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EFFECTS OF DIFFERENT METHODS OF FERMENTATION ON MICROBIOLOGY, PROXIMATE AND MINERAL COMPOSITIONS OF COCOA BEAN *(THEOBROMA CACAO* (L.)

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ABSTRACT

The study investigated effects of different methods of fermentation on microbiology, proximate and mineral composition of cocoa bean (*Theobroma cacao* (L.). Open, close and local methods of fermentation of cocoa beans were carried out separately for five days. Microbial analysis was carried out using pour-plate technique while proximate and mineral analyses were determined using standard methods. The bacterial count of cocoa beans at 5th day of fermentation ranged from 2.0 x 10^4 cfu/ml in local method to 1.0 x 10^2 cfu/ml in close method. The bacteria isolated and identified included *Bacillus subtilis*, *B. cereus*, *B.licheniformis*, *Micrococcus luteus* and *Staphylococcus aureus*. Fungi count also ranged from 1.0^3 x 10^4 sfu/ml in local method to 1.0 x 10^2sfu/ml in close method. Fungi isolated and identified included *Aspergillus niger*, *A. flavus*, *Neurospora crassa* and *Saccharomyces cerevisiae*. The pH of the fermenting cocoa beans ranged from 8.0 in local to 3.5 in close method. Also, the proximate analyses of the fermenting beans showed that the moisture content ranged from 55.56 to 50.89%; fat from 23.56 to 21.93%; protein from 13.46 to 11.06%; ash from 5.94 to 5.91% and carbohydrate from 13.46 to 11.06%. All values ranged from close to local method with open method having higher values than close but lower than local method. There was a significant increase in the concentration of calcium, copper, iron, sodium potassium and magnesium in the fermenting cocoa beans.

Keywords: Cocoa bean, fermentation, microbiology, proximate, mineral

INTRODUCTION

Cocoa, *Theobroma cacao* (L.) belongs to the family sterculiaceae. The word cacao in modern usage refers to the tree which produces the seeds known as cocoa beans (Wood, 1973). The cocoa beans are enclosed inside cacao pods and inside each pod, there are about 30-50 cocoa beans which is called "baba decacao" by some cocoa bean harvesters (FAO, 2007). The cocoa pods attain maturity between 110 to 130 days depending on the variety, from pollination to pod ripening. Hence, production of quality beans requires that only matured and ripe pods are harvested and processed promptly (FAO, 2007). Cocoa was given the name "*Theobroma*" which literally means, 'The food of the gods' by Linnaeus. This indicates that cocoa is used as food and medicine and can be processed in a variety of ways into different products. Cocoa and its processed products are very rich in food nutrients such as fats, protein, carbohydrate and vitamin B complex (Owolabi, 1972). The dried cocoa beans are the principal raw materials for chocolate, cocoa powder and cocoa butter. Also, potash from cocoa pod is used in making soap (Asiedu, 1989). Cocoa beans are source of income and poverty reduction in the Southwest Nigeria and are responsible for 50% of household incomes in Cote d'ivore and 65% of household incomes in Southwest Nigeria

(Levinsion and Levinson, 1978). Fermentation of cocoa beans is carried out in order to obtain a proper taste, colour; flavour associated with cocoa products and also to kill the embryo to forestall germination (Jonfia-Essien, 2004). Fermentation can be carried out in a variety of ways but all methods depend on removing the beans from the pods and pilling them together for 5 to 7 days depending on the weather to allow microorganisms to develop and initiate the fermentation of the pulp surrounding the beans. The quality of commercial beans depends very largely on fermentation (Khoh and Ho, 1992). Therefore, the present study investigated the effects of different methods of fermentation on microbiology, proximate and mineral composition of cocoa beans.

MATERIALS AND METHODS

Sources and collection of samples

The cocoa pods samples of Forastero variety were obtained from Lagba farms, Ondo, Ondo State, Nigeria. The samples were brought to the Laboratory of the Department of Biology, Federal University of Technology, Akure, Nigeria for analyses.

Processing of Cocoa Beans for Fermentation

The cocoa pods were washed in 10% sodium hypochlorite solution for 10 minutes, rinsed in sterile distilled water, drained and allowed to air-dry. The pods were cut opened with a sterile knife and the cocoa beans were aseptically removed from the pods. Subsequently, 500g of the cocoa beans was weighed separately, into a transparent covered plastic of 100mm by 400mm (close method of fermentation) and into another transparent plastic but uncovered (open method) and fermented for five days. Also, local method of fermentation was carried out in which the same gramme of the cocoa beans was placed on a concrete floor and covered with banana leaves and allowed to ferment for 5 days. The fermented samples were analyzed for associated microorganisms, proximate and mineral composition.

Microbial counts and isolation of associated microorganisms

One gramme of the sample was macerated in 9ml of sterile physiological saline and diluted serially to 10^3 dilution factor. One millilitre from the dilution factor was then pipetted separately into sterile Petri dishes. This was followed by the addition of sterile molten nutrient agar (for bacteria count and isolation) and potato dextrose agar (for fungi count and isolation) separately onto each of the plates in triplicates. The plates were gently swirled and allowed to solidity. The nutrient agar plates were incubated at 37^0C for 24hours while potato dextrose agar plates were incubated at 28 ± 2^0C for 72hours. At the end of the incubation period, associated microorganisms were counted and later isolated to obtain a pure culture. Isolated bacteria were identified according to the methods of Holt *et al.* (1994) while isolated fungi were identified with reference to Barnett and Hunter (1972) and Frazier and Westhoff (1998).

Determination of pH

The electronic Jenway pH meter was used. The pH meter was standardized using standard buffer solutions. The glass electrode was then dipped into the sample aliquot of the fermenting solution in a 100ml glass beaker and the pH values were read on the meter and recorded.

Determination of temperature

Mercury in glass thermometer was inserted into the fermenting solution and temperature observed was recorded in degree Celsius.

Proximate analysis

The moisture content of the fermenting cocoa beans was determined by the method of AOAC (1995). The method of Lowry *et al.* (1951) was used to determine the protein content while crude fibre, fat, ash and carbohydrate contents were determined according to AOAC (1990).

Mineral analysis

The minerals analyzed include potassium, calcium, magnesium, iron, zinc, sodium and copper. They were analyzed using the method of Fox *et al* (1984). The respective cocoa bean samples were prepared into solutions after heating inside clean crucibles placed in a muffle furnace at 550^0C to obtain white ashes. Two millitres of concentrated HCl solution was added to each crucible to break-up the ash. The mixture was filtered through acid washed No. 42. What man filter paper into 100mls volumetric flasks. Metals contained in the sample solutions were analyzed by the use of atomic absorption spectrophotometers.

Statistical Analysis
All data obtained were subjected to Analysis of variance (ANOVA) and where significant differences existed, the treatment means were separated using new Duncan's New Multiple Range Test (P=0.05).

RESULTS
By the 5th day of fermentation, local method of fermentation, recorded the highest microbial counts, having $2.0x10^4$cfu/ ml and $1.0 x10^4$sfu/ ml as counts for bacteria and fungi respectively while least microbial population occurred in the close method with bacteria and fungi counts of $1.0x10^2$cfu/ ml and $1.0x10^2$sfu/ ml respectively (Table 1). Also, fungi population was higher than bacteria in all the three methods (Table 1). The bacteria isolates *included Bacillus subtilis, B. Cereus, B. licheniformis, Micrococcus luteus and Staphylococcus aureus* while *Aspergillus niger, A. flavus, Neurospora crassa* and *Saccharomyces cerevisiae* were the fungi isolated by the end of the fermentation period. The pH of the fermenting cocoa beans was in the range of 3.5 (close) to 8.0 (local) while temperature ranged from 28^0C (local) to 31^0C (close) (Table 2).
For proximate analysis, table 3 showed range of values from local to close method with moisture content ranging from 50.88 to 55.56%, fat from 21.95 to 23.56%; ash from 2.95 to 5.94%; fibre from 10.16 to 12.90%; protein from 11.00 to 12.08% and carbohydrates from 3.94 to 13.46%. The proximate values of the fermenting cocoa beans were higher when compared with values obtained for unfermented samples (Table 3). However, the proximate composition of dried fermented cocoa beans (Table 4) was higher except for fibre and moisture contents in comparison with fermented samples. The same trend of results was obtained for mineral compositions. Mineral compositions of fermenting cocoa beans were higher when compared with those of unfermented samples (Table 5) but lower than the mineral compositions of dried fermented samples (Table 6).

Table 1: Microbial counts of fermenting cocoa beans

Fermentation Method	Bacteria (cfu/ml)	Fungi (sfu/ml)
Close	1.0×10^2	1.0×10^2
Open	6.0×10^2	2.0×10^2
Local	2.0×10^4	1.0×10^4

Cfu = colony forming unit
Sfu = spore forming unit

Table 2: pH and temperature of fermenting cocoa beans

Fermentation Method	pH	^{0}C
Close	3.5	31
Open	3.7	30
Local	8.0	28

Table 3: Proximate composition of fermenting and unfermented cocoa beans

Fermentation Methods	Moisture (%)	Fat (%)	Ash (%)	Crude (%)	Protein (%)	CHO (%) Close
	55.56±0.00c	23.56±0.00c	5.94±0.00c	12.90±0.00c	12.08±0.02c	13.46±0.00c
Open	53.78±0.00b	23.46±0.00b	5.91±0.00b	11.56±0.00b	11.06±0.00a	11.06±0.00b
Local	50.88±0.00a	21.95±0.02a	2.95±0.00a	10.16±0.00a	11.00±0.29a	3.94±0.00a
Unfermented	51.49±0.00d	21.13±0.03d	3.92±0.93d	9.42±0.50d	10.10±0.04b	3.94±0.40d

Mean values followed by the same letter along the same column are not significantly different at P>0.05.
CHO – Carbohydrate

Table 4: Proximate composition of fermented cocoa beans after drying

Method	Moisture (%)	Fat (%)	Ash (%)	Crude (%)	Protein (%)	CHO (%)
Close	7.54±0.00b	41.25±0.00a	4.96±0.01bc	8.15±0.00a	15.63±0.01b	22.47±0.00a
Open	6.94±0.01a	42.64±0.00a	4.60±0.01b	8.05±0.00a	15.45±0.00b	22.32±0.00a
Local	6.37±0.00a	42.25±0.00a	4.21±0.01a	9.23±0.01b	14.95±0.00a	22.99±0.00a

Mean values followed by the same letter along the same column are not significantly different at P>0.05.
CHO - Carbohydrate

Table 5: Mineral composition of fermenting and unfermented cocoa beans

Fermentation	Minerals (mg/kg)					
Methods	Zn	Ca	Fe	Na	K	Mg
Close	0.63±0.01a	151.47±0.01a	1.03±0.01a	3.31±0.01c	53.10±0.00a	51.27±0.01b
Open	0.72±0.01c	155.10±0.00b	1.58±0.01b	3.21±0.01b	55.33±0.00c	53.59±0.01c
Local	0.66±0.00b	180.01±0.01c	1.69±0.10c	3.04±0.00a	54.11±0.01b	30.58±0.00a
Unfermented	0.59±0.50d	141.18±0.08d	1.01±0.00a	2.16±0.04d	51.27±0.02d	44.71±0.07d

Mean values followed by the same letter along the same column are not significantly different at P>0.05.

Table 6: Mineral composition of fermented Cocoa beans after drying

Fermentation	Minerals (mg/kg)					
Methods	Zn	Ca	Fe	Na	K	Mg
Close	0.83±0.00a	51.49±0.01c	0.78±0.00a	55.45±0.01c	685.14±0.00b	21.39±0.01b
Open	0.84±0.00a	43.22±0.00a	1.94±0.02c	43.14±0.01b	601.98±0.00a	16.47±0.01a
Local	0.83±0.00a	47.53±0.02b	0.94±0.00b	31.69±0.10a	766.34±0.00c	20.02±0.01b

Mean values followed by the same letter along the same column are not significantly different at P>0.05.

DISCUSSION

The concrete floor where the cocoa beans were placed and the local banana leaves used in covering them must have possibly contributed to the comparative higher microbial counts observed in the local method. This is indicative of the fact that microorganisms are ubiquitous and that so many microorganisms are always associated with non-sterile materials (Ann, 1994). The high microbial counts observed in the three methods cannot but be connected with availability of nutrients and the essential minerals in the cocoa beans during fermentation. This agrees with the findings of Arotupin *et al* (2012) that the high bacterial and fungal counts may be due to the availability of nutrients such as fermentable sugars and essential minerals during fermentation.

Also, fungi population was more than bacterial except in local methods of fermentation. This might be due to the acidic condition of fermentation which does not favour growth of bacterial (Adegunloye, 2012). Even, the pH values obtained attested to this, showing the acidic nature of fermenting cocoa beans and it has been reported (Ann, 1994), that fungi grow best at range pH of 3.5-6.0 while low pH restricts growth of bacteria. Besides, the

temperature range of the fermenting cocoa beans indicated that the associated microbial isolates were mesophiles and fungi are most frequently associated with products stored at ambient temperature (Ann, 1994).

The presence and dominance of the species of *Bacillus* in the fermenting cocoa beans may be due to the nutritional versatility of the organism (Ajayi, 2011) while S*taphylococcus aureus* isolation during fermentation was indicative of human contamination (Awe *et al*., 2009 cited by *Arotupin et al*., 2012). The presence of *Aspergillus niger*, and *Neurospora crassa* was an indication of environmental contamination since their spores are common laboratory contaminants (Arotupin *et al.*, 2003) while *Saccharomyces cerevisiae* has always been implicated in fermentation process (Ann, 1994).
There was a significant increase in the proximate composition of the fermentation cocoa beans when compared with the unfermented bean. The increase in moisture contents of the fermenting cocoa beans could probably be connected to the hydrolysis of its components during the fermentation process (Arotupin *et al*., 2012). The increase in the fat, ash, fibre and carbohydrate might be due to the activities of microorganisms during fermentation (Adegunloye, 2012). The increase in protein content could be traced to the ability of microorganisms to secrete some extra cellular enzymes during fermentation (Arotupin *et al*., 2012). Besides the relatively higher percentages of the moisture, fat, ash, fibre, carbohydrate and protein contents observed in fermented cocoa beans after drying could be attributed to the dryness of the sample. This agrees with the report of Adegunloye (2012) that lower moisture content causes reduction in solubility of nutrients in substrate.

The relative increase in mineral compositions of the fermenting cocoa beans when compared with the unfermented could be due to the activities of fermenting micro organisms. The increase could have resulted from leakage from associated microorganisms (Arotupin *et al.*, 2012).
Hence, the fermented cocoa beans showed higher nutritional quality in terms of the proximate and mineral compositions when compared with the unfermented. Among the three methods of fermentation used in this study, close method proved best, having the highest proximate composition and least microbial counts.

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