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Potential Use of Cassava Peels as Microbiological Culture Media

Ukpong, S. E.[1*]; Magnus, O. C.[2]; Okoye, A. C.[3]; Okeke, N. A.[4]; Mini, P. U.[5]

[1]Department of Microbiology, Faculty of Science, Madonna University Elele, Rivers State.
[2]Department of Pharmacology and Therapeutics, Faculty of Science, Madonna University Elele, Rivers State.
[3] Department of Industrial Chemistry, Faculty of Science, Madonna University Elele, Rivers State.
[4]Department of Microbiology, College of Natural and Applied Science, Michael Okpara University of Agriculture Umudike, Abia State.
[5]Department of Microbiology, Federal University of Environment and Technology, Sakpenwa/Koroma, Ogoni, Rivers State.

Corresponding Author: Ukpong, S. E.[1*]

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Abstract: Investigation on the use of cassava peels as potential microbial growth media was carried out, using standard microbiological procedures. The bacteria, *Bacillus subtilis, Enteobacter aerogenes and Leuconostoc mesenteroides* and the fungi, Saccharomyces *cerevisiae and Aspergillus fumigatus* were isolated from fermented cassava peels, fermented pulp and from Outside environment (soil, palm-wine and stool samples). The measurement of their growth rate, generation time, radial growth, optical density and biomass production on un-supplemented cassava peel extract agar (UNSCPA) and broth (UNSCPB), supplemented cassava peel extract agar (SCPA) and broth (SCPB) was carried out using synthetic media Malt Extract Agar (MEA), malt extract broth (MEB), Nutrient agar (NA) and Nutrient broth (NB) as controls. Results of chemical analysis of the peels showed the following values 5.4%, 11.4%, 15.4%, 2.06%, and 65.74% for ash fraction, fiber fraction, lipid fraction, protein fraction, and carbohydrate fraction respectively. The protein fraction, of the peel was lower than that of the synthetic media (5%) which was then enriched with urea and sodium nitrate before inoculation. The cyanide level of the fresh cassava peels was reduced from 21.692mg/1 00g to 17.463mg/100g. Data analysis was carried using students t-test which revealed no significant difference (P>0.05) in radial growth between molds cultivated on SCPA, MEA, UNSCPA and SCPA, although growth rates on MEA (38.4cm) and SCPA (35.2cm) were better than on UNSCPA (24.5cm). Minor alterations in sizes of asexual spores and reproductive hyphae were also observed between molds grown on MEA than on SCPA. Optical density of the test bacteria and yeast after 24hours incubation statistically revealed significant differences (P<0.05) between the growth of test isolates on (UNSCPB, SCPB, MEB and NB). The results showed attenuated growth on the UNSCPB (0.966nm) but the growth on SCPB (1.114nm) aligned with the control (1.362nm). Cassava peels can, therefore, be used as a functional substitute for expensive synthetic media when integrated with nitrogenous sources.

Keywords: *Cassava Peels, Potential Use, Microbiological Culture Media, Radial Growth, Biomass Production and Optical Density.*

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I. INTRODUCTION

A significant challenge experienced by agro-processing industries in developing countries is the management of bye-products. The release of agricultural wastes on land and into water bodies is common, and has become a crucial source environmental hazard (Ukpong *et al.,* 2025). Poorly managed methods of disposal of solid waste result in serious hazard to public health, including contamination of air and water resources, accident hazards, and increase in rodent and insect vectors of disease, otherwise interfering with community life and development (Raphel *et al.,* 2024). The failure or inability to recover and reuse such materials economically results in the unnecessary waste and depletion of natural/resources (Asghar *et al.,* 2025).

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At present, emphasis is on biological transformation of plant wastes, especially agricultural waste into useful products. Microorganisms especially fungi, are known organic waste degraders and are generally capable of hydrolyzing complex organic compounds as a major source of energy (Abdeen *et al.,* 2025) Cassava peel is an agricultural residue, which is obtained from gari and fufu processing, and are normally disposed as waste and allowed to rot in the open, thus resulting in health crisis. About 10 million tonnes of cassava are processed into gari annually in Nigeria alone. Since these peels could make up to 10% of the net weight of the roots, they make up an important potential resource for animal feeds if properly formulated by bio-system, as a good substrate for microbial growth and for production of enzymes such as amylolytic enzyme (Abdulmumini *et al.,* 2025).

Cassava crop () also known as Yuca or Manioc, it is a perennial shrub with a relatively short lifespan, that is vegetatively propagated. It is grown throughout South America, Afriça and Asia (Reilly, Gomez-Vasquez, Buschmann *et al.,* 2007). Cassava is a core food in these regions of the world. It requires at least eight months of warm weather to produce the tuber, thus it is traditionally cultivated in a savanna, in terms of annual production, it is the fifth most important food crop after maize, raize, wheat and potato (FAOSTAT, 2011). Traditional, cassava roots are processed in several ways that vary from one region to another resulting in different end-products like gari, fufu, lafun, tapioca, pande and yucca. Despite all the usefulness of cassava, its usefulness as food source is limited by its fragility, low protein content and its toxicological risk (Andrew, 2002).

The roots of cassava are very rich in starch and contain significant amounts of calcium (50mg/100g), phosphorus (40mg/100mg) and vitamin C (25mg100g) (Ravindran, 1992). However, they are poor in protein and other nutrients. In contrast, cassava leaves are a good source of protein and rich in the amino acid, lysine though deficient in methionine and possibly tryptophan (Ravindran, 1992). Cassava roots and leaves should not be ingested uncooked because they contain two cyanogenic glucosides, linamarin and lotaustralin. These are degraded by linamarase, a naturally occurring enzyme in cassava, liberating hydrogen cyanide (HCN) (Cliff, Maquingue, Nhassico, Nzwalo, and Bradbury, 2011). Cassava varieties are often grouped as either sweet or bitter variety respectively signifying the absence or presence of toxic levels of cyanogenic compound (Ovie *et al.,* 2025) From the sweet cultivar of cassava, cultivars can produce as little as 20mg of cyanide/kg from fresh roots, whereas bitter ones may produce more than 50 times as much (1g/kg). Cassava grown during dry season is particularly high in these toxins. Both varieties of cassava could be used in making good meals, but there is need to understand that the bitter variety must undergo some detoxication processes such as soaking, cooking and fermentation to avoid getting ill and in some cases death, when they are eaten (Oboh *et al.,* 2003).

A growth medium or microbiological culture medium is a liquid or gel formulated to enhance the growth of microorganisms or cells. There are different types of media for growing different types of cells. The two major types of growth media involve those used for cell culture, which use specific cell types derived from plants or animals, and microbiological media, which are used for culturing microorganisms. The most common growth media for culturing microorganisms are nutrient broths and agar. Purpose built media are sometimes required for microorganisms and cell culture growth (Cooper, 2000). Nutritionally demanding organisms can mainly grow on specialized culture media due to their complicated nutritional requirements (Ryan and Ray, 2004). Some of these media are now too financially demanding in developing countries. There is, therefore, need to explore the possible use of cassava peel as a reliable, low cost readily available, easy to use and substitute media for culturing microorganisms on a commercial scale.

II. MATERIALS AND METHODS

➢ *Sources of Raw Materials*

The cassava peels used for this work was obtained from Safe Foods Processing Farms, a private based agro-processing company working with value edition located in Uyo, Akwa Ibom State, Nigeria.

➢ *Chemical Analysis of Cassava Peels*

The cassava peels were washed with distilled water, sun dried for two weeks and then ground to powder using a domestic (moulinex) optiblend 2000 model AAW 941/7l1-10050) and sieved with 0.5mm mesh. The ground powder was preserved in a sterile airtight glass container. The methods for the evaluation of dry matter, protein fraction, fat fraction, fiber fraction, ash fraction and total carbohydrates as well as the ascorbic acid content were those described by, Kehinde-Olayanju *et al.,* (2025). A known mass of the dry powdered sample was ashed at 600^0C in a muffle furnance for 4hrs. The ash was dissolved in 6ml HCI solution and the resulting solution was made up to a definite volume and used for the determination of mineral elements. Phosphorus was evaluated calorimetrically at 440nm using the molybdovanadate reagent as describe by AOAC (1990). Sodium and potassium were determined with a flame photometer, while other mineral elements were determined using an Atomic Absorption Spectrophotometer (Horwitz, 1980). The determinations were carried out in triplicates model.

➢ *Determination of Cyanide in the Peels*

The determination of cyanide level was carried out using alkaline picrate method as described by Udezi (2025), The cassava peels were washed with distilled water and then ground to paste using domestic blender (moulinex) optiblend 2000 model AAW 941/711 10050). One gramme was meticulously weighed into 10ml of distilled water, in a conical flask and allowed to incubate overnight. It was then filtered to obtain the clarified solution for cyanide determination. One miligramme of the clarified solution was transferred into a corked test tube and then 4ml of alkaline

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picrate were added and the mixture was incubated in water bath for 5 minutes, after which a red-brown color was seen. The absorbances of the mixture was detected in the spectrophotometer at 490nm. The absorbance of the blank containing only one mill distilled water and 4ml alkaline picrate solution was also read. The cyanide content of the sample was estimated from the cyanide standard curve which was made of the mixture of potassium cyanide solution, where lg of KCN was dissolved in 10ml of water while lg of picrate and 5g of sodium carbonate were dissolved in 200ml volume of slightly warm water.

➢ *Preparation of Cassava Peel Media*

Two treatments were carried out. In the first treatment, the washed cassava peels were sun dried for 30 minutes. Five hundred grammes of the dried peels were submerged completely in 1500ml of distilled water for one hour before boiling for one hour to get the extract. After cooling, the extract was filtered to obtain the clarified solution and the original pH of the clarified solution was detected to be 2.65. From the clarified solution, 500ml were measured into 1000ml conical flask and sterilized by autoclaving at 121^0C for 15 minutes. This was used as the cassava peel extract broth. To another 500ml of the clarified solution contained in 1000ml conical flask, 10g of agar powder was measured into it, this served as cassava peel extract agar medium. It was sterilized by autoclaving at 121^0C for 15minutes. The media were allowed to cool, 50ml of the cassava peel extract agar was aseptically transferred into sterile disposable petri dishes and was allowed to set.

In the second treatment, the washed cassava peels were sun dried for two weeks and then ground to powder using a domestic blender (moulinex) optiblend 2000 Model AAW 941/711-10050) before sieving with 0.5mm mesh. The ground powder was preserved in a sterile airtight glass container. The cassava powder (200g) was carefully weighed into 800ml of distilled water with l6g of agar powder added and boiled for 45 minutes, then sterilized by autoclaving at 121^0C for 15 minutes as described by Ukoima *et al.,* (2009). The medium was allowed to cool after which 50ml of the cassava peel extract medium were aseptically transferred into sterile disposable petri-dishes and allowed to set. These served as the un-supplemented cassava peel extract medium, because here the original pH of the extract 3.35 was maintained and no possible additive was added. Furthermore, for the supplemented cassava peel extract agar and broth, the original pH of 2.65 and 3.35 were adjusted to 5.6 for fungal isolates and 7.4 for bacterial isolates, through gradual addition of sodium hydroxide (NaOH) using a pH meter, to obtain the required pH after which a buffer solution of potassium hydrogen phosphate (K H, PO4) was added before sterilization to avoid the fluctuation of the pH of the media either towards acidic or alkaline. One percent each of urea and sodium nitrate were added as necessary nitrogenous supplement before inoculation of the isolates was done.

The conventional media that were used in this research include malt extract agar, MacConkey agar, Malt Extract agar, nutrient agar and Reinforced Clostridium medium were prepared according to manufacturer's specification (Merck KGaA (2023). The content of the media and their preparations are shown in appendix · After preparations, the broth and agar media were sterilized by autoclaving at 121'C for 15 minutes. After cooling, 50ml of each of the media were aseptically poured out into disposable Petri-dishes and were allowed to set. Exactly 9.9ml of each broth were introduced into some sterile test tubes.

➢ *Sources of Test Organisms*

The microorganisms used in this study were originally isolated from fermented cassava peels and pulp, stool samples of patients from the University of Uyo Teaching Hospital Uyo, and soil samples from University of Uyo school garden using the spread plate technique (Heperkan and Alperdan, 1998). The isolated bacteria obtained were identified based the integration of colonial morphology and biochemical tests such as; motility tests, catalase, sugar fermentation test, coagulase and gram staining and so on, as outlined by described by Giwa *et al.,* (2012). Through these tests, the cultural and biochemical attributes of the isolates were evaluated. The fungi isolates were characterized and identified according to procedures by Natalic (2001).

➢ *Determination of Radial Growth Rate and Variation in Asexual Spore size of Mold Grown on Cassava Peel Media and Synthetic Media*

The response of the isolates on cassava peel extract agar and Malt extract agar was assessed by determining the radial growth rates of the culture at room temperature 28°C. During this process, sterile 20cm diameter-petri dishes containing 20ml of the basal nutrient agar (Cassava PEA) per plate were inoculated centrally with 5mm diameter agar plugs (inoculum) with the aid of a sterile cork borer from the margin of 3 days old actively growing colonies of *Aspergillus fumigatus* maintained on the approximate medium (Cassava PEA or MEA) were determined. The length and width of the reproductive hyphae (conidiophores) in *Aspergillus fumigatus* as well as the diameter of the asexual spores (conidia) of the isolates cultured on the un-supplemented cassava peel agar, supplemented cassava peel and malt extract agar was determined using calibrated ocular micrometer.

➢ *Estimation of Biomass Measurement on Cassava Peel and Synthetic Media*

The submerge culture technique was used. Spore suspensions were formulated from slant culture of the test fungi as described by Kowaiski, *et al*. (2022) The cultures were flooded with sterile physiological saline. The asexual spores were dislodged into the solution using a sterile wire loop, the suspension spores were then extracted to remove mycelial aggregates of spores using four layers of sterile cheese cloth, and then diluted to obtain spore count of 1.2 x 10^6 spores per ml. The spore concentrations were determined by direct counting and haemocytometer (Agina, 1991; Essien, 2002). Three sets of twelve 250ml Erlenmeyer flasks (the first containing 50ml of un-supplemented cassava peel broth, (UNSCPB), the second containing supplemented cassava peel broth (SCPB) and third containing malt extract broth. (MEB) all of the same volume), were inoculated with

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lml aliquot of the mold spores, the flasks were incubated at 28^0C in an orbital incubator with agitation at 180 rpm. Biomass production on the UNSCPB, SCPB and MEB substrate was assessed on weekly basis by determining the mycelial dry weight. In this case the mycelia of the cultures were harvested by extracting the clarified solution out, washed with several changes of cold distilled water, dried to a constant weight in an oven at 60°C and then checked in a chemical balance.

➢ *Determination of Bacterial Growth on Cassava Peel Media and Synthetic Media*

The total viable bacterial count using the streak plate method as described by Giwa *et al.,* (2012) was used in estimating the number of microbial colonies formed on the plates after incubation. Two plates were used for each dilution factor, after incubation, each of the plate was divided into four parts, The quadrant with more discrete colonies was counted and the sum was multiplied by four to give the total colony count on the plate. In this calculation, the microbial population in agar plates is usually expressed as colony forming unit/ml (cfu/ml).

➢ *Determination of Optical Density*

This was used as an index of microbial growth in the broth (Mira *et al.,* 2022), using a spectrophotometer (SPECTRONICS® GENESYS), the absorbance was measured at 600nm wavelength. The spectrophotometer was zero at 600nm with a blank (distilled water) in the cuvette (cell); all the sterile broth media used were used as control, three quarters of the cuvette were filled with each control and placed in the cuvette chamber at a wavelength of 600nm. The absorbance of all the control media was taken. After this the cuvette was rinsed with distilled water. The outside walls of the cuvette were wiped with cotton wool. The readings of each dilution factor were taken also at 600nm as in the case of the control. After each reading, the cuvette was rinsed with distilled water and then wiped with cotton wool.

➢ *Influence of Un-supplement Cassava Peel Extract Medium, Supplemented Cassava Peel Extract Medium and Synthetic Media on the Growth of selected Species of Bacteria and Yeasts*

Bacteria and yeast growth and utilization of nutrients was determined by viable cell counts on supplemented cassava peel agar, unfortified cassava peel agar, malt extract agar and nutrient agar respectively using the standard spread plate technique. (Harrigan and McCance, 1990; Zuberer, 1994. Using the densities derived from viable counts, the number of generation (n), generation time (g) and growth rates (Gr) of the microbial consortia were calculated as follows:

$$n = \frac{log_{10}b - log_{10}B}{log_{10}2}$$

Where n is the number of generations, B indicates bacterial count at zero time, while b refers to bacterial count at the end of a given period. Generation time (g) is equal to "t" (time which elapsed between b and B) divided by the number of generations (n) as shown in equation below:

$$G_t = \frac{t}{n}$$

The growth rate (G_r) was estimated as

$$G_r = \frac{n}{t} = \frac{1}{g}$$

Analysis of variance, the Duncan Multiple Range test (DMRT) were carried out to determine the significant differences and the least significant differences (LSD) between treatments using standard statistical procedures as described by Sokal and Rohf (1981).

III. RESULTS

➢ *Chemical, Mineral and Cyanide Composition of Cassava Peels*

The results gotten from the analysis of the chemical and the elemental composition of Cassava peels is presented in Table 2, respectively. The results revealed that the carbohydrate content of cassava peels had the` highest value (65.74) and the protein content recorded the lowest value (2.06%) while comparative concentration of 15.4 % > 11.4% > 5.4% were recorded for total lipid, total fibre and total ash respectively. The mineral content of the cassava peels is also displayed in Table 2, with minerals such as calcium, sodium, phosphorus, potassium, iron, magnesium and ascorbic acid, with correspondent values 51, 0.10, 0.10, 0.25, 16, 3 and 30mg respectively. Sodium recorded the highest value while calcium recorded the lowest value. These elements available in cassava peels shows the possibilities of using these peels as microbial substrates. Also presented in table 2, is the results of cyanide content of fresh, boiled and sun-dried cassava peels. The cyanide content of the fresh cassava peels (21.692mg/100g) was higher than the cyanide content of the boiled (20.191 mg/100g) and sun-dried cassava peels (17.463mg/1 00g).

➢ *Morphological and Biochemical Characteristics of Bacterial Isolates from Fermented Cassava Peels and Pulp*

Table 3. shows the biochemical and morphological features of the bacterial isolates from fermented cassava peels and pulp, respectively. The characteristics of the isolates showed that different bacteria were isolated from fermented cassava peels and pulp such include; *Bacillus subtilis, Bacillus cereus, Streptococcus pyogenes Clostridium perfrigens, Enterobacter aerogenes and Leuconostoc mesenteroide*. The bacteria isolated exhibited two basic shapes which include the rod and spherical shapes.

➢ *Morphological, Characterization and Identification of Yeast and Filamentous Fungal Isolates from Fermented Cassava Peels and Pulp*

Table 4. shows the characterization and identification of the fungal isolates from fermented cassava peels and pulp respectively. The characteristics of the isolates showed that

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different fungi were obtained from the above mention samples to include; *Aspergillus fumigatus, Aspergillus paraticus, Aspergillus glancus, Aspergillus niger, Mucor mucedo, Torula species, Saccharomyces cerevisiae and Fusarium semitechum. Aspergillus spp*. had uneven colonies. It changed color on consistent incubation, at first it was white, later it became yellow and finally to green. Branched mycelium terminating into conidia was observed. It was isolated from the three samples.

➢ *Mean Viable Plate Count*

The results for the mean viable plate count for some organisms obtained from fermented cassava peels, fermented pulp and those obtained from soil, palm-wine and stool sample (outside environment) cultured on un-supplemented, supplemented cassava peel agar and synthetic media (nutrient agar) is presented in Tables 5 and 6. The results confirmed that amongst the two groups of organisms, from within cassava and outside environment, *Bacillus subtilis and Leuconostoc mesenteroides* had a low microbial count on un-supplemented cassava peel medium. Whereas *Saccharomyces cerevisiae and Enterobacter aerogenes* had high microbial count on the supplemented and synthetic media respectively. Enterobacter aerogenes is the only organism that showed a high microbial count on un-supplemented cassava peel medium, supplemented medium and synthetic medium, although it showed more luxuriant growth on both supplemented cassava peels medium and on the synthetic medium when compared.

➢ *Growth Profile*

Figure 1. Shows the growth profile of *Saccharomyces cerevisiae, Enterobacter aerogenes, Bacillus subtilis and Leuconostoc mesenteroides* obtained from fermented cassava peels and pulp on un-supplemented, supplemented cassava peels extract broth and synthetic broths (malt extract and nutrient broths) after 24 hours incubation. The results indicated that *Saccharomyces cerevisiae and Enterobacter aerogenes* exhibited more growth on the un-supplemented cassava peel extract broth than other Isolates.

Figure 2 also showed the growth profile of *Saccharomyces cerevisiae, Enterobacter aerogenes, Bacillus subtilis and Leuconostoc mesenteroides* obtained from outside environment (soil and palm-wine) on un-supplemented, supplemented cassava peels extract broths and on the synthetic broths (malt extract and nutrient broths). *Saccharomyces cerevisiae and Enterobacter aerogenes* also exhibited more growth on the un-supplemented cassava peels extract broth although at the same level as the isolates obtained from inside environments than other isolates which exhibited very poor growth on un-supplemented cassava peels broth.

➢ *Radial Growth of Aspergillus Fumigatus*

The radial growth of *Aspergillus fumigatus* obtained from cassava peels and outside environment (soil) is shown in Figures 3 and 4. They are derived from the linear relationship of colony diameter with time which showed evidence that growth of the organism on synthetic media was still slightly better than on supplemented and un-supplemented cassava peels media.

➢ *Cumulative Biomass Weight of Aspergillus Fumigatus*

The results of the cumulative biomass weight of *Aspergillus fumigatus* obtained from outside environment (soil) and cassava peels grown on un-supplemented cassava peels broth, Supplemented cassava broth and malt extract broth accordingly for 4 weeks is presented in (figures 5 and 6). However, in both cases the biomass produced by the mold increased linearly with growth. The biomass value produced on the malt extract broth (5.95mg) is higher than that produced on the supplemented broth (4.875mg) while that on supplemented broth is greater than that produced on the un-supplemented broth (2.125mg).

➢ *Influence of Un-Supplemented, Supplemented Cassava Peels Extract Media and Synthetic Media on Growth Rates, Generation Time and Number of Generations of Test Organisms*

The results of the influence of un-supplemented, supplemented cassava peels extract media and synthetic media on the growth rates, generation time and number of generations of test microorganisms is presented in (figures 7 - 14). Results obtained has confirmed a general retarding effect of microbial cell multiplication on un-supplemented cassava peels medium when compared with control and supplemented media. Although the effect on *Enterobacter aerogenes and Saccharomyces cerevisiae* obtained from both cassava (pulp and peels) and outside environments (palm-wine, stool and soil samples) was not definitive, Un-supplemented cassava peels extract medium exhibited remarkable impacts on the growth and generation of *Bacillus subtilis and Leuconostoc mesenteroides* obtained from cassava environment cultured on it as shown in figures 13 and 14. Precisely 0.026h', 1.85, 38.92h and 0.026h 1.85 and 38.92h were observed for growth rate, number of generations and generation time respectively. For *Bacillus subtilis and Leuconostoc mesenteroides* with same attributes cultured on supplemented medium as shown in figures I3 and 14. Precisely 0.052h, 3.85. 16.14h and 0.053h. 3.46 and 18.18h were observed for growth rate, number of generations and generation time respectively. *Bacillus subtilis and Leuconostoc mesenteroides* with same attributes cultured on nutrient agar as shown in figures 13 and 14. Precisely 0.059h: 4.25, 15.44h and 0.057h'. 4.11 and 16.44h were observed for growth rate, number of generations and generation time respectively. As seen the microbial cell multiplication on un-supplemented medium decreased while cell multiplication on the nutrient agar and supplemented media increased.

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Table 1 Standard Composition of the Culture Media Used in this Investigation

	MEA	NA	UNSCPA	UNSCPB	SCPA	SCPB
Cassava peel exract powder			200g	500ml	200g	500ml
Distilled water	1000ml	1000ml	800ml	1000ml	800ml	1000ml
Agar/Agar powder	15g	15g	16g	-------	16g	-------
pH	5.6	7.4	3.35	2.65	-------	-------
pH 1					5.6 for fungi	5.6 for fungi
PH 2					7.4 for bacteria	7.4 for bacteria
Dextrose glu.	20g					
Urea					1%	1%
Sodium nitrate					1%	1%
Peptone						
Malt extract	1g	5g				
Sodium chloride	20g	5g				
Beef extract		3g				

Table 2 Chemical, Mineral and Cyanide Composition of Cassava peels

Parameters	**Content (%)**
Ash	5.4
Crude fibre	11.4
Crude lipid	15.4
Crude protein	2.06
Carbohydrate	65.74
Minerals	Content per 100g of product
Sodium	0.10
Calcium	51
Phosphorous	0.10
Potassium	0.25
Iron	16
Magnesium	3
Ascorbic acid	30
Cassava peels	Cyanide Content mg/kg
Fresh cassava peels	31.692
Boiled cassava peels	20.191
Sun-dried cassava peels	17.463

Table 3 Morphological and Biochemical Characteristics of Yeast and Filamentous Fungi isolated from Fermented Cassava Peels and Pulp

Colony Morphology	**Type of soma**	**Nature of hyphae**	**Special vegetative structure**	**Asexual reproductive structure**	**Special reproductive structure**	**Conidial head**	**Vesicle shape**	**Probable organism**
Colonies are creamish white with reddish centre and reddish brown reverse	Filamentous at 28±2^{0}C	Septate	-	Microconidia elevate thick wall smooth microconidia arthroconidia	-	-	-	Trichophyton species 1
Greyish green	Filamentous	Septate	Foot cell	Subglobose conidia	Hydia conidiophores	radiate	subglobose	Aspergillus glancus 2

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Compact white or yellow basal dark colony	Filamentous	Septate	Fppt cell	Globose conidia	Smooth walled erect conidiophores	Globose	Globose	Aspergillus niger 1
Yellowish, slow growing colony	Filamentous	Coenocytic	Vegetative structure	Sporangiophore	Branched sporangiophore	-	-	Mucor mucedo 1
White often with peach tinge	Filamentous	Septate	-	Foot called conidia	Sporodochia absent	-	-	Fusarium
Smoky or grey green colony	Filamentous	Septate	Foot cell	Globose conidia	Short conidiophores	Typically columner	Dome shaped broadly clavate	Aspergillus fumigatus 1
Dark green colony	Filamentous	Septate	Foot cell	Globose conidia	Greenish conidiophore	Radial	Subglobose	Aspergillus parasiticus 2
Pinkish moist colony	Pseudo-hyphae	Septate	Pseudomyceliu m	Blasto conidia	-	-	-	Torula species 1
Moist milky	Large single global cell Filamentous Filamentous at 28C°±2°C	- Septate	Rudimentry pseudomycliu m Well developed Pseudomyceliu m	Budding cells Blastospores	- -	- -	- --	Sacchromyces cerevisiae 2

Table 4 Morphological and Biochemical Characteristics of Bacteria Isolated from Fermented Cassava Peels, Pulp and Outside Environment

Gram rxn	Shape	Catalase	Coagulase	Motility	Starch hyd.	Citrate	Urease	MR	VP	Spore and hyphae	Dnase	Lip	Gala	Glucose	Malt	Xylose	Lactose	Fructose	Sucrose	Manitol	Galactose	Dextrose	Probable organisms
+ve	Rods	+	+	-	-	-	-	-	-	A	+	-	-	A	A	A	A	AG	-	AG	A	AG	*Bacillus spp*
-ve	Rods	+	-	-	-	-	-	+	-	-	-	-	-	A/G	A	A/G	A/G	A	A	-	AG	A	*Salmonella spp*
+ve	Cocci	+	-	+	+	-	-	-	+	±	-	-	-	A	A	A	A	A	A	-	A	A	*Staphylococcus aureus*
-ve	Rods	+	-	+	+	-	-	-	-	±	+	-	-	A	A	A/G	A	A	-	-	AG	-	*Enterobacter spp*
-ve	Rods	+	-	-	-	-	-	+	-	±	-	-	-	A	AG	A/G	A/G	A	AG	-	A	A	*Escherichia coli*
-ve	Rods	+	-	-	+	+	+	+	-	-	-	+	+	A	A	A	A	A	-	A	A	-	*Vibrio spp*

Key: A/G = Acid/Gas positive, A = Acid positive, + = Positive, - = Negative, VP = Voges Proskauer, MR = Methyl red,

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Table 5 Total Viable Count (TVC) of Isolates Obtained from Cassava Peel and Pulp on Different Growth Media after 24hours of Incubation

Media Used	Pure Test Isolates	TVC (CFU/ml)
Unsupplemented cassava peel agar	*Bacillus subtilis*	8.3×10^5
Synthetic medium (nutrient agar)	*Bacillus subtilis*	3.08×10^6
Supplemented cassava peel agar	*Bacillus subtilis*	2.59×10^6
Unsupplemented cassava peel agar	*Leuconostoc mensenteroides*	6.7×10^5
Synthetic medium (nutrient agar)	*Leuconostoc mensenteroides*	2.42×10^6
Supplemented cassava peel agar	*Leuconostoc mensenteroides*	1.67×10^6
Unsupplemented cassava peel agar	*Enterobacter aerogenes*	1.42×10^6
Synthetic medium MacConkey agar	*Enterobacter aerogenes*	TNTC
Supplemented cassava peel agar	*Enterobacter aerogenes*	TNTC

Values are means of duplicate determinations

Key:

TNTC = Too numerous to count

TVC = Total Viable Count

Table 6 Total Viable Count (TVC) of Organisms Obtained from Outside Environment on Different Growth Media after 24hours of Incubation

Media Used	Pure Test Isolates	TVC (CFU/ml)
Unsupplemented cassava peel agar	*Bacillus subtilis*	8.1×10^5
Synthetic medium (nutrient agar)	*Bacillus subtilis*	2.51×10^6
Supplemented cassava peel agar	*Bacillus subtilis*	1.91×10^6
Unsupplemented cassava peel agar	*Leuconostoc mensenteroides*	9.9×10^5
Synthetic medium (nutrient agar)	*Leuconostoc mensenteroides*	2.7×10^6
Supplemented cassava peel agar	*Leuconostoc mensenteroides*	2.61×10^6
Unsupplemented cassava peel agar	*Enterobacter aerogenes*	1.2×10^6
Synthetic medium MacConkey agar	*Enterobacter aerogenes*	TNTC
Supplemented cassava peel agar	*Enterobacter aerogenes*	1.42×10^6

Values are means of duplicate determinations

Key:

TNTC = Too numerous to count

TVC = Total Viable Count

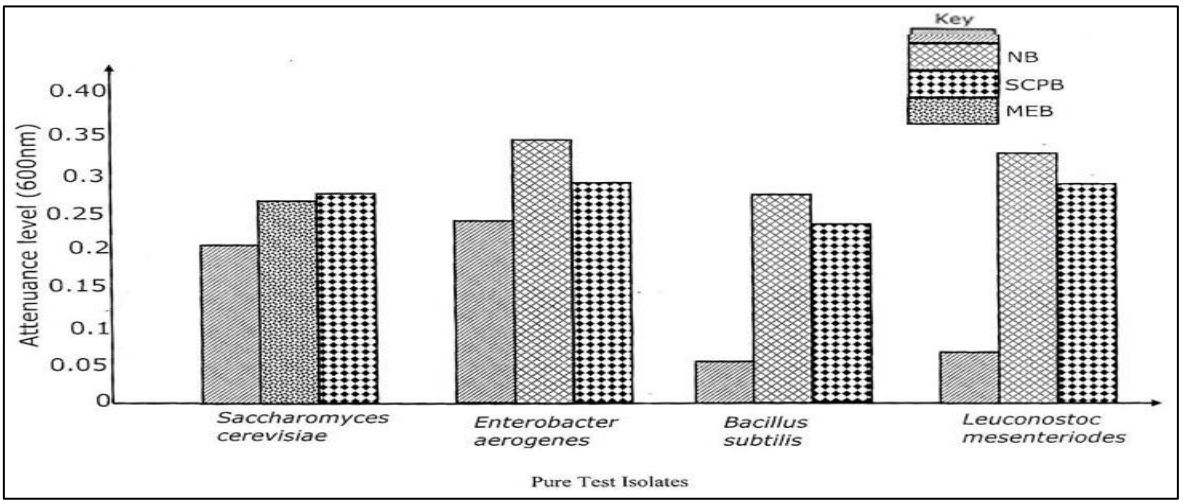

Fig 1 Growth Profile of Isolates from Fermented Cassava Peels and Pulp on Unsupplemented, Supplemented Cassava Peel Extract Broths and Synthetic Broths after 24hours of Incubation

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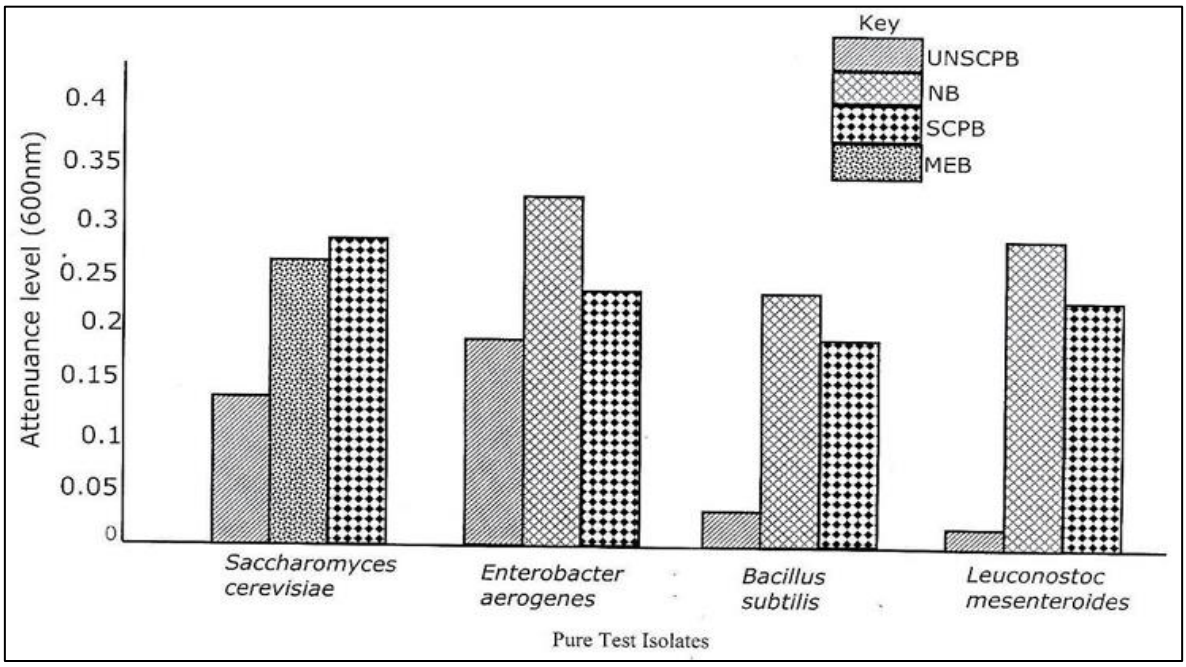

Fig 2 Growth Profile of Isolates from Outside Environment (Soil, Palmwine, and Stool Samples) on Unsupplemented, Supplemented Cassava Peel Extract Broth, Synthetic Broths, Nutrient and Malt Extract Broths after 24hours of Incubation

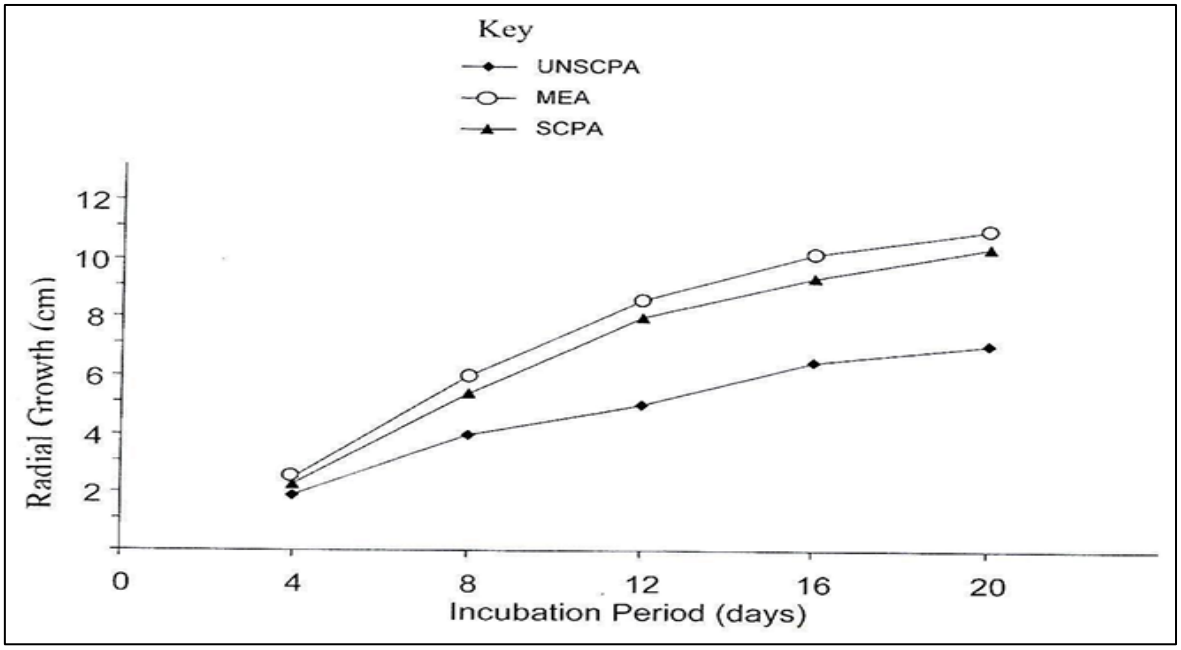

Fig 3 Radial Growth of Aspergillus Fumigatus Obtained from Cassava Peel on Unsupplemented Cassava Peel Agar, Supplemented Cassava Peel Agar and Malt Extract Agar 28±2°C

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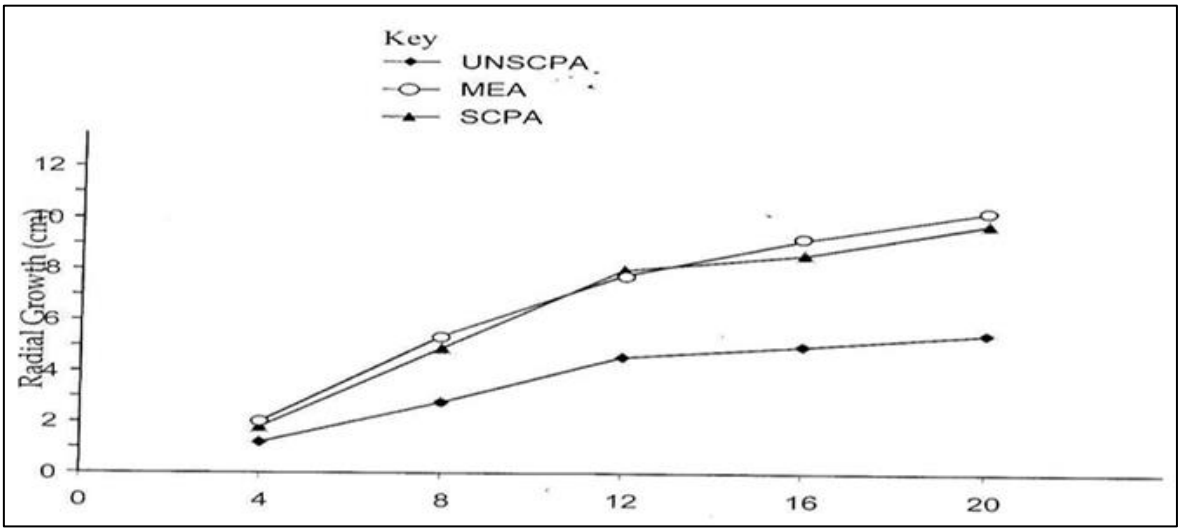


Fig 4 Radial Growth of Aspergillus Fumigatus Obtained from Outside Environment (Soil) on Unsupplemented Cassava Peel Agar, Supplemented Cassava peel Agar and Malt Extract Agar at 28±2°C

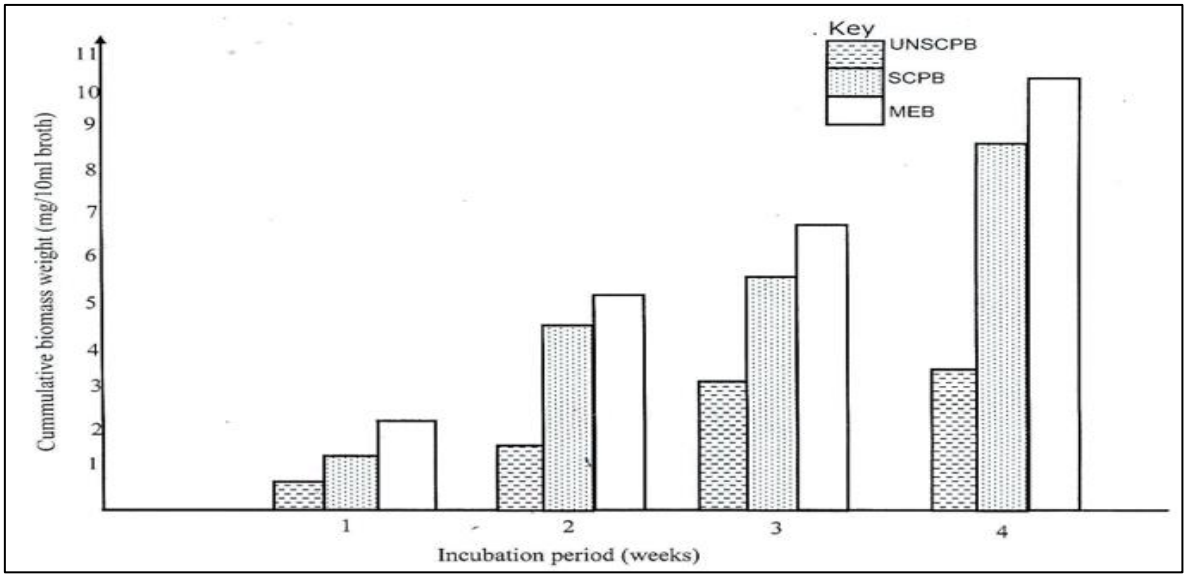
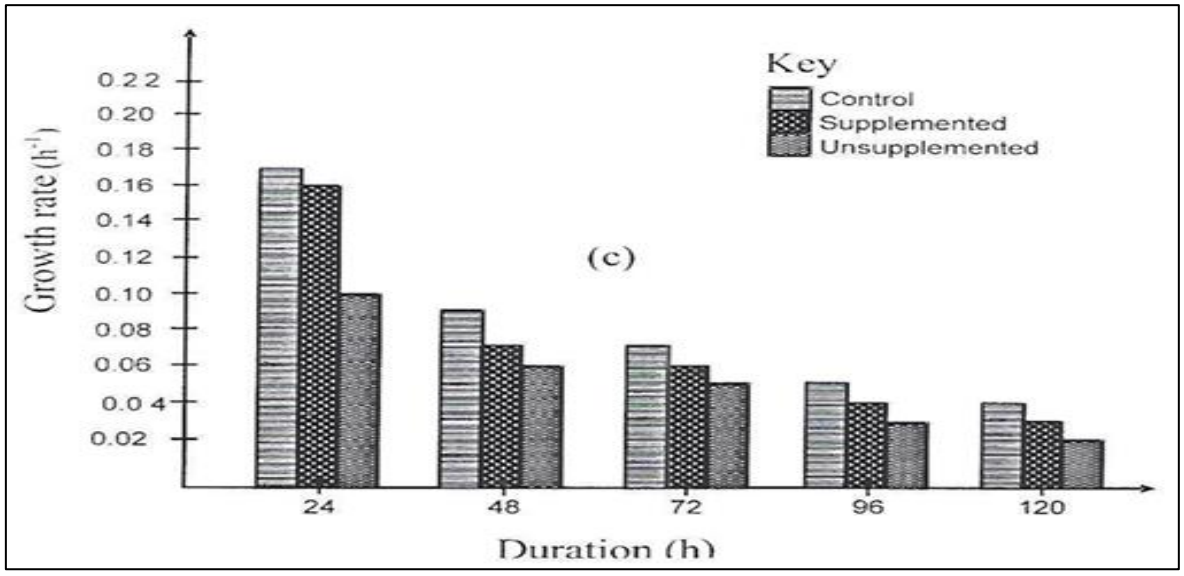


Fig 5 Cummulative Biomass Production by Aspergillus Fumigatus Obtained from Cassava Peels on Unsupplemented, Supplemented Cassava Peel Extract Broth and Malt Extract Broth



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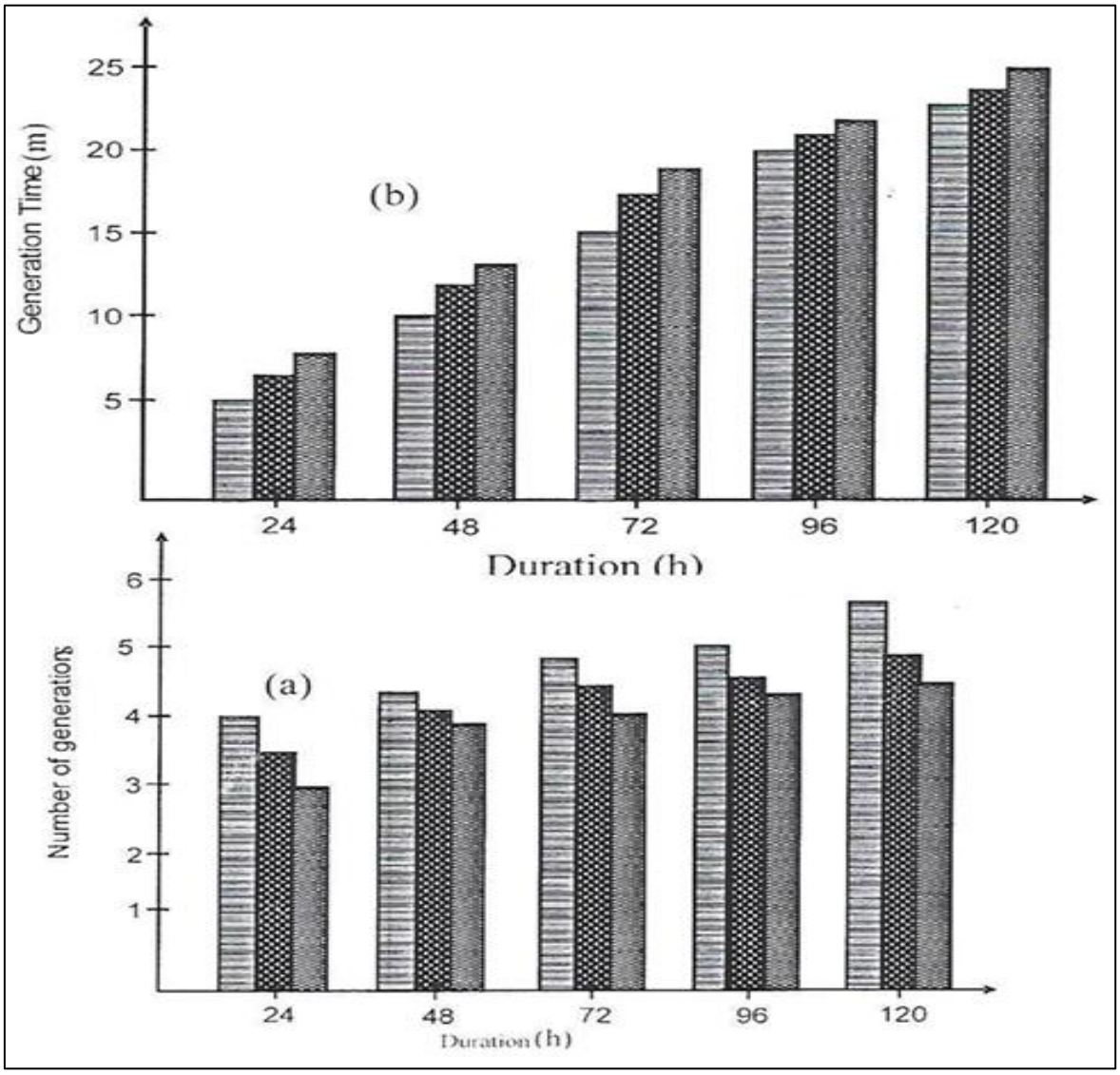
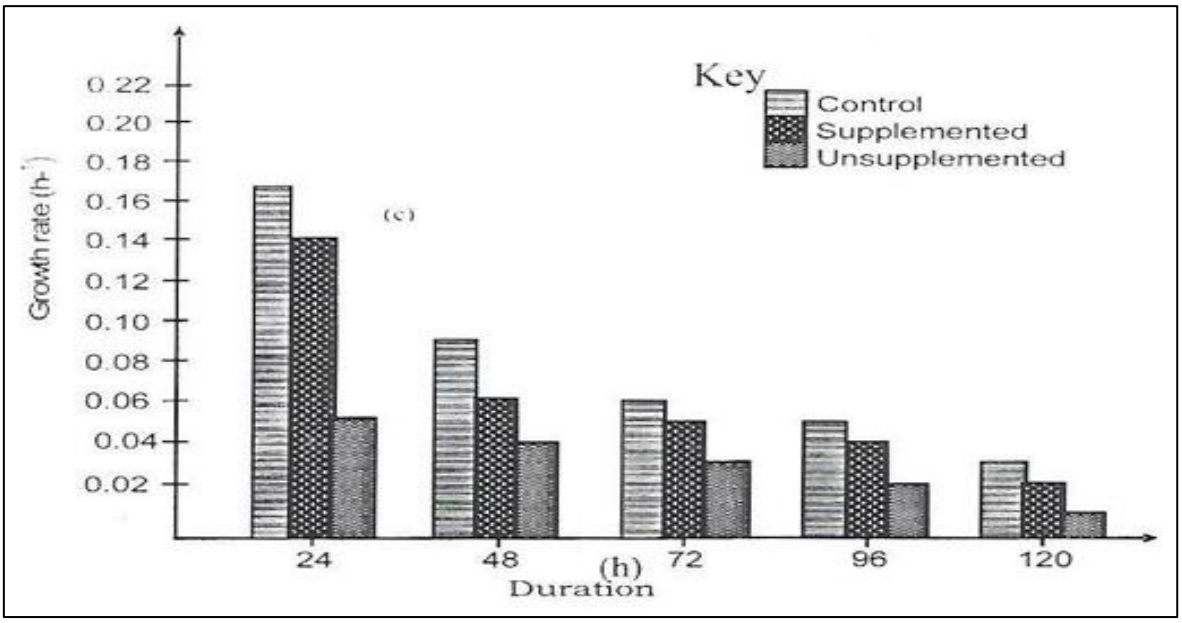


Fig 6 Influence of Supplemented, Unsupplemented Cassava Peel Extract Media and Synthetic Media on Number of Generation (a) Generation Time (b) and Growth Rate (c) of Saccharomyces Cerevisiae Isolated from Fermented Cassava Pulp



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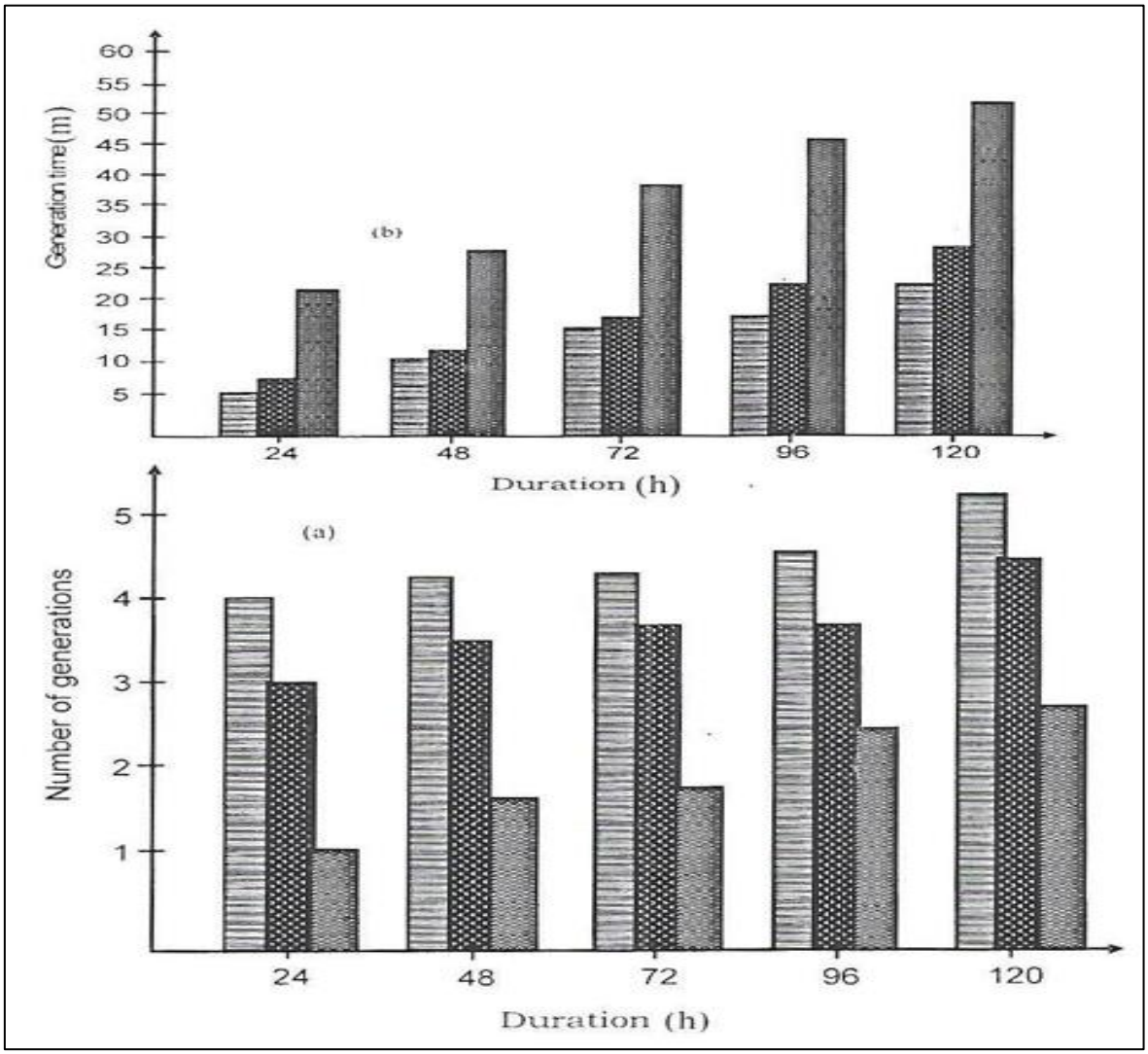
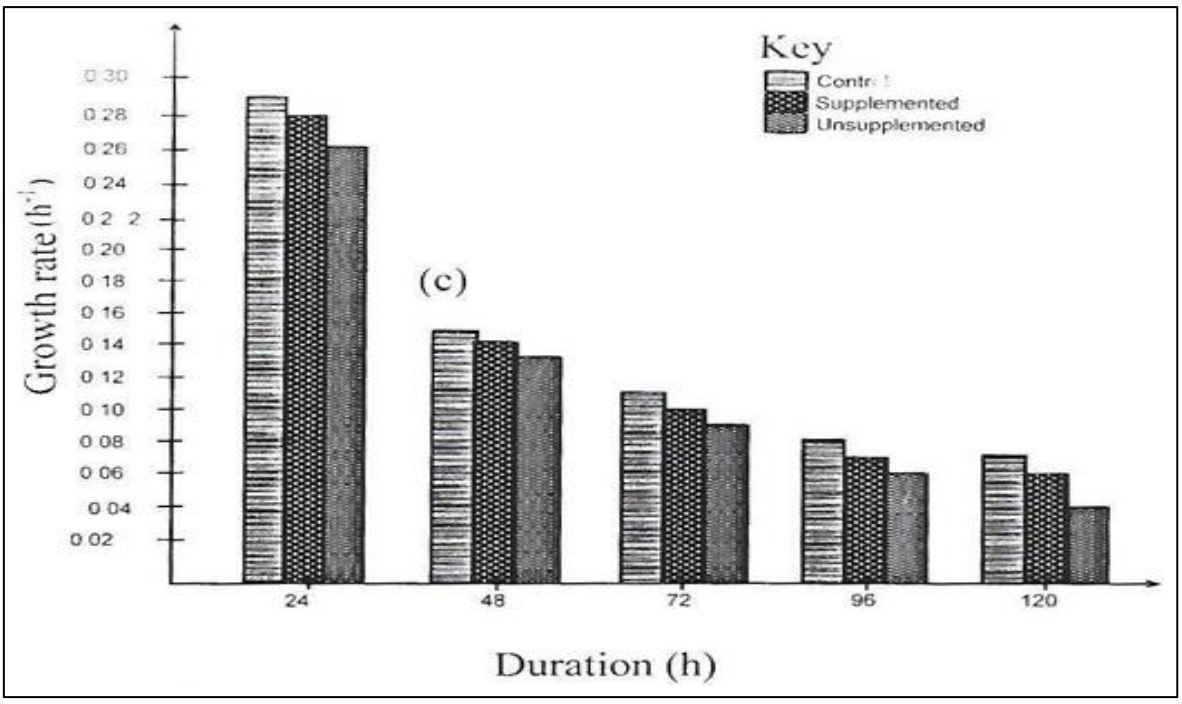


Fig 7 Influence of Supplemented, Unsupplemented Cassava Peel Extract Media and Synthetic Media on Number of Generation (a) Generation Time (b) and Growth Rate (c) of Bacillus Subtilis Isolated from Fermented Cassava Peels



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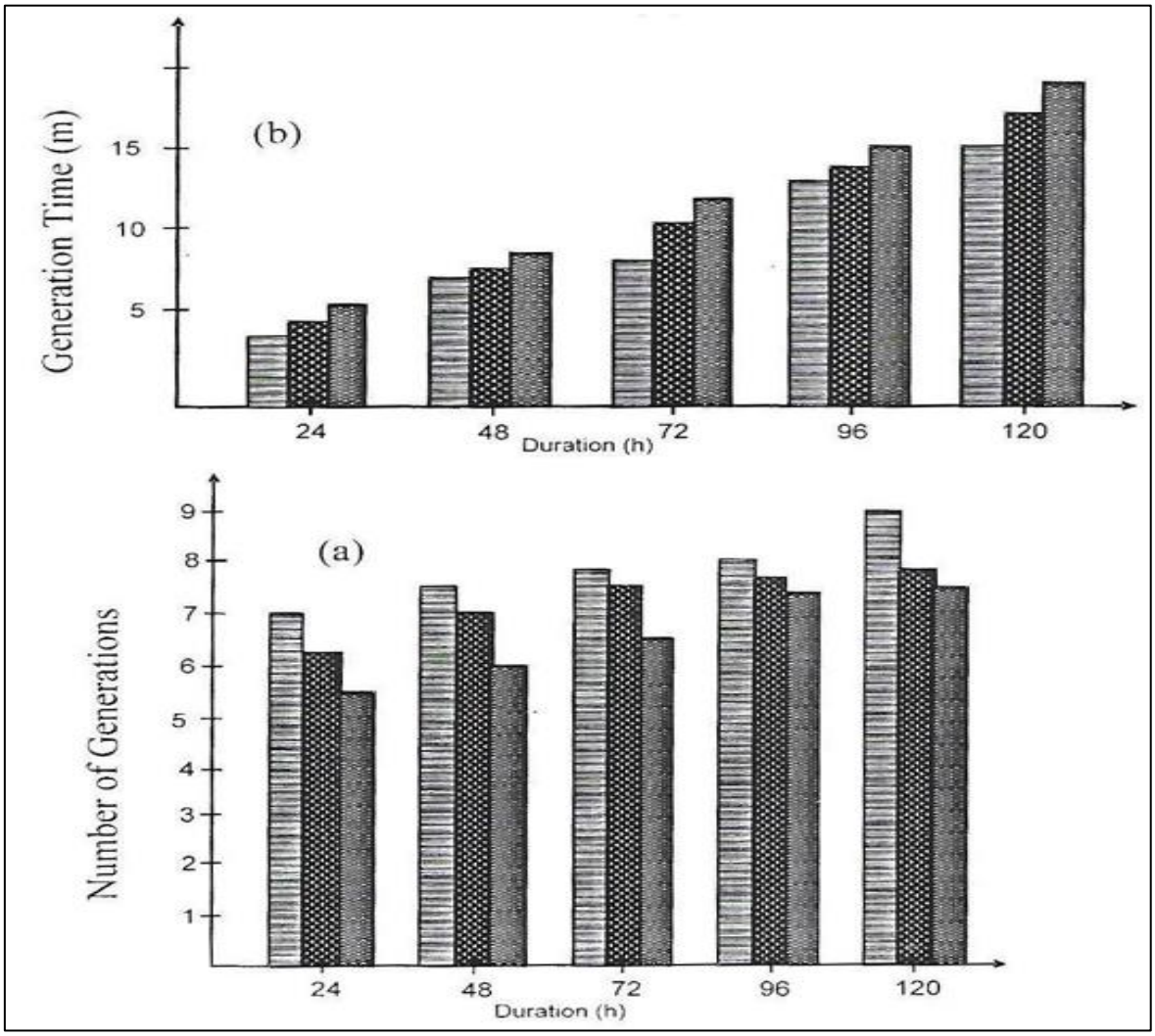

Fig 8 Influence of Supplemented, Unsupplemented Cassava Peel Extract Media and Synthetic Media on Number of Generation (a) Generation Time (b) and Growth Rate (c) of Enterobacter Aerogenes Isolated from Cassava Peel

Table 7 Variation (um±SD) in Sizes of Asexual Reproductive Structures of *Aspergillus fumigatus*

Asexual and special	*Aspergillus fumigatus from soil sample*			*Aspergillus fumigatus from cassava peel*		
reproductive structures	UNSCPA	SCPA	MEA	UNSCA	SCPA	MEA
Diameter of conidium	1.2 ± 03	1.8 ± 0.5	2.1 ± 04	3.2±1.8	4.3± 2.1	6.4±1.3
Length of conidiophore	230 ± 40	245 ± 43	324±45	312±55	340 ± 30	410 ± 78
Width of conidiophore	4.2±1.01	5.1 ± 1.2	7.5 ± 1.50	6.2±2.10	7.3 ± 2.50	9.4 ± 1.51

IV. DISCUSSION

The results of the chemical composition and the elemental properties of cassava peels, indicated that the carbohydrate content of the peels (65.74%) had the highest value while the protein content (2.06%) had the lowest value. The high carbohydrate content of cassava peels points that they can supply enough carbon and energy needed for microbial growth and metabolism, while the low protein content of cassava peels (2.06%) is a pointer that even though they make carbon source available, they might be lacking in nitrogenous sources such as ammonium and urea which can help in increasing protein production when cassava peels are used as culture medium demanded for maximal microbial growth and metabolism, therefore pointing out the necessity of nutrient integration to beef-up their usefulness as a culture medium, This supports the investigation of MPDI (2025) which reported that cassava

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peels are rich in carbohydrates but poor in protein resulting in carbon-to-nitrogen ratio that can reduce yield of microbial cells or metabolites.

Lipid content is higher in cassava peels (15.4%) than in peels of other agricultural products. Lipids are vital to the structure and biological functions of cells and are used as alternative energy source. The high lipid content of cassava peels points that the substrate may supply extra energy and necessary components needed for cell membrane synthesis and metabolic activity, which may enhance biomass production in formulated media by lipid utilizing microorganisms such as Aspergillus species. These results aligned with the observation of Ebu *et al.,* (2025) which observed that cassava peel medium contained measurable lipid content that can contribute to the nutritional composition of the media used for microbial growth. The crude fiber content (11.4%) of cassava peels is higher than (7.68%) and (0.34%) reported in banana and sweet potato peels respectively. The high fiber content of the cassava peels helps microorganisms by making available complex carbon source, supporting fermentation where the fibrous materials are degraded by microorganisms and converted into useful metabolic products like organic acids and enzymes which can be utilize for their growth, offering a surface for attachment, and supplying nutrients gradually to sustain growth. Furthermore, the ash content of cassava peels (5.4%) compares satisfactorily with that of sweet potato peels (4.55%). Ash is a reflection of the quantity of essential mineral elements required for microbial metabolism contained in the sample. Where the nutrient profile of the cassava peels, including fiber and ash added to their efficacy as a source of nutrient manufactured microbial media. This observation agrees with the findings of Ebu *et al.,* (2025). Furthermore, the results obtained from the determination of cyanide content of cassava peels showed that the fresh cassava peels had higher cyanide level (31.692mg/100g) when compared to the cyanide level of the boiled cassava (20.19lmg/100g) and sun-dried peels (17.463 mg/100g), Generally, the high cyanide content of cassava peels can adversely affect microbial growth by stopping respiration, reducing metabolic activity and limiting biomass production of the cultured microorganisms. However, proper processing of cassava peels by some traditional methods such as boiling, fermenting and drying reduces its toxicity, improve palatability and convert these perishable undesirable agricultural fresh peels into useful products such as culture media for culturing both cyanide tolerant and non-cyanide tolerant microorganisms. This study corroborated with the observations of Cooke and Maduagwu (2007) and Padmaja (2010).

However, results obtained from the total viable count (TVC) have confirmed that, the bacteria test isolates were able to grow and form colonies on the available culture media (UNSCPA, SCPA and NA) but their growth potential however varied depending on the media. The number of colonies formed was generally better on the synthetic which recorded the highest bacterial count with a mean of ($2.42x10^6$ – $3.08x10^6$ CFU/mL). This result may be attributed to the fact that synthetic media is produced with balanced nutrients required for maximum bacterial growth. The supplemented media also enhanced substantial bacterial growth with a mean viable count of ($1.67x10^6$ – $2.59x10^6$ CFU/mL). Here the integration of medium with nitrogenous sources have upgraded the nutrient level of the medium thereby enhancing microbial growth and multiplication. The un-supplemented medium indicated the lowest viable bacterial count, with ($1.42x10^6$ – $8.3x10^5$CFU/mL) This may be attributed to a low utilization of medium by bacteria due to it fibrous nature, minimal protein content, high level of hydrocyanic acid and lack of proper blend of ingredients that could enhance the growth of test organisms. Similar observations have earlier been reported by Iyayi and Losel (2001) and Ubalua (2007). Although growth was obtained on the un-supplemented medium, there was retardation in growth after 24hours. Asegbeloyin and Onyimonyi (2005) reported that though there were increase in the total protein content of cassava peels after fermentation, the level was still low, this explains the major reason for the scanty and poor growth of test isolates on un-supplemented cassava peel extract medium. The number of colonies formed by Enterobacter aerogenes was generally better in UNSCPA than other bacteria test isolates as presented on table 5. This is associated with the fact that the organism has the ability to carry out fermentation at low pH which makes it desirable energy source because it will continue energy production without much regulation of the bacterial environment. The limited utilization of UNSCPA and the formation of scanty colonies by test isolates which leads to the supplementation of the cassava peel extract with urea, sodium nitrate and adjustment of the pH from 2.56 and 3.35 to 5,4 for fungi and 6.5 – 7.5 for bacteria (Merck KgaA, 2023), leads to the improvement of its nutritional values with respect to protein content and lowering of its cyanide content. Thereby increasing the colonies formed by test isolates. This result is in agreement with the observation of Ezekiel *et al.,* (2010). The luxuriant growth performance and discrete colony formation of the test isolates on SCPA, NA and MEA are attributed to proximal, nutritional and environmental factors of the culture media (Prescott, Harley and Klein, 2008). However, there was no significant difference ($P < 0.05$) observed in the total viable count of test isolates obtained from both cassava peels and outside environment on both the SCPA and synthetic media, although number of colonies formed on the synthetic media was slightly better than the number formed on supplemented cassava extract media.

The results obtained from optical density analysis also confirmed that in all the treatments, the growth of the test yeast and bacteria varied significantly $P>0.05$ on SCPB, synthetic (MEB and NB) and the UNSCPB. Although, growth on un-supplemented cassava peel medium was lower than the growth that occurred on both supplemented cassava peel medium and synthetic medium, which points to lack of additional nutrients in the UNSCPB. However, growth on supplemented cassava peel medium was comparable to synthetic medium, suggesting that the integration of the nitrogenous sources effectively improved the nutritive value of cassava peel-based media. This result aligns with the observations of Wyatt (1973).

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Furthermore, the superior growth of Saccharomyces cerevisiae and Enterobacter aerogenes in the un-supplemented cassava peel medium can be attributed to their ability to efficiently metabolize the abundant carbohydrates, tolerate residual cyanide and other inhibitory compounds, and adapt to nutrient deficient conditions, whereas Leuconostoc mesenteroides and Bacillus subtilis desire higher nitrogen and amino acids availability, and are more sensitive to inhibitory substances present in cassava peels at the same, they have a low adaptability to low-protein, low-mineral environments.

For a microorganism to be able to grow, a suitable medium should be employed, and to be effective, this medium must contain all the nutrients the microorganism requires for growth. This depends on organism requirements of carbon, energy and hydrogen atoms or electrons Zhang *et al.,* (2021). As seen in the composition of cassava peels, its protein content (2.06%) was low when compared to that of the synthetic media (5%). However, the high content of protein in the synthetic and supplemented media, results in almost double the growth performance of the microorganisms. This agrees with the findings of Antai & Mbongo (1994). It implies that the test microorganisms had more carbon and nitrogenous sources to support their growth. The UNSCPB, being a crude medium with acidic pH and no proper blend of ingredients that can enhance proper growth of the test organisms. Some of the test isolates exhibited scanty growth on UNSCPB but Saccharomyces cerevisiae and Enterobacter aerogenes exhibited significant growth, although not as much as on the SCPB and synthetic broths. This result ascribes to the fact that these microorganisms can grow on the UNSCPB without supplementation of any nitrogenous compound. Moreover, Enterobacter aerogenes is capable of carrying out fermentation on the UNSCPB with the production of energy without regulation of its bacterial environment, while Saccharomyces cerevisiae could utilize the medium for single cell protein production, these findings is in agreement with the observation of Essien *et al.,* (2005). The production of fungal biomass on agricultural wastes shall not only minimize loads of pollutants, but at the same time the malnourished people can have protein supplement at an affordable cost.

In other word, Smucker and Cooney (1981) reported that filamentous fungi grow as heavy mycelial mats or fuzzball in liquid culture and so turbidimetric methods are not accurate measures of its growth, thus in this present research, the influence of un-supplemented, supplemented cassava extract media and malt extract media (control) on filamentous fungi was monitored using radial growth analysis. In all the treatments, the radial growth of the molds increased exponentially with increase in incubation time. These findings are in agreement with the observations of Lopez-malo, Alzamora and Argaiz, (1995), RSC (2025). There was no $P>0.05$ significant difference in the radial growth rate of molds between those cultured on supplemented cassava peel extract agar (35.2cm) and malt extract agar (38.4cm). Slight variation in sizes of asexual spores were also observed between Aspergillus fumigatus cultured on supplemented cassava extract agar and malt extract agar with the later supporting the production of larger spores. The conidia of Aspergillus fumigatus cultured on supplemented cassava peel extract agar were slightly smaller in diameter than the values recorded on malt extract agar. However, it was noted that the biomass produced by Aspergillus fumigatus increased linearly with growth phase of the fungus. This is in agreement with the work of Kowaiski *et al.,* (2022). Who established that fungal biomass increased rapidly after the initial lag phase as the fungus entered the exponential growth phase? Fungi are known to have different enzymatic responses to plant-based substrates. Under suitable conditions the extracellular enzymatic activities are affected by both the components of the medium and temperature of incubation (Aseigbu, Paterson and Smith, 1996). The low biomass production rates of the test mold on the un-supplemented cassava peel extract medium may be ascribed to the presence of the cyanogenic glycosides, low pH of the extract and lack of some nutrients and nitrogenous sources which can support mycelial biomass production, including high enzymatic activities leading to degradation of the substrate.

Finally, the results of the growth analysis, have also revealed a general reduction and retarding microbial cell multiplication in growth rates of the test microorganisms especially, Bacillus subtilis and Leuconostoc mesenteroides grown on the un-supplemented cassava peel extract medium, which is associated with the long generation time (Gt) and lack of ability to produce high number of new cell (number of generations *n*) and grow faster on the medium. Although the effect of the crude medium on the growth and performance of Saccharomyces cerevisiae and Enterobacter aerogenes was not definitive which can be connected to the versatility of their metabolic activity, low nutritional requirements, adaptive physiological mechanisms, possession of robust enzymatic systems that can enhance the utilization of diverse and complicated substrates that are present in refined media, while also tolerating differences in environmental factors and capable inhibitory compounds. These results aligned with the findings of Walker, 1998; Stanbury *et al.,* (2017); Madigan *et al.,* (2018); Prescott *et al.,* (2002), where they all reported that industrial microorganisms possess metabolic and environmental tolerance that can enhance their effective growth in a crude and poorly compounded media. Furthermore, their ability to efficiently assimilate the available nutrients and sustain fast growth to ensure a consistent performance, even under sub optimal medium compositions, which support their widespread application in industrial fermentation process. Comparatively these findings have also shown that there was an increase in growth rates of the test microorganisms on the synthetic media followed by the supplemented medium, though growth on the synthetic media was slightly better. With evidence in the short generation time (Gt) and ability to produce high numbers of new cells (number of generation *n*) then faster growth of isolates in the medium. The implication is that the synthetic and supplemented cassava extract medium were properly formulated where nutrients such as carbon, nitrogen, vitamins and minerals are provided in balanced and controlled proportions to support

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microbial growth. Moreover, optimum environmental and nutritional factors are in proximal proportion. This finding is in agreement with the observation of Zhang *et al.,* (2021). Whereas the un-supplemented cassava peel extract medium is a crude medium and does not have the proper blend of ingredients that can support the growth of microorganisms. Finally, a standard and properly formulated medium is also apparent on the generation time of the test organisms. High generation time is indicative of the period of time needed for the inducible enzymes such as permeases and complexing protein molecules to be synthesized. Higher generation time also affects the number of generations adversely because these organisms with longer generation times exhibits reduced growth rates, leading to reduced population increase compared with organisms that divide faster and thus the microbial populations which are produced on culture media (Jones *et al.,* 2023).

V. CONCLUSION

In respect of the persisting economic recession and high cost of substrate for microbial cultivation, especially in the current centuries, there is an urgent need to explore organic materials as alternative sources of microbial growth medium. This study has confirmed that cassava peel can offer a good option if its growth potential could be improved by integration with inorganic nitrogen sources like urea and sodium nitrate before inoculation which will boost the growth potential of the medium, also adjusting the pH to the optimum pH range of 4-6 and 7-8 for both bacteria and fungi will also improve the ability of the cassava peel extract to be a good microbiological medium for culturing all classes of microorganisms.

RECOMMENDATION

In this study, nitrogenous sources (urea and sodium nitrate) were the possible additives used to upgrade the peels extract media and broth to increase its growth potential for microorganisms. Researches should be carried out so as to uncover more possible nutrients that could be used in upgrading the nutritional status of cassava peels extract agar medium and broth while the pH of the extract which is acidic as a result of the high concentration of the cyanogenic glycoside in the peels be made less acidic. Also, while constituting the cassava extract media, proper blend of ingredients should be considered. Moreover, the potentials of these microorganisms to utilize the substrate could be harnessed for effective waste management, with this, cassava peels extract may become a cheaper, reliable and veritable alternative medium for culturing microorganisms on commercial scale.

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