PROPAGATION OF TEMPEH *RHIZOPUS OLIGOSPORUS* I: USE OF ORGANIC FOOD WASTES

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ABSTRACT

The possibility of using some Nigerian organic food wastes as substrates to propagate tempeh *Rhizopus oligosporus* was investigated. The food wastes tested were peels of cassava, yam, potato and plantain. The ability to propagate the fungus was compared on the bases of radial growth, germination rate and ratio, rate of hyphal elongation and mycelial dry weight. All the substrates supported the growth of the organism; but the potato peel was the best. The order (descending) in which the food wastes supported the growth of the *R. oligosporus* was: potato peels > cassava peels > plantain peels > yam peels.

Key words: Organic food wastes, tempeh, *Rhizopus oligosporus*
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INTRODUCTION

Tempeh is a popular Indonesian food made by fermenting soybeans with *Rhizopus* species (Wang *et al.,* 1975). Several species of *Rhizopus* have been isolated from tempeh, but *Rhizopus oligosporus* is considered most suitable for tempeh making (Hesseltine, 1985; Ko, 1985). Fermentation is considered completed when the beans have been bound tightly by the mold mycelium into compact white cakes, which are customarily consumed within a day or two (Rusmin & Ko, 1974). Tempeh has been noted for its nutritional enhancement and digestibility during consumption. The characteristic delicious flavour has contributed to its being consumed regularly by millions of people (William & Akiko, 1985). Preparation of tempeh requires the use of a starter culture. The latter include natural starters, pure culture starters and semi-pure culture starters (Nout *et al.,* 1992).

Considering the nutritional potential of tempeh, it is necessary to introduce the product into the Nigerian diet. One of the factors necessary for large-scale production of tempeh is the propagation of the fungus. The mass production of *Rhizopus oligosporus* is needed for commercial production of tempeh. There are very abundant organic food wastes from households. Some of these kitchen wastes in Nigeria include peels of yam (*Dioscorea rotundata* Poir), potato (*Ipomoea batatas* Lam), cassava (*Manihot esculentus* Crantz) and plantain (*Musa* spp. L.). Most of these food wastes are dumped in open spaces, constituting litter and sources of environmental pollution. However, these potential sources of health hazard may contain some nutritional components, which can support the growth of *Rhizopus oligosporus.* The wastes may also serve as very cheap and readily available substrates for producing the starter culture.

Thus, the objective of this study was to determine the suitability of some Nigerian organic food wastes as substrates to propagate *Rhizopus oligosporus*. The peels of yam, cassava, potato and plantain were compared on their suitability to be used as substrates.

MATERIALS AND METHODS

Sources of Materials

Yam (*Dioscorea rotundata* Poir), potato (*Ipomoea batatas* Lam), cassava (*Manihot esculentus* Crantz) and plantain (*Musa* spp. L.) were bought from a local market in Ado – Ekiti. The fresh peels obtained using kitcken knife were sundried, ground into powder and sieved (850 um mesh, Pascall Eng. Co. Ltd, Sussex). The mold culture (*Rhizopus oligosporus*) was obtained through Ms. Pharma Wanita of the Indonesian Embassy, Lagos. The mold was subcultured and maintained on Malt Extract Agar.

Preparation of spore suspension

The mold was grown on Malt Extract Agar (Lab M) in petri dishes at 30^0C till it sporulated (~72hrs). The spores were harvested by washing the culture with sterile 0.2% peptone water. Mycelia were removed by filtering through sterile non-absorbent cotton wool into a sterile conical flask. The filtrate was used as inoculum. The spore count was determined using an improved Neubaeur Counting chamber and expressed as count per millilitre.

Preparation of extracts of substrates

A known weight each of the powdered yam, plantain, potato and cassava peels was added to distilled water at the ratio of 1:5 (w/v) in a conical flask. The slurry was stirred occasionally for 30 minutes and then filtered into a clean 250ml conical flask using a Whatman No.1 filter paper. The filtrates were used as extracts of substrates.

Determination of radial growth

Media were formulated using the extracts of the food wastes. To known volume of each extract was added the appropriate quantity of dehydrated agar powder (Oxoid) to make a final concentration of 1.5% agar. The media were autoclaved at 121^0C for 15 minutes. Solidified agar media plates were inoculated with 7mm agar disk from sporulated culture of the *Rhizopus oligosporus*. Plates were incubated at 30^0C and the radial growth was measured at 6-hour intervals. Determinations were done in replicates.

Determination of germination rate and ratio

The same quantity (7.5ml) of the extracts obtained from the substrates (as described earlier) was added into 2.0cm-diameter test tubes and autoclaved at 121^0C for 15 minutes. Each sterile extract was inoculated with 0.5ml of the spore suspension. Samples taken at 2-hour intervals were placed in a Neubaeur Counting chamber and examined under the x10 objective. The total number of spores and the spores, which had germinated, were counted. The percentage

germination ratio was thus calculated. The rate of germination of spores during the eight-hour period were also determined in replicates, and expressed per hour.

Determination of hyphal elongation rate

A known volume (7.5ml) of the extracts of substrates was pipetted into 2cm diameter test tubes and sterilised by autoclaving at 121^{0}C for 15 minutes. Each sterilized extract was inoculated with 0.5ml of spore suspension and the tubes were incubated at 30^{0}C. At regular interval of 30 minutes, samples were placed on a clean microscope slide and the hyphal lengths were measured using an ocular micrometer.

Determination of mycelial dry weight.

Twenty-five millilitres each of the extracts were added into 250ml conical flasks and autoclaved at 121^{0}C for 15 minutes. The content of each flask was inoculated with 0.5ml of spore suspension and incubated at 30^{0}C for 48 hours and 72 hours. The content of each flask was filtered through a pre-weighed dry filter paper. The dry weight of the mycelia was determined after drying the sediment on the filter paper in an oven at 70^{0}C for 48 hours. Determinations were done in replicates.

Proximate Analyses of substrates

The proximate analyses of the food wastes were performed. The parameters determined include the crude protein, fat, fibre, sugar, ash and carbohydrate contents. The moisture content of each sample was also determined. The methods of AOAC (1990) were adopted to determine the moisture, fibre, crude protein (Kjedahl method) and ash contents. The fat content was determined using Soxhlet extractor (Pearson, 1970), while reducing sugars content was assayed by the Dubois *et al.* (1956) method. The percentage carbohydrate content was determined by calculating the difference.

RESULTS

The fungus grew extensively on all the formulated media, showing characteristic white fuzzy mycelia within 24 hours. Radial growth was most extensive on the potato peels formulated medium (Figure 1). Similarly, the germination rate and ratio of the fungal spores was highest in potato peels extract and closely followed by that in the cassava peels extract (Table 1).

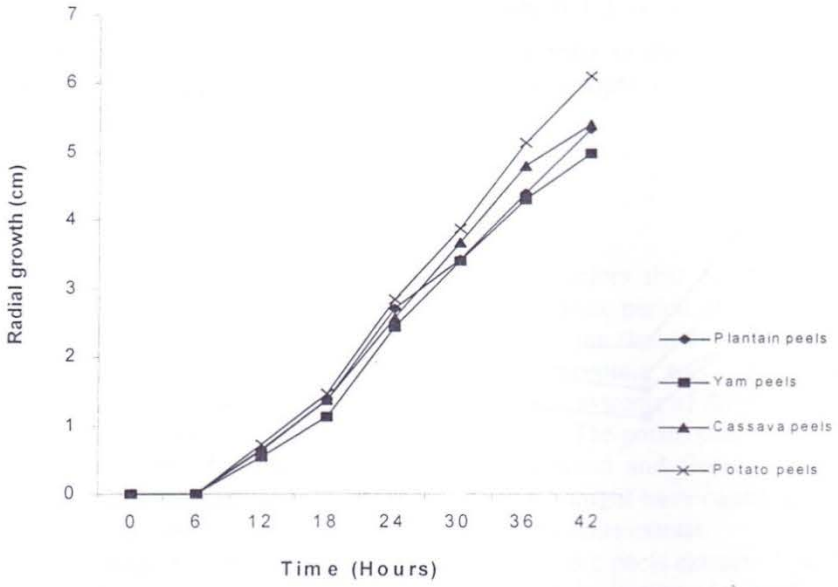

Figure 1: **Radial growth (cm) of *Rhizopus oligosporus* on agar medium formulated with substrates**

Table 1: **Germination rate & ratio of spores of *Rhizopus oligosporus* on the substrates. The ratio is expressed as percentage (%) and rate as percentage per hour (%hr^{-1}) of total no. of spores inoculated.**

Time (hrs)	Plantain peels		Yam peels		Cassava peels		Potato peels	
	Ratio (%)	Rate (%hr^{-1})	Ratio (%)	Rate (%hr^{-1})	Ratio (%)	Rate (%hr^{-1})	Ratio (%)	Rate (%hr^{-1})
0	0.41	-	0	-	0.43	-	0.65	-
2	2.26	0.92	1.73	0.86	3.09	1.33	4.05	1.7*
4	4.93	1.13	3.37	0.84	6.34	1.48	7.61	1.74
6	6.22	0.97	5.45	0.91	10.10	1.61	10.82	1.69
8	7.66	0.91	6.73	0.84	12.66	1.53	12.89	1.53

The rate at which the hyphae elongated was fastest in potato peels extract than any other extract (Figure 2). The dry weight (grams) of the mycelia harvested after incubation for 48 hours and 72 hours confirmed the superiority of the potato peels over the other food wastes in supporting the growth of the *Rhizopus oligosporus* strain (Table 2).

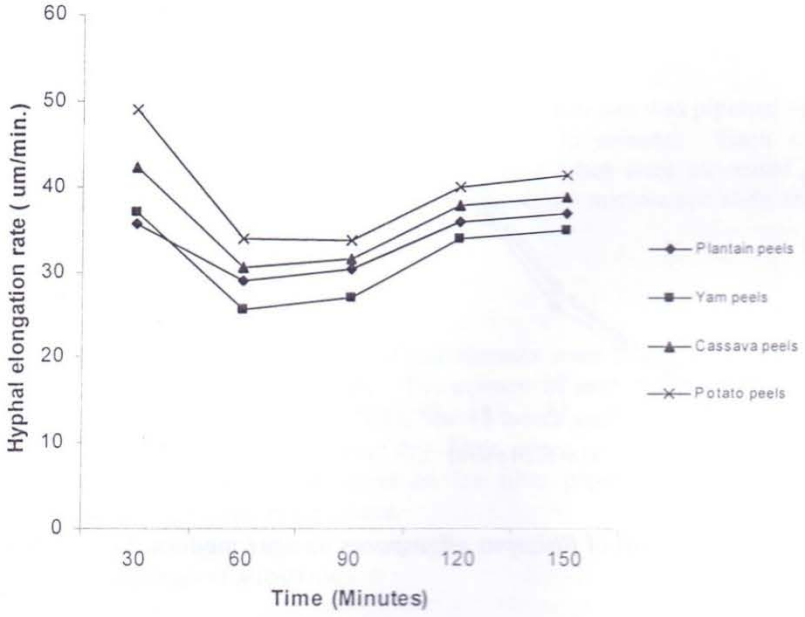

Figure 2: **Hyphal elongation rate (um/min.) of *Rhizopus oligosporus* on extracts of organic wastes.**

Table 2: **Dry weight (g) of mycelia of the *Rhizopus oligosporus* grown on extracts of organic wastes.**

Time (hrs)	Dry weight of mycelia (g) of:			
	Plantain peels	Yam peels	Cassava peels	Potato peels
48	0.067	0.047	0.07	0.08
72	0.073	0.063	0.087	0.093

The proximate composition of the food wastes are shown in Table 3.

Table 3: **Proximate composition of the organic food wastes.**

Parameters (%)	Plantain peels	Yam peels	Cassava peels	Potato peels
Ash	5.08	5.86	6.27	5.79
Moisture content	10.40	11.56	12.91	13.73
Crude protein	7.23	6.33	8.39	6.80
Fat	9.55	1.19	1.03	1.55
Fibre	5.77	10.52	7.01	0.44
Carbohydrate	61.97	64.52	64.39	71.68
Sugar	0.07	0.07	0.02	0.10

The carbohydrate and sugar contents were highest in the potato peels; while crude protein was highest in the cassava peels. Fat content was highest in plantain peels; while yam peels had the highest fibre content.

DISCUSSION

William and Akiko (1985) reported that some factors that can affect the growth of *Rhizopus oligosporus* include the available nutrients, aeration, period of incubation, the ratio of water to substrate and the size of the inoculum. Growth of the strain of *Rhizopus oligosporus* on the formulated media indicated that these food wastes contain sufficient nutrients that can support the growth of the fungus. The versatile enzymatic systems of *Rhizopus oligosporus* can metabolise several types of substrates (Wang *et al.*, 1972). The potato peels had relatively higher sugar and carbohydrate contents than other peels. Medwid and Grant (1984) reported that carbohydrate promotes sporangiospore germination. This might have contributed to the highest germination rate and ratio that were observed in the potato peels extract.

The rate of hyphal elongation was highest in the potato peels extract. Where there is high rate of hyphal elongation, there will be a faster mycelial colonisation of the substrate. From the results of this study, potato peels extract proved to be the best substrate among the food wastes tested for use to propagate *Rhizopus oligosporus*. These food wastes can serve as very cheap and readily available substrates for producing the starter culture. Utilisation of these potential sources of health hazards to propagate *Rhizopus oligosporus* will contribute positively to solve the problem of proper waste management in Nigeria.

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