Original Research Article

Evaluation of Partial Substitution of Cocoyam Starch as Alternative Gelling Agent in Culture Media for Cultivation of Fungi

ABSTRACT

Aim: In view of high cost of agar in Nigeria, the study evaluated the suitability of cocoyam starch and cocoyam starch-agar blends as alternative gelling agents in mycological culture media.
Methodology: Media characteristics and ability of the media to support growth and sporulation of the test fungi *A. niger* and *A. flavus* were determined using standard procedures. Potato dextrose agar (PDA) served as the control medium.
Results: Addition of cocoyam starch to the media did not significantly affect media clarity, viscosity, and ease of pouring. Media in which cocoyam starch made up 57% or less of the gelling agent solidified while those with higher contents of starch were either semi solid or watery. Mean radial growth ranges were 75.00 ± 0.70 – 84.00 ± 1.00 mm and 77.50 ± 0.50 – 86.50 ± 0.50 mm for *A. niger* and *A. flavus* respectively. Cocoyam starch-agar blend (4:3) medium produced significantly higher mean radial growth (84.00 ± 1.00 mm and 86.00 ± 0.50 mm for *A. niger* and *A. flavus* respectively) than all the other treatments (P = .05). Mean spore counts per mycelial disc of the various media ranged from 8.6×10^3 to 9.3×10^3 for *A. niger* and 7.9×10^3 to 9.5×10^3 for *A. flavus*. Though both fungi sporulated well on all the media, the PDA medium and the 2 g of cocoyam starch + 4 g of agar medium had significantly higher mean spore counts compared to the other media.
Conclusion: The findings show that cocoyam starch can partially substitute agar as gelling agent in mycological culture media.

Keywords: agar, cocoyam, culture media, gelling agent, starch

1. INTRODUCTION

Microorganisms have a number of requirements for growth and reproduction. They require nutrients such as carbon, nitrogen, vitamins, mineral elements, and also enzymes for their metabolism. Suitable environmental conditions in terms of pH, temperature, oxygen are also required [1].

In order to study the characteristics of microorganisms, it is important to culture them on microbial culture media in the laboratory. Culture media can be in liquid, semi-solid, or solid forms however microorganisms are better grown and identified on solid media. In addition to nutrients, a solid culture medium usually contains some amount of agar, a solidifying agent, which helps to solidify the medium thus providing a firm surface on which the organism can grow and be observed [2]. A good solidifying agent is expected to possess the following desirable qualities: solidity, reversible colloid-forming ability, and transparency. The solidifying agent should also be able to confer to the medium a level of firmness that will allow the carrying out of common techniques including different types of culture plating. It is also important that the gelling agent be relatively inexpensive [3].

Gelling agents are essential components of both solid and semisolid microbial culture media.. The first gelling agent to be discovered was gelatin. Use of agar as a gelling agent for culture media commenced in the later part of the nineteenth century and because of its unique and superior properties it has since become the most widely used gelling agent in microbiological media [4]. Agar has a uniquely large hysteresis, solidifying at about 32 to 40 °C, but melting at 85°C, a property that provides a suitable balance between easy melting and good gel stability at relatively high temperatures [5].

The good qualities of agar have led to its high demand by many industries, but its continued exclusive use in culture media puts a lot of pressure on it, making it to become expensive. The fact that seaweeds which are natural sources of agar are seasonal plants subjects the production and availability of agar to seasonal constraints [3, 4]. Overreliance on agar could also result in overexploitation of natural resources from the natural ecosystem [6]. Non ready availability of agar, importation of the commodity at the loss of scarce foreign exchange, and consequent high cost of agar which makes up about 70% of cost of solid culture media [7] makes it necessary to search for more readily available and cheaper alternatives to agar. Many new gelling agents such as carrageenan, gellan gum, isubgol, guar gum, xanthan gum, corn flour, coconut powder, and starch has been discovered over time [5, 3].

Starch acts as a gelling agent and can be explored towards reducing the consumption of agar in solid media preparation [8, 7]. Starch makes up a major component of plant foods and also serves as a useful raw material for some industries. It is a versatile raw material which has found uses in food, feed, textile, paper, pharmaceutical and cosmetics. It has also found application as a thickener, colloid, stabilizer, gelling agent, and as an adhesive [9, 10, 11]. Cereal grains such as corn and rice, and root/tuber crops such as yam, cassava, and cocoyam are the main sources of starch in Nigeria.

Cocoyam (*Colocasia esculenta*), a member of the Araceae family, is grown in tropical and sub-tropical countries, with Nigeria being a major producer. Its edible starchy corms and cormels are significant contributors to the carbohydrate content of the diets of many Nigerians [11]. Cocoyam has a reasonably high starch content (about 25% wet weight) [12]. This high starch content together with the abundant availability of cocoyam in Nigeria makes it to be a good source of starch for both domestic and industrial uses. Though there have been some studies on the physicochemical characteristics, functional properties, and possible industrial uses of cocoyam starch [10, 12, 11], there is limited information as to its possible use as a gelling agent in microbiological culture media. The aim of this study therefore was to determine to what extent cocoyam starch can substitute agar as a gelling agent in culture media for cultivation of fungi.

2. MATERIAL AND METHODS

2.1 Sample Collection

Cocoyam tubers used were purchased from Farin Gada Market of Jos North Local Government Area of Plateau State. The tubers were transported in clean polythene bags to the microbiology laboratory of the Department of Science Laboratory Technology of the University of Jos where they were used for subsequent studies.

2.2 Test Organisms

Stock cultures of *Aspergillus niger* and *Aspergillus flavus* which were the test organisms in this study were obtained from the Department of Science Laboratory Technology, University of Jos. The organisms were subcultured from potato dextrose agar (PDA) slants onto fresh PDA plates and incubated at 26 °C ± 2 for 5 days.

2.3 Preparation of Cocoyam Starch

The method employed by [3] was used to obtain cocoyam starch. Cocoyam tubers were peeled, washed and grated. Using a domestic blender, 1 kg of the grated cocoyam was blended into paste. The paste was strained using cheese cloth and the solution obtained topped with 3 L of sterile distilled water. The starch solution was left in the laboratory under ambient conditions for 72 h with daily change of the water, after which the supernatant was poured off to obtain a clean starch paste. The starch was broken into pieces, sun-dried for 48 h, and crushed into powder. The dried starch powder was subsequently used for the study.

2.4 Preparation of Potato Broth and Potato Dextrose Agar

One hundred grammes (100 g) of peeled, washed, sliced potatoes was transferred into a beaker and topped with 100 ml of water and then boiled for 30 minutes until it became tender. Potato broth was obtained by straining the boiled potato using cheese cloth and topping with distilled water to 1000 ml. Five gram weights of agar and glucose were placed in a 500 ml conical flask. A volume of 250 ml of the potato broth was added to the flask to obtain the potato dextrose agar (PDA).

2.5 Preparation of Experimental Media

Potato Dextrose agar was prepared in 250 ml volumes. The agar contents of the PDA media was varied by supplementing with different amounts of cocoyam starch as shown in Table 1 below.

Table 1: Proportions of cocoyam starch and agar used in media preparation

Gelling Agent	Proportion of gelling agent (g)										
Cocoyam Starch	0	1	2	3	4	5	2	4	6	8	10
Agar	5	4	3	2	1	0	4	3	2	1	0

The control used was 250 ml of potato broth treated with 5g of agar and 5g Dextrose to form potato Dextrose agar (PDA). The media preparations were pre-heated separately on a hot plate and stirred continuously with a stirring rod till it thickened. This was to ensure that the resultant mixture would be homogenous and to avoid lumping during autoclaving. The mouths of the conical flasks containing the different media preparations were tightly plugged with cotton wool and the flasks with their contents were autoclaved at 121°C and1 kg/cm^2 pressure for 15 minutes. The sterilized media were separately dispensed into sterile Petri dishes and left to solidify.

2.6 Assessment of Viscosity and Pouring Ability of Media.

Determination of the viscosity of the media was carried out by physical observation. Each of the media was classified as light or thick. Media Pouring ability was determined based on ease of pouring the media into plates. In this determination, the media were classified as easy to pour or difficult to pour. Cooled molten PDA served as the control.

2.7 Determination of Solidity of Media.

The effect of cocoyam starch supplementation on the solidification abilities of the test media was determined using a solidification scale ranging from 0-2 where 0 = not solidified, 1 = semi-solidified, and 2 = solidified. Cooled molten PDA served as the control.

2.8 Assessment of Clarity of Media

Clarity of media was assessed based on how well the test fungi could be seen with the unaided eye on the various media. The cultures were observed from below the plates. The clarity of the growth of fungi on media was compared to that of standard PDA which served as the control medium. Clarity was measured on a scale of 1 to 4 where, 1= Organisms cannot be seen (opaque), 2 = Organism partially seen (Clear), 3 = Organism seen (Very Clear), 4 = Organism absolutely seen (Excellent)

2.9 Determination of Colony Diameter of Test Fungi

In determining the colony diameter of the test fungi, a flame-sterilized 5 mm diameter cork borer was used to cut out mycelial plugs from the periphery of 4-day old PDA cultures of *A. niger* and *A. flavus*. With the aid of flame-sterilised mounting needles, each mycelial plug was placed centrally on each of the media with the mycelia touching the surface of the medium. The plates were covered and left inverted on the laboratory bench at ambient temperature ($25^{o}C \pm 2$) for seven days. At the end of seven days, colony diameter was measured with the aid of a ruler in two directions perpendicular to each other on the bottom side of the Petri plates. The mean of the two measurements was used as the colony diameter per plate. Means of triplicate plates were used as final colony diameter which was expressed in millimetres.

2.10 Assessment of Sporulation of Test Fungi

A 5 mm-diameter cork borer was flame-sterilized after dipping in alcohol and used to cut out three mycelial discs from the edges of 5-day old cultures of the test organisms, *A. niger* and *A. flavus*. Each set of three mycelial discs from each culture plate was bulked in a 20 ml McCartney bottle and serially diluted with distilled water up to the 10^{-5} dilution which was used to determine the spore count of the discs with the aid of a haemocytometer. Sporulation of each fungus was expressed as mean spore count per disc.

2.11 Statistical Analysis

The effect of cocoyam starch supplementation on fungal growth and sporulation were statistically analyzed using one-way analysis of variance (ANOVA) with the aid of Microsoft Excel Version 2010. P-values less than 0.05 were considered significant. LSD was used to separate means with significant differences.

3. RESULTS

3.1 Viscosity and Pouring Ability of Culture Media

Potato dextrose agar (PDA) which served as the control medium was of light viscosity and was easy to pour into plates. The culture media in which agar had been supplemented with various amounts of cocoyam starch were slightly lower in viscosity than the control PDA medium and were also easy to pour into plates.

3.2 Solidification of Culture Media

Of all the media tested for solidification, it was observed that seven of them solidified, two were in semi-solid form, and two others could not solidify. The solidified media included the

control PDA medium and six of the media to which cocoyam starch had been added. It was observed that media that contained higher amounts of agar (3 g and above) turned out to be solid whereas those with lower amounts of agar were either semi-solid or watery. Media that contained only cocoyam starch as gelling agent remained watery. The semi-solid and watery media were discarded and were not used for subsequent experiments. Details of the effect of cocoyam starch supplementation on the solidity of the different culture media are presented in Table 2.

Table 2: Solidification of Culture Media Supplemented with Various Concentrations of Cocoyam Starch as Gelling Agent

Media (Amount of gelling agent added to 250 ml of potato dextrose broth)	Score (1-4)*
5 g of agar (control media)	2
1 g of Cocoyam Starch+4 g of agar	2
2 g of Cocoyam Starch+3 g of agar	2
3 g of Cocoyam Starch+2 g of agar	1
4 g of Cocoyam Starch+1 g of agar	0
5 g of Cocoyam Starch+0 g of agar	0
2 g of Cocoyam Starch+4 g of agar	2
4 g of Cocoyam Starch+3 g of agar	2
6 g of Cocoyam Starch+2 g of agar	1
8 g of Cocoyam Starch+1 g of agar	0

*0 = not solidified, 1 = semi solidified, 2 = solidified

3.3 Clarity of Culture Media

Table 3 shows the clarity of the culture media. All five media used in this experiment were generally clear and allowed the organisms to be clearly seen and measured. PDA which served as the control medium had the highest clarity whereas the 4 g of cocoyam starch + 3 g of agar medium had the least clarity.

Table 3: Clarity of culture media supplemented with different amounts of cocoyam starch as gelling agent

Media (Amount of gelling agent added to 250 ml of potato dextrose broth)	Score (1-4)*
5 g of agar (control media)	4
1 g of Cocoyam Starch+4 g of agar	3
2 g of Cocoyam Starch+3 g of agar	3
2 g of Cocoyam Starch+4 g of agar	3
4 g of Cocoyam Starch+3 g of agar	2

*1 = opaque, 2 = clear, 3 = very clear, 4 = excellent

3.4 Mean colony diameter of test fungi

There were significant differences ($P < .05$) in the mean colony diameters of *A. niger* and *A. flavus* with respect to the media treatments. The colony diameters of *A. niger* on the different media ranged between 75 and 84 mm whereas that of *A. flavus* was in the range of 76 - 86.5 mm. The 4 g of cocoyam starch + 3 g of agar medium produced the highest colony diameters of 84 mm and 86.5 mm for *A. niger* and *A. flavus* respectively. Whereas the 1 g of cocoyam + 4 g of agar medium produced the lowest colony diameter of 75 mm for *A. niger*, the 2 g of cocoyam starch + 3 g of agar medium brought about the lowest colony diameter (76 mm) for *A. flavus.* Details of the mean colony diameters of *A. niger* and *A. flavus* on the various media are presented in Table 4.

Table 4: Mean colony diameter of *A. niger* and *A. flavus* on test media

Media (Amount of gelling agent added to 250 ml PDA	Colony Diameter	
	A. niger	*A. flavus*
5 g of agar (control PDA)	81±0.50^{b}	82.5±1.00^{bc}
1 g Cocoyam Starch+4 g of agar	75±0.70^{d}	77.5±0.50^{d}
2 g of Cocoyam Starch+3 g of agar	78±0.80^{c}	76±1.50^{e}
2 g of Cocoyam Starch+4 g of agar	83±0.50^{a}	83.5±0.50^{b}
4 g of Cocoyam Starch+3 g of agar	84±1.00^{a}	86.5±0.50^{a}

Values are Mean ± standard deviation of triplicate readings
Values with different letters in the same column are significantly different ($P = .05$), Anova test

3.5 Sporulation of *A. niger* and *A. flavus* on culture media

Mean number of spores of *A. niger* per disc ranged from 8.6×10^3 for 1 g of cocoyam starch + 4g of agar to 9.3×10^3 for the PDA medium. Mean number of *A. flavus* spores per disc ranged between 7.9×10^3 for 2 g of cocoyam starch + 3 g of agar medium and 9.5×10^3 for PDA. The control PDA medium supported higher sporulation of both fungi compared to the other media. Differences in the mean number of *A. niger* spores produced on the different media were significant ($P < .05$). There were also significant differences in the mean number of *A. flavus* spores produced on some of the different media. There was however no significant difference between the control PDA medium and the 2 g cocoyam starch + 4 g agar medium in terms of mean number of *A. flavus* spores. Details of the sporulation of the test fungi are presented in Table 5.

Table 5: Mean spore count of *A. niger* and *A. flavus* per disc of test media

Media (Amount of gelling agent added to 250 ml PDA	Colony Diameter	
	A. niger	*A. flavus*
5 g of agar (control PDA)	$9.3\times10^3 \pm 1.4\times10^{2a}$	$9.5\times10^3 \pm 3.5\times10^{2a}$
1 g Cocoyam Starch+4 g of agar	$8.6\times10^3 \pm 3.5\times10^{2d}$	$8.7\times10^3 \pm 0.8\times10^{2b}$
2 g of Cocoyam Starch+3 g of agar	$8.8\times10^3 \pm 0.7\times10^{2c}$	$7.9\times10^3 \pm 7.8\times10^{2c}$
2 g of Cocoyam Starch+4 g of agar	$9.1\times10^3 \pm 1.9\times10^{2b}$	$9.4\times10^3 \pm 3.1\times10^{2a}$
4 g of Cocoyam Starch+3 g of agar	$9.0\times10^3 \pm 1.3\times10^{2b}$	$8.6\times10^3 \pm 3.7\times10^{2b}$

Values are Mean ± standard deviation of triplicate readings
Values with different letters in the same column are significantly different ($P = .05$), Anova test

4.0 DISCUSSION

In this study, the feasibility of substituting the gelling agent, agar, with various amounts of cocoyam starch in culture media for cultivating fungi was investigated. Media to which cocoyam starch substituted agar at various levels were assessed for desirable qualities of gelling agents used in culture media including viscosity, ease of pouring, solidity, ability to support fungal growth and sporulation.

Though addition of cocoyam starch to the culture media resulted in media with lighter viscosity than the PDA media which served as the control. All the media were easy to pour into plates. According to [13], the viscosity of an agar broth at constant temperature and concentration relates directly to its average molecular weight. [3] similarly reported that substitution of agar with moderate levels of cassava starch did not negatively affect medium viscosity. The authors however stated that high levels of cassava starch produced a thick medium which was difficult to dispense. Findings from the present study imply that the combined gelling property of the mixture of agar and moderate amounts of cocoyam starch does not significantly differ from that of pure agar. Higher concentrations of cocoyam starch and decreasing levels of agar resulted in media of very light viscosity which could not solidify. The lower viscosity of the culture media that had high contents of cocoyam starch

could be due to weaker gelling ability of starch compared to agar [14, 15]. This can be explained by the fact that sulphate and pyruvate groups of agar form stronger interactions with water molecules as against the weaker interactions between the hydroxyl moieties of starch and water molecules [16].

Four of the media that contained cocoyam starch and also the PDA medium which served as the control were able to solidify. These media were found to contain higher proportions of agar than the others that did not solidify. The findings showed that agar can be substituted with cocoyam starch up to 57% without negatively affecting media solidity. This implies that agar should not be lower than 43% of the gelling mixture. [7] similarly reported that culture media in which both 24 g/L of cocoyam powder and 24 g/L of sweet potato powder substituted 5 g /L of agar retained their gelling property and were able to solidify. [3] in their study on use of cassava starch-agar blend as alternative gelling agent for mycological culture media equally reported that either 80 g/L or 160 g/L of cassava starch in combination with 8 g/L of agar brought about solidification of potato dextrose broth. According to the report of [13], the gel strength of agar varies with the concentration used. As the ratio of cocoyam starch to agar increased, the media tended towards a watery consistency. Such media were eventually not used for subsequent evaluations.

PDA had the highest clarity among all the media. Media in which agar had been partially substituted with cocoyam starch up to 57% remained clear enough for the test fungi to be clearly seen. Above the 57% level of inclusion of cocoyam starch, media clarity was poor. The finding on clarity of media is in line to that of [3] who reported that media in which cassava starch partially substituted agar were clear even though not as clear as the control (PDA) medium.

Though all the experimental media produced good radial growth of the test fungi, the 4 g cocoyam starch + 3 g agar medium produced the highest mean radial growth for both *A. niger* and *A. flavus* thus performing better than all the other media including the control PDA medium. This excellent performance of the 4 g cocoyam starch + 3 g agar medium could be due to supply of additional nutrients in form of cassava starch in an appropriate proportion. The starch could have served as additional source of carbon for the fungi thereby enhancing rapid growth. The ability of the media that contained cocoyam starch to support rapid radial growth of the test fungi implies that cocoyam starch can be used to substitute agar in the ratio 4:3 in mycological culture media without negatively affecting good growth of the test fungi. In line with the finding on radial growth, [3] reported that fungal culture media in which cassava starch partially substituted agar as gelling agent supported good radial growth of *A. niger* and *Fusarium oxysporium* with the radial growth of *A. niger* being comparable to that on potato dextrose agar.

All the media supported good sporulation of the fungi. The highest sporulation for both *A. niger* and *A. flavus* was however recorded for PDA which produced significantly higher mean number of spores (P = .05) than all the other media except for the 2 g cocoyam starch + 4 g agar medium in which case there was no significant difference for *A. flavus*. Fungal sporulation is dependent on environmental factors including physical, chemical, and nutritional factors. The observed differences in sporulation of the fungi on the different media could be due to differences in available nutrients resulting from addition of cocoyam starch at different concentrations. Addition of cocoyam starch to the media probably made them to be either too rich or not rich enough in nutrients necessary for sporulation of the fungi. However, [17] and [18] stated that due to lack of information on the chemical and physical status of natural microenvironment inhabited by fungi, relating experimental observations on the control of fruiting to natural conditions that stimulate sporulation is often difficult.

5.0 CONCLUSION

Though cocoyam starch possessed some level of gelling ability, it cannot be used alone as gelling agent for culture media. Cocoyam starch can substitute agar in culture media up to 57% without significant negative effects on culture media characteristics such as viscosity, pouring ability, solidity, and clarity. Blending cocoyam starch and agar at the appropriate ratio results in higher mean radial growth and lower sporulation of the test fungi. The findings have shown that cocoyam starch-agar blends can be used as gelling agent for mycological culture medium. Also, it is expected that substitution of agar with cocoyam starch which is cheaper than agar will result in culture media of lower cost and easier affordability for research and other purposes.

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