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Effect of Different Processing and Supplementation on Maize Cob as Microbiological Growth Medium for Fungi

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Abstract: Waste cobs from variously processed (premature, uncooked, roasted, cooked salted, cooked unsalted) maize (*Zea mays*) were used as raw materials to prepare growth agar media for six moulds: *Rhizopus nigricans, Aspergillus niger, Trichoderma viride, Alternaria tenuis, Fusarium* sp*, Penicillium* sp. and one yeast: *Debaryomyces* sp. All the formulated media supported the growth of the microorganisms tested. *Fusarium* sp. produced the widest diameter of growth ranging from 40.0 mm on unsalted cooked cob agar (UCCA) to 54.0 mm on fresh cob agar (FCA). The least diameter of growth was observed with *Debaromyces* sp. and it ranged from 0.1 mm on premature cob agar (PCA) to 0.9 mm on UCCA. The addition of dextrose showed that except with *Debaromyces* sp., the diameter of growth of fungi on media were significantly ($p<0.05$) wider as compared to the formulated media without dextrose. Correlation between addition of dextrose and diameter of growth was significant at 95% confidence interval. The diameter of growth was increased with increasing in time of incubation for all the media tested. For *Fusarrium* sp., there were no differences in the diameter of growth at 48h of incubation and at 72h on all the media tested. Pearson bi-variate correlation showed that at 95% confidence interval, significant positive correlations occurred between incubation time and the media used. This study showed that the supplementation of maize cobs with dextrose provide an alternative cheaper media for fungi cultivation in the laboratory.

Key words: Processing · Supplement · Moulds · Yeasts · Dextrose · Media · Waste cobs

INTRODUCTION

There are many types of microorganisms; hence, there are various types of growth media with varying composition. Generally, any acceptable growth medium should meet the nutritional requirement of the microorganism such as suitable carbon, energy sources and growth factors [1]. Frequently a medium is used to select and grow specific microorganisms or help identify a particular species. In such cases the function of the medium will also determine its composition [2]. Basically, a medium for microbial cultivation contains various organic and inorganic constituents which the microbe degrades and utilizes with the aid of enzymes it secretes so as to carry out its metabolic activities.

Fungi are chemoheterotrophs that grow on chemically undefined medium in the laboratory. They require both macro and micro nutrient and some fungi may have other requirement like thiamine, biotin, steroids, riboflavin, nicotinic acid and folic acid, however, the minimum nutritional requirement of most fungi is mineral salts and glucose [3].

Maize (*Zea mays* L.) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production [4; 5]. In sub-Saharan Africa, maize is a staple food for an estimated 50% of the population. It is an important source of carbohydrate, protein, iron, vitamin B, and minerals. More than 40 different ways of consuming maize had been recorded in many countries in Africa [6]. Africans consume maize as a starchy base in a wide variety of porridges, pastes, grits, and beer. Green maize (fresh on the cob) is eaten parched, baked, roasted or boiled with or without salt and plays an important role in filling the hunger gap after the dry season [3]. Every part of the maize plant has economic value: the grain, leaves, stalk, tassel, and cob can all be used to produce a large variety of food and non-food products. The maize cob though unpalatable for human is sometimes used as animal feed and if not dried it becomes mouldy within a few days in a hot climate.

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A preliminary experiment conducted in our laboratory showed that maize cob which is an agricultural by-product supported the growth of some moulds. As part of the effort to provide cheaper and readily available laboratory media that can compare favorably with the internationally accepted microbiological growth media for fungi, further studies were carried out using variously processed maize cobs supplemented with dextrose to further enhance the growth of fungi.

MATERIALS AND METHODS

Collection of Samples and Microorganisms: Waste cobs from roasted maize, cooked salted maize, cooked unsalted maize, uncooked maize and premature maize were collected fresh from different location within Abeokuta Ogun State during the peak of maize harvest. The cobs of variously processed maize were kept in sterile polythene bags in groups depending on their sources.

Pure cultures of seven moulds (*Rhizopus nigricans, Aspergillus niger, Trichoderma viride, Alternaria tenuis, Fusarium* sp., *Penicillium* sp., *Neuropora crassa*) and a yeast (*Debaryomyces* sp.) were obtained from IITA Ibadan, Oyo State

Proximate Analysis: The moisture, ash, fat, crude protein, lipid or crude fat and fibre of the different cob substrates were determined using the procedures described by the Association of Official and Agricultural Chemists [7].

Media Preparation: All the cobs were air dried and then dried in the oven at 50°C to constant weight. Each category of cob was scrapped off their rachis with a sharp knife and ground to powder in a disinfected dry milling machine. The powders were separately kept in clean specimen bottles for use as fungi growth medium.

Formulation of Fungi Growth Medium Agar: From each labeled cob powder, two different growth media were formulated

Process 1: Each maize powder MP was mixed with only agar - agar in the ratio 5:7 to form Maize powder Agar (MPA).

Process 1: Each maize powder (MP) was mixed with dextrose and agar- agar in ratio 5:10:7 to form Maize powder dextrose agar (MPDA)

Table 1: Codes of formulated and commercial media used for the growth of fungi

S/N	Medium	Code
1	Premature Cob Agar	PCA
2	Roasted Cob Agar	RCA
3	Fresh Cob Agar	FCA
4	Unsalted Cooked Cob Agar	UCCA
5	Salted Cooked Cob Agar	SCCA
6	Premature Cob Dextrose Agar	PCDA
7	Roasted Cob Dextrose Agar	RCDA
8	Fresh (uncooked) Cob Dextrose Agar	FCDA
9	Unsalted cooked Cob Dextrose Agar	UCCDA
10	Salted Cooked Cob Dextrose Agar	SCCDA
11	Potato Dextrose Agar	PDA
12	Sabouraud Dextrose Agar	SDA

Sample Analysis: Twenty five grams of each of the formulated growth medium was mixed with 500 ml distilled water in 1000 ml conical flask, boiled to melt the agar and then sterilized at 121°C for 15 minutes. The media were allowed to cool to about 45°C, aseptically poured into sterile petri-dishes and allowed to set. On the whole, 10 different growth media were formulated (Table 1). Commercially prepared potato dextrose agar (Lab M) and Sabouraud Dextrose agar (Oxoid) prepared according to the manufacturers procedures were used as control.

Preparation of Inoculum: Fresh and active (18-24h) colony of each test organism was picked using a sterile loop, placed in a sterile test tube and mixed thoroughly by shaking for 2 mins with sterile water. It was the transferred into sterile spectrophotometer cuvette and the wavelength of the spectrophotometer adjusted to 550 µm. the content of each cuvette was adjusted with sterile water until an absorbance reading of 0.05Å was obtained.

Using a sterile calibrated syringe, an aliquot (0.01 ml) of the standardized inoculum was dropped aseptically on the centre of each of the solidified formulated medium and the controls. Incubation was at 25°C and the diameters of growth of the organisms were taken at 24h intervals for 3 days using a calibrated vernier caliper.

Data Analysis: Data obtained were analyzed with analysis of variance (ANOVA) test using SPSS 10.0 version. Least significance difference (LSD) test was used to compare differences in means; while correlations between factors were analyzed using Pearson bi variate correlation.

RESULTS

Table 2 shows the proximate composition of the different types of maize cobs used as substrates. The crude protein ranged from 1.58±0.01% (PC) to 2.45±0.05% (FC). The fat, carbohydrate and fiber content of FC were significantly ($p<0.05$) higher than that of the other cob tested. The same trend was observed for crude protein. Unsalted maize cob (SC) had the highest moisture content (10.96±1.12%) while roasted cob (RC) had the lowest moisture content. The highest dry matter (89.96±1.11%) was obtained with RC while the premature corn (PC) had the least (80.92±0.02%).

The mean growth diameter (mm) of the isolates on formulated media and commercial media at 24 h of incubation are presented in Table 3. Results showed that most of the formulated media were able to support the growth of all the isolates tested. The mean growth diameter for *Rhizopus nigricans* ranged from 36.0 mm for Salted cooked cob agar (SCCA) to 48.0 mm for premature cob agar (PCA) while mean growth diameter of *A. niger* ranged from 21.0 mm for PCA to 45.0 mm for FCA. Salted cooked cob agar (SCCA) did not support the growth of *Alternaria tenuis* and *Debaromyces* sp. since no growth was observed after 24 h of incubation. Similar result was observed for *Debaromyces* sp. on Roasted cob agar (RCA).

Least significance difference (LSD) used to compare the means showed that the mean growth diameter of *A. niger* and *R. nigricans* on the commercial media were significantly ($p<0.05$) higher than that of the formulated media. However, for the other isolates tested, the diameter of growth of some formulated media were not significantly different from that of the control especially Sabouraud dextrose agar (SDA). Irrespective of the growth media used, the least mean diameter of growth was observed with *Debarommyces* sp.

Table 4 presents the effect of the incorporation of dextrose into the formulated growth media on the growth of the isolates tested. The mean growth diameter of *R. nigricans* ranged from 56.0 mm on unsalted cooked cob dextrose agar (UCCDA) to 79.0 mm on premature cob dextrose agar (PCDA). With the exception of *Debaromyces* sp., the diameter of growth on media with dextrose were significantly ($p<0.05$) wider at 24 h as compared to the formulated media without dextrose (Table 3). No growth was observed for *Alternaria tenuis* on Fresh cob dextrose agar (FCDA), Unsalted cooked cob agar (UCCDA) and salted cooked cob agar (SCCDA). The least diameter of growth was observed with *Debaromyces* sp. irrespective of the media used. Correlation between addition of dextrose and diameter of growth was significant at 95% confidence interval. With the addition of dextrose, the diameter of growth of some of the isolates on the formulated media were significantly ($p<0.05$) higher than that of the control media especially SDA. Comparison of means showed 65.0 mm obtained for A. *niger* on SCCDA was significantly higher than 55.0 mm obtained on SDA.

Table 5 and 6 show the effect of time of incubation on the growth diameter of the isolates. For all the media tested, the diameter of growth was increased with increasing in time of incubation. For *Fusarium* sp., there were no difference in the diameter of growth at 48h of incubation and at 72 h on all the media tested. Pearson bi-variate correlation showed that at 95% confidence interval, significant positive correlations occurred between incubation time and the media used.

At 24h and 48 h of incubation, wider growth diameters were observed on media with dextrose as compared to media without dextrose (data not shown). At 72 h, the diameter of growth was 90.0 mm for *R. nigricans, Fusarium* sp. *and N. crassa* on all the media tested. There were no significance difference in the diameter

Table 2: Proximate composition of the different maize cobs used as substrates for media formulation

Parameters	PC±SE*	RC±SE	FC±SE	UC±SE	SC±SE
Crude protein	1.58±0.01	1.75±0.05	2.45±0.05	2.10±0.10	1.93±0.01
Crude fat	0.26±0.01	0.28±0.02	0.38±0.01	0.32±0.02	0.35±0.01
Crude ash	1.26±0.02	1.62±0.02	1.58±0.01	1.34±0.02	3.47±0.02
Crude carbohydrate	48.5±1.01	49.2±0.20	55.2±1.0	52.1±0.15	50.4±0.41
Crude fibre	31.74±1.04	32.64±0.01	35.26±1.61	34.86±1.42	33.72±1.52
Dry matter	89.92±0.02	89.96±1.11	89.86±0.14	89.04±4.01	89.75±0.25
Moisture content	10.08±0.01	10.04±0.04	10.14±0.12	10.96±1.12	10.25±0.26

*- Mean of triplicate values±Standard error. PC- Premature cob; RC- Roasted cob; FC- Fresh cob; UC- Unsalted cob; SC- Salted cob

Table 3: Growth diameter of isolates on different formulated growth media

Growth medium	*Mean growth diameter of isolates (mm)±S.E							
	R nigricans	*A. niger*	*T. viride*	*Fusarium* sp.	*N. crassa*	*Alternaria tenuis*	*Penicillium*	*Debaromyces* sp.
PCA	48.0c	21.0a	23.0b	48.0c	40.0b	20.0b	15.0b	0.1a
RCA	40.0b	20.0a	21.0a	44.0b	45.0c	20.0b	17.0b	NG
FCA	47.0c	45.0c	23.0b	54.0d	45.0c	30.0c	27.0c	0.9b
UCCA	50.0c	45.0c	21.0a	40.0a	50.0d	20.0b	17.0b	0.9b
SCCA	36.0a	41.0b	24.0bd	45.0bc	40.0b	NG	10.0a	NG
PDAc	65.0d	73.0e	26.0d	54.0d	53.0e	36.0d	30.0c	1.2c
SDAc	85.0e	55.0d	21.0a	54.0d	30.0a	30.0c	18.0b	1.0bc
**SE	1.51	1.1	1.1	1.51	0.9	1.06	1.41	5.56

Means with the same superscript along columns are not significantly different. *Mean of triplicate determinations, [c]- Control (commercial media), NG- No growth, ** Standard Error

Table 4: Effect of dextrose on the growth diameter of isolates on the formulated growth media

Growth medium	*Mean growth diameter of isolates (mm)±S.E							
	R nigricans	*A. niger*	*T. viride*	*Fusarium* sp.	*N. crassa*	*Alternaria tenuis*	*Penicillium*	*Debaromyces* sp.
PCDA	79.0^{d}	30.0^{a}	20.0^{a}	58.0^{bc}	55.0^{b}	30.0^{c}	35.0^{d}	0.1^{a}
RCDA	66.0^{bc}	30.0^{a}	23.0^{cd}	55.0^{ab}	80.0^{e}	25.0^{b}	45.0^{e}	NG
FCDA	57.0^{a}	41.0^{b}	25.0^{de}	60.0^{c}	55.0^{b}	NG	22.0^{b}	1.0^{b}
UCCDA	56.0^{a}	60.0^{d}	26.0^{e}	52.0^{a}	65.0^{d}	NG	19.0^{a}	0.1^{a}
SCCDA	70.0^{c}	65.0^{e}	17.0^{a}	60.0^{c}	60.0^{c}	NG	20.0^{ab}	0.1^{a}
PDA^{c}	65.0^{b}	73.0^{f}	26.0^{e}	54.0^{a}	53.0^{b}	36.0^{d}	30.0^{c}	1.2^{c}
SDA^{c}	85.0^{c}	55.0^{c}	21.0^{bc}	54.0^{a}	30.0^{a}	30.0^{c}	18.0^{a}	1.0^{b}
**SE	±2.05	2.25	1.23	1.60	2.09	1.15	1.67	4.36

Means with the same superscript along columns are not significantly ($p<0.05$) different. *Mean of triplicate determinations, [c]- Control (commercial media), NG- No growth, ** Standard Error.

Table 5: Effect of incubation time (48 h) on the growth of isolates on the formulated media

Growth medium	*Mean growth diameter of isolates (mm)±S.E							
	R nigricans	*A. niger*	*T. viride*	*Fusarium* sp.	*N. crassa*	*Alternaria tenuis*	*Penicillium*	*Debaromyces* sp.
PCDA	85.0^{d}	45.0^{a}	70.0^{c}	90.0^{a}	87.0^{b}	87.0^{d}	90.0^{e}	0.2^{ab}
RCDA	70.0^{b}	56.0^{b}	67.0^{b}	90.0^{a}	90.0^{c}	88.0^{d}	90.0^{e}	0.1^{a}
FCDA	60.0^{a}	68.0^{c}	71.0^{c}	90.0^{a}	90.0^{c}	25.0^{b}	48.0^{c}	0.5^{b}
UCCDA	62.0^{a}	85.0^{d}	74.0^{d}	90.0^{a}	90.0^{c}	4.0^{a}	42.0^{a}	2.0^{c}
SCCDA	75.0^{c}	90.0^{e}	65.0^{b}	90.0^{a}	88.0^{bc}	60.0^{c}	45.0^{b}	2.0^{c}
PDA^{c}	71.0^{a}	90.0^{e}	75.0^{d}	90.0^{a}	90.0^{c}	89.0^{d}	90.0^{e}	3.0^{d}
SDA^{c}	90.0^{c}	90.0^{e}	60.0^{a}	90.0^{a}	62.0^{a}	88.0^{d}	50.0^{d}	2.0^{c}
**SE	1.43	1.17	1.28	1.02	1.20	1.41	0.93	1.17

Means with the same superscript along columns are not significantly ($p<0.05$) different, *Mean of triplicate determinations, [c]- Control (commercial media), ** Standard Error.

Table 6: Effect of incubation time (72 h) on the growth of isolates on the formulated media

Growth medium	*Mean growth diameter of isolates (mm)±S.E							
	R nigricans	*A. niger*	*T. viride*	*Fusarium* sp.	*N. crassa*	*Alternaria tenuis*	*Penicillium*	*Debaromyces* sp.
PCDA	90.0^{a}	55.0^{a}	88.0^{b}	90.0^{a}	90.0^{a}	90.0^{c}	90.0^{c}	0.9^{ab}
RCDA	90.0^{a}	65.0^{b}	88.0^{b}	90.0^{a}	90.0^{a}	90.0^{c}	90.0^{c}	0.2^{a}
FCDA	90.0^{a}	82.0^{c}	90.0^{b}	90.0^{a}	90.0^{a}	60.0^{a}	75.0^{a}	1.5^{b}
UCCDA	90.0^{a}	90.0^{d}	90.0^{b}	90.0^{a}	90.0^{a}	75.0^{b}	80.0^{b}	2.8^{c}
SCCDA	90.0^{a}	90.0^{d}	89.0^{b}	90.0^{a}	90.0^{a}	90.0^{c}	75.0^{a}	2.8^{c}
PDA^{c}	90.0^{a}	90.0^{d}	90.0^{b}	90.0^{a}	90.0^{a}	90.0^{c}	90.0^{c}	5.0^{d}
SDA^{c}	90.0^{a}	90.0^{d}	81.0^{a}	90.0^{a}	90.0^{a}	90.0^{c}	82.0^{b}	4.0^{d}
**SE	1.02	1.28	0.87	1.02	1.02	1.21	1.41	0.47

Means with the same superscript along columns are not significantly ($p<0.05$) different, *Mean of triplicate determinations, [c]- Control (commercial media), ** Standard Error

of growth on the formulated media and the commercial media tested.

DISCUSSION

The differences observed in the proximate composition of the maize cobs can be attributed to the different processing methods they have passed through.

The highest moisture content observed in the cooked maize cobs suggests that they acquired the moisture from the water used for cooking. However, the highest moisture content of the unsalted cooked maize cob as compared to the salted cooked cob may be due to the fact that the moisture introduced to the maize cob during cooking with water was tied up by the presence of salt in the cooking medium. Solutes and ions are known to tie up water in solution [8].

Ten different growth media were formulated from differently processed maize cob (Table 1) and were used as growth media for eight different fungi (*Rhizopus nigricans, Aspergillus niger, Trichoderma viride, Neurospora crassa, Fusarium* sp. *Penicillium* sp., *Alternaria tenuis,* and *Debaryomyces* sp.). All the fungi tested grew on the growth media with or without the addition of dextrose and showed various diameter of growth at 24 hours of incubation and the diameter of growth increased with increase in incubation time.

Generally, wider diameters of growth were observed with the incorporation of dextrose into the formulated media. Dextrose, a monosaccharide probably acted as a simple source of carbon which the variously processed maize cobs did not posses, their carbon being complex. However, *Trichoderma* sp. and *Alternaria* sp. did not show significance difference to the presence of dextrose probably because they have the enzymes to readily digest the complex fibre of the raw material.

Five of the seven molds exhibited similar growth diameters on both the internationally recognized and the formulated growth media. This probably suggests that for the growth of these five molds, the formulated media can perform equally as the internationally recognized growth media.

A. niger is known to grow well in salted environment [9]. This explains its flourish growth on Salted cooked cob dextrose agar (SCCDA) which compared favourable with PDA medium for its growth.

The formulated media did not encourage the growth of *Debaryomyces* sp. Comparing internationally recognized media with formulated media, PDA was the best growth medium but all the formulated media were better than SDA. Hence, in the absence of PDA, these formulated media can be used. The raw material is a waste maize residue readily available and cheap. It can be processed into powder and kept in a cool dry place for use.

CONCLUSION

Utilization of agrarian residues like corn cobs offers on the one hand possibilities for the development of a decentralized process industry. This is important for many developing countries that are in need for new stimuli for their economic development. On the other hand, the utilization of this side products from agriculture offer farmers new channels of income and also offers advantages in terms of reduced pressures on the environment.

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