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NUTRIENT ENRICHMENT OF SWEET POTATO (*Ipomoea batatas L.*) BY SOLID SUBSTRATE FERMENTATION USING FOUR FUNGAL SPECIES

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ABSTRACT

Sweet potato (*Ipomoea batatas L.*) tubers and flour were fermented using pure strains of four fungal species: *Aspergillus niger, Rhizopus stolonifer, Neurospora sitophila* and *Saccharomyces cerevisiae* using solid substrate fermentation at 30°C for 72 hrs. The protein content of the samples increased significantly ($p \leq 0.05$) from 2.34±0.07% in the unfermented tuber to values ranging from 11.46±0.01 and 12.62±0.09% in the fermented tuber and 6.26±0.05% in the unfermented flour to values ranging from 7.83±0.06 and 9.70± 0.02 in the fermented flour. Fat content was also increased in the fermented tubers. However, fermentation decreased the fat content of the flour except in *Aspergillus niger*- fermented flour. Fermentation increased the ash content of the samples (except in *Saccharomyces cerevisiae*- fermented tuber, *Rhizopus stolonifer*-fermented flour and -fermented flour). The crude fibre, carbohydrate, sugars (reducing, non-reducing and total) and starch contents of both flours and tubers reduced after fermentation. Mineral elements such as Mg (except in *Saccharomyces cerevisiae* fermented tuber and *Aspergillus niger* fermented tuber), Ca (except in *Aspergillus niger* - fermented tuber), P (except in *Rhizopus stolonifer* fermented flour), and K (except in *Aspergillus niger* fermented flour) and decreased significantly; while Zn (except *Saccharomyces cerevisiae* fermented tuber and flour and *Aspergillus niger* fermented flour), Fe (except in *Saccharomyces cerevisiae* ferment tuber and flour and *Rhizopus stolonifer* fermented tuber) and Na (except in *Rhizopies stolonifer* fermented tuber) contents increased. Overall, *Aspergillus niger* appeared to be the most nutritionally-enriching compared to other fungi, while the sweet potato tuber proved to be a better fermentable substrate than the flour.

KEYWORDS: Fungi, Nutrient enrichment; Solid substrate fermentation; Sweet potato.

INTRODUCTION

Sweet potato (*Ipomoea batatas L.*) has been identified as a crop with great potential to meet the food requirements of the world, particularly in the tropics due to its ease of cultivation and its nutritional value (Lan and Ilangantileke, 1993). It has considerable untapped potential as a nutritious food crop particularly for the poor and the more vulnerable groups in the society in developing countries.

Woolfe (1993) stated that sweet potato is often the only crop surviving extremes of drought, flooding or typhoons because of its growth requirements and that it is also capable of yielding in marginal conditions, hence making it one of the most efficient of crops in terms of the amount of food produced per unit area per unit time.

However, the limiting nutritional qualities in the utilization of sweet potato as an important biomaterial both for food and feed are the low protein and lipid contents as well as the presence of antinutritive thioglucoside (Tewe, 1992).

Fermentation has been reported as one of the oldest biotechnologies used for preservation and enhancement of the nutrient content of food through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein and fibre digestibility and degrading antinutritional factors (FAO, 1998).

According to Raimbault (1998), the great socio-economical potential of solid substrate fermentation is the raising of living standards through the production of protein-rich foods for human consumption. Enhancement of nutrient content (especially protein and lipid) by solid substrate fungal fermentation in starchy substrates has been severally reported (Diamante, 1985; Sasson, 1988; Abu *et al.*, 1998, 2000; Akindahunsi *et al.*, 1999).

This study is therefore aimed at enriching the nutrient content of sweet potato by solid substrate fermentation using four fungal species.

MATERIALS AND METHODS

Sample collection and preparation

Fresh sweet tubers of the pale-yellow skinned variety were collected from the research

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farm of the Federal University of Technology, Akure, Ondo state, Nigeria Processing of the fresh tuber to flour was done by the method described by Odaga and Wanzie (1993) which involved peeling, washing, slicing (2-3mm), blanching in boiling water (for 10 minutes) and cooling in cold water (5minutes). The cooled slice were oven- dried at 60°c for 24 hours and dry-milled using a hammer mill to 212μm mesh size. The flour samples were stored in clean sterile transparent polythene bags at 5°C. The pulp was prepared by washing, peeling and crushing of the tubers. Triplicate flasks containing 50g of the samples were autoclaved at 121°C for 15 minutes.

Source and cultivation of fungal isolates.

Pure strains of the fungi used- *Aspergilius niger, Rhizopus stolonifer, Neurospora sitophila* and *Saccharomyces cerevisiae*, were obtained from the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. the cultures were cultivated on malt extract agar (MEA) at 25°C as slants. The spores were harvested with sterile physiological saline (10ml, 0.85% NaCl). The harvested spores were then cultivated in a defined fermentation medium (Solomon *et al.*, 1981) consisting of glucose, 10g/L; $(NH_4)_2SO_4$, 1g; KH_2PO_4, 0.8g; K_2HPO_4.0.07g;$MgSO_4$. $2H_2O$, 0.20g; $CaCl_2$. $2H_2O$, 0.05g; $ZnSO_4$. $7H_2O$, 0.02g; $CuSO_4$. $5H_2O$, 0.005g; $FeSO_4$. $7H_2O$, 0.1g; Na_2SO_4, 1g; and yeast extract, 0.05g., incubated at 30°C for 72 hrs providing oscillation of 45rpm in a Gallenkamp shaker water-bath for 12hrs.

Solid substrate fermentation

A fifty gram (50g) portion of sterile sweet potato flour and pulp were each aseptically inoculated with 25ml of 3-day old spore suspension containing 10^7 spores per ml of each microorganism. The flasks were incubated at 30°C for 72hrs. Control experiments were also set up by incubating 2 flasks each containing 50g of sterile uninoculated sweet potato pulp and flour respectively at 30°C for 72 hrs. The products were dried at 60-80°C for 3 and 8hrs for flour and pulp respectively (Abu *et al* 1998, 2000).

Sample analyses

The proximate composition of the samples was analyzed by the standard AOAC method (1995). Protein was determined using the micro-Kjedahl method (NX6.25) while carbohydrate determination was by difference. Minerals were determined using atomic absorption spectrophotometer for Ca, Mg, Fe and Zn, flame photometer for Na and K while P was determined colorimetrically using the phosphovanado-

Table 1: Proximate % composition of fermented sweet potato samples by different microbial inocula

Inoculum	Flour or tuber % composition of:											
	Moisture		Protein		Ash		Crude fibre		Fat		Carbohydrate	
	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber
A. niger	40.93±0.26^b	50.00±0.34^d	9.70±0.02^e	12.62±0.09^h	1.81±0.14d^e	1.43±0.10^{bc}	1.14±0.03^c	0.77±0.04^a	5.40±0.08^{gh}	5.57±0.14^h	41.05±0.41^c	29.61±0.45^a
R. stolonifer	40.82±0.66^b	50.10±0.61^d	8.46±0.06^d	12.01±0.11^g	1.55±0.21^{cd}	1.14±0.05^{ab}	1.16±0.01^c	0.96±0.02^b	4.97±0.17^f	5.43±0.09^h	42.71±0.55^d	30.36±0.32^a
N. sitophila	42.10±0.42^b	49.81±0.65^d	7.83±0.06^c	11.60±0.09^f	2.55±0.12^f	2.00±0.04^e	1.36±0.01^d	1.18±0.04^c	3.38±0.22^c	3.77±0.11^d	42.78±0.32^d	32.59±0.74^b
S. cerevisiae	41.28±0.39^b	49.21±0.31^d	8.38±0.03^d	11.46±0.10^f	2.48±0.10^f	1.05±0.05^a	1.72±0.03^e	1.20±0.04^{cd}	0.71±0.06^a	4.56±0.08^e	45.44±0.39^e	31.57±0.46^b
Uninoculated	8.69±0.03^a	45.31±0.67^c	6.26±0.05^b	2.34±0.07^a	1.93±0.02^e	1.12±0.03^{ab}	1.84±0.09^e	1.69±0.09^e	5.03±0.25^{fg}	2.89±0.04^b	76.25±0.17^g	46.65±0.19^f

Values are means of triplicate determinations.
Values in the same column having different superscripts are significantly different.

molybdate method (AOAC, 1995). Reducing sugars were determined by the method of Lane and Eynon (1923) and starch using the AOAC (1995) procedure of direct acid hydrolysis.

RESULTS AND DISCUSSION.

Results of proximate analysis (Table 1) showed increased protein content. Values ranged from 2.34±0.07% in the unfermented tuber to 11.46±0.10% (*Saccharomyces cerevisiae*), 11.60±0.09% (*Neurospora sitophila*), 12.01±11% (*Rhizopus stolonifer*) and 12.62±0.09% (*Aspergillus niger*) in the fermented tubers. In the flour, values ranged from 6.26+.05% in the unfermented flour to 8.38±0.03% (*Saccharomyces cerevisine*), 7.83±0.06% (*Neurospora sitophila*), 8.46+0.06% (*Rhizopus stolonifer*) and 9.70+0.02% (*Aspergillus niger*) in the fermented flours. Fungal fermentation has been severally reported to increase the protein content of starchy substrates (Rajagopal, 1978; Diamante, 1985;

Raimbault *et al.*, 1985; Yang, 1988; Yang *et at.*, 1993; Raimbault, 1998; Abu *et al.*, 1998, 2000, Rodolfo *et al.*, 2000; Akindahunsi et al., 1999) According to Kuo *et al.* (1995) protein increase could result from slight protein synthesis by the proliferation of the microorganisms used and a synthesis of enzyme proteins or from a rearrangement of the composition following the degradation of other constituents. Formation of proteases by the moulds during fermentation may result in increase in protein amino acids (Burnett, 1976).

Protein enrichment was highest in *Aspergillus niger*-fermented samples as compared to others. This corroborates the findings of Abu *et al.* (1998, 2000) and Oboh (2002). This could be attributed to the great enzymatic activities of this fungus. Frazier and Westhoff (1988) stated that *Aspergillus niger* contain a number of enzymes that enable it carry out many metabolic activities. Protein enrichment was also higher in the pulp than the flour. This may be as a result of differences in the moisture content of the samples. Diamante (1985) reported that fresh cassava and sweet potato roots yielded more protein than the dried ones. This was corroborated by the findings of Rodolfo *et al.* (2000) that high moisture sweet potato pulp was a better fermentable substrate with the resulting feed containing higher percentage of crude protein than that produced from low moisture pulp.

The fat content of the pulp increased significantly ($p \leq 0.05\%$) from 2.89±0.04% in the uninoculated tuber to values ranging from 3.77±0.11 to 5.57±0.14% in the fermented tuber

Table 2: Reducing, non-reducing, total sugar and starch contents (%) of fermented sweet potato samples by different microbial inocula.

Inoculum	Flour or Tuber % composition of							
	Reducing sugar		Non- Reducing sugar		Total sugar		Starch	
	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber
A. niger	6.52±0.04^{c}	4.57±0.05^{a}	12.77±0.04^{f}	13.73±0.53^{g}	19.29±0.05^{f}	18.30±0.06^{e}	21.76±0.02^{e}	11.31±0.13^{a}
R. stolonifer	4.65±0.05^{a}	6.63±0.07^{cd}	9.20±0.03^{b}	9.25±0.19^{b}	13.85±0.04^{a}	15.88±0.02^{d}	28.86±0.04^{h}	14.98±0.02^{c}
N. sitophila	5.01±0.03^{b}	8.05±0.23^{e}	9.89±0.01^{bc}	11.65±0.07^{e}	14.90±0.03^{b}	19.70±0.11^{g}	27.88±0.03^{g}	12.89±0.63^{b}
S. cerevisiae	6.25±0.05^{c}	6.15±0.49^{c}	13.04±0.01^{g}	8.92±0.03^{a}	19.29±0.02^{f}	15.07±0.17^{bc}	26.15±0.03^{f}	16.50±0.11^{d}
Uninoculated	7.24±0.04^{d}	8.96±0.12^{f}	14.12±0.02^{h}	10.99±0.06^{d}	21.36±0.03^{h}	19.95±0.04^{g}	54.89±0.03^{i}	26.70±0.07^{f}

Values are means of triplicate determinations.
Values in the same column having different superscripts are significantly different

Table 3: Composition (mg/100g) of minerals in fermented sweet potato samples by different microbial inocula

helen	Flour or Tuber mg/100g composition of:															
Inoculum	Zn		Fe		Mg		Na		Ca		P		K			
	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber	Flour	Tuber		
A. niger	0.57± 0.01^{a}	1.18± 0.01^{c}	0.97± 0.02^{ab}	3.22± 0.04^{e}	22.90± 0.11^{e}	23.49± 0.02^{f}	40.38± 0.31^{h}	17.75± 0.09^{c}	17.32± 0.05^{e}	17.51± 0.16^{e}	32.24± 0.02^{d}	32.31± 0.52^{d}	633.33± 0.04^{i}	322.68± 0.01^{e}		
R. stolonifer	1.20± 0.01^{c}	7.64± 0.05^{f}	0.96± 0.02^{ab}	2.33± 0.13^{c}	25.67± 0.11^{g}	12.10± 0.06^{a}	34.63± 0.04^{g}	8.76± 0.08^{a}	18.46± 0.17^{f}	12.00± 0.27^{a}	33.71± 0.04^{g}	32.99± 0.01^{e}	553.77± 0.19^{j}	192.75± 0.07^{a}		
N. sitophila	1.98± 0.01^{e}	1.60± 0.22^{d}	0.89± 0.01^{a}	3.67± 0.03^{f}	26.29± 0.05^{h}	21.41± 0.07^{d}	42.10± 0.06^{i}	19.49± 0.31^{d}	26.38± 0.04^{g}	13.77± 0.01^{b}	27.19± 0.04^{b}	25.99± 0.32^{a}	493.13± 0.59^{g}	251.41± 0.02^{c}		
S. cerevisiae	0.55± 0.23^{a}	0.98± 0.04^{bc}	0.78± 0.01^{a}	2.39± 0.06^{c}	26.53± 0.06^{h}	16.88± 0.07^{b}	32.25± 0.08^{f}	19.36± 0.12^{d}	18.45± 0.08^{f}	15.35± 0.09^{c}	32.18± 0.01^{d}	30.21± 0.61^{c}	423.44± 0.32^{f}	235.65± 0.18^{b}		
Uninoculated	0.79± 0.01^{ab}	1.01± 0.05^{bc}	0.81± 0.01^{a}	2.95± 0.43^{d}	29.93± 0.02^{i}	17.85± 0.03^{c}	27.11± 0.01^{e}	14.57± 0.06^{b}	38.97± 0.02^{h}	16.06± 0.01^{d}	32.83± 0.02^{e}	33.42± 0.05^{f}	500.99± 0.10^{h}	268.11± 0.08^{d}		

Values are means of triplicate determinations.
Values in the same column having different superscripts are significantly different.

with *Asergillus niger* giving the highest increase. However, the fat content of the flour was reduced after fermentation from 5.03±0.25% in the unfermented flour to 0.71±0.06 and 4.97±0.17% in the fermented flour, although flour samples fermented with *Aspergillus niger* gave increased fat content (5.40±0.08%). Weete (1980) and Weete and Ghandi (1992) stated that *Aspergillus niger* can increase fat content of a sample despite not being an oleaginous fungus. Akindahunsi *et al.* (1999) recorded increased fat content in gari and reduced content in cassava flour when Rhizopus oryzae was used to ferment cassava. Leung (1992) had earlier reported decreased fat content in the fermentation of soyabean for tempeh production using *Rhizopus oligosporus*. According to Lin (1980), *Rhizopus* spp have high lipolytic activity and preferentially use lipids for energy. Although the reason for other decreases may not be explainable, the increase may be due to the possible transformation of carbohydrates to fat; as reported by Akindumila and Glatz (1998) that certain fungi could produce microbial oil during the course of fermentation.

Ash (except in a few cases), crude fibre and carbohydrate contents of the samples were significantly ($p \leq 0.05$) decrease as a result of fermentation. El- Tinay *et al.* (1979) reported that fibre tends to decrease during fermentation. The decrease in carbohydrate content could be attributed to the selective utilization of carbohydrate as an energy source by fermenting microorganisms (Dike, 2001).

The reducing non-reducing and total sugars of the samples were reduced following fermentation (Table 2). The reports of Khetarpaul and Chauhan (1991), Kingsley (1995) and Raimbault (1998), corroborate these results. It was attributed to the utilization of some of the sugars by fermenting microorganisms for growth and metabolic activities, hence resulting in a decrease in sugar content (Kingsley, 1995). Results obtained also showed higher non-reducing sugar content in all the samples as compared to the reducing sugar. Solomon (1987) stated that usually the carbohydrates are hydrolyzed to glucose and then used as a source of carbon and energy for microbial growth. This raises the percent protein in the residue. This may have accounted for the decrease in carbohdrate and sugar contents and increased protein content observed in all the fermented samples investigated in this study.

Fermentation caused significant ($p \leq 0.05$) decrease in the starch content of the samples (Table 2). Highest reduction rates were recorded

in sample fermented with *Aspergillus niger*, thereby showing the more amylolytic activity of this organism as compared to others used in this study. Ekundayo (1986) reported that fungi asscciated with flour deplete starch. El –Tinay *et al.*(1979) stated that starch and fibre tend to decrease during fermentation as a result of being utilized by the fermenting microbes. Raimbault (1981) also attributed this depletion to transformation of starch and mineral salts by fungi (especially Aspergilli) into fungal proteins. More reduced starch content was recorded in the pulp than the flour. This could still be traced to the moisture differences with the pulp being a better fermentable substrate.

Results of the mineral analysis (Table 3) showed significant decreases in the potassium, phosphorus, magnesium and calcium contents (except in a few cases) and increased zinc, iron and sodium contents. Oboh (2002) recorded increased Zn content in *Aspergillus flavus*-fermented cassava products. Although these increases cannot be explained, Burnett (1976) stated that the minor and major metallic element requirements of fungi are very similar to those of other organisms. Raimbault (1998) also stated that during fermentation, the fermenting fungi utilize mineral salts for metabolic activities.

In conclusion, the results obtained in this study showed that solid substrate fungal fermentation of sweet potato enriched its protein and lipid contents. Overall, *Aspergillus niger* proved to be most nutritionally-enriching of the four fungi used and also the pulp was a better fermentable substrate than the flour.

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