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MICROBIOLOGY OF NATURAL FERMENTATION OF COWPEA AND GROUNDNUT FOR DAWADAWA PRODUCTION

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ABSTRACT

Traditionally, locustbean is fermented naturally for *dawadawa* production. Scarcity of locustbean indicated a need for using other legumes as substitutes for producing *dawadawa*. The feasibility of using cowpea and groundnut was therefore investigated. The microorganisms associated with natural fermentation of cowpea and groundnut were isolated in three separate fermentations lasting 72h. Sensory techniques were used to evaluate the flavour of the finished product. *Lactobacillus casei* and *L. fermentum* were responsible for the fermentation of cowpea. *Corynebacteria equi*, *C. xerosis* and *Mycobacteria segmeatis* were identified as the most predominant microorganisms during groundnut fermentation. A significant increase ($P < 0.05$) in titratable acidity from 1.05 mg/g to 6.80mg/g and from 1.20mg to 6.60mg/g was observed for cowpea and groundnut fermentation respectively. As fermentation progressed, pH increased significantly ($P < 0.05$) from 7.04 to 7.83 in groundnut, but decreased significantly ($P < 0.05$) from 7.14 to 6.60 in cowpea. Changes in pH suggest that proteolytic activity was dominant in groundnut fermentation. Bacillus species, primarily responsible for the flavour in naturally fermented locustbean, were not detected in fermented cowpea and groundnut. *Dawadawa* made from cowpea and groundnut were less acceptable to sensory panelists than *dawadawa* made from locustbean. Overall, cowpea and groundnut were not suitable for producing *dawadawa* with acceptable organoleptic quality.

KEY WORDS: Natural fermentation, titratable acidity, Lactobacillus, Corynebacteria, Mycobacteria

INTRODUCTION

Fermentation involves complex transformations of organic materials by using the metabolic activities of microorganisms(Mittal, 1992). Locustbean (*Parkia biglobosa*) is fermented naturally for the production of *dawadawa*, a popular food condiment in West and Central Africa (Campbell-platt, 1980a and Mebrahtu and Hahn, 1986). Besides its unique flavour, *dawadawa* contributes significantly to the protein available to low socio-economic groups in Nigeria. However, locustbean has become increasingly scarce and expensive and thus, the potential of using other legumes for *dawadawa* production is being investigated.

This work was undertaken to identify the microorganisms associated with natural fermentation of cowpea and groundnut for *dawadawa* production and also to assess the organoleptic qualities of the *dawadawa* produced from cowpea and groundnut.

MATERIALS AND METHODS

Processing methods

Groundnut was purchased from a local market at Ibadan, Western Nigeria while cowpea was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. The seeds were cleaned to remove foreign materials and then stored at 4°C until needed. Two hundred grams (200g) each of cowpea and groundnut seeds were roasted over low heat temperature (150°C) for 10 min, followed by dehulling.

The dehulled cowpea and groundnut were both cooked for one hour, drained and spread (while hot) on a calabash lined with papaya leaves. The seeds in each calabash were covered first with papaya leaves and then with jute bags to allow for heat building. The legumes were allowed to ferment at room temperature (25-°C) for 72h. Three replicate fermentations were carried out and samples used for the analysis were drawn at 12hour intervals.

Analysis

Viable Count Determination

About 10g of legume seeds were removed with a sterile spatula. A homogenate was prepared according to the method of Ikenebomeh *et. al* (1986), using 90ml sterile 0.1% peptone water as diluent. Serial dilutions (10^{-2}, 10^{-3}, 10^{-4} etc) were prepared from this homogenate by adding serially, 10ml of solution from preceeding concentration to 90ml of the diluent. Pour-plate method (Harrigan and McCance, 1976), was used

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Table 1
Characterization of bacterial isolates from fermenting cowpea and groundnut

	Bacterial Isolates				
	Cowpea		Groundnut		
	I	II	I	II	III
Cell Characteristics					
Gram stain	+	+	+	+	+
Shape	rods	rods	rods	rods	rods
Motility	-				
Endospore	-	-	-	-	-
Acid-fast	-	-	-	-	-
Biochemical Characteristics					
Catalase	-	-	-	-	-
Hemolysis				-	-
Nitrate reduction	-	-	+	+	+|
Gelatin			-	-	-
Urease			-	-	-
Growth in <7 days			-		
Growth on 5% and 8% Nacle	+	+	+	+	+
Growth on MacConkey jar	-	-	-	-	-
Twen hydrolysis <5 days			+		
Tellurute reducing in 3 days			+		
Niacin			-		
Carbohydrate fer/mentation					
Glucose	+	+	-	-	+
Maltose	+	+	-	-	+
Sucrose	+	-	-	-	+
Arabinose	+	-			
Cellobiose	+	-			
Fructose	+	+			
Galactose	+	+			
Lactose	+	+			
Mannitol	-	+			
Melozitose	+	-			
Melibiose	+	-			
Raffinose	+	+			
Rhamnose	+	-			
Ribose	+	+			
Sorbitol	+	+			
Trehalose	+	+			
Xylose	+	+			
Probable identiy:	*Lactibacillus Casei*	*L. fermentum*	*Mycobacteria segmatic*	*Corynebacteria equi*	*C xerrosis*

for enumerating the microorganisms. For viable bacterial count, 0.1ml of the inoculum was added into sterile petri dish and 10 – 15ml of sterile medium poured into the plate under a laminar air flow. The media used for the preliminary work were nutrient agar, malt extract and plate count agar. Petri dishes containing these media were incubated at 37°C for 24h. Plate count agar was found to support distinct growth of colonies and was subsequently used for enumeration of microorganisms. Plates with 30 – 300 colonies were counted and colony forming units per unit mass of sample (cfug^{-1}) were computed from these counts. Plating was done in 4 replicates.

Determination of Fungi and Yeast Count

Suitable serial dilutions of 1ml were plated out into petri dishes containing sabourand dextrose agar (oxoid), maltose agar and acidified potato sugar (APDA) (oxoid) and then spread with a

sterile glass rod. These plates were subsequently incubated at 27°C for 1 week. Only plates containing APDA had growth at the end of the incubation period. Suspensions of APDA culture plates were made with sterile distilled water and then aliquots of 1ml were used for counting on a Hemacytometer (Neverbauer).

Isolation and Identification of Bacteria

Ten grams of sample were ground and then serially diluted. One ml of suitable dilutions were plated out in four replicates in petri dishes containing plate count agar using spread plate method. The morphology of the colonies arising from the different batches was observed. Colonies that showed growth from 0 – 48h were assumed to represent the most predominant microorganism. The colonies were subsequently streaked on nutrient agar plates for isolation. Isolates were grouped on the basis of their morphology. Isolates were purified by repeated streaking of the single colonies and thereafter stored in screw capped bottles at 0°C. Bacterial charactersation tests were carried out on the isolates using definitions given in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Aseptic conditions were maintained throughout the study

Objective evaluation

The temperature of each of the substrates was monitored by inserting a thermometer in the center of each fermenting batch. Method of Ikenebomeh *et. al.* (1986), was used for determining titratable acidity and pH of the substrate. Ten (10)g of fermented bean taken at 12h intervals were ground in a porcelain mortar and 90ml distilled water were added. The mixture was stirred for 2min at maximum speed. The resultant pH was read with a single electrode (model pHm 82).

For titratable acidity (TA), distilled water was boiled to expel carbondioxide, cooled to room temperature and then used to prepare a 10^{-1} homogenate. To prepare a homogenate, 10g of sample were added to 90ml decarbonated distilled water and then blended for 90s in a Warring blender (Philips, H. R. 1707). The slurry was filtered through No 1 whatman paper and a 10ml aliquot of the filtrate was titrated with 0.1N NaOH. Phenolphthalein (1%) was used as end point indicator. 10ml of decarbonated distilled water was also titrated and the water titre value was subtracted from the sample titre. Titratable acidity was calculated as mg lactic acid per g of sample.

Subjective evaluation

The fermentation end products of cowpea and groundnut were subjected to sensory analysis and data were evaluated using multiple comparison analysis (Larmond, 1979). Nine Panelists were asked to smell and rate the samples using *dawadawa* produced from the traditional locustbean fermentation as reference. A scale of 1 – 9 was used, where 9 was extremely inferior and 1 was extremely superior. The scores were subjected to analysis of variance using SAS (1985).

RESULTS AND DISCUSSION

Total viable counts of the microorganisms steadily increased during the 72h fermentation period with a peak at 48h (Fig. 1). Both yeast and fungi were detected in low counts during the first 24h. Cowpea had yeast and fungi counts of $3.0 \times 10^2 \pm 28.0$ and 93.0 ± 10.0, respectively. Yeast and

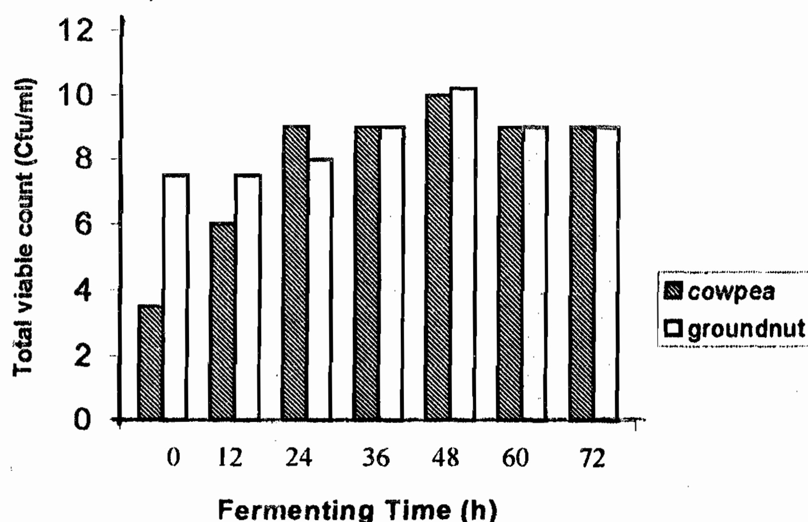

Fig. 1: Total Viable Count of Bacteria in Cowpea and Groundnut during Fermentation

fungi counts for groundnut were 2.7 x 10^2 ± 42.0 and 1.0 x 10^2 ± 12.0, respectively. This shows that the coupea were about twice heavily contaminated with fungi compared to groundnut. Fungi and yeast were probably contaminants in the legumes (Ikenebomeh (1982), reported fungi in *dawadawa* production as contaminants, whereas Barber *et. al* (1988) Ogbadu and Okagbue (1988) as well as Popoola and Akueshi (1984) did not detect any fungi or yeast in their studies. The temperature of the cowpea and the groundnut increased significantly ($P < 0.05$) during fermentation (Fig 2). This observation is in agreement with Odunfa (1986) who observed a similar increase in temperature during locustbean fermentation. Titratable acidity of the legumes also increased significantly ($P < 0.05$) as fermentation progressed (Fig. 3). A significant increase in pH was observed for groundnut

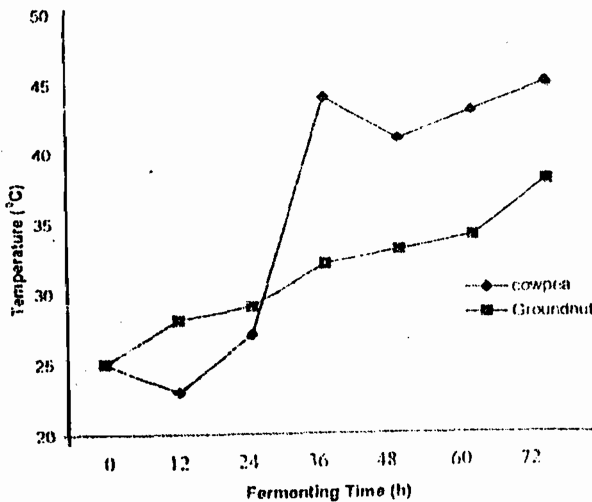

Fig. 2: Effect of fermenting time on temperature in fermenting substrates

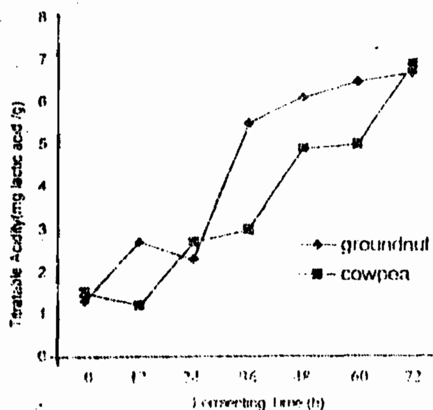

Fig.3: Effect of fermenting time on titratable acidity development (as lactic acid) in fermenting substrates

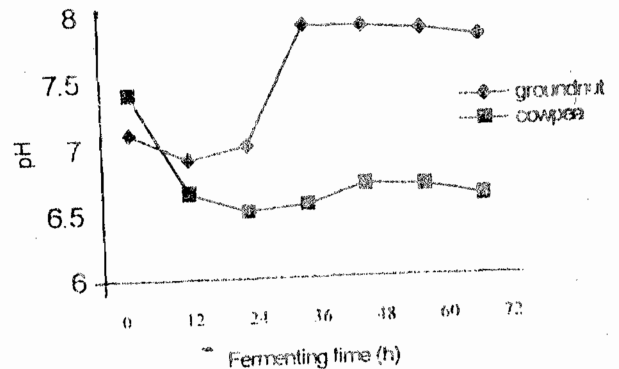

Fig. 4: Effect of fermenting time on pH values of fermenting substrates

fermentation but not for cowpea fermentation where a decrease in pH was evident (Fig. 4). It was observed that final pH of groundnut remained almost neutral, whereas pH of the cowpea dropped from neutral to acidic levels. Changes in pH of cowpea and groundnut fermentation differed from that reported for locustbean fermentation in that pH increased from acidic to alkaline levels in the latter (Onyejegbu and Oguntunde, 1993). Changes in pH suggests a proteolytic activity dominant in groundnut fermentation as opposed to carbohydrate fermentation in cowpea. This is presumably so because the low pH observed during cowpea fermentation is usually associated with lactic acid fermentation, commonly found in carbohydrate rich food substances Odunfa (1986) reported a high proteinase activity with resultant rapid amino acid production and also stated that the most important biochemical activity during locustbean fermentation was proteolysis.

Lactobacillus casei and *L. fermentum* were predominant in cowpea fermentation (Tabl. 1). Bacteria belonging to this genera have been associated with fermentation of many food products in different parts of the world. Various workers have reported the involvement of *Lactobacillus spp.* in African fermented foods. During fermentation of maize for ogi production for example, Akinrele (1970), and Odunfa and Akinyele (1985), identified *Lactobacillus species. Lactobacillus spp.* were also identified during fermentation of maize for Koko and Kenkey, Uji, Pito and Busaa (Christian, 1970; Mbugua; 1981; Ekindayo; 1969 and Nout, 1980). *Lactobacillus spp.* occur principally in substrates rich in carbohydrate, hence *Lactobacillus spp.* were predominant in cowpea with high carbohydrate content.

Corynebacteria equi, C. xerqsis and *Mycobateria segmatic*, were the principal microorganisms in groundnut. *Corynebacterium spps.* have also been identified in high carbohydrate foods. Okafor (1977), identified *Corynebacterium* during cassava fermentation for garri production.

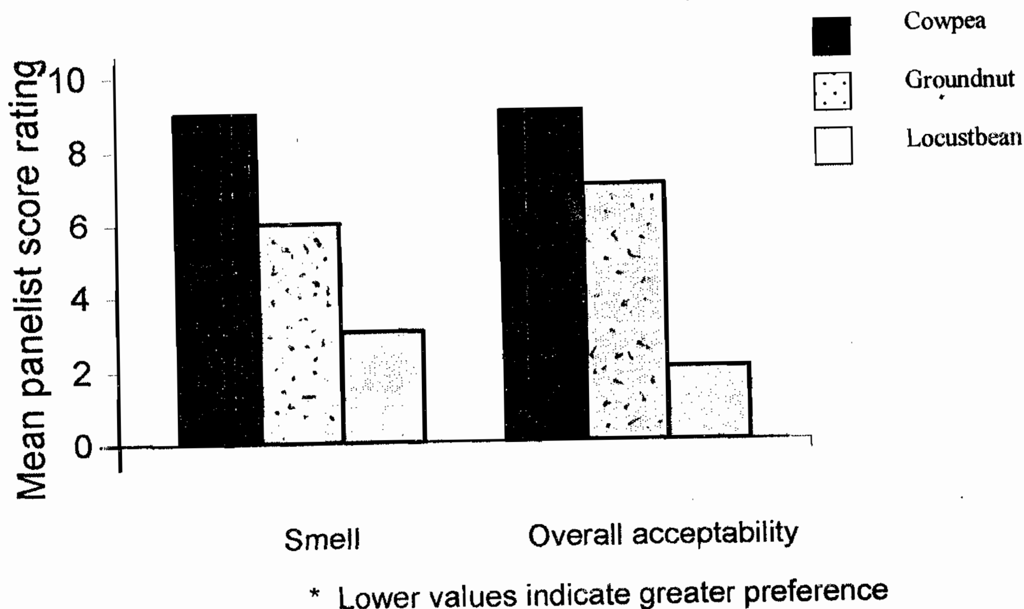

Fig.5: Mean score of sensory evaluation of fermented end-product (dawadawa) of cowpea and groundnut

Similarly *Corynebacterium spp.* was identified in groundnut fermentation which has a high carbohydrate content. In addition to the high carbohydrate content, the high fat content of groundnut provided an additional carbon source for the metabolism of microorganisms. *Corynebacterua spp.* and *Mycobacteria* which have high affinity for carbohydrates and lipids, were thus identified from groundnut fermentation. Odunfa (1986), reported that the predominant microorganism during locustbean fermentation was *Bacillus spp.* and identified *B. pumilus,* and *B. Subtilis* in the fermentation of locustbean, though *B. subtilis* was the most predominant. Similar findings have been reported been by Campbell-platt (1980b), as well as Ikenebomeh (1982). The acceptable flavour of the *dawadawa* produced from locustbean could be attributed to the Bacillus spp. in the locustbean alkaline ferment. No *Bacillus spp.* was identified in this study. Lactobacillus spp., Mycobacteria and *Corynbacteria* which were identified have not been associated with acceptable *dawadawa* flavour.

Flavour of the end-products of cowpea and groundnut fermentation were significantly different ($P \geq 0.05$) from that of locustbean (Fig 5.). The characterisitic dawadawa flavour, acceptable to the panelists, failed to develop in cowpea and groundnut. The fermentation end-product of cowpea was highly objectionable while groundnut *dawadawa* was less acceptable than locustbean *dawadawa* to the panelists. The highly objectionable flavour of the cowpea fermentation end-product could be due to the predominance of *Lactobacillus spp.*

CONCLUSIONS

This research revealed that fermentation of groundnut and cowpea did not result in acceptable *dawadawa* flavour. In addition, cowpea fermentation indicated carbohydrate metabolism by the microorganisms as shown by changes in pH levels. Cowpea and groundnut may therefore not be regarded as suitable substitutes for locustbean in *dawadawa* production.

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