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Bacteriological examination and proximate analysis of *'pupuru'* (a fermented cassava product)

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ABSTRACT Freshly prepared and market samples of *'Pupuru'*, a fermented cassava product was analysed to determine the bacterial load and proximate composition. The bacteria were identified as *Bacillus, Escherichia, Staphylococcus* and *Pseudomonas*. The average bacterial load for the samples ranged from 1.9 x 10^3cfu/g to 2.0 x 10^5cfu/g while the average coliform count ranged from 0 to 4.2 x 10^2cfu/g.

Only the sample from Agbabu market was contaminated with coliform. The chemical analysis on the samples revealed the following composition: Moisture content (11.47 ± 0.06% to 19.41 ± 0.38%); Ash content (0.55 ± 0.14% to 1.13 ± 0.03%); Crude protein (1.54 ± 0.04% to 1.66 ± 0.99%); Fat content (2.02 ± 0.11% to 3.75 ± 0.15%); Crude fibre (1.13 ± 0.14% to 1.80 ± 0..11%); Carbohydrate (74.51 ± 0.47% to 82.53 ± 0.10%). The energy values also varied from 330.99 ± 0.72 Kcal to 354.82 ± 0.79 Kcal.

Key Words: Cassava; *Manihot esculenta*; Fermentation; *'Pupuru'*; Proximate analysis; Microbial load.

Introduction

Cassava is a single species (*Manihot esculenta, Crantz*) belonging to the family *Euphoribiaceae.* Fresh cassava, like cocoyam and yam, is primarily a source of carbohydrate with a typical value of the composition as starch, 20-30%; water, 75-80%; protein, 2-3%; fat, 0-1%; fibre, 1.0%; ash, 1-1.5% respectively (Ihekoronye and Ngoddy, 1985). The food use of cassava cannot be over emphasised ranging from the tubers to leaves. Cassava is used mainly for human consumption, although small proportions of total production are in livestock feed and as industrial materials.

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Cassava in its processed form is used to prepare a wide range of meals in Nigeria. *'Pupuru'*, prepared from cassava fermented in water is very popular in the south-western states of Nigeria especially in the riverine areas of Ondo State. This food is one of the important native delicacies of the Ilajes in Ondo State. It is also widely eaten in places like Irele, Agbabu and Okitipupa and some African countries. At least as many as 4.6 million in Nigeria and more in some African countries eat *Pupuru*. It is a high carbohydrate food material with very low protein content (Okpokiri *et al*; 1995).

Traditionally, *'Pupuru'* is produced from cassava by fermentation. Mature cassava tuber is harvested while the bark peeled off. This is closely followed by steeping the cassava in water inside a container for 4-7 days to allow complete fermentation. Draining off water is followed in a jute bag while the shafts are removed subsequently. It is then moulded into ball-like shapes and fire-dried. The dried 'balls' look brownish at the surface as a result if oxidative browning. This can be kept and sun-dried often to avoid deterioration until is ready to be processed into a meal. The brownish surfaces are scrapped off with a knife, pulverised and finally sieved into *'Pupuru'* flour (Odetokun, *et al*; 1998). Preparation of meal involves pouring the already sieved flour into hot or boiled water, proper stirring until a fine paste-like semi solid formed which is a resemblance of pounded yam in its texture and appearance except for its taste. It is served with stew.

Abba-kareem and Okagbue (1991) observed that the traditional method of processing and sale of ready-to-eat foods expose them to high microbial contamination. Different species of microorganisms including normal microflora and contaminants from air or due to processing and handling methods have been reported by Aboaba (1982) from some fermented foods and beverages.

'Pupuru' can be stored for a few weeks, depending largely on the method of storage employed. It harbours bacterial that may release toxins (heat stable) and eventually affect the keeping quality and consumption effect on the consumers. This study is aimed at comparing he bacteria load of marketed *'Pupuru'* with that of freshly prepared sample to ascertain the presence of any microorganism whose existence is ecological and determine their effects on the quality of samples.

Materials and Methods

Collection of Samples

Samples of *'Pupuru'* were obtained from three different towns Viz: Okitipupa (sample A), Irele (sample B), and Agbabu (sample C) in Ondo State, Nigeria. The samples were pulverised and sieved using sterile seive (450um) into separate sterile polythene bags and stored in dessicator until used. Control sample (sample D) was prepared from fresh cassava tubers. The peeled, fermented, drained, and moulded balls of cassava were oven-dried at 90^{o}C (Odetokun *et al*; 1998).

Microbial examination of Samples

One gram of each samples was diluted serially with 9ml sterile normal saline. Subsequent dilutions were made to 10^{-5} level. Using pour plate method, plating was done in replicated with Nutricnt Agar and MacConkey Agar. Following incubation at 37^{o}C for 24 hours, the total microbial load and coliform count were calculated and expressed as colony forming units per gram (cfu/g) of sample. Bacteria isolates were identified according to Holt *et al* (1986).

Proximate Analysis of Samples

Samples were subjected to proximate analysis. Moisture, Fat, Fibre, and Ash contents were determined by the methods described by AOAC (1980). Nitrogen was determined by the micro-Kjeldahl method described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying by 6.25. Carbohydrate content was calculated by difference. The energy contents were calculated by multiplying protein, fat, and carbohydrate value by 4, 9, and 4 respectively (Iwe and Onuh, 1992).

Results and Discussion

Microbial load of the *'Pupuru'* samples (Table 1) showed that C alone did not conform to microbiological standard. The International Microbiological Standard recommended limits for bacteria contaminants in food are in the range of 10^1-10^2 cfu/g for coliforms and less than 10^5 cfu/g for total bacterial plate count (Refai, 1979). Similar results were reported by Abba-Kareem and Okague (1991). Different organisms were isolated from different sources of sample collection. Seven organisms identified as *Bacillus cereus, B. subtilis, B. firmus, Pseudonomas putida, P. flourescens, Escherichia coli* and *Staphylococcus roseus* were obtained only from sample C. *Bacillus cereus* in food causes food intoxication and also capable of causing non-gastrointestinal and systemic infection (Umoh *et al*; 1995). A population of more than 10^5 *Bacillus cerus* per gram of food is required for food poisoning and infection to occur (Gaepfert *et al*; 1972). It can therefore be deduced that the samples except sample C are safe for consumption since the counts obtained are not high enough to be of health hazard.

Table 1: Microbial load of *'Pupuru'* samples from different sources (cfu/g)

Source	Sample code	Total aerobic count	Coliform count
Okitipupa	PO	2.0×10^3	-
Irele	PI	1.10×10^4	-
Agbabu	PA	2.0×10^5	4.2×10^2
Control	PC	-	-

Most of the organisms isolated are contaminants from water and air. The difference in the species isolated from various samples could be due to environmental factors and the processes in the preparation of the various samples. Heat resistant endospores of *B. cereus* may survive the cooking process even though *'Pupuru'* is cooked before consumption. Yusuf *et al* (1992) reported that surviving *B. cereus* endospores in 'tuwo' increased to a level of 10^6 cfu/g in 36 hours of storage at room temperature. It is a common practise to prepare flour-based foods and stored for period ranging from few to 48 hours before consumption both in public places and in the home. *Pseudomonas spp* are known to be natural inhabitants of soil and water. The isolation of *S. roseus* and *E. coli* from only sample C is an indication of the poor level of hygiene maintained by the sellers at Agbabu market. Differences in the processing methods and storage of samples in ill-ventilated or non-ventilated stores are also factors responsible. *Staphylococci* are normal flora of the body and may be introduced into the flour upon contact with the hands of the sellers. They are known to produce enterotoxins causing staphylococcal food poisoning. This poses problems from the public health point of view. Greater attention should therefore be paid to the level of hygiene maintained during processing and storage of consumable flours. Using good source of water for processing would be also improve the microbiological value.

The moisture content of *'Pupuru'* is relatively high (19.41± 0.368%) except for the control sample (11.47± 0.06%_. The modified method of oven drying reduced the moisture content. High moisture content, hence the need to improve on the method of drying. Proximate compositions of the *'pupuru'* flour from different sources are presented in Figures 1-6. The crude fibre contents (1.13-1.80%) fall within the nutritional maximum level of 3.0% (Purseglove, 1968). *'Pupuru'* is a good source of carbohydrate with the value as high as 82.53% and low protein content. It is referred to as a fuel food. Similar observation was

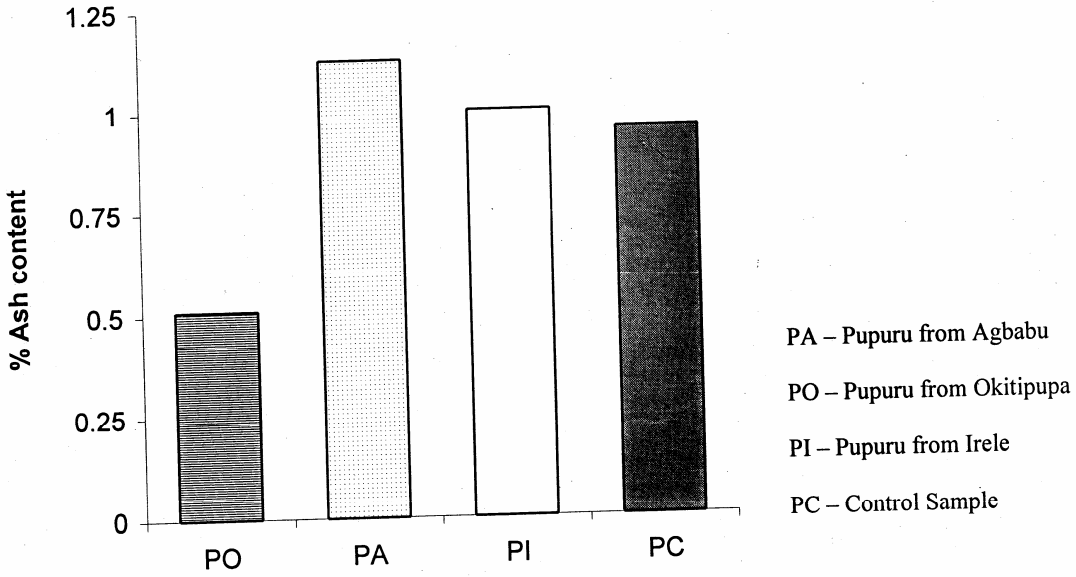

Figure 1: Ash content of *Pupuru* samples (in %)

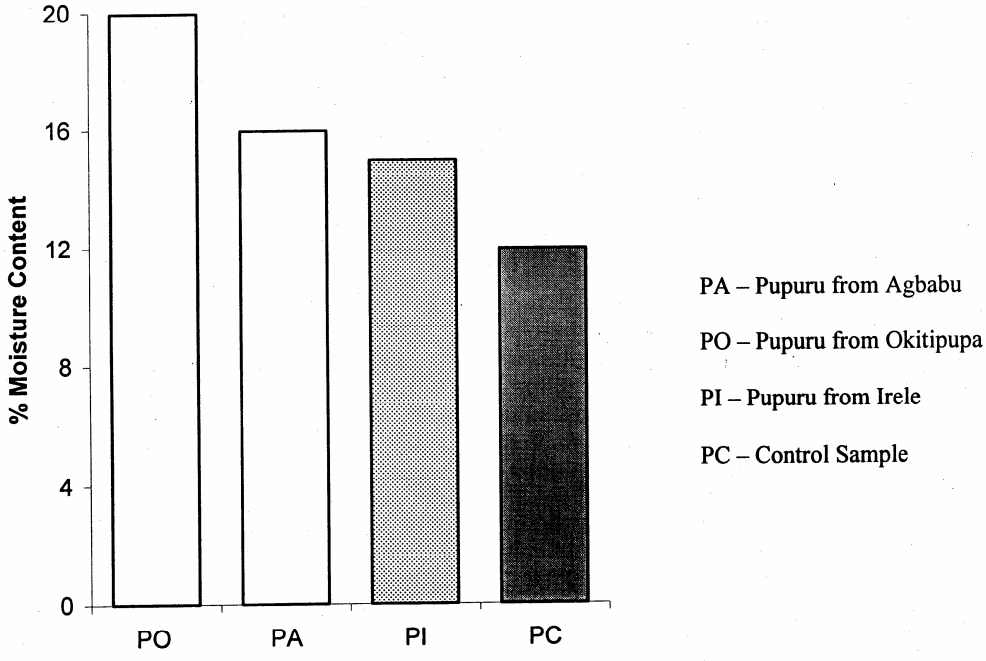

Figure 2: Moisture Content of *Pupuru* samples (in %)

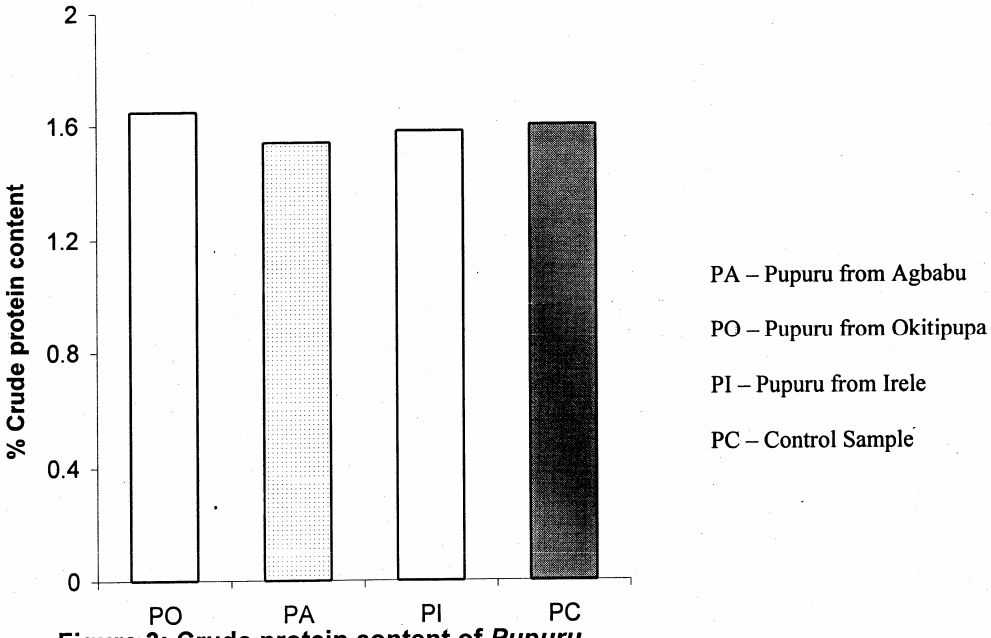

Figure 3: Crude protein content of *Pupuru* sample (in %)

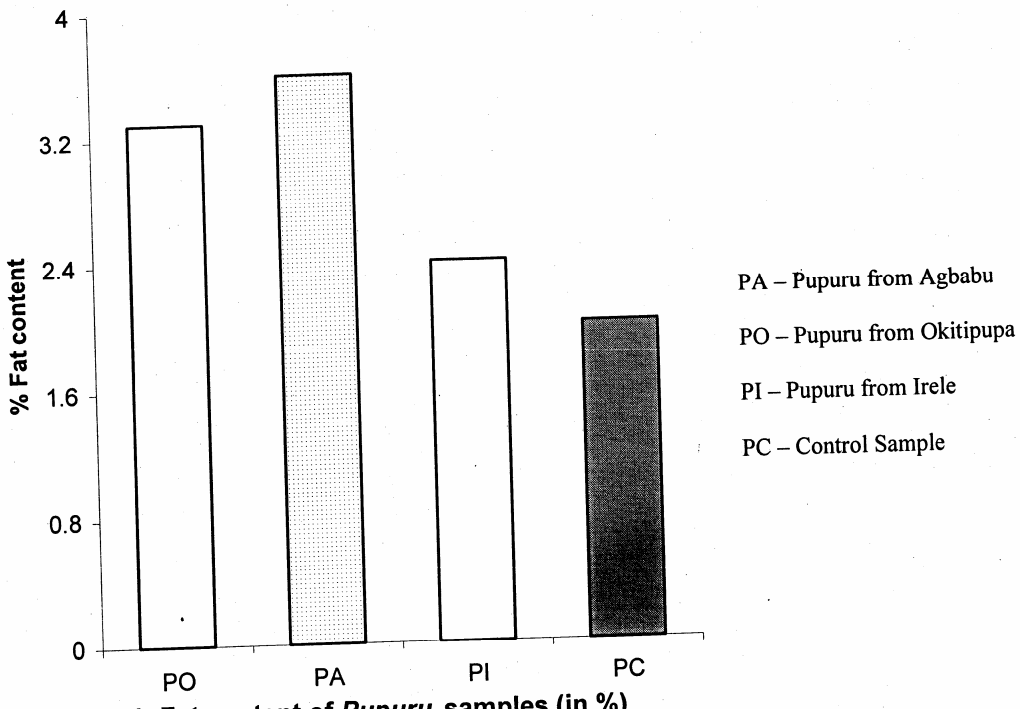

Figure 4: Fat content of *Pupuru* samples (in %)

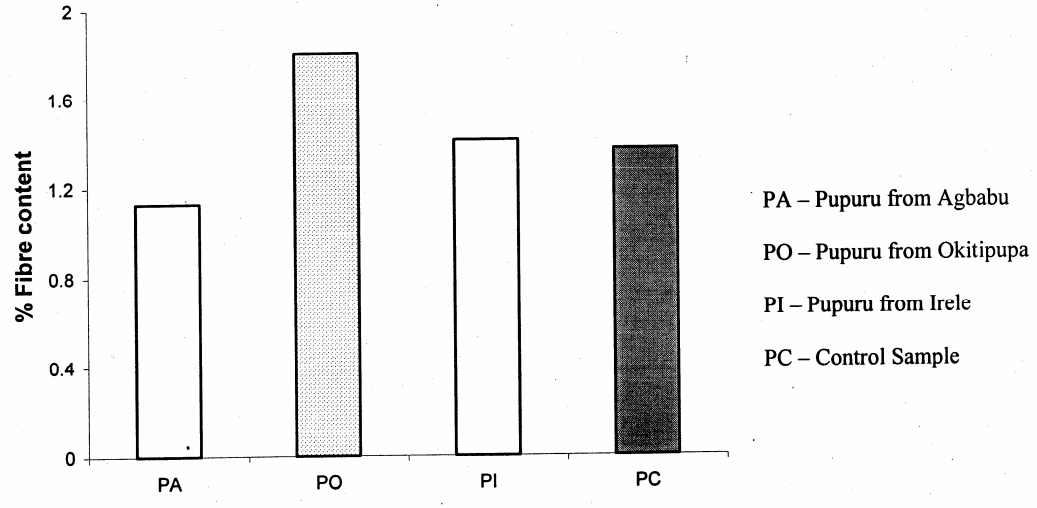

Fig. 5: **Fibre Content of *Pupuru* samples (in %)**

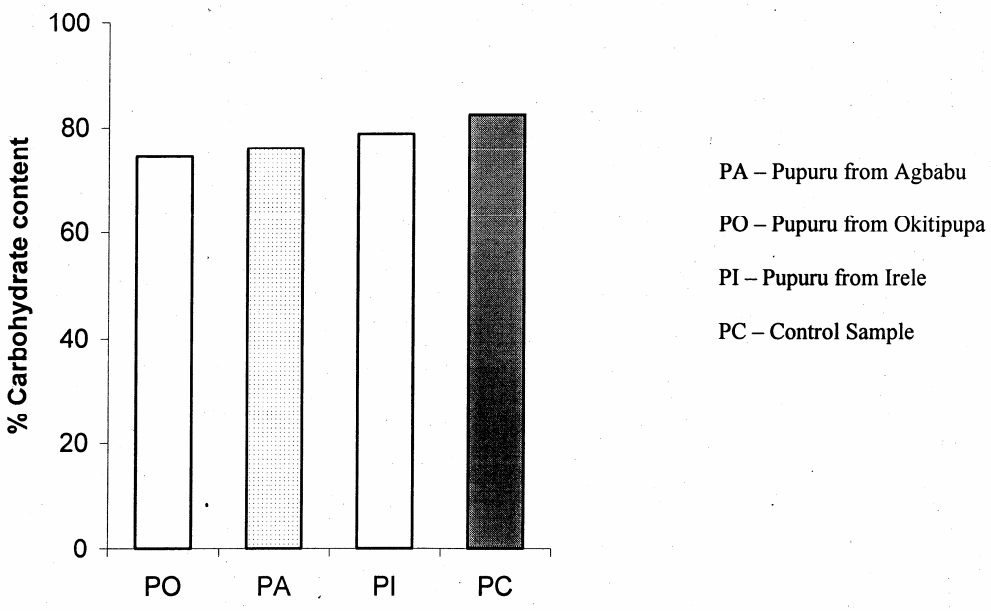

Figure 6: Carbohydrate content of *Pupuru* sample (in %)

reported by Odetokun *et al* (1998). The control sample had the highest proximate composition of carbohydrate and calorific value, which are needed for body energy.

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