STUDIES OF CYANIDE CONTENT AND MICROORGANISMS INVOLVED IN CASSAVA (*MANIHOT ESCULENTA*) FERMENTATION

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ABSTRACT

Fresh cassava cortex, peels and leaves were studied for their cyanide content and microorganisms involved in their fermentation process. The total bacteria and fungi count, using the standard plate count, the cyanide content of each of the samples and their pH were evaluated. The results obtained showed that the species of Citrobacter feundii, Providentia, Acetobater and Lactobacillus were the bacteria isolated and identified during the fermentation while Aspergilius. Pencillium, Rhizopus, Candida and Geotrichum species were the fungi identified. The total viable bacteria count increased between 5.96 to 9.6, 6.23 to 9.9 and 5 to 9.85 (log 10cfu/ml) respectively for the cortex, peel and leaves while the total viable fungi count increased also between 6.23 to 9.56, 6.15 to 8.58 and 5.3 to 7.94 (log 10cfu/ml) respectively for the same samples throughout five days of fermentation. It was found that the cyanide content decrease with increase in the days of fermentation with the highest cyanide content of 120CN/g recorded in the peels and the lowest of 93.3CN/g in the cortex. The pH of the three samples also decreased with increase in the days of fermentation. It is apparent from the findings that microorganisms are involved in cassava fermentation and the cyanide content of cassava can be considerably reduced by the process of fermentation.

Keywords: Cassava, Fermentation, Microorganisms, Cyanine.

INTRODUCTION

Cassava (Manihot esculenta crantz) is the only edible cultivated species in the genus Manihot comprising about 25 species. Manihot esculenta is cultivated primarily for its tubers as roots, but young shoots and leaves are also edible and are used as food in some parts of the tropics (Kobaurila *et al.,* 2006).

The cassava tuber consists of three layers, a hard brownish scaly layer, a more succulent inner layer and an inner fleshy part usually whitish in colour which makes up the greater bulk of the tuber. The later is the part of the cassava tuber most commonly used for food and provide major source of calories for million of people. The growing use of cassava for human food and animal feed can be hampered by the presence of the toxic substance, cyanide in its tubers (Brandbury and Denton, 2010).

The cyanide in the cassava can occur as three types of compounds; the cyanogeic floucosiders, linamarines and lotaustralin which are hydrolysed to hydrogen cyanide. Due to this toxic substance, cassava needs to be processed by the traditional methods which include peeling, grating, fermenting, frying, roasting, steaming or boiling inorder to reduce the cyanide content to safe level for human consumption (Cumbara *et al.,* 2007; Achi and Akomas, 2008).

This study is therefore designed to determine the cyanide content of the cassava plant and the effect of length of period of fermentation on the cyanide content of the plant.

MATERIALS AND METHODS

A piece of fresh cassava tuber was peeled and 20g each of cortex, peels and leaves was cut into small pieces and washed thoroughly with distilled water before being soaked in distilled water in three separate sterilized conical flasks. Each flask was tapered with cotton wool wrapped with aluminium foil and labeled clearly. The flasks and their contents were left on a clean table in the labo-

ratory undisturbed for 5 days for the contents of the flasks to ferment.

Microbiological Analysis

Ten fold dilution of 1ml of liquid from each of the fermented samples were made to achieve a dilution factor of 10^{-4}, 10^{-6}, 10^{-8}. Exactly 1ml of the diluent from each factor was pour plated in triplicate plate on nutrient agar. The plates were incubated for 24h at 37^0C. Pure colonies of each isolate were obtained by streaking the specific colonies on the surface media and incubated 370^C. Identification was achieved using the methods of Buchanan and Gibbson (1974), Cowan (1985) and Macfaddin (1986). The fungi were cultured using the potato dextrose agar (PDA) and identified based on the cultural and morphological characteristics as described by Barnet and Hunter (1972).

Cyanide Determination

Twenty grams each of cassava cortex, peel and leaves were cut into small pieces, washed and blended in 60ml of $0.1mH_2SO_4$ using a household blender to obtain their extracts. The extracts obtained were filtered through the Wassaman No.1 filter paper fitted into a thistle funnel in order to obtain a clear solution. This process was repeated each day of the fermentation period.

For each of the extracts, 20ml was pipetted into three separate 250ml conical flasks after which 10ml alkaline pictrate was added and mixed thoroughly. Each of the flask was toppered with cotton wool and placed in a water bath preheated to 94^0c for 5 minutes. A blank was similarly prepared but with distilled water substituted for the extract.

All the flasks were removed after 5min, cooled and read at 495nm against a blank. All determinations were made in triplicate.

RESULTS AND DISCUSSION

The results obtained during the fermentation of cassava cortex, peels and leaves showed that there were changes in total microbial count as shown in Tables 1. The total bacteria viable count increased steadily throughout the 5 days period of the fermentation of the cortex, peels and leaves (Table 1). Similar trend of growth was observed in the total fungi count. This is because during fermentation, enough organic acid including lactic acid were released into the fermenting medium which led to the decrease in the pH thereby providing a favourable environment for the growth of these fungi. The pH at which different fungi can grow vary widely, but fungi usually grow better in acid pH of 5.0 or lower (Nester *et al.,* 1993).

The bacteria isolated based on morphological, cultural and biochemical characteristics were Citrobacter freundii, Providential species, Acinetobacter species, and Lactobacillus species.

Citrobacter freundii and Providential species were isolated throughout the period of fermentation. Acinetobacter species, were isolated within the first two days while Lactobacillas species was isolated on the fifth day.

Fungi were also found to be involved in the fermentation of cassava cortex, peels and leaves as shown in Table 2. Fungi isolated include species of Aspergillus, Penicillium, Rhizopus, Candida and Geotrichum (Table 2). Aspergillus and Rhizopus species were isolated throughout the fermentation period except on the fifth day.

Aspergillus and Penicillum are consistently associated with the root surface of cassava hence, they are referred to as root surface mycoflora of cassava (Ikediugwu and Ejale, 1980). It was observed that the moulds disappear after the fourth day of the fermentation, as they appear to be occasional contaminants of the cassava tubers. The moulds disappearance may be due to the low oxygen tension developed in the steeping water. On the fifth day, Candida and Geotricum species were isolated from the cortex, Candida species from the leaves on the fourth and fifth day while Candida tropicalis was isolated from the peels on the fifth day. During the process of fermentation, carbohydrate or starch is broken down into sugar, which encourages the growth of yeast, Candida and Geotricum species.

Table 3 shows changes in the pH value and cyanide content during the fermentation of cassava cortex, peels and leaves. The importance of the fermentation stage in cassava processing is linked to the reduction of cynogenic glucosides and the lowering of the pH in

the fermented product. Oke (1994) discovered that the hydrolysis of the endogenous linamarase enzyme is a reversible reaction facilitated by the growth of microorganisms that subsequently results in the gradual decrease of the pH of the medium. This is confirmed in Table 3 where the pH of the cortex decreases from pH5 to pH4. Table 3 also shows decrease in pH value during the fermentation of cassava peels and leaves. It is generally believed that the lower the final pH of the cassava mash the better the quality of the gari obtained, and also fermented cassava mash while a pH 4 or higher is considered undesirable (Akinrele, 1984).

Fermentation by soaking in water is the most effective method of reducing the cyanide of cassava. Wesby (1991) found out that by increasing the soaking time of the cassava tubers, the cyanogens removal process could be improved significantly. Thus more cyanide can be eliminated by allowing the fermentation to proceed for a longer period. It could be seen from Table 3 that there was a decrease in the cyanide level of the cassava cortex, peels and leaves as the time of fermentation increased. The table shows that the cyanide level of the fresh cassava cortex on the first day of fermentation was estimated to be 126.7CN/g fresh weight of cassava tissue but decreased to 93.3CN/g fresh weigh of cassava tissue on the fifth day. The cyanide level in the peels is higher compared to the cortex and leaves as shown in Table 3. This agrees with the findings of Tewe and Kasall (1986) who stated that generally the cassava peels contain higher cyanide content than the pulp. The findings in this study revealed that the concentration of cyanide in the cassava decreases with the length of period of fermentation. To avoid the danger associated with cyanide poisoning, it is necessary to ensure that our cassava is properly fermented before processing and subsequent consumption.

Table 1:
Total bacteria and fungi counts obtained during the fermentation of cassava leaves

	Bacterial Counts (Log10cfu/ml)					Fungal Counts (Log10cfu/ml)				
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Cortex	5.98	11.76	1.58	5.95	9.6	6.23	6.32	6.38	9.51	9.56
Peels	6.28	6.56	6.88	9.9	9.9	6.15	6.26	6.32	8.52	8.58
Leaf	5	7.35	8.42	9.69	9.85	5.3	7.23	7.48	7.68	7.94

Table 2: Fungi isolated during the fermentation of cassava cortex, peels and leaves for the period of five days

Days	Cortex	Peel	Leaves
1.	*Aspergillus sp., A. flavis, A. fumigatus, A. niger, Rhizopus sp.*	*Rhizopus sp., A. fumigatus.*	*Rhizopus sp., Aspergillus sp, A. fumigatus.*
2.	*A. fumigatus, Rhizopus sp.*	*A. niger*	*Rhizopus sp.*
3.	*Apergillus sp., Penicillium sp., Rhizopus sp.*	*Rhizopus sp., A. fumigatus, Penicillum sp.*	*Rhizopus sp., Penicilluum s.*
4.	*Penicillium sp., A. fumigatus, rhizopus sp.*	*Rhizopus sp., Penicillium sp., A. fumigatus*	*Candida sp., A. fumigatus*
5.	*Candida sp. Geotrchum sp.*	*Candida tropicalis*	*C. tropicalis*

Table 3: pH cyanide content of cassava cortex, peels and leaves during fermentation.

(Days)	Cortex		Peels		Leaves	
	pH	**Cyanide (CN/g)**	**pH**	**Cyanide (CN/g)**	**pH**	**Cyanide (CN/g)**
1.	5	126	6	163	6	143
2.	4	116	6	163	6	123
3.	4	103	5	146	5	123
4.	4	100	5	126	5	110
5.	4	93.3	5	120	5	100

CONCLUSION

Cassava is an important food crop in the tropics and will continue to be a major stable food for millions of people. It will continue to play a significant role in food security and economy, as well as socio- cultural and industrial lives of people, particularly in Africa. Cassava in the fresh form contain different levels of cyanide depending on the variety and the environment in which it was grown; therefore

the roots need to be well processed before consumption. Fermentation process helps in this regard since it reduces the cyanide level and decreases the pH value while the microorganisms enhance flavour development and product stability. Since cassava and its products formed the staple food of many of the African society, it is recommended that it be allowed to ferment properly in water before being processed into products such as Gari, Fufu, Akpu, Lafun, etc. Most of the cyanide is lost during soaking process. In many areas cassava leaves are consumed as vegetable. These leaves are good source of protein, mineral and vitamins A and C. Although their cyanide content exceed those of the cortex (roots), but simple traditional processing methods such as pounding or baking would reduce the cyanide to very low levels.

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