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ENHANCEMENT OF PROTEIN IN CASSAVA (IN VITRO)

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Target Audience: Research biotechnologists, farmers

ABSTRACT

A study was conducted to determine the level to which protein content can be increased in cassava by anaerobic fermentation with Aspergillus niger in the presence of two different nitrogen sources. Poultry manure and urea which served as the two nitrogen sources were mixed under aseptic conditions with cassava substrate and incubated along with Aspergillus niger for periods of 0,2,4 and 6 days. Results showed that increase in protein content occurred in samples containing either poultry manure or urea. Maximum increase in protein content in the presence of poultry manure was by day 2 (69%) while in he presence of urea it was by day 3 (131%). This shows that although urea was better utilized than poultry manure as a nitrogen source, poultry manure itself has potential for use as a nitrogen source in enhancing protein content in cassava.

Key words: Protein enhancement, cassava, anaerobic fermentation

DESCRIPTION OF PROBLEM

The use of cassava as a substitute for maize in livestock feeding has been well established. However, one of its major limitations is its low protein content in relation to that of maize. Several methods have been adopted for processing cassava before consumption either by man or animal. One of these methods is by aerobic/anaerobic fermentation which results in nutrient enrichment of the fermentated product (1). Garri and lafun are the main products of anaerobically fermented cassava and they constitute the staple foods of majority of the people in South Western Nigeria (2). During aerobic fermentation, increased protein content has been reported in the products within a period of three days (3).

The inoculation of ground cassava with microorganisms in the presence of a nitrogen source was first studied about two decades ago (4). Since then, various species of microorganisms have been used to inoculate cassava substrates in order to obtain an enhancement of protein content in the fermented product (5,6). A study on solid state fermentation of cassava with

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growing *Aspergillus niger* in a cassava medium enhanced protein content from 5% to 18%(7). The following study was undertaken to determine the increase in protein content of cassava subtrates inoculated with *Aspergillus niger* and anaerobically fermented in two different nitrogen sources.

MATERIALS AND METHODS

Preparation of Cassava Substrates

Cassava tubers of the local variety OFEGE were harvested early in the morning, washed, peeled and sliced. The chips were washed with distilled water and heat sterilized. Twelve jumbo-sized tins were also thoroughly washed with detergent, rinsed with distilled water and heat sterilized on an electric heater for 5 minutes. After cooling, the sterilized cassava chips were spread at the bottom of the tins.

Preparation of Medium and Cultivation of Fungi

Potato dextrose agar was used as the medium 39g of powdered potato dextrose agar was accurately weighed and dissolved in 100ml of distilled water in a conical flask and heated until it was boiled. The open end of the flask was sealed with aluminum foil and sterilized in an autoclave under high pressure for 15 minutes and cooled. Twelve petri dishes were thoroughly washed with detergent, rinsed with distilled water and wrapped in aluminum foil. Drying was completed in an oven at 110°C for 45 minutes. After cooling, the potato dextrose agar was poured into the petri dishes placed on clean sterile table tops. A needle sterilized with ethanol and heat was cooled and dipped into *Aspergillus niger* colonies present on mouldy bread and inoculated onto the petri dishes containing the medium. The process was repeated for each of the twelve petri dishes. The dishes were covered and taped to prevent outside contamination and placed in a dark cupboard at room temperature. After 2 weeks a dark biomass of mycelia had spread on the surface of the agar.

Mixture Preparation for Protein Enhancement of Cassava

Three separate mixtures were prepared using the cassava substrate present in the jumbo-sized tins as shown in Table 1. Poultry waste was obtained by sun-drying poultry droppings collected from caged laying hens and freed of extraneous materials and feathers. It contained approximately 2.8% nitrogen (17.15% protein). The urea used was fertilizer grade which contained 45% nitrogen. The poultry droppings and urea served as nitrogen sources. Dilute sulphuric acid was used to adjust the pH of each mixture to a range of 4.5-5.0

Table 1: Composition of Mixtures for Protein Enhancement

Components	Mixture 1	Mixture 2	Mixture 3
Raw Cassava	100g	100g	100g
Aspergillus niger	+	+	+
Distilled water	20ml	20ml	20ml
Sulphuric acid	2ml	2ml	2ml
Poultry waste	-	5g	-
Urea	-	-	2g

Measured quantities of the materials shown in Table 1 were poured into the tins containing the cassava substrate. Each mixture was prepared four times so that a total of twelve samples was available. The *Aspergillus niger* spores were introduced into the mixtures by gently tapping the inverted petri dishes with the colonies into the substrates. The twelve samples were placed in a cupboard to ferment for six days and fermentation was controlled by removing samples at 0,2,4 and 6 days for each mixture. Each sample was sun-dried for three days and ground in a hammer mill. All samples obtained were examined for physical changes and two replicates from each sample were analyzed separately for dry matter and protein content (8).

RESULTS AND DISCUSSION

Physical changes

The cassava substrates in the three mixtures were initially soft and moist but these became tender as fermentation progressed and by the fourth day, were pulpy and friable (Table 2). The physical changes observed may be attributed to increased absorption of moisture and biochemical changes which took place during fermentation. A major limitation to the inclusion of cassava flour in poultry feeds is the characteristic dustiness which causes irritation in the respiratory tract of the animal(9). The change in the texture of the fermented product may therefore be advantageous in enhancing the utilization of cassava as poultry feed. Other physical changes observed were in the colour and odour of the cassava substrates. The substrates mixed with poultry waste or urea were white before fermentation (day 0) but they became grey by day 4 while the odour changed from sweet smelling to offensive. The odour of mixture II was that of poultry waste which was extremely irritating by the sixth day. The change in the colour of mixtures II and III was due to increased mycelial growth in these samples(10), while the odours recorded were all due to various gases released during the fermentation processes.

Table 2: Physical Changes in Samples

Days	Parameter	Mixture 1	Mixture 2	Mixture 3
0	Texture	Soft & moist	Soft & moist	Soft & moist
	Colour	Whitish	Whitish	Whitish
	Odour	Sweet	None	Ammoniacal
2	Texture	Soft, moist & tender	soft, moist & tender	Soft, moist & tender
	Colour	Whitish	Slight off coloured	Whitish
	Odour	Slight fermented	Fermenting odour	Strong smell of ammonia
4	Texture	Soft, moist &pulpy	Soft, moist & pulpy	Soft, moist & pulpy Grayish Reduced smell of ammonia
	Colour	Whitish	off coloured	
	Odour	Fermenting odour	Poultry waste odour	Soft, moist and friable Grayish Irritating
6	Texture	Soft, moist & friable	Soft, moist and friable	
	Colour	Whitish	Grayish	
	Odour	Irritating	Irritating	

Moisture and Dry Matter contents

The cassava substrates from all three mixtures recorded an increase in moisture contents over a period of time (Table 3). Consequently, a decrease in dry matter content was observed. Changes in chemical composition during microbial fermentation was found to be responsible for the increased moisture content (10). The moisture contents in substrates from mixtures II and III were slightly less than from mixture I. This was probably due to the presence of poultry manure and urea respectively which contributed to a higher DM content. However, the increase in moisture content for all three samples were 2.8, 2.3 and 1.2% respectively showing that the biochemical changes observed in the three mixtures were different. The reduction in dry matter is an indication that some of the carbohydrates (major component of cassava) in the chips were broken down during fermentation.

Table 3: Composition of Fermented Samples

Days	Sample	%DM	%Moisture	%Nitrogen	%Protein
0	Mixture 1	37.7	62.3	0.37	2.30
	Mixture 2	40.1	59.9	0.45	2.80
	Mixture 3	40.2	59.8	2.30	14.36
2	Mixture 1	36.5	63.5	0.34	2.10
	Mixture 2	40.0	60.0	0.74	4.73
	Mixture 3	40.0	59.9	3.28	20.50
4	Mixture 1	35.0	65.0	0.42	2.62
	Mixture 2	38.9	61.1	0.60	3.78
	Mixture 3	39.6	60.4	5.30	33.13
6	Mixture 1	34.9	65.1	0.38	2.36
	Mixture 2	36.8	63.2	0.39	2.45
	Mixture 3	39.0	61.0	4.40	27.53

Nitrogen and Protein Contents

Changes in nitrogen and protein contents of the fermented samples are shown in Table 3. In samples from Mixture 1, it was observed that the protein content decreased slightly by day 2 and thereafter increased by day 4 and again decreased by day 6. It is possible that some protein synthesis occurred between days 2 and 4 which resulted in 14% protein increase Manidal *et al* (5) and Eruvbetine and Adegboyega (2) similarly observed some synthesis of protein in cassava under natural aerobic fermentation up to three days followed by a decline in protein content.

In samples from mixture II there was an increase in protein content by day 2 (69% increase) followed by a progressive decline in protein content by day 6. This shows that protein synthesis was favoured up to 48h. Samples from Mixture III however showed an increase i protein content (43%) by day 2 and 13% by day 4. Noomhorn *et al* (9) recorded an increase in content by 40h from cassava inoculated with *Aspergillus niger* and provided with a nitrogen source from uric acid as against urea in Mixture 111 which provided inorganic nitrogen. They also observed that filamentous fungi grow to a significant extent under solid-state fermentation. Fungi are believed to be chemorganotrophs whereby organic compounds are used as energy and carbon sources. Part of the organic matter is used for the synthesis of protein together with the nitrogen provided either by poultry manure or urea. In agreement with the results of this study, increased protein content from 5 to 48% and a decline in carbohydrate content from 65 to 28% during solid state fermentation have been reported (6). A process for protein enrichment of cassava using *Candida utilize* resulted in a product containing 40% crude protein after 2h fermentation (10) and this generally agrees with the observations of this study. Poultry manure contains organic nitrogen in the form of uric acid and did not seem to have been as efficiently utilized as inorganic nitrogen from urea which was readily assimilable and easily utilized by *Aspergillus niger* for the synthesis of protein.

CONCLUSION AND APPLICATIONS

The findings of this study indicate that:

i. The inclusion of a nitrogen source to cassava substrates in the presence of a fungus like *Aspergillus niger* resulted in increased protein content.

ii. Poultry manure as a possible nitrogen source seemed to have the potential for increased protein levels but urea was better utilized as a nitrogen source.

iii. It is advisable to improve the processing technique of the poultry manure and increase the level of inclusion so that more uric acid will be made available for utilization by the microorganisms.

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