FUNGAL MASS PRODUCED BY GROWTH OF *ASPERGILLUS FLAVIPES* ON ROTTEN FRUITS AND VEGETABLES – A CHEAP SOURCE OF PROTEIN

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Growth of *Aspergillus flavipes* a new substrate of rotten fruits and vegetables was found to be more economical than on Czapeck's synthetic medium. The resultant fungal mass was analysed for the presence of protein and amino acids, and the cell-free broth for other metabolites. The results of preliminary studies are being reported.

INTRODUCTION

The nutrient medium usually used for the growth of *Aspergillus flavipes* is Czapeck's sucrose nitrate agar [1]. We used rotten tomatoes, bananas and spinach as a substrate and found that it was not only cheaper but the yield of fungal mass was much higher. The primary and secondary metabolities of *Aspergillus flavipes* have been studied by a number of workers [2,3]; some of the metabolites such as proteases and glactosidase are employed in industry [4,5]. Our experiments have shown that the fungal mass contained a high proportion of protein, though the substrate on which it was grown had only a negligible protein content.

EXPERIMENTAL AND RESULTS

We have recently reported the average daily waste of fruits and vegetables in Karachi [6]. The percentage that goes waste is shown in Table 1.

Growth of the fungal-mass. Rotten banana were liquidized in an electric blender. The mixture was freeze-dried and suspended in water in concentrations of 1, 5, 10 and 20 per cent in four sets of flasks. Each set of flasks, with a blank was sterilized by autoclaving at 80° C for 30 minutes. After cooling they were inoculated with *Aspergillus flavipes* strain 240 and placed in an incubator at 37°. One set of flasks was taken out of the incubator on day 1/,5, 10 and 15. The fungal mass was weighed in each case. The growth rate of fungal mass with respect to time and concentration is shown in Figs. 1 and 2.

Table-1: The average daily waste of vegetable/fruit in Karachi

Vegetable/fruits	Spoilage	% waste of vegetable/fruit
Tomatoes	8.5	9.75
Potatoes	2.2	2.52
Onion	5.5	6.31
Peas	6.0	6.88
Spinach	18.0	20.65
Banana	20.0	22.94
Mangoes	14.0	16.06
Water melon	8.0	9.18
Cauliflower	5.0	5.78

Acid treatment. Acid treatment was tried as an alternative to steam-sterlization which is an expensive method. A 10 per cent mixture of rotten vegetable/fruit in water was taken in six conical flasks; three of these were steam-sterilized as described earlier; the remaining three were acidified to pH-2 with 10 per cent H_2SO_4, and heated in an oven at 80° for 20 minutes. On cooling the pH was adjusted to that of the particular fruit/vegetable with ammonia solution. There was no microbial growth or distinct visible change during a period of 40 days on either of the two sets, showing that acid treatment is as effective as steam-sterilization.

Effect of ammonia as nitrogen source. Four sets of six conical flasks each containing 50 ml of 10 per cent vegetable/fruit were taken. Three flasks in each set were steam sterilised as described earlier and remaining three treated with acid and adjusted to pH-5.2 with ammonia solution. Two flasks from steam sterilised and two from acid treated

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flasks were inoculated with *Aspergillus flavipes* strain; remaining were used as blanks. These were incubated for two weeks. The fungal mass was removed and dried to constant weight; the results are shown in Table 2 along with approximate material costs. The raising of pH in acid treated rotten fruit/vegetable with ammonia solution to pH 5.2 increases the fungal growth as it also supplements the nitrogen required for the growth.

Combination of rotten banana, spinach and tomatoes as substrate. Rotten tomatoes, banana and spinach were blended in different proportions for use as substrate. Samples were treated with acid, pH adjusted and inoculated as described earlier. The results of fungal-mass growth are shown in Table 3.

Electrophoretic protein patterns. Electrophoresis[7] of water-soluble portion of the cell-free extract was carried out using TRIS-HCl buffer pH 8 and trisglycine buffer pH 8.8. The gels stained with Coomassie R 250, showed the presence of new protein bands when compared to a blank in case of tomato, banana and spinach. The band intensity increased in nitrogen-supplemented medium.

Amino acid composition. The fungal-mass grown on banana substrate was washed with water and ground in a motor to a thick paste. The mixture was given a sucrose shock and shaken on a mechanical stirrer for eight hours at room temperature (28°). The solids were separated by centrifugation; the supernatant fluid was concentrated in a vacuum evaporator at 40°C and its amino acid composition determined by paper chromatography[8].

The fungal mass obtained on banana substrate was hydrolysed with 6N HCl in a sealed tube at 100° for 18 hours; HCl was removed in vacuum and amino acid composition was determined by paper chromatography.

Table-2: Cost of the fungal-mass grwon on various media

	Media	g of Fungal-mass /100g of substrate	Approx. cost of substrate/ kg Rs.	Approx. cost of fungal-mass/ kg Rs.
1.	Rotten banana+ tomato + spinach	14.953	0.29	3.80
2.	Banana: tomato: Spinach+Nitrogen Supplementation	15.316	0.30	3.5
3.	Rotten tomatoes	2.947	0.30	15.50
4.	Rotten banana	9.845	0.25	4.00
5.	Rotten spinach	2.016	1.00	15.50
6.	Czapek's-sucrose nitrate medium (100 ml)	7.612	30.00	45.00

*Rs.100 = approx. $ 7.6.

Similarly the spore-free extract was analysed for amino acid composition before and after the hydrolysis. The results are shown in Table 4.

Alkaloids. Aspergillus flavipes was grown on 10% vegetables/fruits for 35 days after acid treatment as described earlier. The cell free broth was concentrated and basified with ammonia solution and extracted twice with chloroform. The extract was evaporated and the residue was tes-

Table-3: The production of bio-mass in various substrates (conc. approx. 10% by weight) and time period

No. of days	Banana g	Banana spinach 1:1 g	Banana tomatoes 1:1 g	Banana tomatoes spinach 1:1:1	Synthetic medium g
1 day	Traces	Traces	Traces	Traces	Traces
2 days	.0312	.0829	.0716	.0912	.0428
3 days	.4102	.4986	.5002	.6123	.1010
4 days	.6208	.7128	.7011	.8231	.2918
5 days	.8420	.9063	.8243	.9826	.4023
6 days	.9764	1.0120	.9800	1.0692	.5870
7 days	.9826	1.1627	.9891	1.3810	.6281
15 days	1.0110	1.2863	1.0922	1.4531	.7621

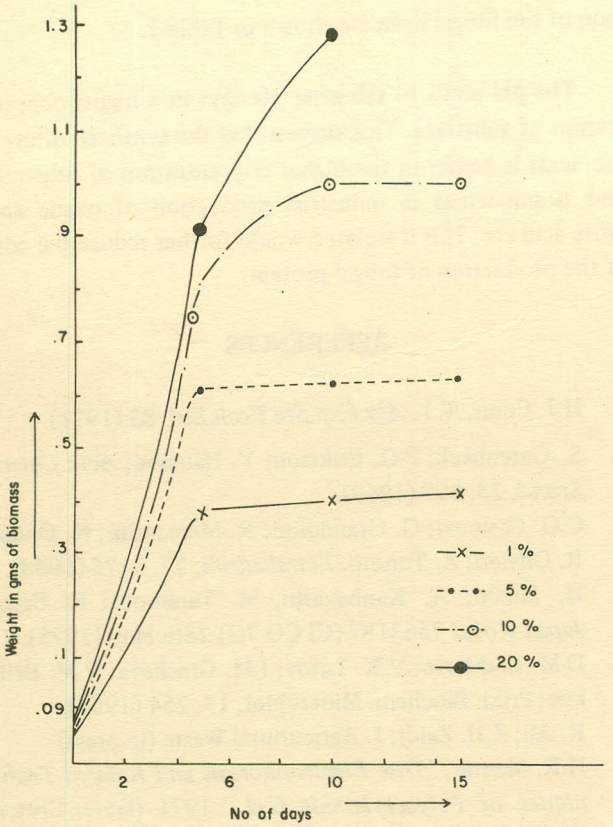

Figure 1. Rate of growth of *Aspergillus flavipes* on rotten banana substrate (concentration 1,5,10 and 20%).

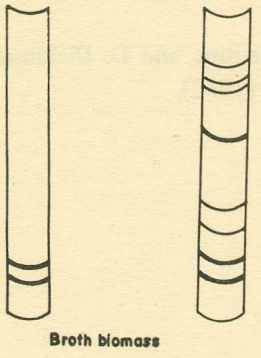

Figure 3. Electrophoretic pattern of soluble proteins from the cell-free broth and bio-mass of *Aspergillus flavipes.*

ted for alkaloids. The cell-free broth of *Aspergillus flavipes* grown on banana was found to contain four alkaloids on a TLC plate 20cm; using $CHCl_2$: CH_3OH: (8:2) solvent system and Dragendorff as spraying agent. Further studies are in progress. No alkaloids were isolated from banana and spinach.

Organic acid.The cell-free extract was found to contain citric acid[9]. The identification was carried out by M.Pts 156° and 189° respectively and paper chromatography[10].

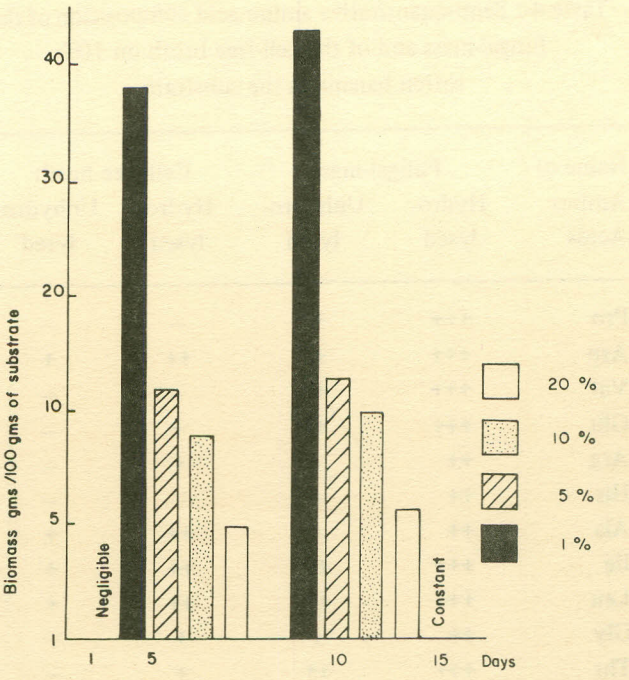

Figure 2. Total production of biomass per 100 gms of substrate having different concentration of rotton banana as substrate over various time period.

DISCUSSION

We are reporting for the first time the use of decaying fruits and vegetables as a susbstrate for the growth of *Aspergillus flavipes.* We found the growth rate of fungal mass on this substrate to be much higher than on a synthetic medium such as Czapeck's sucrose nitrate agar (Table 2). It is significant that although the subtrate contained a negligible amount of protein, the fungal mass had a high protein content.

Acid treatment used as an alternative to steam sterlization of the substrate, hydrolysed the cellulose, which was more easily assimulated by the fungus resulting in a higher yield of fungal mass. Secondly, ammonia used to basify the substrate for the optimal growth of the fungus also supplemented the nitrogen source, thus increasing the growth of fungal mass and protein content.

The substrate concentration is an important factor, the biosynthesis of the fungal mass is slow in concentrations lower than 5% and the rate of growth increases with increase in substrate concentration upto 20%, after which there is negligible increase (Fig. 1).However production of the fungal mass in concentration of substrate is 7g/100g of substrate and on 1% concentration of substrate is 43g/100g. Therefore although the rate of growth of the fungal mass is fastest on 20% substrate concentration the amount on

Tavle-4-: Semi-quantitative amino acid composition of the fungal-mass and of the cell-free broth on 10% rotten banana as the substrate

Name of Amino Acids	Fungal-mass		Cell-free broth	
	Hydro-lysed	Unhydro-lysed	Hydro-lysed	Unhydro-lysed
Pro	+++	++	–	–
Asp	+++	++	++	+
Val	+++	++	–	–
Glu	+++	++	+	–
Arg	++	–	+	–
His	++	–	–	–
Ala	++	+	++	+
Ile	+++	++	++	+
Leu	+++	++	++	+
Gly	++	+	+	–
Thr	+++	++	+	–
Try	–	+	++	+
Lys	+++	++	++	+
Phe	+++	++	++	+
Ser	+++	+	++	+
Tyr	+++	++	–	–

weight-to-weight basis is the most on 1% concentration (Fig. 2). Thus 1% concentration is the most economical and may be the most suitable for production of fungal mass on industrial scale. The approximate costs involved in preparation of the fungal mass are shown in Table 2.

The pH tends to fall after 10 days in a higher concentration of substrate. This suggest that the synthesis of organic acids is higher in the higher concentration of substrate and is important in industrial production of oxalic and citric acid etc. This if isolated would further reduce the cost of the production of fungal protein.

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