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Microorganisms Associated with Fermentation of Cowpea, Enriched Gari

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ABSTRACT:

This study investigated the microorganism associated with the fermentation of cassava and cowpea produced into gari and the shelf life stability of the final products. A total number of 14 microorganisms were isolated which are: *Bacillus cereus, Bacillus subtilis, Corynebacterium manihot, Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus fermentum, Penicillium chrysogenum, Aspergillus niger, Articulospora inflate Aspergillus flavus, Aspergillus saprophticus, Saccharomyces cerevisiae, Trichoderma viridae and Geotrichum candidium.* The microbial population was found to reduce with fermentation time. The pH decreased with fermentation time from 0 – 48hours and later increased at 72 hours for all the samples. Titrable acidity increased with decrease in pH.

Keyword: Fermentation; Cassava; Cowpea; Microorganisms

INTRODUCTION

Fermentation is a metabolic process in which an organism converts carbohydrate, such as starch or sugar, into alcohol or acid. For example, yeast performs fermentation to obtain energy by converting sugar into alcohol. Fermentation usually implies that the action of microorganisms is desirable and the process is used to produce alcoholic beverages such as wine, beer, and cider. Lactic acid bacteria (LAB) and yeasts are responsible for most of these fermentations (Adeleke et al. 2010). Cassava *(Manihot esculenta* Crantz*)* is an important root and tuber crop that is grown throughout the tropics and sub-tropics, where it contributes a considerable proportion of the total caloric intake and ranks fourth after rice. To underline this fact, Africa accounts for more than 90% of the global cassava production in 2004 (FAO 2005) with Nigeria contributing 38.4 million metric tonnes and coming up from the third position behind Brazil and Zaire in the Eighties to being the largest producer of cassava in the world today. Cassava is a very cheap source of carbohydrate and is the main carbohydrate source in the diet of the teeming population of the third world countries where it is largely grown. The chemical composition of fresh cassava roots showed that it is made up of water (62%), carbohydrate (35%), protein (1%) and mineral salt (1%). Gari is the most popular cassava product consumed in West Africa and the most important food product in the diet of millions of Ghanaians and Nigerians (Oduro et al. 2000; Kordylas1 990; Edem et al.2001). Thus, continuous dependence on "gari" without enrichment with meat, fish and/or other protein-rich sources would result in protein deficiency. Cowpea (*Vigna unguiculata*), also known as blackeye pea, southern pea, and crowder pea, is a legume of African origin that is useful as a rotational cover crop to help meet a cash crop's nitrogen needs, control erosion and improve soil properties. Cowpea provides majority of low-income populations the main nutritious source of high and inexpensive protein and combined with cereals, cowpea for example gives a balanced amino acid intake (Inaizumi et al. 1999).The microorganisms associated with the fermentation of cowpea-enriched gari is reported in this study.

MATERIALS AND METHODS

Fresh Cassava tubers (*Manihot esculenta krantz)* were collected from a village close to the Federal University of Technology Akure, Ondo State,while cowpea (*Vigna unguiculata* L. Walp.) was purchased from Oba market in Akure, Ondo State.

Processing of Cowpea into Flour

A 600g of cowpea seeds were cleaned by removing stones, sticks, damaged beans and then washed using tap water. The seeds were dehulled by soaking in clean tap water (1:10 w/v seed to water ratio) at room temperature (28°C) for 5hours, followed by hand-rubbing (within two palms) to remove the testa. The floating testa were removed by decanting until no testa was present. The dehulled seeds were boiled for 30 min with clean tap water (three times the weight of dry seeds) to inactivate the trypsin inhibitor. The boiled samples were then dried in a hot air-circulating oven (Stuart Scientific HT Oven Size 1, Surrey, England) at 60°C to constant weight. The seeds were then milled into cowpea flour.

Processing of Cassava Tubers

A 3400g of Cassava tubers were processed as follows; Cassava tubers were washed with clean water to remove the dirt on it after which the tubers were peeled using a clean knife. The tubers were then washed using clean water and then milled using a grinding machine. The milled cassava was divided into 1000g , 900g, 800g, 700g and put into 4 different clean polythene bags with each of the bag labeled Sample A,B,C and D respectively.

Preparation of Gari

A 1000g Unfortified milled cassava represents Sample A, 10% cowpea flour was added to 900g of milled cassava (Sample B), 20% cowpea flour was added to 800g of milled cassava (sample C), 30% cowpea flour was added to 700g of milled cassava (Sample D). All the samples were mixed properly. Sample A, B, C and D were put into clean sac each, the samples were then put under hydraulic press to remove the water (dewatering). The samples were fermented for 72hours. The samples were sieved using a mesh to remove fibre waste. The samples were then fried

in hot metal dish with continuous stirring to produce gari. Cowpea – gari was formed from Sample (B, C, and D).

Microbiological Analysis

Microbiological analyses were carried out on the fortified and un-fortified samples, during fermentation (0, 24, 48 and 72hours) and after frying into gari (week0, week2, week4 and week16). The following media were used for the isolation of microbial load of un-fortified fermented and fortified fermented cassava samples: Nutrient agar for the enumeration of total viable mesophilic bacteria count, De Man Rogosa sharpe agar for lactic acid bacteria count and Sabauroud Dextrose agar for fungi. The colonies from the analysis were subcultured until a pure culture was obtained. The purified cultures were kept on slants of Nutrient agar for bacteria and De Man Rogosa sharpe agar for lactic acid bacteria and Sabauroud Dextrose agar for fungi.

Chemical Analysis

Determination of pH

The changes in pH of the samples during fermentation period was determined by pounding 10g portion of the root with 10ml of the supernatant and then homogenized into 100ml sterile distilled water. The pH of the resulting suspension was measured using an A Tioron meter (model 310) equipped with a glass electrode. The electrode sensor of the pH meter was inserted directly into 20ml sample in a clean 50ml glass beaker. Triplicate determinations were made in all cases.

Determination Of Total Titratable Acidity (TTA)

The total titratable acidity of the fermenting samples and final products were determined at different time interval by the method described by AOAC (2005). 2 grams of the samples were each weighed into 20ml of distilled water in a beaker. 10ml of the filtrate was put in a beaker, and a few drops of phenolphthalein indicator was added. This was then titrated with 0.1M sodium hydroxide (NaOH) solution. Readings of the titre values were obtained in triplicates. Acidity was expressed as lactic acid based on the conversion of 1ml of 0.1M NaOH being equivalent to 9.008×10^{-3}g of lactic acid.

Statistical Analysis

All data obtained from the analyses were subjected to one way analysis of variance (ANOVA) using SPSS (version 17) software package. The difference between means was separated using Duncan's multiple range test.

Results

Microbial Growth on the Samples During Fermentation

A total number of six bacteria were isolated from the unfortified and fortified cassava sample. The bacterial isolates are: *Bacillus cereus, Bacillus subtilis, Corynebacterium manihot, Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus fermentum.* Eight fungi were isolated these include; *Penicillium chrysogenum, Aspergillus niger, Articulospora inflate Aspergillus flavus , Aspergillus saprophticus, Saccharomyces cerevisiae, Trichoderma viridae, Geotrichum candidium.*

Fungal growth during fermentation

The fungal growth during fermentation is shown in figure 1. The fungal population decreased with fermentation days, with values ranging from ($2.49\ X10^4$ – $1.26X10^4$) cfu/g, ($1.95\ X10^4$-$0.28X10^4$) cfu/g, ($1.07x10^4$-$0.35X10^4$) cfu/g and ($0.87X10^4$-$0.21X10^4$) cfu/g, for samples A, B, C and D respectively. Sample A had the highest fungal population from 0 to 72hours while sample D had the lowest at 0, 24 and 72hours, sample C had the lowest Population at 48hrs.

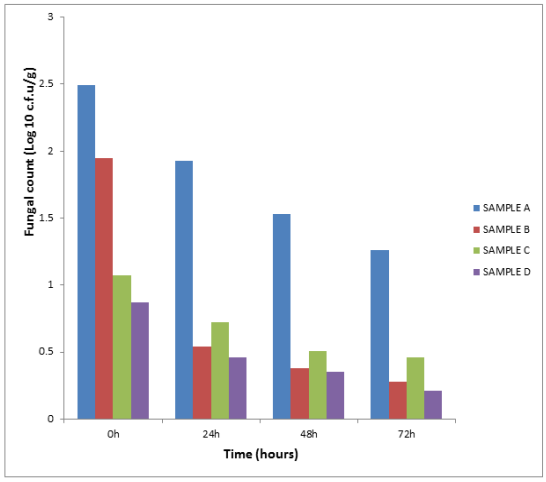

Figure 1: Fungal population during fermentation
Key: Sample A - 0% fortification, Sample B - 10% fortification, Sample C – 20 % fortification, Sample D - 30% fortification

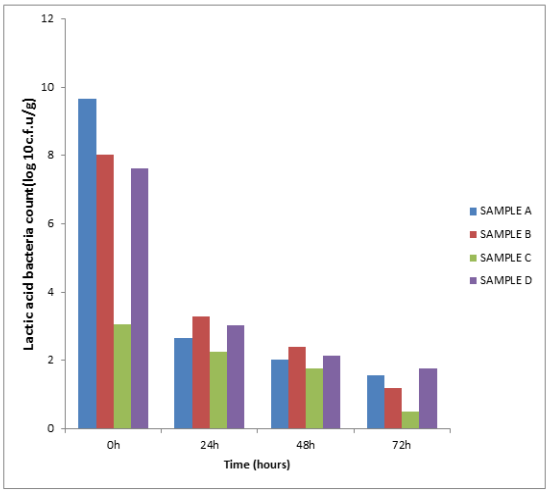

Figure 2: Lactic acid bacteria population during fermentation
Key: Sample A - 0% fortification, Sample B - 10% fortification, Sample C – 20 % fortification, Sample D - 30% fortification

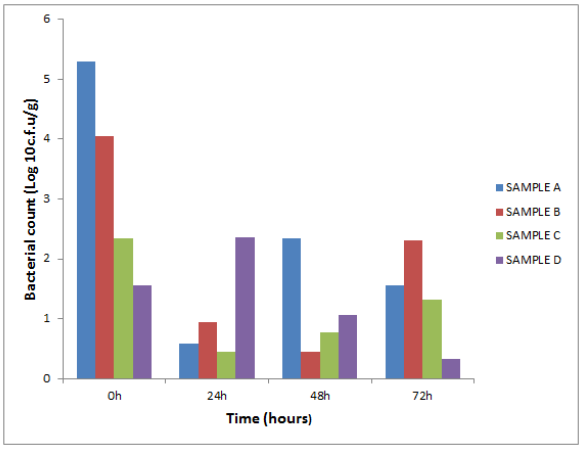

Figure 3: Bacterial population during fermentation
Key: Sample A - 0% fortification, Sample B - 10% fortification, Sample C – 20 % fortification, Sample D - 30% fortification

LAB Growth During Fermentation

Lactic acid bacteria growth decreased with increase in fermentation days in all the samples, LAB growth is shown in figure2. Sample A had the highest count of $9.66x10^6$ and $1.56x10^6$cfu/g at 0 and 72hours while sample C had the lowest count of ($3.07 x10^6$ to $0.51X10^6$)cfu/g from 0 to 72hours.

Bacterial Growth During Fermentation.

The bacterial growth is shown in figure 3. The population of bacteria decreased from 0 to 24hours in all the samples except in sample D where the population increased from 0 to 24hours with decrease at 48hours and 72hours. Sample C increased from 48hours to 72hours.The population decreased in sample B throughout the fermentation process. The population tends to increase in sample A at 48hours with slight decrease at 72hours.

Table 1: Changes in Total Titratable Acidity During Fermentation

SAMPLE	0h	24h	48h	72h
A	0.0865 ± 0.0250^a	0.1034 ± 0.025^a	0.3534 ± 0.513^a	0.0856 ± 0.0253^a
B	0.3298 ± 0.4827^a	0.3385 ± 0.439^a	0.351 ± 0.4569^a	0.162 ± 0.028^a
C	0.0627 ± 0.0250^a	0.0857 ± 0.0253^a	0.172 ± 0.025^b	0.052 ± 0.2671^a
D	0.0597 ± 0.0250^a	0.0875 ± 0.256^a	0.180 ± 0.025^b	0.0558 ± 0.0250^a

Key: Sample A - 0% fortification, Sample B - 10% fortification, Sample C – 20 % fortification, Sample D - 30% fortification.

DISCUSSION

The fungi isolated during fermentation are; *Aspergillus flavus, Aspergillus niger, Penicillum chrysogenium, Saccharomyces cerevisae, Geotrichum candida, Aticulosporium inflata and Trichoderma viridae*. No data has been found on the involvement of *Aticulosporium inflata* during cassava fermentation. It could be a contaminant from the environment. In this study, it was observed that lactic acid bacteria had the highest population, the activities of LAB which led to the production of organic acid thereby causing a decrease in pH could be responsible for the rapid decrease in the population of other microorganisms present in the samples. The decrease in pH during the fermentation of cassava roots results from the production of organic acids by lactic acid bacteria which constitute the dominant microflora (Malonga et al. 1993; Malonga et al. 1996). A fast decrease in pH was observed from 0 to 48hours in all the samples as fermentation progresses. The decrease in pH during the fermentation was due to the presence and activity of Lactic acid bacteria. Lactic acid bacteria hydrolyse the starch present in the cassava to produce simple sugar and organic acids which will impact the aroma and taste of the end products. Amoa and Jakobsen (1996) have reported the fermentation of cassava during gari and agbelima production in Ghana to be largely lactic acid fermentation. The pH values decrease from 4.43 – 4.10, 4.37-3.88, 4.26 – 3.81, 4.44 – 3.94 for unfortified,10%, 20% and 30% fortification respectively. The fortified samples had a lower pH than the unfortified sample at 48hours.This could be as a result of excess nutrient availability which enhance the growth and metabolic activities of the microorganisms. More acids will be produced since the added cowpea flour is also undergoing fermentation. Mbata (2009) reported that Bambara-nut fortification increases acid production which could be due to availability or more nutrients for microbial proliferation and enhances metabolic activities. Cowpea fortification is also reported by Afoakwa et al. (2007) to have great significance of influencing acid production and enhanced the development of the characteristics sour taste and flavour of cereals based food. The acids produced during fermentation help to hinder the growth of undesirable microorganisms. Sea-Dedeh (2004) have reported that lactic acid fermentation exhibits antimicrobial effects on pathogenic microorganisms due to the presence of acid. The pH tends to increase gradually in all the samples at 72hours, this was due to the reduction in the population of Lactic acid bacteria at 72hours as the environment becomes less acidic. Hence, the growth of proteolytic Bacteria (*Bacillus cereus* and *Bacillus subtilis)* was favoured. The alkaline pH during the fermentation of cassava leaves could be due to amines produced by *Bacillus* (Louembe et al.2003). The pH values after 72hours were 4.22, 4.29, 5.05, 5.20 for the unfortified, 10%, 20% and 30% fortification respectively. The titrable acidity increased with the fermentation time and with increase in fortification. The titrable acidity increased with decrease in pH.

This study reveals the successful fermentation of cowpea enriched cassava produced in to gari, it equally documents that the microorganisms involved in the fermentation are heterogeneous.

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