NAUB JOURNAL OF SCIENCE AND TECHNOLOGY
(NAUBJOST)
e-ISSN:2811-2350
Email: jost@naub.edu.ng http://journal.naub.edu.ng/naubjournal/

POTENTIAL OF USING FOOD WASTE FOR THE LABORATORY CULTIVATION OF FUNGI

[1]Ogbeba, J.,[2]Udosen, I.E., [3]Adeosun, F.F., [4]Samuel, E. and [1]Ufomba G.C.
[1,2,5]Department of Science Laboratory Technology (SLT), Federal Polytechnic Bauchi, Bauchi State, Nigeria. [3]Department of Nutrition and Dietetics (NUD), Federal Polytechnic Bauchi, Bauchi State, Nigeria. [4]Department of Food and Science Technology (FST), Federal Polytechnic Bauchi, Bauchi State, Nigeria
Corresponding Author's Email: jerrielife2012@gmail.com
Phone: +234 803 8690 148.

Abstract

A major problem experienced by both developed and developing countries is the management of wastes. Thus, there is a growing interest regarding the conversion of organic wastes generated by the food processing sector and through other human endeavors into useful forms. An investigation was carried out to test the suitability of food crop wastes (yam, sweet potato, and Irish potato peels) for the formulation of media for cultivating fungi. Three formulated media which included Yam Peel Dextrose Agar (YPDA), Sweet Potato Peel Dextrose Agar (SPPDA) and Irish Potato Peel Dextrose Agar (IPPDA) were used to determine the growth of two test fungi isolated from the spoilt food stuff. Mean colony count (MCCs) of the isolates on the formulated waste media were determined and compared with that of Sabauraud Dextrose Agar (SDA) which served as control. The fungal isolates used in the study were *Aspergillus niger* and *Aspergillus flavus.* The test organisms were aseptically inoculated unto the three different formulated media and the control medium in triplicates. The cultures were incubated for a period of six days and the colonies were counted thereafter.All the formulated media supported the growth of the test fungi at various level with Sweet Potato Peel Dextrose Agar (SPPDA) having the highest MCCs of 7.4 X 10^6 cfu/ml for *A. niger* and 8.8 x 10^6± 0.03 cfu/ml for *A.flavus* . *Irish* Potato Peel Dextrose Agar (IPPDA) yielded the lowest MCCs (3.2±0.06x 10^6 cfu/ml and 4.2±0.03 x 10^6 cfu/ml) values for *A. niger* and *A. flavus* respectively. The physical and morphological characteristics of the test fungi on the formulated and standard media were similar in colour, shape and size of the colonies, except for SPPDA which had bigger sized colonies than all other media. The proximate composition of the crop waste had significant carbohydrate, protein, fat, fibre, moisture and ash content. This study reveals that the waste materials contain nutrients that can be utilized as culture media for *invitro* growth of fungi.

Keywords: **Food waste, Fungi, Formulated media, Agar.**

Introduction

Wastes are materials that have not yet been fully utilized. They are leftovers from production and consumption. However, waste is an expensive and sometimes unavoidable result of human activity. It includes plant materials, agricultural, household, industrial and municipal wastes and residues (Okonkwo *et al .,* 2006). Large amount of wastes are generated every year from the industrial processing of agricultural raw materials and individual homes. Most

of these wastes are used as animal feed or burned as alternative for elimination (Smith *et al*., 2009).

However, such wastes usually have a composition rich in sugars, minerals and proteins, and therefore, making them useful for other processes directly or indirectly (Pelczar *et al*., 2009). The presence of carbon sources, nutrients and moisture in these wastes provides conditions suitable for the development of microorganisms and this opens up great possibilities for their reuse (Ofuya and Nwajuiba, 2004). The economical aspect is based on the fact that such wastes may be used as low-cost raw materials for the production of other value-added compounds, with the expectancy of reducing production costs (Smith *et al*., 2009). The environmental concern is because most of the agro-industrial wastes contain phenolic compounds and/or other compounds of toxic potential; which may cause deterioration of the environment when the waste is discharged to the nature (Theisen, 2010).

Microbiological studies depend on the ability to cultivate and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favorable environmental conditions (Okonkwo *et al*., 2006). A nutrient material prepared for the growth of microorganisms in a laboratory is called culture media (Gao *et al*., 2007). Microorganisms can obtain energy directly from sunlight while carbon can be made available in organic forms such as carbohydrates, or inorganic forms such as carbon dioxide and water (Ruth *et al*., 2012). The need to develop alternative media to various culture media has become imperative as the conventional media used are either not readily available or relatively expensive in most developing countries like Nigeria and other developing countries of the world (Pelczar *et al*., 2009).

Fungi are a group of eukaryotic spore bearing microorganisms. They generally reproduce asexually and sexually.Some are agents of diseases in plant and animals (parasitic) while some are saprophytic and play a major role in nutrient recycling (Gomez *et al*., 2005). Saprophytic fungi tend to be responsible for most of the disintegration of organic materials, and some of them render food material toxic (Pelczar *et al*., 2009).

Generally fungi are grown on Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Rose Bengal Agar (RBA) and Corn Meal Agar (CMA) which are very expensive (Okonkwo *et al*., 2006). Basically, every fungus requires carbon, nitrogen and energy source to grow and survive (Ainsworth and Bisby, 2005). Utilization of agricultural waste as a substrate for fungal cultures for the production of value added products has been reported (Walker and White, 2005).

Routine practicals require large amount of media on regular basis for streak plate, pour plate and spread plate experiments (Barnett *et al*,. 2009). Availability of low cost media providing rich in nutrients is much warranted. The search for alternative, cheap media for use in laboratory agents for routine microbiological experiments is on going (Okonkwo *et al*,. 2006). Recent research has been focused on finding alternatives to gelling agents of media, (agar in particular) and media, in general, because of its exorbitant price (Ravimannan *et al*., 2014).

The accumulation of this food waste contribute to environmental and health hazard as they decompose in the environment. Hence the need to utilize home food wastes (yam peel, sweet potato peel, and Irish potato peel) in the production of cost effective and readily available culture media.

This study aims to formulate microbial growth medium using home food wastes (yam peel, sweet potato peel and Irish potato peel) for the isolation of fungi (*Aspergillus niger* and *Aspergillus flavus*).

Materials and Methods

Collection of Sample.
Wastes (peels) were obtained by cutting off the outer surface of Yam, Irish potato, Sweet potato were collected from some houses in Gwallameji, Bauchi state, Nigeria.

Treatment of Sample
The wastes were sorted out and each type of waste material were washed, sun-dried and then milled into powdery form. The milled peels were stored in sterile plastic airtight containers.

Proximate analysis:
Determination of moisture content
The AOAC, (2006) method was adopted. The crucible was heated at 100 ^{o}C in an oven for 1hour cooled in desicators and weighed. 2grams of the samples each were weighed into the clean crucible and dried in an oven at 100 ^{o}C for 1 hour and then cooled.The weight was expressed as follows: % moisture=$\frac{\text{Loss in weight due to dryness x 100}}{\text{Weight of sample}}$

Determination of Ash content
The AOAC, (2006) Method was adopted. 2 grams of each dried sample was weighed into the crucible and was first burnt on a burnsen burner to remove the smoke to decarbonize it. It was transferred into the muffle furnace at temperature of about 550 ^{o}C for 4 hours until the residue was completely charred. The heating was discontinued, the crucible was removed from the muffle furnace and allowed to cool in a desiccator. The crucible was then weighed with the residue. % Ash content =$\frac{\text{Weight of Ash}}{\text{Weight of Sample}}$ x 100

Determination of Fat Content
The method described by Harold and Kirk (2004) was used in the determination of fat content. Clean thimble was filled with the dried sample of 10g.The thimble was blocked with cotton wool and placed into the extractor of the extraction soxhlet barrel of the soxhlet kit filled with a small round bottom flask of known weight containing the solvent (ethanol). The condenser was joined tightly in place and the round bottom flask fitted to the soxhlet extractor kit and was placed on the apparatus and the flask was dried in an oven at 100 ^{o}Cuntil constant weight was obtained.
% crude fat extracted = $\frac{\text{Weight of Extract}}{\text{Weight of Sample}}$ x 100

Determination of crude protein
The method described by Harold and Krik(2004) was used in the determination of the crude protein and these involved three stages: digestion, distillation and titration.

Determination of Crude fibre content
The AOAC, (2006) method was Adopted. Two grams of the samples each were weighed and transferred into conical flask. 50ml of 0.3M sulphuric acid was added and boiled for 30minutes. 25ml of 1.5M Sodium hydroxide was equally added and transferred into filter

paper for filtration. The residue was washed for 5minutes with distilled water before neutralizing with 50ml of 0.3M HCl. It was later rewashed for 10minutes with hot distilled water before washing with 50ml acetone. The crucible was dried in the oven with the sample at 100 ^{o}C for an hour, cooled and weighed. Then it was later ignited in a muffled furnace at 700 ^{o}C for an hour. It was cooled and weighed.

$$\% \text{ Crude fibre} = \frac{a-b}{c} \times 100$$

Where a= weight of sample + crucible before ashing
b= weight of sample + crucible after ashong
c= weight of sample

Determination of carbohydrates

The method described by AOAC (2006) was used. The total percentage of moisture, protein, fat, crude fiber and ash content was substracted from 100 %.The remainder account for carbohydrates content. Carbonhydrate = 100 % (Crude protein % + Fat % + ash % + crude fibre % + moisture %)

Test Organisms

Aspergillus niger and *Apergillus flavus* were isolated from onion bulbs showing symptoms of rot and discolouration. The onions were randomly sampled from various traders at Muda Lawal market, Bauchi, Bauchi state, were stripped of their outer dry scales and small pieces of the infected onion bulbs were removed with a sterile knife and surface sterilised in 1% Sodium Hypochlorite for one minute. The pieces of the scale tissues were rinsed three times in sterile distilled water and allowed to dry in a sterile laminar flow cabinet. The scales were plated on PDA in 90mm diameter sterilised petri dishes and incubated at 28°C for seven days in an incubation room. The developing fungal colonies was sub-cultured on fresh PDA plates to obtain pure cultures. The fungal isolates were dentified based on their cultural and morphological characteristics (Barnet *et al*., 2009).

Characterization and identification of fungal cultures

A small amount of aerial growth of each fungus was removed using mounted needles and transferred to a drop of lactophenol cotton blue stain on a clean slide. The hyphae was teased apart with the needles and a cover slip was placed over the preparation taking care not to trap air bubbles. The preparation was viewed under the microscope. Morphology and cultural characteristics was useful in the identification of the fungi. Magnification ×100 oil immersion objective was used for observing features such as the nature of hyphae, fruiting structures, spore types and spore attachment. The fungal isolates were identified by making references to Barnett *et al*. (2009) and Domsch and Anderson (2007).

Media formulation and determination of fungal radial growth

Three different media were formulated, namely Sweet Potato Peel Dextrose agar (SPPDA), Irish Potato Peel Dextrose Agar (IPPDA) and, Yam Peel Dextrose Agar (YPDA). Ten grammes of each milled waste was introduced into separate 500 ml conical flasks. 5g of glucose and 7.5g of agar were added. Distilled water was added to bring the volume to 500 ml. The pH of each medium was then adjusted with NaOH to 5.6. The combined ingredients was shaken vigorously so as to obtain a homogenous mixture. After autoclaving, each of the media was allowed to cool to about 45°C before adding 1 ml of 0.005g of chloramphenicol (this was added to inhibit bacterial growth). For comparative analysis, a conventional mycological media, SDA was also prepared according to manufacturers (Oxoid) specification, and used as control. The suitability of the formulated media was estimated by culturing the

test fungi on them. With the aid of a sterile cork borer, a 5mm mycelial disc of each fungus was obtained from a 5-day old culture of the fungus and used to centrally inoculate triplicate plates of the formulated media and the control SDA medium. The plates was incubated at 25°C for 6 days and the radial growth of each fungus on the various media was measured and recorded at the end of the incubation period. The mean radial growths of the fungi on the formulated media were compared with their mean radial growths on SDA which was the control. Growth rates of the test fungi on the different media was also calculated from the results of the mean radial growths (Barnett *et al*., 2009).

Sterility Test

The media was prepared and autoclaved at pressure 15psi at a temperature of 121ºC .After sterilization, it was plated and tested for sterility by incubation at 37 ºC in an incubator for 24hours. After incubation, it was observed for any visible colony.The absence of visible colonies indicated that the formulated media was sterile (Baker *et al*., 2004).

Results

The proximate composition of the different waste materials are shown in Table 1. Carbohydrate was highest in the three crop wastes (94.67g, 91.24g, 92.34g for YP, SPP and IPP respectively) used in the study. Crude protein, fat, fibre, moisture and ash were present in all the samples at varying measures. The fungal isolates from this study were *Aspergillus niger* and *Aspergillus flavus.* All the formulated media supported the growth of the test fungi at various counts. There were significant differences in the number of colonies produced on the different media for the test fungi. Sweet Potato Peel Dextrose Agar (SPPDA) gave the highest mean colony count of 7.4 x 10^6±0.1cfu/ml for *A. niger*. The MCCs of this fungi on SPPDA was found to be higher than the MCCs on all the other media including the control medium (SDA). The difference in the MCCs of *A. niger* on SPPDA (7.4 x 10^6 ±0.1cfu/ml) and on the control medium (7.3 x 10^6 ± 0.06cfu/ml) were however not significant. The control SDA yielded the highest MCCs (8.8 x 10^6 ± 0.03cfu/ml) for *A. flavus* and was closely followed by SPPDA (8.5 x 10^6 ± 0.06cfu/ml). Though higher, the MCCs of the control medium was also not significantly higher than that of SPPDA. Irish Potato Peel Dextrose Agar (IPPDA) yielded the lowest MCCs 3.2±0.06x 10^6 cfu/ml, 4.2±0.03 x 10^6 cfu/ml) value for *A. niger* and *A. flavus* respectively. Details of mean colony count of the fungi on the various media are presented in Table 3.

Table 2 shows the physical and morphological characteristics of the test fungi on the formulated media and the commercial media. The color, shape of colony and size of colony were recorded.

Table 1 : Proximate composition of crop wastes (Yam peel, sweet potato peel and Irish potato peel).

Component	YP(%)	SPP(%)	IPP(%)
Moisture	0.06	0.06	0.06
Crude protein	1.5	1.6	1.7
Fat content	0.17	3.9	2.9
Fibre	1.3	2.4	1.5
Ash	1.3	2.4	1.5
Carbohydrate	94.67	91.24	92.34

YP = Yam Peel, SPP = Sweet Potato Peel, IPP = Irish Potato Peel,

Table 2 : Physical and Morphological Characteristics of the Test Fungi on the different formulated media and on the commercial medium

Medium	Test organism	Colour of colony	Shape of colony	Size of colony
YPDA	*A. niger*	Black	Round	Small
	A. flavus	White	Round	Small
SPPDA	*A. niger*	Black	Shapeless	Big
	A. flavus	White	Round	Big
IPPDA	*A. niger*	Black	Round	Small
	A. flavus	Black	Round	Small
SDA	*A. niger*	Black	Round	Small
	A. flavus	White	Round	Small

YPDA = Yam Peel Dextrose Agar, SPPDA = Sweet Potato Peel Dextrose Agar, IPPDA = Irish Potato Peel Dextrose Agar, SDA = Sabouraud Dextrose Agar

Table 3. Mean fungal growth count on the formulated media and Commercially sold media.

Medium	Mean colony count (x 10^6 cfu/ml)	
	A. flavus	***A. niger***
YPDA	4.6± 0.1	3.4± 0.1
SPPDA	8.5± 0.06	7.4±0.1
1PPDA	4.2±0.03	3.2±0.06
SDA	8.8± 0.03	7.3± 0.06

YPDA = Yam Peel Dextrose Agar, SPPDA = Sweet Potato Peel Dextrose Agar, IPPDA = Irish Potato Peel Dextrose Agar, SDA = Sabouraud Dextrose Agar

Discussion

Results of the study revealed that all the formulated media supported the growth and sporulation of all the test fungi, though, at varying degrees. This is in conformity with the findings of Weststeijn and Okafor (2012), Adesemoye and Adedire (2005), Tharmilla and Thavaranjit (2011) and Ruth *et al*. (2012) who reported the use of alternative culture media for growing fungi.

The growth of the fungi on the formulated media implies that the wastes (peels) which were used in formulating the media contained the required nutrients for fungal growth (Table 1). This is in line with the findings of Akinyele and Adetuyi (2010) and Silva *et al*. (2009), which shows that agricultural waste materials supports the good growth of fungi. Microbiological studies depend on the ability to grow and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favourable conditions (Domsch and Anderson, 2007; Beever and Bollard, 2009).

The nutrients in the wastes included protein, carbohydrate and minerals. Protein constitutes a significant portion of microbial cells and thus is necessary for the growth of microorganisms (Prescot and Harley, 2012). The protein content of the formulated media must have ensured a good supply of nitrogen while the carbohydrate content served as additional carbon source both of which are essential for good fungal growth. The mineral content of the wastes in the formulated media was probably useful for some aspects of the fungi metabolism. Although

moisture (water) is required by all organisms for their life processes and fungi in particular require water for extracellular digestion of nutrients (Pelczar *et al*., 2009). The moisture content of each of the samples has negligible or no effect on the growth of fungi tested because they were grown in the media containing water.

The MCCs for the control SDA yielded the highest ($8.8 \times 10^6 \pm 0.03$cfu/ml and $7.4 \times 10^6 \pm 0.1$cfu/ml) for both *A. flavus* and *A. niger,* which was closely followed by SPPDA ($8.5 \times 10^6 \pm 0.06$cfu/ml and $7.3 \times 10^6 \pm 0.06$cfu/ml) for the respective fungi growth. This implies that Sweet Potato Peel Agar (SPPDA) was found to be the best formulated media for growing *A. niger* out of all the media used in the study.

Conclusion

This study has revealed that waste materials contain nutrients that can meet the nutritional requirements of fungi, thus they can be utilized as alternative materials in the formulation of culture media for the *in-vitro* cultivation of fungi for teaching and research purposes. An important advantage of the food crop peels used in formulating the various media is that it is readily available in Nigeria . In solving the problem of the shortage of culture media for laboratory practical, the result of this research will go a long way in ameliorating this problem.The fact that the fungi grown on SPPDA performed better in most cases than when they were grown on the conventional SDA shows that SPPDA could serve as a good and possibly cheaper alternative medium for the cultivation of some fungi .

Recommendations

Further studies should be employed to improve the cultivation potential of these formulated medium. Also, other microorganisms' cultivation could be studied on using these formulated media. Further studies to improve the formulated media's efficiency, it's preservatives ability could be carried out such as potency after long media storage.

References

Adesemoye, A. O. and Adedire,C.O. (2005). *World journal of microbiology and biotechnology*, 21; 329-336.

Ainsworth, R. and Bisby, T. (2005). Dictionary of Fungi Sedition Oxon, CAB International Uk. 6.

Akinyele, B.J. and Adetuyi, F.C. (2010). Effect of agro-wastes pH and temperature variation on the growth of Volvariella volvacea. *African. Journal of Biotechnology*, 4(12):1390-1395.

A.O.A.C. (2006).Association of Official Method of Analysis of the AOAC. W. Horowitz, 18th Edition :Washington D.C.,AOAC.

Baker, F. I., Kilshaw, D. and Silverton, R. E. (2004).Preparation of Culture Media. Introduction to Medical Laboratory Technology, 6th Edition, Butter Worth and Co-Publisher Limited. 262-274.

Barnet, J.A., Payne, R.W. and Yarrow, D. (2009). Yeast,Characteristic and Identification. Cambridge Uni. Press. 811.

Beever, S. and Bollard, C.V. (2009). The Nature of the Stimulation of Fungal Growth by Potato Extract. *Journal of General Microbiology*, 60: 273-279

Domsch, K.H. and Anderson, T. (2007). Compendium of soil fungi. Academic Press Limited Mc Graw-Hill Publishers London. 105-106

Gao, L.M., Sun, H., Liu, X.Z. and Che, Y.S (2007).Mycology Resources. Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of everal bio-control fungi. 111: 87-92.

Gomez, J., Pazos, M., Cuoto,S.R. and Sanroman, M.A., (2005). Chestnut and barley bran as potential substrate for lactase production by *Coriolopsis rigida* under solid state conditions. J. Food Eng. 68, 315–319. (Google Scholar)

Ofuya, A. and Nwajuiba, C.J. (2004). Microbial dedgradation and utilization of cassava peels, *Word Journal of Microbiolorgy Biotechbiology.* 6:144-148.

Okonkwo,I.O., Oiabode, O.P. and Okeleji, O.S. (2006). The role of Biotechnology in the Socio-economic Advancement and National Development. *African Journal Biotechnology,* 5(19): 2354-2366.

Pelczar, M.C., Chan, E.C.S. and Krieg, N.R. (2009). Microbiology. 5th ed. Tata McGraw-Hill, New Delhi, India. 917.

Prescot, L.M. and Harley, D.A. (2012). Microbiology. 5th Edition McGraw-Hill Publishers London. 105-106

Ravimannan, N., Arulanantham, R., Pathmanathan, S. and Niranjan, K. (2014). Alternate Culture Media for Bacteria Growth using different Formulation of Protein Sources. *Annals of Biological Research*, 5 (1), 36-39.

Ruth, A.O., Gabriel, A. and Mirrila, E.B. (2012). Basal media formulation using Canavalia ensiformis as carbon and nitrogen source for the growth of some fungi species. *Journal Microbiology Biotechnology Food Science* 1 (4) : 1136-1151.

Silver, R., Lago, E.S., Merheb, C.B., Macchione, M.M., Park, Y.K. and Games, E. (2009). Production of xylanage and cincase on substrate fermentation in different residue by Thermoascus aurantiacus. *Journal of Microbiology.* 36:235-241.

Smith, J.E., Anderson, J.G. and Aidoo, E.K. (2009). Bio-processing of Lignocellulose. *Philosophical Transaction of the Royal Society of London Series A.* 321: 507-521.

Tharmila, E. C. Jayaseelan and A. C. Thavaranjith (2011). *Archives of Applied Science Research,* 3; 389-393.

Theisen, K.B.(2010). *African Journal of Microbiology Research*, 4; 2626-2632.

Walker, G.M and White, N.A. (2005). Introduction to Fungal Physiology. McGraw-Hill Publishers London. 10.

Weststeijn, G. and Okafor, N. (2012). Comparison of Cassava, Yam and Potato Dextrose Agar as Fungal Culture Media. Nether. *Journal of Plant Patholorgy*. 11:134-139.

Fig 1:Growth of the test fungi on SPPDA

Fig 2:Growth of the test fungi on YPDA

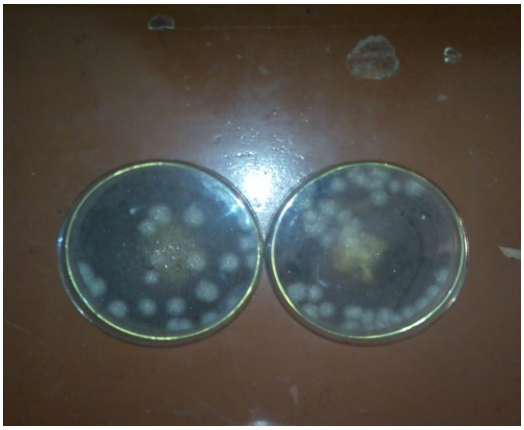

Fig 3:Growth of the test fungi on SDA

Fig 4: Growth of the test fungi on IPPDA