantly different ($p > 0.05$) from the corresponding values before inoculation.
[c]Values of protein content that are significantly different ($p < 0.05$) from YPD values.
[d]Values of protein content that are not significantly different ($p > 0.05$) from YPD values.

The optimum growth of yeast as shown by researches is best in the presence of carbon and nitrogen sources; and glucose residues (or reducing sugars), as well as proteins, in particular, have been notable as important carbon and nitrogen sources for the growth of microorganisms including yeast in which these molecules play a major role in biosynthetic pathways, energy generation and growth of microbes[5,23]. From the results of growth of raffia and palm wine yeast cells observed in this study (Table 3), it could be said that formulations 3:2, 4:1, and 5:0 [PM (g)/CS (g)] are highly suitable for the growth of palm wine and raffia wine yeasts when compared with the standard medium (Yeast extract-Peptone-Dextrose medium) in the growth of the raffia and palm wine yeast cells are comparable. This appears to be so since these particular formulations, 3:2, 4:1, and 5:0 [PM (g)/CS (g)], are very rich in reducing sugar and total protein contents (Tables 1, 2) that probably served as sources of carbon and nitrogen for the growth of the raffia palm (*R. farinifera*) wine and African oil palm (*E. guineensis* Jacq.) wine yeasts (*S. cerevisiae*). Aside the nitrogen content of poultry manure (a major constituents of these formulations), researches have also proved that it contains some vital mineral such as phosphorus, potassium, calcium and magnesium which can as well aid in the growth and development of yeast and fungi[24,25,26]. Hence, yeast cells in these formulated media having high amount of hydrolyzed poultry manure (3:2, 4:1, and 5:0 [PM (g)/CS (g)]) probably have harnessed the presence of minerals in the media to optimize their growth as compared to other media where the PM is relatively lower (0:5, 1:4 and 2:3). The findings of this study therefore suggest the recycling of poultry droppings (PM), a readily available environmental waste, since it appears to be an efficient substitute for peptone in the formulation of growth media for yeasts (*S. cerevisiae*).

Table 3. Growth of raffia wine and palm wine yeasts (*S. cerevisiae*) in the locally formulated media and YPD.

PM (g):CS (g) formulated medium	Growth of palm wine yeast (OD_{600nm})	Growth of raffia wine yeast (OD_{600nm})
0:5	0.087	0.113
1:4	1.140	1.447
2:3	1.143	1.412
3:2	2.101	2.912
4:1	2.830	2.859
5:0	3.131	4.000
YPD	2.057	2.110

The optical density at 600 nm is a measure of the microbial (yeast) growth. PM, Acid-hydrolyzed poultry manure (g); CS, acid-hydrolyzed cassava flour (g).

Table 4. pH values before and 36 h after inoculation with raffia wine and palm wine yeasts.

Formulated medium [(PM (g):CS (g)]	pH value before inoculation	pH value 36 h after inoculation with palm wine yeast	pH value 36 h after inoculation with raffia wine yeast
0:5	7.0 ± 0.1	7.7 ± 0.1[a,c]	8.8 ± 0.1[a,c]
1:4	7.0 ± 0.2	8.5 ± 0.1[a,c]	9.2 ± 0.1[a,c]
2:3	7.0 ± 0.1	9.4 ± 0.1[a,c]	10.4 ± 0.2[a,c]
3:2	7.0 ± 0.1	9.6 ± 0.1[a,c]	10.5 ± 0.2[a,c]
4:1	7.0 ± 0.1	9.6 ± 0.1[a,c]	10.7 ± 0.1[a,c]
5:0	7.0 ± 0.2	9.7 ± 0.2[a,c]	10.7 ± 0.2[a,c]
YPD	7.0 ± 0.1	5.5 ± 0.1[a]	7.4 ± 0.1[a]

Results are expressed as mean ± standard deviation of mean. PM represents acid-hydrolyzed poultry manure (g); CS, acid-hydrolyzed cassava flour (g).
[a]Values of pH that are significantly different ($p < 0.05$) from the corresponding values before inoculation.
[b]Values of pH that are not significantly different ($p > 0.05$) from the corresponding values before inoculation.
[c]Values of pH that are significantly different ($p < 0.05$) from YPD values.
[d]Values of pH that are not significantly different ($p > 0.05$) from YPD values.

Variation in pH values of the locally formulated hydrolyzed cassava flour/poultry manure media after the period of growth of the raffia and palm wine yeast cells was also determined using a sensitive pH meter. The result shows that the lesser the proportion of cassava flour (g) in each formulation, the less acidic the media were (i.e the higher the pH value) (Table 4). It was also noted that the local growth media formulations with lower amounts of hydrolyzed cassava flour (and higher amounts of hydrolyzed poultry manure) supported the optimum growths of yeasts observed (Tables 3 and 4). Results of some former studies show that yeast cells grow better at neutral pH but their growth slightly declines as pH tilts towards alkalinity[6,11]. Maximal growth declination, however, is usually obtained at acidic pH especially when the medium pH is lower than 4. The findings of this study are in agreement with the previous reports[6,11].

Conclusion

The method described in this research work can be applied on an industrial scale for the production of yeasts (*S. cerevisiae*) isolated from African oil palm (*E. guineensis* Jacq.) wine and raffia palm (*R. farinifera*) wine. The research has taken the advantage of using cassava flour, in its rich content of starch as a carbon source, and poultry manure extract as an alternative nitrogen source to peptone because of its ready availability, for the production of raffia wine and palm wine yeast. Formulations 3:2, 4:1, and 5:0 [PM (g)/CS (g)] appeared to be highly suitable for the growth of palm wine and raffia wine yeasts when compared with their growth in the standard medium (YPD). The result of this study showed that Raffia and palm wine yeasts production can be achieved using these readily available local raw materials thus reducing the dependency on the importation of standard growth media constituents like Peptone, yeast extract and dextrose sugar.

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