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Formulation and Evaluation of Dehydrated Microbiological Media from Avocado Pear (*Peasea americana* Cmill)

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Abstract: Avocado pear (*Peasea amaricana* Cmill) has an excellent nutritional quality that can support the growth of microorganisms. Different media were formulated from both defatted and undefatted dehydrated avocado pear. The proximate analyses of the pear flour show that defatted samples were better in term of minerals contents than their corresponding undefatted samples. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* in that order thrived very well in the composed media. The test bacteria grew better in media composed with defatted pear than their corresponding undefatted samples. Undefatted samples seem to support fungal growth than defatted samples. *Trichoderma* sp. grew better than *Aspergillus flavus* and *Penicillium notatum*. Comparing with the performance of conventional bacteriological and mycological media, avocado pear is a good and cheap media material for the cultivation and isolation of both bacteria and fungi.

Key words: Avocado pear, pathogens, formulated media, proximate composition, mineral composition, microbial growth

INTRODUCTION

Microbiological media have no doubt played a major role in the cultivation and studying of microorganisms (Anonymous, 1985). Media are very expensive particularly in resource-poor nations (Eleke *et al*., 2006) including Nigeria. The need for the cultivation and propagation of microorganisms on locally available agricultural products becomes imperative (Achinewhu, 1986).

Plant materials have been used to recover both fungi and bacteria from different samples sources. Groundnut (Akinola *et al*., 2004), sorghum extracts (Akinola *et al*., 1997), local food stuff waste (Ogundana and Odoh, 1984), cassava whey (Adesina and Akinyosoye, 2006; Ikenebomen and Chikwendu, 1997), cereals (Kadiri, 1998), three-leaf yam (Eleke *et al*., 2006), African oil bean (Egenu and Njoku, 2006) cassava (Nzeribe and Gugnani, 1984), yam (Westeijn and Okafor, 1971), Maize and beans (Oloke and Famurewa, 1991), pigeon pea (Laleye *et al*., 2007) among other agricultural products have been used as both mycological and bacteriological media.

A single medium cannot totally support the growth of all organisms, but organism thrives optimally in a medium that satisfies their minimal nutritional requirement(s) (Oloke and Famurewa, 1991). The use of dehydrated media has gained a global acceptance in both mycological and bacteriological laboratories (Franhauser, 2005).

Avocado pear (*Peasea americana* Cmill) is an edible and highly nutritious fruit (Keay *et al*., 1964) that is very common in the tropics. The size varies from only a few centimeters to 25 cm in length. It consists as much as 30% digestible fat and appreciable vitamins (Olumese, 2005). Once mature fruit is picked, it perishes quite quickly (Hutchjnson and Dalziel, 1958).

To the best of our knowledge there has not been any report on formulation of microbiological media using avocado pear. This makes this work innovative. Information is available on the formulation

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of media using agricultural materials. It is also known that defatting has different effect on the growth of microorganisms. However, there is no information on growth assessment of both fungi and bacteria on media containing avocado in any form (defatted and undefatted). This study is aimed at providing this information.

MATERIALS AND METHODS

Source and Preparation of Pear Sample

Ripe and unripe (but mature) pear fruits were purchased from different markets in Ado-Ekiti, Nigeria between April and December, 2006.

The method of Umechuruba and Elenwo (1999) was used to prepare pear flour. The fruits were washed thoroughly and each of the groups (ripe and unripe) was further divided into two subgroups: unpeeled (epicarp with the mesocarp) and peeled (mesocarp only). Four formulations were made from pear as follows: Ripe Unpeeled (RU), Ripe Peeled (RP), Unripe Unpeeled (UU) and Unripe Peeled (UP). The seeds were cut manually into 3 mm thickness. The slice were arranged in separate stainless trays which were put into an oven at 90°C for 10 h and later reduced to 80°C for 24 h until chips were completely dried.

The chips were pulverized into fine powder using an electronic blender (SBG-320, Lagos, Nigeria) and sieved into a fine powder. The powder was stored separately in sterile transparent containers. Each of the fine powder was further divided into two portions. The method of Adisa and Odutuga (1998) was used to de-fat a portion of the final groups.

Preparation of Pear Agar Media

The solid medium used was prepared by adding 0.35 g of each of the samples separately inside a conical flask containing 0.1 g K_2HPO_4 and 0.3 g KH_2PO_4 and made up to 100 mL with distilled water. The salts were added to prevent degradation of protein and to act as buffer. To each of the conical flasks, 1.5% agar-agar was added to serve as a solidifying agent. The compounded medium was melted to ensure even distribution of the agar. The medium was sterilized at 121°C for 15 min. Approximately 20 mL of the sterilized medium was distributed into each of the sterile Petri dishes.

Source of Inocula

The microorganisms used for this research were collected from the stock culture collection of the Department of Microbiology, University of Ado-Ekiti, Nigeria. The bacteria used were *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*. They were grown (in separate tubes) at 37°C in Mueller-Hilton (Oxoid) broth for 16-18 h with shaking and diluted to an optical density of 0.1 (0.5 McFarland Standard) at optical activity of 625 nm with Mueller-Hilton (Oxoid) broth and stored at 4°C to arrest further bacterial multiplication. The fungi used included the following: *Aspergillus flavus*, *Trichoderma* sp. and *Penicillium notatum*. They were grown on Malt Extract Agar at 25±2.0°C for 48 h.

Cultivation and Enumeration of Test Organisms

A loopful of the standardized pure culture of each of the test bacteria was inoculated by streaking directly on the solid media. The degree of growth was determined by comparing with the growth on the conventional medium (nutrient agar, Oxoid).

Fungal inoculum was obtained with sterilized 0.7 mm cork borer from the growing edge of 48 h-old mycelia mat. The fungi had previously been grown for 48 h on Malt Extract Agar (Fluka) at 25±2°C. Each of inoculation disc was inverted on the surface of each of the composed media and the control, (malt extract agar). The plates were incubated at 25±2°C for 48 h. The diameter of mycelia was taken as an index of growth and was measured at every 24 h of incubation.

Proximate Analysis and Mineral Composition of Pear Flour

The standard method of AOAC (1984) was used to determine moisture content, crude protein, crude fibre, ether extract (crude lipid). The method of Pearson (1976) was used to determine the protein content of the samples. Phosphorus was determined by the phosphovanado-molybdate by the method of AOAC (1984).

RESULTS AND DISCUSSION

The results of mineral and proximate analyses of both defatted and undefatted samples are shown in Table 1 and 2, respectively. Defatting increased the mineral contents of the samples, (i.e amount of individual element per one gram of sample). The amount of phosphorus was highest ranging between 1148.89 to 1693.00 and 1790.22 and 3170.22 ppm in undefatted and defatted samples, respectively. Avocado is very rich in minerals and vitamins (Olumese, 2005; Kirschmann, 1979) that can support bacterial growth. The essential elements for microbial growth were present and agreed with Cooke (1975). The defatted samples had higher amounts of moisture, ash, protein and carbohydrates than their corresponding undefatted samples.

Escherichia coli grew poorly on undefatted sample; this may be due to its poor lipolytic activity (Akinola *et al*., 2004). *Pseudomonas aeruginosa* thrived very well in all the media which justifies the ability of the organism to utilize a variety of carbon sources (Brooks and Antai, 2006). The three bacteria grew better on media composed with defatted than undefatted pear. This may be due to the preference of the organisms for carbohydrate (Ikenebomen and Chikwendu, 1997).

Unpeeled sample supported the growth of fungi than the peeled samples. Undefatted pear also supported fungal growth than their corresponding defatted samples. *Trichoderma* sp. grew excellently in all the media except DRU that inhibited its growth. This suggests that the lipolytic activity of fungi was better than that of bacteria used in this study (Table 3). The result of this study is similar to the findings of Umechuruba and Elenwo (1999) on defatted and undefatted groundnuts.

Table 1: Proximate analysis of different pear flour used for the formulation of avocado pear

Samples	Moisture content	Ash	Fat	Crude fibre	Protein	Carbohydrate
URU	4.00±0.06	4.48±0.16	42.96±0.55	1.85±0.05	19.77±0.20	26.95±0.60
URP	3.50±0.61	5.14±0.09	38.84±0.50	1.40±0.14	24.84±0.18	26.31±0.49
UUU	4.16±0.50	3.11±0.10	39.66±0.19	1.15±0.03	18.99±0.13	32.47±0.13
UUP	4.38±0.03	3.70±0.02	38.61±1.20	1.88±0.02	16.38±0.12	35.06±1.23
DRU	6.68±0.96	6.02±1.29	1.04±0.02	3.35±1.57	32.98±0.53	49.93±0.86
DRP	5.56±0.42	4.01±0.53	0.78±0.03	2.28±0.60	40.58±0.10	46.93±2.93
DUU	6.80±1.86	6.16±1.08	0.92±0.01	1.74±0.81	29.94±1.65	54.44±2.21
DUP	6.17±0.78	6.54±1.31	1.01±0.05	3.24±0.82	26.00±2.25	57.04±2.99

Data are the mean±SEM of three determinations

URU = Undefatted ripe unpeeled, URP = Undefatted Ripe Peeled, UUU = Undefatted Unripe Unpeeled, UUP = Undefatted Unripe Peeled, DRU = Defatted Ripe Unpeel, DRP = Defatted Ripe Peeled, DUU = Defatted Unripe Unpeeled, DUP = Defatted Unripe Peeled

Table 2: Mineral composition of different pear flour used for the composition of avocado pear

Samples	Ca	K	Na	Mg	Fe	Zn	Mn	Cu	P	Na/K	Ca/P
URU	381.20	409.19	426.81	336.56	4.180	6.89	1.59	1.01	1693.07	1.04	0.23
URP	315.83	313.83	301.99	333.13	5.110	7.15	3.21	0.82	1148.89	0.96	0.98
UUU	425.83	319.38	304.75	298.40	3.810	5.14	2.16	1.84	1543.21	0.78	0.28
UUP	240.20	330.52	294.58	207.24	2.290	4.58	1.15	0.74	1235.59	0.98	0.19
DRU	655.83	708.83	753.48	589.56	7.299	11.89	2.72	2.09	2917.38	1.06	0.22
DRP	504.44	490.39	483.69	590.59	7.970	11.15	5.05	1.83	1791.22	0.99	0.28
DUU	668.18	663.34	610.67	475.53	6.040	8.26	3.36	2.87	2501.43	0.92	0.27
DUP	629.28	530.40	475.04	332.64	3.880	7.84	1.90	1.20	3170.22	0.90	0.19

URU = Undefatted Ripe Unpeeled, URP = Undefatted Ripe Peeled, UUU = Undefatted Unripe Unpeeled, UUP = Undefatted Unripe Unpeeled, DRU = Defatted Ripe Unpeel, DRP = Defatted Ripe Peeled, DUU = Defatted Unripe Unpeeled, DUP = Defatted Unripe Peeled

Table 3: Assessment of bacterial growth on media formulated from avocado pear

Test organisms	URU	UUP	URP	UUU	DUU	DUP	DRU	DRP	NA
Staphylococcus aureus	+	++	++	++	+++	+++	+++	+++	+++
Escherichia coli	+	NG	+	NG	+++	+++	+++	+++	+++
Pseudomonas aeruginosa	++	++	+++	++	+++	+++	+++	+++	+++

Data are the modal values of three determinations
URU = Undefatted Ripe Unpeeled, URP = Undefatted Ripe Peeled, UUU = Undefatted Unripe Unpeeled, UUP = Undefatted Unripe Peeled, DRU = Defatted Ripe Unpeeled, DRP = Defatted Ripe Peeled, DUU = Defatted Unripe Unpeeled, DUP = Defatted Unripe Peeled, +++ = Good growth, ++ = Fair growth, NG = No Growth, +++ = Excellent

Table 4: Mycelia spread (mm) of fungi on media formulated from avocado pear

		Extension diameter (mm)								
Time (h)	Test fungus	URU	UUP	URP	UUU	DUU	DUP	DRU	DRP	MEA
0	*Aspergillus flavus*	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
	Trichoderma sp.	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
	Penicillium notatum	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
24	*Aspergillus flavus*	2.0	2.0	1.0	2.0	0.7	0.7	0.7	0.7	4.0
	Trichoderma sp.	2.0	2.0	2.0	0.7	0.7	1.0	0.7	0.7	4.0
	Penicillium notatum	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	28.0
48	*Aspergillus flavus*	18.0	18.0	12.0	10.0	0.7	0.7	0.7	0.7	20.0
	Trichoderma sp.	19.0	18.0	10.0	11.0	12.0	18.0	0.7	5.0	30.0
	Penicillium notatum	12.0	10.0	12.0	10.0	8.0	6.0	8.0	4.0	28.0
72	*Aspergillus flavus*	33.0	27.0	28.0	20.0	12.0	12.0	14.0	10.0	48.0
	Trichoderma sp.	45.0	25.0	14.0	23.0	36.0	35.0	10.0	18.0	45.0
	Penicillium notatum	16.0	14.0	14.0	14.0	10.0	10.0	12.0	8.0	42.0

Data is the modal value of triplicate determinations
URU = Undefatted Ripe Unpeel, URP = Undefatted Ripe Peeled, UUU = Undefatted Unripe Unpeel, UUP = Undefatted Unripe Peeled, DRU = Defatted Ripe Unpeel, DRP = Defatted Ripe Peeled, DUU = Defatted Unripe Unpeel, DUP = Defatted Unripe Peeled

Oil encourages the metabolism in fungi (Guirrard, 1958; Franhauser, 2005). The oil in the avocado flour serves as bacterial repressor, during cultivation and isolation of fungi. Avocado pear can be used to cultivate both bacteria and fungi. Media composed from the defatted samples can be used to cultivate bacteria while undefatted samples serve as media for cultivation and isolation of fungi (Table 4).

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