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LIVESTOCK PRODUCTS: CREATING DEMAND IN A DEPRESSED ECONOMY

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Solid State Fermentation of Sweet Potato Using Two Monoculture Fungi: Changes In Protein, Fatty Acid And Mineral Composition

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ABSTRACT

This study evaluated the protein fatty acid and mineral changes in sweet potato root meal inoculated with two selected fungi by solid state fermentation procedure. The total protein content was raised from 4.95% to 11.83% and 6.69% within 48 hrs for Aspergillus oryzae and Aspergillus niger respectively. The total lipid dropped from 1.93% in the control to 1.36 and 1.19% for *A. niger* and *A. oryzae* respectively. The fatty acids C16:0 and C18:2 were preponderant over other fatty acids with *A. niger* and *A. oryzae* recording 18.94 and 28.17% for C16:0 and 43.20 and 37.88% for C18:2 respectively. The ash content values were 3.53% for the control, 3.54 and 4.51% for *A. niger* and *A. oryzae* respectively. The mineral changes analysed were for Calcium, Potassium, Iron, Manganese, Sodium, Zinc and Phosphorus. The pH changed from 5.26 for the control to 3.80 and 5.39 for *A. niger* and *A. oryzae* respectively.

INTRODUCTION

The use of root and tuber crops is becoming prominent in the feeding of livestock in the tropics. However the inherently low protein (Woolfe, 1992) content of these crops and their by-products necessitates supplementation with protein sources. Micro-organisms have been grown on roots and tubers in other to increase their protein contents Raimbault et al., (1985), Smith et al, (1986), Yang et al., 1993 and Balagopalan, 1996. These results obtained from studies using cassava as substrate has been promising Raimbault (1985), Smith et al., (1986) and Balagopalan, (1996). The other limitation of the use of sweet potato as animal feed is its high sugar content that has been suggested as inducing diarrhoea in livestock so fed (Oboh, 1986). Some of these organisms have also been found as suitable agents for the removal of toxic components (Essers et al., 1994). Presently sweet potato is still a minor crop in Nigeria and its utilisation as livestock feed is limited to the use of vines and leaves in the fresh and sun-dried forms for rabbits, sheep, goats and cattle while

only small sized or damaged tubers are fed (Tewe, 1997). The thrust of this study is to investigate the protein, fatty acid and mineral changes in inoculated sweet potato tuber using two *A. niger* and *A. orzae*.

MATERIALS AND METHODS

Test Organisms:

Aspergillus niger and *Aspergillus oryzae* were obtained from the culture collection of the Department of Animal and Plant Sciences, University of Sheffield, United Kingdom. The organisms were cultivated at 25°C on malt extract agar slants; containing (g/1): lab malt extract agar, 20.0; lab agar No 2, 10.0; $NaNO_3$, 2.0; KH_2P0_4, 1.0; $MgSO_4.7H_20$, 0.5 and were sub-cultured every four weeks. The spores were harvested with a Tween 80 solution (10 ml, 0.01% v/v) which was then adjusted to give 10^7 to 10^8 spores per ml with sterile water.

Inoculation technique:

Sweet potatoes were purchased from a supermarket in Sheffield. An inquiry revealed that the sweet potatoes were imported from South Africa. The sweet potatoes were washed, peeled, and cut into about 1-cm cube. The peeled tubers and the peels were then used as the substrate for cultivating the micro-organisms. The fermentation's were carried out in 250 ml Erlenmeyer flasks. The minimal nutrient medium contained 20.0; $NaNo_3$, 2.0; KH_2PO_4, 1.0; $MgSO_4.7H_20$, 0.5 g/litre:. The flasks were then inoculated aseptically with 5 ml of the separate inocula accordingly. A control without inoculum but sweet potato was allowed. Triplicates flasks were prepared. The flasks were incubated at 25oC for 48 hrs. At the end of the fermentation period samples were collected and analysed for total protein, mineral, total lipid and fatty acid changes. The samples were blended in a Waring blender for 3 min before analysis.

Determination of pH

10 g of the wet sample was Vortex mixed with 100 ml of distilled waterfor 30 - 45 sec. The slurry was allowed to stand for 3 min before the pH was read using a pH meter (Metler Toledo 320 ph meter)

Chemical analysis

Total protein

Total protein content was determined by the method of Lowry et al., 1951. The Lowry reaction for protein determination is a modification of the bluret procedure. The first step is the formation of a copper-protein complex in alkaline solution. This complex then reduces a phosphomolybdic-phosphotungstate reagent to yield an intense blue colour that is then measured by a spectrophotometer at 660 nm.

Total lipids:

The method of Marsh and Weinstein (1966) was applied for total non specific (neutral lipid) determination. The lipid extraction was based on Christie, (1993) method and washed according to the method of Folch et al., 1957. 20µl of each lipid extract and for the calibration curve, of standard solutions of 0, 10, 20, 30, 40 and 50 mg/ml of olive oil in chloroform were transferred to pairs of thick-walled test tubes. The solvents were then removed under a flow of nitrogen. After evaporation of the solvents, 2 ml of concentrated sulphuric acids were added to each tube, mixed then heated for 15 min, then 3 ml of distilled water carefully added to each tube and mixed thoroughly. The tubes were removed from ice and left standing for 10 min until all bubbles had disappeared. The optical density was measured with a CECIL CE 1020, series 1000 spectrophotometer at 375 nm.

Fatty acid analysis

Fatty acid methyl ester (FAME) derivatives were prepared using methnolic based reagent directly from total lipid extracts. Gas chromatography of the samples was performed using a Varian 3500 gas chromatograph equipped with 8300 auto injector, Australia. The generating conditions were: A 30 x 0.25 mm 30m silica column (DB- 23, J and W scientific, USA). The column was heated from 140°C to 240°C at 6°C min^{-1} with injector set at 275°C and flame ionisation detector (FID) set at 275°C. The carrier gas was nitrogen. Standard FAME samples of fatty acids were injected in the column for identification of fatty acids from the samples against their retention times. The results showed the amount of each fatty acid as a percentage of the total fatty acids in the samples. Pure fatty acids standard (Oil reference standard, AOCS for low Erucic Rapeseed oil, Sigma, 1 amp (100 mg) Lot 65H83681) dissolved in hexane were used as standardsl 1ul of the extract diluted in hexane was injected.

Mineral analysis

The biomass produced were analysed for total nitrogen and minerals. The ash was determined gravimetrically by burning the oven-dried material at 660 C for 6 hr. 100 mg of the samples were digested with 5ml of salicyclic acid/H_2SO_4 mix at 370°C for 4 hr. Li_2SO_4 and $CuSO_4.5H_20$ in the ratio of 10g; lg was used as catalyst. Concentrations of Ca, Total P, Mg, Zn, Fe and K in the ash were determined by a Perkin Elmer-flame atomic absorption Spectrophotometer.

RESULTS AND DISCUSSION

The changes in the pH, total protein and total lipid content of inoculated sweet potato root substrate after 48 hrs of fermentation are shown in Table 1. The pH changed from 5.26 to 3.80 and 5.39 for *A. niger* and *A. oryzae* respectively. These resultant pH values are within range suitable for fungal growth. Litchfield, 1979 reported a pH range of 3.0 and 7.0 as suitable for growth of most fungi. The total protein was raised from 4.95% to 6.69 and 11.83% for substrates inoculated with *A. oryzae* and *A. niger* respectively. These val-

Table 1: pH, total protein and total lipid changes (%, DM basis) of inoculated sweet potato tuber grown in static solid-state fermentation for 48 hours at 25°C

Fungi	pH	Total protein	Total lipid
Control	5.26	4.95 ± 0.10	1.93 ± 0.01
Aspergillus niger	3.80	11.83 ± 0.23	1.36 ± 0.12
Aspergillus oryzae	5.39	6.69 ± 0.24	1.19 ± 0.06

Table 2: Identified fatty acid composition (%) of inoculated sweet potato tuber grown in static solid-state fermentation for 48 hours at 25°C

fatty acids (%)

Fungi	C16:0	C16:3	C18:0	C18:1	C:18.2	C18:3	C20:1
Control	25.12	9.40	3.90	3.90	44.53	8.60	4.57
A. niger	18.94	8.98	4.62	10.77	43.20	5.04	8.46
A. oryzae	28.17	6.82	6.82	9.59	37.88	4.94	4.94

Table 3: Mineral composition of inoculated sweet potato tuber grown in static solid-state fermentation for 48 hours at 25°C

Minerals (mg/Kg)

Fungi	Ca	K	Fe	Mn	Na	Zn	P
Control	8.69	909.13	81.84	10.69	92.82	11.29	45.05
A. niger	2.28	1248.25	54.16	19.81	271.96		70.29
A. oryzae	3.16	1053.55	64.71	14.59	183.52	15.54	56.02

ues represented increases of 35.15% and 134.98% respectively. Compared with the control all the fungi tested showed increased protein content. The results obtained for A. niger were comparable to those of Raimbault et al., 1985 who observed upgraded final protein contents of fermented cassava products, using different strains of A. niger to vary between 10-16.5%. Yang *et al.*, 1993 obtained highest protein content of 32% w/w with a co-culture of *A. niger* and *Rhizopus* sp at 30°C after 4 days fermentation period when compared with either of the fungus used independently. The two fungi showed decrease in total lipid from 1.93% to 1.36 and 1.19% for *A. niger* and *A. oryzae* respectively. The level of total lipid reported for the two fungi were lower than value reported for maize. There is however reported increase in the total lipid level for substrate inoculated with *A. niger* if the fermentation period is allowed to last for more than 72 hours (Abu and Tewe, 1996). Table 2 shows the fatty acid composition of the inoculated sweet potato roots. Generally C16:0 and C18:2 were preponderant when compared with other fatty acids identified. *A. oryzae* is a better palmitic acid producer than the non-inoculated substrate and the substrate inoculated with *A. niger*. Table 3 shows the mineral changes in the inoculated substrates.

An earlier study revealed that *A. niger* recorded a higher radial growth rate when compared with other selected fungi (Abu, 1996). Radial colony growth rate, a function of biomass production, is not a sufficient indicator for selecting fungi for solid-state fermentation (Bull and Trinci, 1977). The rate of starch utilisation is therefore estimated by the rate of clearing. The rate of clearing however increased with increase in the inoculation period (Abu, 1996).

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Determination of Crude Protein Content, Invitro Digestibility of Crude Protein and Tannin Content of Varieties of Raw Cowpea and the effects of cooking on them.

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ABSTRACT

Protein content of both raw and cooked samples of 5 varieties of cowpea were determined. Crude protein value varied significantly ($P<0.05$) among the five varieties and ranged between 21.88% in black eye cowpea and 22.25% in light brown cowpea with a mean of 22.02%. Cooking generally increased the protein content between 3.1 and 14.2% except for white brown streak cowpea which had a higher value 27.09%.

The invitro digestibility values ranges between 43.31% in light brown cowpea and 46.6% in TV x 3236 cowpea except for both raw white seeded cowpea and white brown streaks cowpea which had 62.34 and 70.1% respectively. The varietal means were significantly ($P< 0.05$) different.

Cooking also increased in vitro digestibility values of samples to between 106.2 and 126.7% except for white brown streak cowpea and white seeded which had 40.0% and 56.0% respectively.

Tannin was present in all varieties of cowpea. The light brown cowpea had the highest value of 2.59 mg/g/ and 1.96 mg/g respectively for both raw and cooked samples. Black eyed cowpea had the lowest value of 0.47 and 0.9mg/g respectively for both raw and cooked samples. White seeded had the highest reduction in tannin content of 1.98 and 0.37 mg/g for both raw and cooked samples respectively (about 81.3% reduction). Other varieties of cowpea apart from the highly competed for by man could serve as an alternative source of protein in livestock feeding. Simple detoxification process like cooking improves invitro digestibility and content of their crude protein and also reduced their tannin content.

INTRODUCTION

Much work has been done on the black eyed variety of cowpea (Del Rosario et al 1981). This variety is highly competed for by man. Other varieties of cowpea are culti-