Microbiological and Biochemical Changes in the Production of *Akyeke* -A Fermented Cassava product

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Microbiological and Biochemical Changes in the Production of *Akyeke* -A Fermented Cassava product

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DECLARATION

This thesis is the result of research work undertaken by ERIC MANTEY OBILIE in the Department of NUTRITION AND FOOD SCIENCE, University of Ghana, under the supervision of Dr. Kwaku Tano Debra and Dr. Wisdom Amoa-Awua.

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DR. KWAKU TANO-DEBRAH

SIGN.....

DR. WISDOM AMOA-AWUA

DEDICATION

TO:

GOD,

JANET,

MUM and SUSANA

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ABSTRACT

Utilization of cassava, which contributes 22% of the gross agricultural product in Ghana, is limited by its high perisability, low protein content and toxicity due to the presence of the cyanogenic glucoside, linamarin and lotaustralin. *Akyeke*, a fermented and granulated, steamed-cooked cassava meal consumed extensively along the southwestern parts of Ghana is one of several products prepared from cassava in order to reduce postharvest loss of the crop. Fermenting grated cassava for 5-7 days with an inoculum, water is added several times to remove some amount of starch and colour from the fermented cassava mash, dewatered, sifted and sun dried before steaming. During fermentation, a breakdown of the texture of cassava dough occurs to give a smooth and non-rubbery texture.

The microflora of *Akyeke* inoculum and the inoculated fermenting cassava dough were examined to determine microbial and biochemical changes associated with fermentation of cassava into *Akyeke* as well as detoxification of the product during fermentation.

The microflora in *Akyeke* inoculum and during fermentation were dominated by *Bacillus* spp., lactic acid bacteria and yeasts.The *Bacillus* species identified were *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Bacillus pumilus* and *Corynebacterium* sp. Isolated lactic acid bacteria were *Lactobacillus plantarium, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus salivarius and Leuconostoc mesenteroides* at levels of 10^9 to 10^{10} in both laboratory and field

samples. Yeasts species *Candida tropicalis, Candida krusei, Zygosaccharomyces florentinus and Geotrichum candidum.*

Bacillus sutilis, Candida tropicalis and *Zygosaccharomyces florentinus* were found to be responsible for the disintegrated cassava tissues when plated directly on sterile cassava slices

Titratable acidity expressed as lactic acid increased from 0.38%-0. *32%* to 1.67%-1.11% after fermentation for field and laboratory samples respectively. pH dropped from 5.15 to 3.21 after fermentation. The large number of lactic acid bacteria, low pH and high acidity confirms the fermentation of cassava in the production of *Akyeke* to be lactic acid fermentation.

There was substantial reduction of total cyanide from 69.3 and 110.3 mg/kg to 1.4 and 2.8 mg/kg for field and laboratory samples respectively.

1.0 INTRODUCTION

Cassava (*Manihot esculenta* Crantz), is grown in many diverse environmental conditions. In recent years it has become the most widely cultivated tropical root crop (Aalbersberg and Limalevu 1991) and is one of the most important source of food in West Africa. Cassava is currently the most widely cultivated root crop in Ghana with the total annual production of about 7.11 million metric tonnes, contributing 22% of agricultural gross domestic product, 19% of dietary energy intake and averaging 380 Kcal/day per person (Dosoo and Amoa-Awua 1992;Al-hassan 1989, Ministry of Food and Agriculture, Ghana 1999).

Despite the importance of cassava in the food supply system of Ghana, utilisation of cassava tubers as food is limited by its high perishability, low protein content and toxicity due to the presence of the cyanogenic glycosides, linamarin and lotaustralin (Cooke and Coursey, 1981). Fortunately these factors, which limit utilization of cassava as food can be overcome by processing cassava into various shelf stable products, a practice, which occurs extensively, in Ghana practised both as domestic and commercial activities (Sefa– Dedeh 1989; Amoa-Awua 1996).

Traditional processing of cassava often involves a stage of fermentation. The major fermented products are *gari, agbelima* and *kokonte*. *Gari* is a fermented cassava product. In the processing of *gari,* peeled cassava tubers are grated and packed in polythene sack and dewatered. It is allowed to ferment for 2 to 4 days. The fermented dewatered mash is sieved to break up the cake and fibre strands removed. The granulated dough is roasted in an open pan. *Konkonte*, which is fermented cassava flour, is prepared by washing peeled cassava tubers and cutting them into chips. The

chips are sundried for a period of7 to 14 days depending on the weather. The chips ferments during drying. The dried cassava chips are pounded or milled into *konkonte. Agbelima* is a high moisture cassava meal. Peeled cassava tubers are grated together with an inoculum and fermented for 2 to 3 days. A less known product in most parts of Ghana is *Akyeke* which is extensively produced in the southwestern part of the country and in neighbouring La Cote d'Iviore.

In the processing of cassava into *Akyeke*, cassava tubers are peeled, washed and milled in the presence of an inoculum in a cassava grater. The meal is packed into polythene sacks and weights are placed on top to dewater for 5 to 7 days. During this period, the meal ferments. The fermented cake is granulated by sieving through a cane sieve and steamed into *Akyeke.* The inoculum is prepared by submerging peeled cassava chunks in water and leaving it to ferment for 3 days in a warm place.

The fermentation of *gari* and *agbelima* have been the subject of intense scientific study over the last couple of years, whilst *kokonte* is being investigated in some laboratories in Ghana (Okafor 1974, Sefa-Dedeh 1995, Budu 1994, Amoa-Awua and Jakobsen 1995). Unfortunately there is very little information on *Akyeke* in the literature and the few publications limited to investigation of the biochemical changes, which occur during fermentation (Aboua 1993).

The microbial species present in inoculum and those responsible for the fermentation are not known and it was the objective of this study to evaluate the microflora of *Akyeke* inoculum and the microbiological as well as biochemical changes, which takes place during fermentation.

OBJECTIVES

1. To characterize and identify the dominant microbial species present in *Akyeke* inoculum and during *Akyeke* fermentation.

2. To determine the biochemical changes which take place during the fermentation of *Akyeke*.

3. To investigate the possible role of the dominant microorganisms in the breakdown of cyanogenic glucoside during *Akyeke* fermentation.

2.0 LITERATURE REVIEW

2.1 History of Cassava

Cassava is strongly believed to have been introduced into Africa by the Portuguese slavers during the latter half of the 16th century. From the areas of introduction on both the East and West Coasts, the crop spread throughout the tropical areas of Africa. The crop was apparently introduced into South Africa by southward -migrating Tonga tribesmen, who settled in the Eastern Transvaal and Northern Natal. Elsewhere on the mainland West Africa it was of little importance prior to the 19th century and its spread and use increased during the 20th century.

At a time when variety of approaches to poverty alleviation are being considered by governments in developing countries and their development partners, increase attention is being paid to the potential of cassava as a food security crop as well as a cash crop. For the full potential of the crop to materialise, there is general agreement that, there is the need for global strategy for cassava development (IFAD 1996).

2.1.1 The Cassava Plant

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae, which is made up of 7200 species. Table 1 below shows the classification of cassava.

Table 1. Classification of Cassava

Class	Dicotyledoneae
Subclass	Archichlamydeae
Order	Euphorbiale
Family	Euphorbiaceae
Subfamily	Manihotea
Genus	Manihot
Species	Manhihot esculenta Crant

The cassava plant is a tropical perennial woody shrub crop, but the storage roots are harvested during the first or second year. The plant grows between 30° N and 30° S in areas where annual rainfall is greater than 500 mm and mean temperature greater than 20° C. However, according to Grace (1977), some cassava varieties grow at 2000 m attitudes or in sub-tropical areas with annual mean temperatures as low as 16 °C

.

The cassava plant grows under cultivation to a height between 2-4 m. The large, palmate leaves have 5-7 lobes borne on a long slender petiole. The leaves grow only towards the end of the branches. As the plant grows, the main stem forks, usually into three branches, which then divide similarly. The root radiate from the stem just below the surface of the ground. Feeder roots grow vertically from the stem and form the storage root, which penetrate the soil to a depth of 50-100 cm. This capacity of the cassava plant to obtain nourishment from some distance below the surface may help explain its growth on inferior soil (Grace 1977).

Cassava plant is mostly propagated from stem cuttings. When planted in moist soil under favourable conditions, the stem cuttings sprout and produce roots within weeks. However

propagation by seed is common under natural conditions and in cassava breeding. After planting, flowering and tuberization starts six and eight weeks respectively. Some of the factors which can affect the growth of cassava plant are rate of photosynthesis, respiratory activity, size of leaf surface available for photosynthesis, capacity to translocate assimilates from leaves to storage roots and capacity of storage roots to accept assimilates.

There are hundreds of cassava varieties in existence in many tropical countries, however little is known of the nomenclature and identification of these varieties. Morphological characteristics (colour of stem, petioles, leaves and tubes) are used to differentiate the varieties. The numerous varieties of cassava are usually grouped into two categories: *Manihot palmata and Manihot aipi* or sweet and bitter cassava. The distinction between the two categories is based on the content of hydrocyanide acid that causes toxicity in cassava root.

2.2 Importance of Cassava as Food

Cassava is a major source of dietary energy for low-income consumers in many part of tropical Africa, including major urban areas (Dahniya, *et al* 1991; Berry 1993; Nweke 1994). Table 2 shows that farmers in a third of village in the cassava growing areas of Sub-Sahara Africa (SSA) rated it as the most important crop. Cassava provides the daily food for more than 200 million Africans (Cock 1982; Maleley 1993).

The plants' starchy root is a source of valuable inexpensive calories. Also in many parts of Africa, cassava leaves and tender shoots are consumed as vegetable. Cassava leaves contain about 7% protein (fresh weight) and high levels of lysin. The fresh cassava roots according

Table 2 Percentage distribution of villages in which farmers reported that selected crops are the most important crop in cassava growing areas of Sub-Sahara Africa (SSA), by country, 1991.

Important crop	Cote d'Ivoire	Ghana	Nigeria	Tanzania	Uganda	Zaire	Zambia	Malawi	Burundi	Kenya	Weighted Mean
Cassava	8.4	35.0	20.5	51.6	42.5	80.0	60.6	12.4	9.6	7.6	32.8
Yams	17.8	21.3	35.8	-	-	-	-	-	-	-	7.5
Cocoyam	-	-	--	-	-	-	-	--	2.9	-	0.3
Potato	-	-	-	-	-	-	-	-	5.8	-	0.6
Plantain	-	16.3	-	-	-	-	-	-	-	-	1.6
Bananas	-	-	-	5.7	1.9	-	-	-	-	-	0.8
Maize	-	12.5	15.3	21.7	13.2	12.1	12.1	82.8	31.7	68.2	27.0
Rice	16.8	-	3.2	2.8	-	3.6	-	-	-	4.6	3.1
Other food crop	-	3.8	25.3	12.3	34.9	4.3	27.3	4.8	50.0	4.6	16.7
Cash crop	57.0	11.3	-	5.7	7.6	-	-	-	-	15.2	9.7

Source: COSCA (1991)

to Latham (1997) contain 62% water, 149 k cal energy, 1.2 g proteins, 0.2 g fat, 35.7 g carbohydrates and 1.1 g fibre.

The importance of cassava in Ghana is confirmed in terms of crop area, total production, and contribution to Agricultural Gross Domestic Product (AGDP) and food expenditure. The average area planted to cassava, which was about 387,000 ha in 1986 increased to 630,000 ha in 1998 (MOFA 1999) showing average annual increase of 20,250 ha. During the same period cassava production increased from 2.9m MT to 7.17m MT (Table 3). Cassava is by far the largest agriculture product commonly produced in Ghana and represent 22% of AGDP compared to 5% for maize, 2% for rice, sorghum and millet, 14% for cocoa, 11% for forestry, 7% for fisheries and 5% for livestock (Al-Hassan 1989).

2.2.2 Cassava as a Food Security Crop

Food security may be defined as physical and economic access to and consumption of safe and nutritious food at all times by all people to maintain a healthy and active life.

Cassava is increasingly important as a food and income source for the rapidly expanding rural and urban poor population of Sub-Sahara Africa. Between 1992-1994 for example, 50.6% of the global cassava storage root production (162.3 million tonnes) occurred on the continent (Anon 1997). Between 1965 and 1995, the biggest increase in the share of production on the continent occurred in Nigeria, which increased its share from 22% to 38%, and in Ghana, which increased its share from 4% to 8% (Neuenschwander and Braima 1998).

The reliability of cassava in times of famine or hunger, year round availability, effective production of energy and the crops comparatively better tolerance to drought and pest attack as well as civil disturbances are important reasons for the observed trend of increasing

Table 3 Comparison by region of 1998 and 1999* production of major crops (in '000mt)

REGION	Cassava			Cocoyam			Yam			Plantain		
	1998	1999	Change (%)	1998	1999	Change (%)	1998	1999	Change (%)	1998	1999	Change (%)
Western	605570	817280	34	192720	280100	45	74650	109090	46	325370	458750	40
Central	608200	848440	40	70300	78950	12	19100	21050	10	47700	52900	10
Eastern	2236000	2075800	(8)	463000	456900	(2)	690000	669300	(3)	817000	767000	(7)
Greater Accra	65298	-	-	-	-	-	-	-	-	-	-	-
Volta	115800	1161520	-	28650	28050		232992	232800	(1)	27760	31450	13
Ashanti	1168862	1160000	(1)	610731	608400	(3)	201546	236600	17	476355	469180	(2)
Brong Ahafo	1168622	1611720	37	211286	255000	20	904086	1331070	47	218463	266960	22
Northern	160000	170680	6	0	-	-	409700	413000	-	0	-	
Upper East												
Upper West	-	-		0	-	-	-	-		0	-	
	-	-		-	-		170783	236130	38	0	-	
TOTAL	7,171,452	7,845,440	9	1,576,687	1,707,400	8	2,702,857	3,249,040	20	1,912,648	2,046,240	

( ) :Deficit *As at October,1999

production. This supports the view that cassava is planted as a food security crop. The crops ability to provide food base is a function of its flexibility in terms of planting and harvesting strategies (FAO, 1999).

Cassava is widely appreciated as a low cost carbohydrate source for urban consumers especially where it is available in convenient forms for working urban housewives. The crop can therefore be seen as playing a major role in effort to alleviate the African food crisis.

2.3 Problems Associated with Cassava Utilization

Cassava roots provide an important source of dietary carbohydrate but they have some limitations. First fresh cassava roots are readily perishable if they are not processed and cannot be stored like cereals, yams and potatoes. Secondly, cassava roots from many cultivars cannot be consumed raw and are therefore unsuitable for roasting, boiling as fresh roots due to the high levels of the potentially toxic cyanogenic glucoside linamarin.

2.3.1 Perishability

About 50% of the root crop production in the tropics is provided by cassava, which cannot normally be kept in the fresh state for more than 2-5 days after harvest (Booth 1973). As a result of this the crop is commonly left in the ground until required for consumption or processing. Deterioration usually manifest in loss of quality and quantity as a result of pathological, physiological or physical damage or various combinations of all three.

Physical injury takes many forms and arises in stages from pre-harvest operations, through harvesting handling such as grading, packing and transporting to exposure to the mercy of the whether in the market and finally in the home of the consumer.

It has also been suggested (Ingram and Humpheries 1972) that chilling damage may occur in cassava stored at 12° C. The extent of chilling damage is usually dependent on a time temperature interaction and damage manifest in many ways but the common symptoms are internal tissue break down, increase water loss, susceptibility to deterioration, and changes in culinary qualities. Damage may also results from exposure to excessive high temperatures during harvest, handling and storage.

Cassava is alive and because of this, natural endogenous respiratory loss of dry matter together with transpiratory or wilting losses of water do occur. As a result stored cassava produce turn to lose water through this means. Physiological deterioration is observed as discolouration of the vascular tissues and stored parenchyma, and is accompanied by biochemical changes that are typical of wound response in other plant systems (Beeching *et al* 1994).

Microorganisms also attack cassava root and this is probably the most serious cause of losses in cassava roots. According to The berge (1985), three major diseases caused by pathogens that result in losses in cassava roots are:

Disease: Cassava dry root rot

Pathogen: *Armillariella mellea* (vahl.) Pat., *Rosellinia necatrix* Prill and other genera.

Symptoms: The disease usually occurs on land, which has recently been cleared of trees and shrubs. Infected tubers are typically covered with thick strands of hyphae. Infected plants wilt but do not shed their leaves. In time the drooping leaves and the entire plant dehydrate, turn brown and takes on a scorched appearance.

(ii) Disease: Cassava soft root rot.

Pathogen: *Phytophthora* spp., *Pythinm* spp and *Fusarium* spp

Symptoms: Usually expressed under wet soil conditions and cooler temperatures. Depending on the amount of soil inoculum, the rolling can take from several days to weeks. Typically many of the smaller feeder roots are dead and necrotic brown lesions are present on older roots. As the roots begin to decay, they in turn affect the tubers. With time the entire plant becomes wilted, defoliates and dies. The infected roots emit pungent foul odour.

(iii) Disease: Cassava tuber rot.

Pathogen: *Sclerotium rolfsii* Sacc

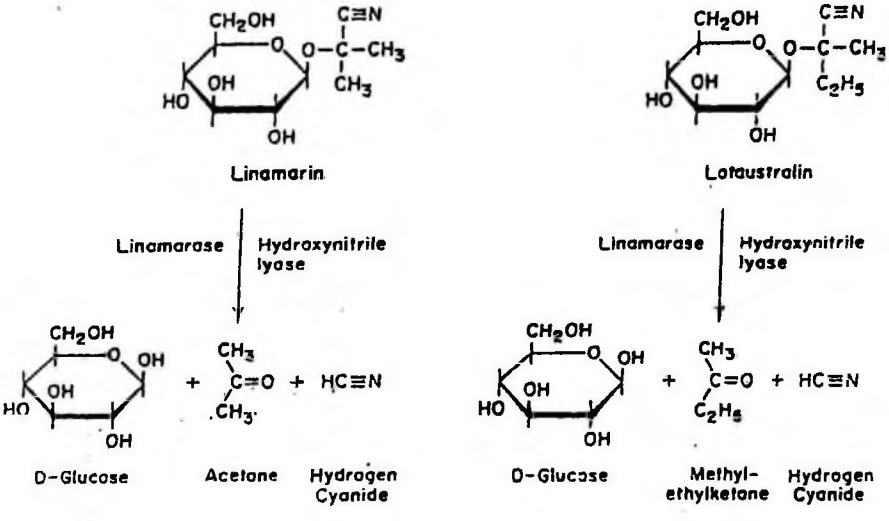
Symptoms: *Sclerotium rolfsii* can attack cassava roots at all stages of development and is readily recognized by the appearance of a white mycelia growth on infected cassava roots.

2.3.2 Cyanogenesis and Detoxification

Cassava is a food crop of great importance for the nutrition of over 500 million people in the tropical world (Bokanga 1994). However, cassava contains certain toxic factors, which has been known since its cultivation. Cassava has a cyanogenic potential which means that though the cyanide is not actually present, it (hydrocyanide acid HCN) is produced through enzymatic processes when cassava plant cells are bruised, grated or bitten (Fig 1).

The toxicity of cassava is due to the presence of cyanogenic glucosides (Linamarin and Lotaustralin), which liberate hydrogen cyanide upon hydrolysis by endogenous enzyme, linamarase. Hydrolysis occurs when the plant tissue is damaged or looses its physiological

Fig 1. Breakdown of linamarin and lotaustralin by β-glucosidase.



integrity. This hydrolysis yields glucose and the corresponding cyanohydrins, the decomposition of which releases the potent toxin HCN (Conn 1969). All the cassava varieties contain cyanogenic glucosides, but the concentration varies greatly between varieties and with edaphic condition (de Bruiyn 1973). This is illustrated in Table 4

Table 4. Cyanogenic potential of major cassava varieties and products in Ghana.

	CYANOGENIC POTENTIAL OF VARIETIES (mg HCN/100g)		
	Afisiafi TMS 30572 (GC/88-07)	Gblemoduade TMS 50395 (GC/88-05)	Abasa Fitaa TMS 4(2) 1425(GC/88-03)
Unpeeled Fresh Root	13.0 -25.5	19.8 - 23.8	8.4 - 24.0
Peeled Fresh Root	15.2	18.8	17.5
Ampesi (boiled roots)	3.3	6.6	4.6
Fufu	N.D	4.2	2.1
Kokonte	8.2	4.2	3.5
Agbelima	2.7	2.1	3.8
Gari	1.1 - 5.2	0.5 -2.0	04 - 2.3

Source: Root and Tuber Improvement Programme(RTIP) FACTSHEET NO. 1 OCTOBER 1999.

ND represent not determined

2.3.2.1 Occurrence and Distribution of Cyanogenic Glucoside in Cassava

Cyanogenic glucoside (linamarin and lotaustralin) produced by cassava occur in the ratio of 10:1. Valine and isoleucine are the precursors used in the synthesis of lianmarin and

lotustralin respectively (Bokanga 1995). The metabolic pathway for converting valine linamarin has recently been elucidated (Conn 1969) and the leaf has been identified as the main plant organ where the synthesis takes place (Koch *et al* 1992).

Although they are synthesized in cassava leaves, linarmarin and lotaustralin are translocated to and accumulated in all plant tissues in various proportion. For example the cyanogen content of cassava roots vary considerably from <10mg kg^{-1} to >500mg kg^{-1}, measured as hydrogen cyanide, on a fresh weight basis (HCN, fwb). Within the cells, they are mostly stored inside the vacuoles. Linamarase, a β-glucosidase capable of hydrolysing the cyanogenic glucosides is located in the cell wall (Mkpong *et al* 1991). This indicates that in intact cells, cyanogenic glucosides breakdown would not occur.

According to Grace (1977), the distribution of the cyanide acid in the cassava root varies in different varieties. In sweet varieties, the major part of the acid is located in the skin and in the exterior cortical layer, while in bitter varieties the acid is uniformly distributed in all parts of the roots.

The cassava tuber contains enzymes, which are capable of breaking down linamarin. In the naturally occurring tuber in its intact form, these enzymes are so compartmentalized in such a way that they cannot come into contact with their substrate. When the intact form of the cassava tuber is destroyed so as to bring the enzymes and the substrate together, the expected transformation of the poison occur.

Linamarin is first attacked by the enzyme linamarase to produce acetone cyanohydrin and glucose. At a pH of 5.0 acetone cyanohydrin decompose spontaneously to give a hydrogen cyanide and acetone but an enzyme hydroxynitrile lyase is also able to bring about the

decomposition of acetone cyanohydrin. The acetone and the hydrocyanic acid are both volatile and readily soluble in water.

Conn (1985) reported that fermentation of crushed tubers in sufficient volumes of water, followed by boiling to remove the volatiles, makes the tuber safe as food.

In countries where cassava is consumed, a variety of ways are employed to remove or at least to reduce to safe levels in cassava leaves and roots. The original owners of cassava, the Amerindians, grate, press and heat the tubers to eliminate the poison (Roger 1965). This procedure is adopted in cassava consuming countries of West Africa.

In 1992, Akintonwa and Tunwashe reported that insufficient removal of the cyanogenic substance may result in dietary cyanide exposure, which can cause acute poisoning. Aggravate iodine deficiency disorders and induce paralytic diseases tropical ataxic neuropathy (Osuntokun 1981) and konzo (Tylleskar *et al* 1992) have also been reported. Sub-lethal acute exposure to cyanide may cause non-specific symptoms such as headache and drowsiness. Cyanide exposure may also aggravate protein malnutrition due to the preferential use of the essential sulfur amino acid to provide the sulfur needed for enzymatic detoxification of cyanide to thiocyanate (Tylleskar *et al* 1992). However the knowledge about the dose rate of exposure and the degree from cassava that will induce or aggravate these diseases is still uncertain.

There are conflicting reports as to the role fermentation plays in detoxification. Oyewole (1992) and (Campbell-Platt 1994) reported that root fermentation plays important role in detoxifying bitter cassava varieties. It contributes to the release of the endogenous linamarase from the plant tissue. This enzyme is involved in the breakdown and hydrolysis

of cyanogenic glucosides HCN release and thus plant detoxification. Bokanga (1992) and Hahn (1992) reported fermentation in water leads to faster tissue disintegration and appears to be more efficient for reducing both free and residual cyanide in cassava roots and products.

Amoa-Awua *et al* (1996) also reported that the fermentation of cassava mash with a traditional inoculum into *agbelima* in Ghana, accomplishes three objectives, a souring of the dough, reduction of the content of cyanogenic glucosides and production of volatile aroma compounds.

Some authors Brauman *et al* (1994) have the view that during cassava fermentation, enzymatic detoxification may over ride high microbial action. Indeed, although high linamarase lactic acid bacteria are involved in cassava retting, it appears that the degradation of cyanoglucosides largely depends on the endogeneous linamarase of cassava roots. Aidoo (1992) reported that traditional cassava fermentation by itself is an unreliable detoxification method, as it does not achieve total cyanide elimination.

Sokari (1992) also noted that adding water to the grated cassava reduced linamarin content up 99% with little or no change in pH of mash. Sokari, asserted that fermentation has nothing to do with detoxification and that linamarin breakdown is essentially a hydrolytic process which is catalyzed by the endogeneous linamarin and enhanced by adding water. To Sokari, fermentation is only involved in flavour development.

2.4 Cassava Processing

The raw cassava roots and leaves are not palatable therefore there is the need to process cassava into various products which have added advantages such as improved palatability, contain less cyanide, increased shelf life, easier to transport and market.

Improvement of cassava processing and utilization methods would greatly increase labour efficiency, productivity, incomes and improve life of cassava farmers as well as increase efficiency of land use by releasing land after harvest for other crops or for fallow to sustain soil productivity. Processing also reduces food losses and stabilizes seasonal fluctuation in supply of cassava crop.

2.4.1 Current Non Traditional Uses of Cassava

In cassava growing households, approximately 26% of cash income from all food crops can be derived from sale of cassava (Nweke 1996). In such areas cassava frequently forms the basis of cottage industries to produce cassava for domestic consumption and local export market. Export of cassava, mainly through increased private sector awareness of international market opportunities for cassava chips and pellets as animal feed ingredient is strengthening the value of the crop and provides another tool for the fight against poverty (Neuenschwander and Braima 1998).

Cassava composite flour is now being studied extensively to replace wheat flour in many tropical countries. Ababio (1998) and Grace (1977) reported that bread of acceptable quality was obtained by the use of 30% cassava flour and 70% wheat flour.

Starch and starchy products are used in many food and nonfood industries and as chemical raw material for many other purposes, such as plastics, tanning of leather and in the textile industry. Non-food uses of starches or flour such as coating sizing and adhesives accounts for about 75% of the output of the commercial starch industry (Grace 1977).

In the sugar industry, cassava flour rather than cassava starch is being encouraged due to ease of production of the flour. Cassava flour or starch can be used to produce maltose (sugar) and the sugar further fermented to produce industrial alcohol.

There is little utilization of cassava for industrial purpose in Ghana. Cassava starch is used for laundry purposes making of adhesives and as substitute for user industries in Ghana namely battery, pharmaceutical, paints and glue.

2.4.2 Problems Associated with Cassava Processing

Processing of cassava can cause pollution in terms of its large quantities of solid waste (peels fiber and wastewater). The biological upgrading of cassava waste may be achieved with anaerobic fermentation techniques for a safer environment, which is less, polluted.

The following lines have sprung up in research to help address these pollution problems:

i) Production of biogas example methane by anaerobic methanogenic microorganisms.

ii) Conversion of solid waste to ethanol by amylolytic yeast *Schwaniomyces castelli* or into lactic acid by the action of amylolytic *Lactobacillus plantarum*.

iii) Conversion of cassava solid waste into high –value lipidic biomas by the action of *Trichosporum* sp., which is resistant to high cyanide, content (Wosiacki *et al* 1994).

iv) Cassava wastewater may undergo an anaerobic biofiltration on natural support such as bamboo straw or cassava fiber for biocompost production (Gotin *et al* 1994).

2.4.3 Food Fermentation

Fermentation involves action of microorganisms and/or enzymes that cause desirable biochemical changes and significant modification of the food. In the cause of fermentation, microorganisms breakdown carbohydrate, protein and lipids in the raw material being fermented by releasing hydrolytic enzymes into the medium. The breakdown products (fatty acids, amino acid and simple sugars) liberated are converted into microbial structural components, secondary metabolites and odoriferous volatile molecules (Fook - Min Yong 1992; Paredes - Lopez 1992).

Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylase, protease and lipase hydrolyze the polysaccharide, proteins and lipids to non-toxic products with flavors, aromas and textures that are pleasant and attractive to the human consumer (Steinkraus 1997).

Fermented foods can be divided into two: i) submerged deep fermentation (SDF). In SDF microbial action occurs at a relatively low biomass concentration in the liquid phase. SDF is used in industrialized countries for production of large industrial enzymes. ii) Solid state fermentation (SSF). In SSF microbial growth and product formation occur spontaneously or by adding inoculum on the surface of the solid substrate. SSF is mainly used in developing countries for the fermentation of indigenous foods.

Traditionally, microorganisms acting alone or with others, produce products which are usually found most acceptable by consumers. These organisms are gradually selected and used repeatedly. The selection is based on the nature of the raw material from which the fermented food product is derived, the local environmental conditions and the local practice used in the preparation of the fermented product. However,

microorganisms used in modern fermentation are selected, preserved and used as starter culture.

Indigenous food fermentation is one of the oldest forms of biotechnology. Many agricultural products involve fermentation techniques leading to a wide variety of fermented foods examples dairy products - cheese, yoghurt, cereals, rice, sorghum and maize, legumes - soybean, roots and tubers - cassava, meat, fruits and vegetables - grapes, pickles and others - coffee, tea, cocoa etc.

Lactic acid bacteria and yeasts have been reported as being the dominant microorganisms in the natural fermentation of a variety of traditionally fermented African foods (Akinrele 1970; Nout 1980; Odunfa and Adeyele 1985; Adegoke and Babaloa, 1988; Halm *et al* 1993 and Amoa - Awua 1996).

2.4.2.1 Importance of fermentation

The importance of fermentation are that it:

1) adds variety to diet.

2) contributes to general nutrition of large population by improving digestibility and bio-enrichment in proteins and vitamins.

3) destroys undesirable factors like cyanide making foods safe.

4) reduces amount of energy used by traditional women in cooking.

5) gives specific organoleptic characteristics (desire aroma, texture and sour taste).

6) ensures protection of the environment example in cassava waste treatment.

2.4.3 Fermented Cassava Products

Cassava roots are processed traditionally by a variety of methods into many different food products and used in different ways based on local custom and preference. It is believed that traditional method of processing in Africa probably originated in Brazil.

The processing method is made up of combination of a number of activities such as peeling, slicing, grating, steeping, fermenting, drying, milling, boiling, steaming, pounding, roasting and pressing.

The major cassava fermented food products are *gari* (Nigeria and Ghana), *eba* (Nigeria), *akyeke* (L'Cote d'Iviore and Ghana), *chickwangus* (Zaire) *myondo* and *bobolo* (Cameroon), *lafun* (Nigeria) *mangbela* (Central African Republic), *agbelima* (Ghana) and *fufu* (Central Africa).

2.5 Microbial Changes during Cassava Fermentation

There are a lot of cassava fermented food products, but the ones in which extensive microbial work have been done are *gari* and *wet fufu* (Nigeria) and *agbelima* dough (Ghana). Early studies done by Collard and Levi (1959) on the microbiology of *gari* fermentation has it that *Corynebacterium* sp. and *Geotrichum candidum* were responsible for the acid and flavor production. In 1979, Ngaba and Lee reported differently, they found out that of the microorganisms isolated from *gari, Lactobacillus plantarum* produced the most typical *gari* flavor. In 1977 Okafor isolated organisms of five genera from *gari* fermentation: *Leuconostoc, Alcaligenes, Corynesbacterum, Lactobacillus and Candida.*

A study on *wet fufu* by Okafor *et al* (1984) reported the following genera: *Bacillus, Lactobacillus, Klebsiella, Leuconostoc, Corynebacterum and Candid. Agbelima* dough has been extensively studied in the laboratories of Nutrition and Food Science of the University of Ghana (Budu 1990; Sefa-Dedeh 1995) and Food Research of CSRI (Amoa-Awua and Jakobsen 1995; Amoa-Awua *et al* 1996; Amoa-Awua 1996; Amoa-Awua *et al* 1997). The work done by Amoa-Awua (1996) identifies fermentation to the species level. He found out that *Bacillus sibtilis* and other *Bacillus* spp. including *B. mycoides, B. punilus, B. cereus, B. amyloliquefacieus* and *B.licheniformis* are the tissue degrading micro-organisms in three types of inocula examined and the moulds, *P. nodulum, P.* citrinum and *Geotrichum candidum* is a forth type of inoculum whose biota is dominated by moulds. He further reported that for souring of agbelima, *Lactobacillus plantarum* and other lactic acid bacteria including *Lactobacillus brevis and Leuconstoc mesenteroides* are the dominant microorganisms. Yeasts are also dominated by *Candida krusei, Candida tropicalis* and *Zygosaccharomyces spp*.

2.7 Akyeke, Fermented Cassava Produce

Akyeke is one of the most common forms of fermented cassava product consumed in L'Cote d'Ivoire and southwestern part of Ghana. Production of *Akyeke* involves fermentation.

In the processing of cassava into *Akyeke*, cassava root tubers are peeled, washed and milled in the presence of inoculum in a cassava grater. The cassava is packed in a perforated polythene and allowed to ferment for 5-7 days depending on the proportion

of inoculum added. Water is added to the fermented meal and then screened. The water helps to decolour the fermented meal. Pressing is done to dewater the fermented meal. The dewatered fermented meal is screened again and to obtain the large granular size, it is stirred several times with the hand. The fermented meal is sundried and steamed to obtain *Akyeke.*

Aboua (1995) investigated condition under which Akyeke is processed. Inoculum ratio moisture content, temperature, pH and cassava varieties were tested in order to evaluate their influence on the fermentation process. He reported that the best combinations were 8% inoculum ratio, 28-30^{0}C, pH 3.9-4.0 using cassava variety Bonoua, Yace or a mixture and 59-62% moisture. He further reporter that when cassava was fermented in fiberglass bags draining the liquor, the *Akyeke* had good sensory properties.

Among the cassava food products *Akyeke* is ranked next to *gari* and *kokonte* in terms of food energy (Table 5).

2.8 Lactic Acid Bacteria

Lactic acid bacteria are Gram positive, non- sporing, cocci, coccobacillus or rods, dividing in one plane only with the exception of the pediococci, lacking catalase, devoid of cytochromes, non-aerobic habitat but aerotolerant, fastidious, acid tolerant, requiring a fermentable carbohydrate for growth and converting glucose mainly to lactic acid or lactic acid, CO_2, ethanol and or acetic acid (Sharpe 1979; Axelsson 1993).

Table 5 Composition of cassava products commonly consumed in Ghana

	Food Energy		Moisture	Protein	Fat	Carbohydrate (total % fibre)	Fibre	Ash	Calcium	Phosphate	Iron
	Calories	Kilojoules	%	g	g	g	g	g	g	mg	mg
Starchy root, Tuber Cassava; raw	171	715	56.7	1.0	0.4	40.8	0.8	1.1	27.0	58.0	0.9
Cassava; cooked	147	615	61.6	0.7	trace	36.1	0.3	1.6	2.0	45.0	0.7
Chips	352	1474	10.8	1.3	0.4	85.6	-	1.9	50.0	72.0	1.4
Dough	198	828	50.8	0.8	0.5	47.5	0.6	0.4	25.0	21.0	0.9
Akyeke	216	904	46.8	0.3	1.8	49.2	0.6	1.4	18.0	15.0	4.3
Fufu	136	569	65.2	0.9	0.2	32.8	0.3	1.0	10.0	51.0	0.7
Gari	350	1464	11.7	1.5	0.2	85.3	1.3	1.3	43.0	66.0	1.7
Kokonte	346	1448	12.2	1.4	0.4	84.3	1.1	1.7	50.0	107.0	2.5

Source: K.K. Eyeson and E. K. Ankrah (1975)

Classification of lactic acid bacteria into different genera has been based largely on the following; morphology, mode of glucose fermentation, growth at different temperatures, configuration of lactic acid produced, ability to grow at high salt concentrations, acid or alkaline tolerance, and fermentation of different carbohydrates. The taxonomy of lactic acid bacteria is under going major changes with advent of new classification schemes such as rRNA sequence analysis and other phylogenetic techniques, fatty acid composition cell wall analysis and mobility (Chassy and Murphy 1993; Axelsson 1993). Axelsson (1993) listed the current genera of lactic acid bacteria to consist of *Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Aerococcus, Carnobacterium, Lactococcus, Tetragenococcus* and *Vagococcus* .

Lactic acid bacteria play an important role in food fermentation. Their primary role in food fermentation used to be to effect preservation by converting sugars to organic acid mainly thus causing reduction in pH, by removing carbohydrates as nutrient source and lastly by producing antimicrobial compounds like hydrogen peroxide, bacteriocins, diacetyl and secondary reaction products. In recent times, lactic acidic bacteria are being used to play more important role in food system by proving diversity in food supply by altering flavour, texture and appearance of raw commodities in a desirable way. The sour aromatic flavours imparted by lactic acid fermentation are desirable trait in fermented food products and gives a natural image to the food product. Lactic acid bacteria (Gilliland 1985; Daeschel 1989; Chassy and Murphy 1993; Davidson 1993; Salovaara 1993). Lactic acid bacteria are also known to colonize the human intestinal mucosa leading to beneficial effect since they often have antagonistic effects against other bacteria (Molin *et al* 1993).

Lactic acid bacteria play another role in providing microbial safety of many African foods. Nout *et al* (1987) showed that *Salmonella typhimumrium* was unable to survive in lactic acid fermented sorghum based porridges during storage at 30^0C for 24hr. Mensah *et al* (1990) demonstrated that lactic acid fermentation significantly contributes to the microbial safety of Ghanaian maize porridges if the pH of the ready to eat porridge is less than 4.5. In 1991, Lorri and Svanberg found a growth inhibition rate of 10^3 for the pathogens *Escherichia coli* (ETEC) and *Campylobacter jejui* after 3 hr and *Shigella* and *Salmonella* after 7 hr, when the pathogens were inoculated into fermented cereal gruels in Tanzania. Mbugua and Njenga (1991) demonstrated that the number of *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Shigella dysenteriae* decreased when inoculated into fermenting *uji*, a Kenyan fermented cereal porridge during fermentation and storage.

2.9 *Bacillus* Species

Wolf and Barker (1968) defined *Bacillus* species as rod-shaped spore bearing organisms, usually Gram-positive, catalase producing and capable of sporulating aerobically. The ability to sporulate aerobically is used to differentiate the species from some aerotolerant clostridia which have the ability to produce catalase but fails to sporulate under aerobic condition.

Several authors have reported the presence of *Bacillus* species during the fermentation of many African foods. In African fermented foods involving the alkaline fermentation of legumes such as the African locust bean (*Parkia biglobosa*), melon seeds (*Citrullus vulgaris*), oil bean (*Pentaclethra macrophylla*), sesame seed (*Sesamum indicum*), castor oil bean seed (*Ricinus communis*) and fluted pumpkin

bean (*Telfaria occidentale*). *Bacillus* spp. mainly *Bacillus subtilis* are reported to be responsible for the fermentation. This fermentation in which alkaline pH in combination with ammonia and are related to the extensive hydrolysis of protein to amino acids and peptides (Odunfa 1985; Antai and Ibrahim 1986; Steinkraus 1991). These fermentation products are not only used as condiments to flavour stews and soups but also as a source of low protein in the diet.

Other alkaline fermented products by *Bacillus spp.* included the West African *dawadawa, iru* or *suombala* made by fermentation of cooked African locust bean, Nigerian *ogiri* made by fermentation of melon seed, Nigerian *ugba* made by the fermentation of oil bean, Sierra Leonian *ogiri-saro* made by the fermentation of sesame seed, Nigerian *ogiri-nwan* made by the fermentation of the fluted pumpkin bean and Nigerian *ogiri-igbo* made by the fermentation of castor oil bean seeds. Odunfa (1985) demonstrated that in the fermentation of African locust bean, the primary role of the *Bacillus* spp. is to serve as a source of proteolytic enzymes leading to an increase in soluble products such as amino acids, thus improving the digestibility of the locust bean which is not normally used as food in its unfermented form.

Industrially, Fogarty *et al* (1974) have reported that *Bacillus* spp. represent one of the most important group of bacteria which produce a range of industrially important enzymes such as amylase, protease, isomerases, cellulase, hemicellulase, pectinases, pencillinases, cell wall lytic enzymes as well as a number of cyclic or linear polypeptide antibiotics.

3.0 MATERIALS AND METHOD

3.1 Methodology

A visit was made to Adjeza in the Western Region to study the processes involved in the preparation of *Akyeke* (Fig 2). Samples of the intermediate products were taken at the various stages of the processing of cassava into *Akyeke* for analysis in the laboratory. *Akyeke* was also prepared in the laboratory and samples were taken of the inoculum and the final products. Fermenting Akyeke was also sampled at 0, 24, 48, 72, 96 and 120 h of fermentation. All experimental work on the samples and tentative identification of yeasts and bacteria were carried out at CSIR-Food Research Institute, Accra, Ghana.

3.1.1 Preparation of *Akyeke* Inoculum

Akyeke inoculum was prepared by steeping chunks of peeled cassava tubers in water for 3 days followed by 3 days of sun drying.

3.1.2 Preparation of *Akyeke*

Cassava tubers were peeled and washed. The inoculum was added to the fresh peeled cassava root tubers and grated in an exclusively cassava grater. The grated cassava mash was packed in a perforated polythene bag (Fig 3) and left to ferment for 5-7 days depending on the amount of inoculum added. After fermentation three volumes of water was added to the fermented cassava mash (Fig 4) to remove the yellowish colour and some amount of starch from the fermented cassava mash. The wet cassava mash was dewatered (Fig 5), screened and kneaded to obtain medium size granules. This was followed by sun drying for 1-2 hours depending on the weather (Fig 6). The dried cassava mash was then steamed (Fig 7) to obtain

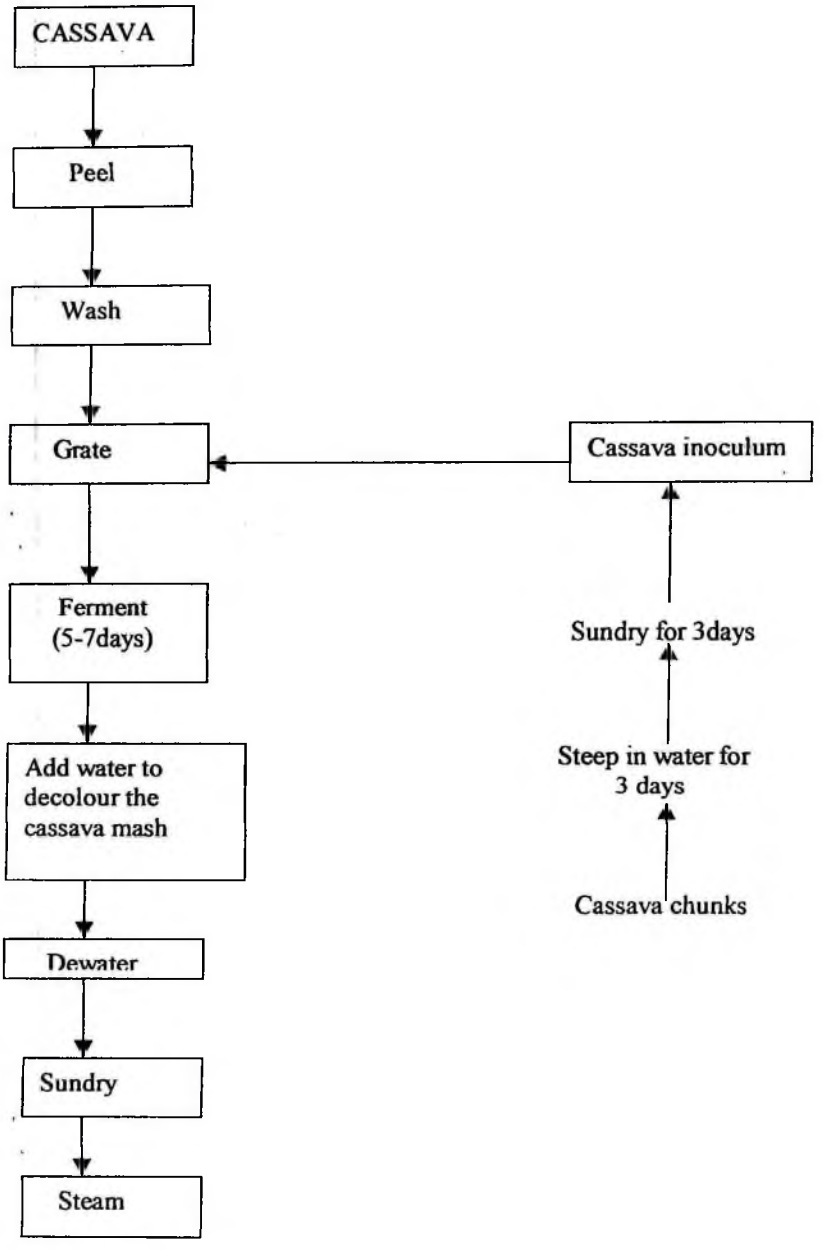


Fig.2 Flow diagram of processing of cassava into akyeke.

Fig.3 Fermentation of cassava substrate during Akyeke processing

Fig.4 Decolouration of fermented cassava substrate

Fig.5 Dewatering of decoloured fermented cassava meal in Akyeke processing

Fig.6 Sundrying of dewatered,decoloured fermented cassava meal in Akyeke processing

Fig.7 Steaming of fermented cassava meal in Akyeke

the final product *Akyeke*.

3.2 Microbial Analyses

10g of all samples were added to 90.0 ml sterile diluents containing 0.1% peptone, 0.8% NaCl, with pH adjusted to 7.2 and homogenized in a stomacher blender (Lab Blender, Model 4001, Seward Medical) for 30 sec at normal speed. From appropriate ten-fold dilutions; enumeration of aerobic mesophiles was carried out on Plate Count Agar (PCA, DIFCO, 247940,USA) incubated at 30^0C for 3d. Lactic acid bacteria were enumerated on deMan Rogosa and Sharpe (MRS Merck, Germany) medium, incubated anaerobically in an anaerobic jar with anaerocult A at 30^0C for 5d. Yeasts count were enumerated on Malt Agar (MA, Oxoid,CM 57, UK) medium containing 100mg chloramphenicol (Chloramphenicol Selective Supplenment Oxoid) and 50mg chlorotetracycline (Sigma no C-4881, St. Louis, MO, USA) per litre and incubated at 25^0C for 5d.

3.2 Isolation of Dominating Microorganism

About 20 colonies from a segment of the highest dilution or appropriate agar plates were sub-cultured in corresponding broth medium and streaked onto the agar substrate until pure cultures were obtained.

3.4. Initial Characterization of Isolates

PCA isolates were sub-cultured in nutrient broth or agar. Isolates were examined by colony and cell morphology, Gram reaction and catalase test. MRS isolates were sub-culture in MRS medium and examined by Gram reaction, catalase production, oxidase test, aerobic and anaerobic growth, colony and cell morphology.

MA isolate were subcultured in yeast-malt-peptone-glucose broth and agar (MYPG) containing g/l; 3, yeast extract (Difco 0127-17-9), 3, malt extract (Difco 01886-17-7), 5, bacteriological peptone (Oxoid Code L37), 10, glucose (Merck 1.08342) with or without 15, agar (Merck 1.01614). These were examined by colony and cell morphology.

Gram reaction was carried using modification of the method of Lillie (1928) by Parry *et al* (1983). Isolates were smeared on slides and covered with crystal violet solution for 20s. Crystal violet solution was washed off with distilled water and excess water drained. The smear was covered with gram iodine solution and left to stand for 60 s and then poured off. The smear was then flooded with 95% ethyl alcohol for 10 to 20 s. The alcohol was rinsed off and the smear covered with safranin for 20 s. It was washed gently for a few seconds and blotted with paper towel and left to dry at room temperature. The slide was examined under oil immersion.

For catalase production a loopful of culture was mixed into a drop of 3% hydrogen peroxide on a microscopic slide and observed for the production of gas bubbles to indicate production of catalase.

The oxidase test was carried out as follows, a piece of Whatman No. 2 filter paper was placed in a petri dish and several drops of oxidase test reagent was added. A loopful of the organism from one of the colonies was smeared on a small area of the filter paper. The result was observed and recorded on Descriptive chart after 10 - 15s.

3.5 Maintenance of Isolates

PCA isolates were maintained on PCA slants and stored at 4^0C. MRS isolates were preserved by subculturing in MRS broth and 1ml of 50% glycerol was added to 1ml of culture broth in a cryotube and frozen immediately at -20^0C. Malt Agar isolate were maintained on MYPG agar slants and stored at 4^0C.

3.6 Identification of *Bacillus* spp.

Different *Bacillus* species were recognized from the test of PCA isolates as Gram positive, catalase positive rod with or without phase bright spores.

Bacillus species were identified according to Claus and Berkeley (1986) and Parry *et.al* (1983). Test carried out included morphological examinations and biochemical test. For the morphological examination, the shape and position of spore were observed. The biochemical test included anaerobic growth, acid production from D - glucose, D-xylose, L anabinose and D - mannitol, hydrolysis of casein, starch and gelatin, nitrate reduction, indole formulation, growth at pH 5.7, in 7% (w/v) NaCl, at 10^0C, 50^0C, 65^0C.

For the hydrohysis of casein, plates of skim milk agar containing g/l distilled water; 100, skim milk powder and 20, agar were inoculated with single streaks, and incubated at 30 ^{0}C and examined for clear zones around growth at 2,7 and 14d indicating deposition of casein and proteolytic activity.

For starch hydrolysis, starch agar containing g/l distilled water; 10, starch and 23, nutrient agar were inoculated with the test organism and incubated at 30 ^{0}C for 5d.

Hydrolysis of starch was determined at 3 and 5d by flooding plates with 95% ethanol. Clear zones around colonies indicated hydrolysis of starch. Unhydrolysed starch became white and apaque within 15 to 30min.

For indole production, tubes containing 5ml of 1% tryptone broth were inoculated with isolates and incubated at 30 ^{0}C for 14d. 2ml of Kovacs' reagent containing per 150ml; 5g 4 dimethyl aminobenzaldehyde, 75ml, iso-amyl alcohol and 75ml, concentrated HCl were added to the tube and vigorous shaken. Positive cultures developed pink to red colour indicating production of indole.

To test for growth in 5%, 7% and 10% sodium chloride tubes containing 3ml of nutrients broth with 5%,7% and 10% (w/v) sodium chloride respectively were inoculated with the test organism and incubated at 30 ^{0}C and observed for growth after 7 and 14d.

To test for growth at pH 5.7, sabouraud dextrose agar slants containing g/l distilled water, 10, peptone, 40, dextrose and 15, agar, pH 5.6 and saboraud dextrose broth containing g/l distilled water; 10, peptone, 20, dextrose, pH 5.7 were inoculated with isolates which had previously been grown in nutrients broth. The tubes were observed for growth for up to 14d after incubation at 30 ^{0}C.

For acid production test from carbohydrate, 3ml slants containing 150 microlitres of 10% filter sterile solution of either D (+) Glucose, L (+) Arabinose, D(+) xylose or D(-) manitol and a basal medium containing g/l distilled water; 1, diammonium hydrogen phosphate, 1, potassium chloride, 0.2, magnesium sulphate, 0.2, yeast

extract and 15, agar, 0.006, bromcresol purple, pH 7.0 were inoculated with the test organism and incubated at 30 ^{0}C. A change in colour of the medium from purple to yellow indicated acid production.

For fermentation of carbohydrate, a basal medium was prepared from 10.0g Tryptone, 5.0g Sodium chloride, 2.5ml Bromocresol purple (1% solution), 1 litre Distilled water. All the ingredients (except the indicator) were added to the water and dissolved by steaming. pH adjusted to 7.2 and then the indicator was added. 3.0ml were distributed into test tubes with inverted Durban tubes. Sterilization was done at 121^{0}C for 20 minutes. 10% solution of 14 different carbohydrates (celliobiose, fructose, galactose, lactose, maltose, melibiose, raffinose, salicin, sorbitol, melezitose, trechalose, arabinose, xylose and mannitol) were sterile filtered into sterile bottle. 0.5 ml of each carbohydrate was added to the 14 different tubes with the basal medium. The 14 tubes with the different carbohydrates were inoculated with pure colony mass only from single colonies. After incubation at 30^{0}C for 7d, change in colouration indicated assimilation of the carbohydrate as a result of the acid, which changed the indicators colour. For each carbohydrate one non-inoculated tube was also incubated to serve as control. The species of isolates were identified by determining their pattern of carbohydrate fermentation and comparing them to known carbohydrate fermentation profile (Claus and Berkeley 1986).

3.7 Identification of Lactic Acid Bacteria

MRS isolates, which were regular gram positive rods, coccoid or cocci, catalase negative, oxidase negative were examined by gas production from MRS broth with Durham tube in which glucose was replaced with gluconate as sole carbon source,

growth at 45 ^{0}C and Hugh and Leifson test (Hugh and Leifson 1953).

Test for the production of air from glucose were carried out by inoculating MRS - broth containing a Durham tube with the test organism and incubating at 30 ^{0}C for at least 72 h. Presences of air bubble trapped in the inverted Durham tube were indicative of gas production.

For growth in 6.5% and 18% sodium chloride, tubes containing 3.0 ml of MRS broth with 6.5% and 18% sodium chloride were inoculated with the test organism and incubate at 30^{0}C and observed for growth after 7 and 14 d.

For growth at 45 ^{0}C, two tubes containing MRS - broth were inoculated with the test organism and incubated at 45 ^{0}C for up to one month and examined for significant growth.

For Hugh and Leifson's test, two tubes containing per litre distilled water; 2g peptone, 5g NaOH, 0.3g K_2HPO_4, 15ml of 0.2% bromothymol blue and 1% glucose added filter sterile, pH 7.1 were inoculated with each organism and one of the tubes topped with paraffine oil to obtain anaerobic conditions.

For fermentation of carbohydrate Modified MRS-broth was prepared from 10g peptone, 5g Yeast extract, 1ml polyoxyethylene sorbitan mono-oleate(Tween 80),2g K_2HPO_4 5g $CH_3COONa.3H_2O$, 2g triammonium citrate, 0.2g MgSO4. $7H_2O$, 0.05g $MnSO_4$. H_2O, 1000 ml distilled water, 0.2% chlorophenol red, pH 6.2.4.5 ml of modified MRS broth was dispensed into tubes and sterilised at 121°C for 15 minutes.

10% solution of 17 different carbohydrates (amygdalin, arabinose, cellubiose, fructose, galactose, glucose, gluconate, lactose, maltose, mannitol, melezitose, melibiose, raffinose, salicin, sorbitol, sucrose and trehalose) were sterile filtered into sterile bottles. 0.5 ml of each carbohydrate was added to the 17 different tubes with the modified MRS broth. The 17 tubes with the different carbohydrates were inoculated with pure colony mass only from single colonies. After incubation at 30^0C for 7 d, change in colouration from red to yellow indicated assimilation of the carbohydrate as a result of the production of acid, which changed the indicators colour from red to yellow. This was recorded as positive result. Non-change in colour after 7 days was recorded as negative. For each carbohydrate one non-inoculated tube was also incubated to serve as control. The species of isolates were identified by determining their pattern of carbohydrate fermentation and comparing them to known carbohydrate fermentation profile (Kandler and Weiss 1986).

Gram positive, catalase - negative cocci were tentatively identified by mode of glucose breakdown, tetrad formation, growth at 10^0C and 45^0C, growth at pH 4.4 and 9.6, growth in 6.5 and 18% (w/v) NaCl.

3.8 Identification of Yeasts

3.8.1 Assimilation of various carbohydrates

Yeasts isolates were examined by colony and cell morphology. Yeasts assimilation of carbohydrate was done as described by Kreger-Van Rij (1984). Basal medium was prepared from 4.5 g yeast extract, 7.5 g peptone, 1.0 ml Bromthymol blue solution and 1000 ml distilled water. The pH was adjusted to 6.4. Inverted Durban tubes were inserted into test tubes containing 4.0 ml of basal medium and autoclaved at 121°C

for 15 min. 12% of raffinose, 6% each of glucose, celliobiose, xylose, trechalose galactose, maltose, lactose, meliobiose, and sucrose were prepared and sterile filtered into sterile bottles. 2.0 ml of each sterile filtered carbohydrate solution was added per 4.0 ml of the basal medium. The 10 tubes with different carbohydrates were each inoculated with a single colony of the test organism and incubated 30°C for 14 d. Change in colouration from red to yellow indicated assimilation of the carbohydrate and production of acid, which changed the indicators colour from red to yellow. This was recorded as positive result. Non-change in colour after 14 days was recorded as negative. For each carbohydrate one non-inoculated tube was also incubated to serve as control.

3.8.2 Fermentation of various carbohydrates

For fermentation of carbohydrates by Yeasts, the same method as described above was used. The presences of gas bubbles trapped in the inverted Durban tube indicated fermentation of carbohydrate by the test organism and thus the production of gas, which was collected in the inverted Durban tube. The presences of gas in the inverted Durban tube was recorded as positive result. The absence of gas in the inverted Durban tube was record as negative since the test organism could not ferment the carbohydrate.

3.9 Chemical analyses of Inoculum and Fermenting Cassava mash

3.9.1 pH

Inoculum and fermenting cassava mash samples weighing 40 g were homogenized in a blender with 40ml of distilled water and the pH determined with a pH meter.

3.9.2 Determination of Titratable Acidity

A sample weighing 10g was dissolved in 200ml of distilled water and filtered using filter paper. 80 ml of the filtrate was titrated against 0.1m NaOH using 1% phenolphthalein as indicator.

3.9.3 Determination of Cyanogenic Compounds

The cyanogenic potential (total cyanide), cyanogenic glucocides (Linamarin and lotaustralin), cyanohydrins and free hydrogen cyanide content of the fermented cassava mash samples were determined by modification of the enzymatic assay of Cooke (1978) and O'Brien *et al* (1991) as described by Essers *et al* (1993)

Cyanogenic compounds were extracted by homogenizing 50 g of sample with 250 ml of refrigerated 0.1M-orthophosphric acid in a blender for about 3 - 5 min. Homogenates were centrifugated at 10000 x g for 15 min and supernatants used for assays. The cyanogenic potential of a sample was determined by adding 0.1 ml of extract to 0.4 ml of sodium phosphate buffer, pH 7, and 0.1 ml of linamarase solution (5 unit /ml) and incubated at 30 ^{0}C for 15 min. 0.6ml of 0.2M NaOH was added to the digested extract followed after 5 min by 2.8 ml of sodium phosphate buffer pH 6. Colouration of the sample was carried out by adding 0.1 ml of 1%(w/v) chloramine T (N-choro-p-toluene-sulphonamine sodium salt, Sigma no C-9887) followed after 5min by addition of a colour reagent containing per litre; 18.5g NaOH, 35g 1, 3 - dimethyl barbituric acid (Merck ART 800133) and 28.5 g isonicotinic acid

(Sigma no I-1137), pH 7 - 8. The absorbance was measured with Uvikon 940 spectrophotometer (Kontron AG Instruments, Zurich, Switzerland) at 605 nm after 15 min.

The non-glycosidic cyanogens content of the samples were determined by adding 0.1 ml of extract to 0.6 ml of 0.2M NaOH followed after 2min by 3.3ml of sodium phosphate buffer pH 6. The treated extract was assayed calorimetrically by colouration and measurement of absorbance as described above. Free cyanide content of the samples were determined by diluting 0.1 ml of extract with 3.9ml sodium phosphate buffer pH 4 and assayed calorimetrical as described above.

Calibration curves were drawn using 5, 10, 25, 50 and 75 nmol per tube of linamarin, acetone cyanohydrin or potassium cyanide for the determination of cyanogen potential, non-glycosidic cyanogens and free hydrogen cyanide using methods described above. Cyanogen levels were calculated in mg CN equivalent per kg same on dry weight basis as follows;

$$\text{mg CN eq/kg} = \frac{a(v + s \times m/100) \times 0.026}{s(1 - m/100) \times d}$$

where S is the sample weight in g

V is the volume of extraction medium in ml

M is the moisture content of sample in percentage

a is the quantity of cyanogen (nmol) in the tube and is derived from the

calibration curve

$a = (A_{605} - i)/$ slope, where i is the intercept of the curve.

The cyanogenic potential = total cyanide

Cyanogens = non glucosidic cyanogens - free cyanide

Cyanogenic glycosides = cyanogenic potential - non-glucosidic cyanogens.

3.10 Test for Breakdown of Cassava Tissue by Isolated Microorganisms

To test for the capability of isolated microorganisms to break down cassava tissue was examined by the method described by Amoa-Awua and Jakobsen (1995).Cassava slices of dimension 1 cm x 1 cm x 2 cm were surfaced sterilized by dipping the slices in 70 % alcohol for 15 min. and dried at 45°C overnight. A few loop full of 24-h-old pure colonies of the isolated microorganism were streaked directly onto the surface of the cassava slices and then incubated at 30°C. A sterile glass rod was used to prod the cassava slices on daily bases. A control experiment of sterile cassava slices free of any microorganism was also set up.

4. 0 RESULT

4.1 Field Study

Observation made in the field studies revealed that, in *Akyeke* processing, inoculum is added at a point and the processors interviewed confirmed that the inoculum helps in the breakdown of the texture of the cassava dough and gives *Akyeke* its characteristic aroma. Some processors in L'Cote d'Ivoire reported that with the use of inoculum the product obtained has a rubbery texture (Amoa-Awua, Personal communication).

The inoculum used in the preparation of *Akyeke* is prepared by steeping cassava chunks in water for three days followed by sun drying for another three days.

4.2 Culturing of *Akyeke* Samples on Plate Count Agar (PCA)

Growth of the two samples of inocula and during fermentation on Plate Count Agar (PCA), referred to as aerobic mesophiles, were dominated by phase bright spores, Gram-positive catalase-negative rods and cocci. Count of aerobic mesophiles of both samples of inoculum were to the level of 10^9 (Table 6).

Majority of the *Bacillus* isolates which were Gram positive, catalase positive, produce acid from glucose, arabinose, xylose, mannitol, hydrolysed starch and casein, grew at pH 6.8 and 5.75 NaCl and 30^0 C (Table 7). The most occurring *Bacillus* isolates had small cells with centrally placed spores and fermented cellobiose but not lactose and melezitose. These isolates were identified as *Bacillus subtilis*. The second most common *Bacillus* isolate had rough opaque star-shaped colonies, which were very difficult to

Table 6 The microbial population of bacteria and yeasts (cfu/g) during the processing of cassava into Akyeke

	Sample from Traditional processors	Sample prepared in the laboratory
Inoculum		
Aerobic mesophiles[a]	4.8×10^9	6.1×10^9
Lactic acid bacteria[b]	5.0×10^9	8.0×10^8
Yeasts[c]	3.2×10^{10}	4.2×10^9
Start of Fermentation		
Aerobic mesophiles[a]	nd	4.1×10^7
Lactic acid bacteria[b]	nd	3.3×10^8
Yeasts[c]	nd	3.0×10^7
After 24 hr		
Aerobic mesophiles[a]	nd	2.5×10^8
Lactic acid bacteria[b]	nd	2.9×10^9
Yeasts[c]	nd	2.1×10^7
After 48 hr		
Aerobic mesophiles[a]	nd	3.8×10^9
Lactic acid bacteria[b]	nd	4.4×10^9
Yeasts[c]	nd	4.8×10^7
After 72 hr		
Aerobic mesophiles[a]	nd	6.8×10^9
Lactic acid bacteria[b]	nd	5.0×10^9
Yeasts[c]	nd	9.6×10^7
After 96 hr		
Aerobic mesophiles[a]	nd	4.8×10^6
Lactic acid bacteria[b]	nd	6.2×10^9
Yeasts[c]	nd	7.0×10^6
After 120 hr		
Aerobic mesophiles[a]	9.0×10^9	4.6×10^8
Lactic acid bacteria[b]	8.0×10^{10}	6.2×10^9
Yeasts[c]	5.0×10^8	7.0×10^6
Wet cassava mash		
Aerobic mesophilesa	3.0×10^8	nd
Lactic acid bacteriab	9.7×10^8	nd
Yeasts[c]	5.0×10^7	nd
Dried cassava mash		
Aerobic mesophiles[a]	3.0×10^7	nd
Lactic acid bacteria[b]	1.6×10^6	nd
Yeasts[c]	2.4×10^6	nd

Key; a : Microbial count on PCA b: Enumeration on MRS c: Determined on Malt Extract Agar
nd: Not determined

Table 7 Morphology and Biochemical Characteristics of *Bacillus* Isolates

	Isolates						
Test	1	2	3	4	5	6	7
Shape	R	R	R	R	R	R	R
Acidity	-	-	-	-	-	-	-
Mobility		-	-	-	-	-	-
Growth in Air	+	+	+	+	+	+	+
Gram reaction	+	+	+	-	+	+	+
Catalase	+	+	+	+	+	+	+
Oxidase			-	-	-		-
Growth at:							
10°C			-	-	-		+
15 °C	-	-			-	-	
30 °C	+	+	+	+	+	+	+
50 °C		+			-	+	
65 °C	-	-			-	-	-
Indole formation	-			-			
Starch hydrolysis	+	+	+	+	+	+	+
Growth in NaCl at;							
5%	+	+	+	+	+	+	+
7%	+	+	+	+	+	+	+
10%	+	+	+	+	+	+	+
Nutrient broth pH;							
6.8	+	+	+	+	+	+	+
5.7	+	+	+	+	+	+	+
Gas from glucose	+	+	+	+	+	+	+
Hydrolysis of casein	+	+	+	+	+	+	+

scrape from the surface of the nutrient agar. These isolates fermented cellobiose, fructose, galactose, lactose, maltose, raffinose, salicin, sorbitol and trahalose and melezitose and were identified as *Bacillus licheniformis*. Isolates identified as *Bacillus pumilus* were cream in colour and moist. *Bacillus pumilus* fermented cellobiose, fructose, galactose, raffinose, salicin and trehalose but not lactose, melibiose, sorbitol and melezitose. The colonies of *Bacillus cereus* had oval shape and fermented cellobiose, fructose, galactose, maltose, raffinose and salicin (Table 8). The bacteria, which appeared after 96 h of fermentation, were curved rods. They fermented glucose, fructose, maltose, sucrose, trehelose and salicin. These isolates were identified as *Corynebacterium spp*.

Bacillus species were present at a level of 10^6-10^8 cfu/g at the beginning of fermentation time and rose to 10^7-10^9 cfu/g after 96 h of fermentation (Table 9). However, *Bacillus subtilis* occurred in the highest levels of 10^9 in the inoculum processed in the laboratory

The result of this work affirms earlier reports that *Bacillus* species are one of the types of microorganisms which dominate fermentation of cassava product (Amoa-Awua *et al* 1996). In the present study *Bacillus* species dominated the fermentation period from the 0 h up to the 96 h. However, *Corynebacterium* species appeared in the 96 h and completely dominated the fermentation by the 120 h.

Table 8 Sugar fermentation pattern of *Bacillus* isolates

	Isolates						
Sugars	1	2	3	4	5	6	7
Cellobiose	+	+	+	-	-	+	+
Fructose	+	+	+	+	+	+	+
Galactose	+	+	+	-		+	+
Lactose		+	+	-	+	+	
Maltose	+	+	-	+	+	+	+
Melibiose	+	+	+	+		-	
Raffinose	+	-	+	-		+	+
Salicin	+	+	+		+	+	+
Sorbitol	+	+	+			+	
Melezitose					-		-
Trehalose	+	+	+	-	+	+	+
Arabinose	+	+	+	+	+		+
Xylose	+	+	+		+	+	+
Mannitol	+	+	+	-	+	+	+

Species identified: 1 – *B. subtilis*, 2 – *B. licheniformis*, 3 - *B. subtilis*, 4 – *Corynebacterium*, 5 – *B. cereus*, 6 - *B. licheniformis*, 7- *B. pumilus*

Table 9 **The composition and population of Bacillus spp. (cfu/g) of inocula and fermenting cassava Akyeke processing.**

	Composition and population of Bacteria species				
	Bacillus subtilis	*Bacillus lichniferomis*	*Bacillus cereus*	*Bacillus pumilis*	*Corynebacterium Spp.*
Inoculum	2.4×10^9	2.8×10^8	2.3×10^8	1.6×10^8	
Fermenting time					
0 hr	1.1×10^8	1.6×10^7	2.1×10^7	3.6×10^6	
24 hr	1.9×10^8	1.0×10^8	2.2×10^7	1.0×10^7	
48 hr	1.2×10^9	1.3×10^8	2.8×10^7	1.6×10^7	
72 hr	2.0×10^9	1.1×10^9	1.8×10^9	1.9×10^7	-
96 hr	3.5×10^9	9.1×10^8	8.6×10^7	2.0×10^7	6.1×10^7
120 hr			-		4.8×10^8

4.3 Culturing on De Mann, Rogosa, Sharpe (MRS)

Gram- positive, catalase-negative rods dominated growth of all samples taken during the *Akyeke* processing on MRS. These colonies were also oxidase-negative, non-spore forming and strictly fermentative. They were suspected to be lactic acid bacteria. The counts of all samples ranged from 10^5 and 10^{10} cfu/g.

There was general increase in lactic acid bacterial population from 10^6-10^7 cfu/g at the start of the fermentation to 10^8 -10^9 cfu/g in the fermented cassava mash. This decreased to 10^7 cfu/g in the wet cassava mash and the dried cassava mash (Tables 10 and 11).

4.3.1 Characterization of Lactic acid Bacteria

The gram-positive, catalase negative, oxidase negative and fermentative rods isolates on MRS from both field and laboratory samples taken during *Akyeke* processing were found to be either facultatively heterofermentative or obligatively heterofermentative lactobacilli by their ability to produce carbon dioxide from glucose.

The lactobacillus isolates were also found to be non-oxidative, utilized glucose fermentativley in Huge and Leifson medium (Table 12).

Most of the facultatively heterofermentatve lactobacilli utilized amygdalin, celloboise, fructose galactose, lactose, maltose mannitol, melezitose, melibiose, raffinose, salicin, sorbitol, sucrose and trehalose and were identified as *Lactobacillus plantarum* (Table 13).

Table10 Biochemical Characteristics of Lactic Acid Bacteria isolated from samples of *Akyeke* at various stages of Processing

Isolates													
Test	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}	L_{12}	L_{13}
Tetrad formation	-	-	-	-	-	-		-		-	-	-	-
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-				
Anaerobic growth	+	+	+	+	+	+	+	+	+	+	+	+	+
CO_2 from glucose						+		+				+	
CO_2 from gluconate		-	-	-	-	+		+		-	-	+	-
Growth at 45^0C						-		-				+	
Growth in 6.5% NaCl	-												
Growth in 18% NaCl	-	-	-	-	-	-	-	-	-	-	-		-
Fermentation(H&L)	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidative (H&L)	-	-	+	-	+	-	-	-	-	-	+	-	-

Species identified after further test by the pattern of carbohydrate fermentation
Isolate number 1 – *Lactobacillus plantarum, 2 - Lactobacillus plantarum, 3 – Lactobacillus salivarius,*
4 - Lactobacillus plantarum, 5 - Lactobacillus salivarius, 6 – Leuconostoc mesenteroides
7 - Lactobacillus plantarum, 8 –Lactobacillus brevis, 9 - Lactobacillus plantarum, 10 - Lactobacillus plantarum, 11 - Streptococcus salivarius, 12 – Lactobacillus fermentum, 13 - Lactobacillus plantarum

Table 11 Pattern of Carbohydrate Fermentation of Lactic Acid Bacteria isolated during *Akyeke* processing

Isolate no	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}	L_{12}
Amygdalin	+	+	-	+		-	+	-		+		
Arabinose	-		-	+	-		-	+		-	-	w+
Cellubiose	+	+	-	+		-	+	-		+	-	-
Fructose	+	+		+		-	+	+		+		+
Galactose	+	+		+			+			+	-	w+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Gluconate	+	+		+		-	+	+		+		+
Lactose	-	+	-	+	-	-	+	w+		+		+
Maltose	+	+	-	+	-	-	+	+	-	+		+
Mannitol	+	+	+	+			+		-	+		+
Melezitose	+	+	+	+			+	+		+		+
Melibiose	+	+	+	+		+	+		+	+		+
Raffinose		-	+	+	-	-	+			+	-	+
Salicin	+	+	+	+		-	+	-	-	+		
Sorbitol	+	+	+	+	-	-	+			+	-	+
Sucrose	+	+		+	+	-	+	+		+	+	+
Trehalose	+	+	+	+			+	-		+		+
Growth 45°C			+	+			-	-	-			+

Table12 The Population of various species of Lactic Acid Bacteria (cfu/g) isolated during Laboratory processing of Akyeke

Composition and Population of Lactic Acid Bacteria					
	L. plantarum	*L. brevis*	*L. fermentum*	*L salivarius*	*Leucounostoc meseteroides*
Inoculum	2.5×10^9	5.7×10^8	1.5×10^8	1.5×10^7	4.6×10^8
0 hour fermentation	3.1×10^8	2.4×10^7	1.3×10^6	1.0×10^6	3.0×10^9
24 hour fermentation	1.5×10^9	3.0×10^8	1.1×10^7	2.6×10^7	3.8×10^7
48 hour fermentation	2.1×10^9	2.1×10^8	2.1×10^8	1.4×10^8	2.1×10^8
72 hour fermentation	2.9×10^9	6.2×10^8	2.9×10^8	5.8×10^7	1.2×10^8
96 hour fermentation	2.5×10^9	7.4×10^8	2.5×10^8	nd	1.7×10^8
120 hour fermentation	1.9×10^9	3.3×10^9	9.5×10^8		3.4×10^8
Wet cassava mash	5.8×10^7	2.4×10^7	nd		1.5×10^7
Dried cassava mash	5.9×10^7	2.7×10^7	nd		1.4×10^7

nd- not detected

Table 13 The Population of Lactic Acid Bacteria species (cfu/g) in different stages of traditional Akyeke processing

	Samples			
	Inoculum	Fermented cassava mash	Wet cassava mash	Dried cassava mash
Lactobacillus plantarum	2.46×10^8	5.10×10^9	5.82×10^7	5.91×10^7
Lactobacillus brevis	7.69×10^7	2.04×10^9	2.43×10^7	2.70×10^7
Lactobacillus fermentum	1.54×10^7	5.10×10^8	nd	nd
Leuconostoc mesenteroides	4.62×10^7	1.53×10^7	1.46×10^7	1.36×10^7
Lactobacillus salivarius	1.54×10^7	nd	nd	nd

nd = not determined

There were three different types of obligately heterofermentative isolates. The first type utilized arabinose, fructose, glucose, lactose, maltose, melezitose and sucrose and not amygdalin, cellibiose, galactose, mannitol, melibiose, raffinose, salicin, sorbitol and trehalose and was identified as *Lactobacillus brevis*. The second type utilized arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, melezitose, raffinose, sorbitol , sucrose and trechalose and not amygdalin, cellubiose and salicin. These were identified as *Lactobacillus fermentum*. The third type, which is a *Leuconostoc spp.*, utilized only galactose, glucose, and melibiose out of the 17 carbohydrates and was identified as *Leuconostoc mesenteriod spp cremoris*. The fourth type, utilized glucose and sucrose out of the 18 carbohydrates and was identified as *Lactobacillus salivarius*.

4.3.2 Composition and Population of Lactic Acid Bacteria

The most dominant lactic acid bacteria isolated from both field and laboratory samples during the processing of *Akyeke* was *Lactobacillus plantarum*. Of the Eighty-six cultures isolated from samples taken from the field (Adjeza), 51 isolates representing 59.3% were *Lactobacilus plantarum*, 20 isolates representing 23.3% were *Lactobacillus brevis*, 12 isolates representing 14.5% were *Leuconostoc mesenteroides*, 2 isolates representing 2.32% were *Lactobacillus fermentum* and 1 isolate representing 1.16% was *Lactobacillus salivarius*. In the laboratory samples, 114 culture isolates were identified. 62 isolates representing 52.3% were *Lactobacillus plantarum*, 26 isolates representing 22.8% were *Lactobacillus brevis*, 18 isolates representing 15.8% were *Leuconostoc mesenteroides*, 5 isolates

representing 4.38% were *Lactobacillus fermentum* and 3 isolate representing 2.6% were *Lactobacillus salivarius*.

4.4 Growth on Malt Extract Agar (MEA)

Growth of the two types of inoculum (from traditional processors and laboratory preparation) on Malt Extract Agar (MEA) was made up of yeasts. Counts from cultures of both inocula samples were between the levels of 10^9 to 10^{10} cfu/g. Yeasts were determined by colony morphology, cell morphology and biochemical tests.

4.4.1 Characterization of Yeasts Population

Cell and colonial morphology of isolates were examined (Table 14). Yeasts cells were present in all samples examined. The highest yeasts population occurred in the inoculum from Adjeza, which was to the level 10^{10} cfu/g.

4.4.2 Morphology, Cultural and Sexual Characteristics

Four main types of yeasts were isolated from samples taken during the production of *Akyeke*. Table 14 shows the morphology, culture and sexual characteristic of yeasts isolates. The cells of the first type of yeasts were single, few pairs and subglobose to ovoidal. On malt agar their colonies were filamentous, smooth, soft and off – white. Their mode of vegetative reproduction was by binary fission. In liquid medium, there was sediment at the bottom of the tube and ring growth at the air-liquid –glass junction but an absence of pellicle. The ascospores observed were big, rounded and not liberated. The number of ascus per ascospore were between 1-4 for all isolates.

Table 14 Cultural Properties, Cell Morphology and Ascospore Formation of Yeasts isolated during Akyeke processing

Isolate group	Growth on malt extract agar after 3 days	Cell morphology	Ascospore formation	Growth in liquid medium
Y_1	Circular, 1-4mm raised, undulating edge and white	Single, subglubose and mono-polar budding	Two ascospore per ascus	No pellicle formation Bottom sediment Ring growth at air-liquid-glass junction
Y_2	Filamentous, 1-4mm,raised, filamentous edge, soft and creamy	Single, oval, subglobose cylindrical and binary fission	One to two ascospore per ascus	Dry winkled pellicle formation. Bottom sediment
Y_3	Curled, 2-3mm, flat, dry and white.	Single, paired, chained(polymorphic chain) cylindrical and arthrocondia.	One ascospore per ascus	No pellicle formation Bottom sediment Ring growth at air-liquid-glass junction
Y_4	Filamentous, 2-4mm,raised, filamentous edge, soft and off-white	Single, Subglobose, oval, cylindrical and binary fission.	Two to three ascopore per ascus	No pellicle formation Bottom sediment
Y_5	Circular, 1-3mm raised, undulating edge, white with faint ester-like odor	Single, oval, subglubose and mono-polar budding	Two to three ascospore per ascus	No pellicle formation Bottom sediment
Y_6	Circular, 1-4mm raised, entire edge, white with faint ester-like odor	Single, subglubose and mono-polar budding and bipolar budding	Two to three ascospore per ascus	No pellicle formation Bottom sediment Ring growth at air-liquid-glass junction
Y_7	Circular, 1-4mm raised, lobate edge, white with faint ester-like odor	Single, subglubose and mono-polar budding and bipolar budding	Two to three ascospore per ascus	No pellicle formation Bottom sediment Ring growth at air-liquid-glass junction
Y_8	Filamentous, 2-4mm,raised, filamentous edge, soft and off-white	Single, Subglobuse, oval and binary fission.	Two to three ascopore per ascus	No pellicle formation Bottom sediment
Y_9	Filamentous, 2-4mm,raised, filamentous edge, soft and off-white	Single, reinform, Subglobuse, oval, cylindrical and binary fission.	Two to three ascopore per ascus	No pellicle formation Bottom sediment

The second types of yeasts were mostly single,(few occurring in pairs), ovoidal to elongated in shape. Their colonies were filamentous, butyrous and light cream in colour. Their mode of vegetative reproduction was by binary fission. In liquid medium, they formed a sediment at the bottom of the tube and a heavy dry pellicle on the top of the broth. The ascospores observed were big, rounded and not liberated. The number of ascus per ascospore were between 1-4 for all isolates. The third type of yeasts isolates, occurred mostly in single, few pairs and were spherical to ovoidal in shape. Their colonies were circular, slightly raised and creamy. Their mode of vegetative reproduction was by binary fission. In liquid malt broth, bottom sediment was observed and an incomplete ring formed at air liquid-glass junction. No pellicle was observed. Asci were persistent in all the isolates. The number of ascus per ascospore was two.

The fourth type of yeasts isolates occurred in singles, pairs, chained (polymorphic chain) and were cylindrical and arthrocondia in shape. Their colonies were flat, dry and white.

They formed sediment in liquid malt broth and a ring at air –liquid –glass junction. There was no pellicle formation. Asci were subspherical in all isolates. The number of ascus per ascospore was one.

4.4.3 Fermentation and Assimilation of Carbohydrate

Tables 15 and 16 show fermentation and assimilation of carbohydrate. The first type yeast isolates assimilated glucose, maltose, trehalose and xylose and not sucrose, lactose, cellobiose, meliobiose and raffinose. These isolates also fermented glucose, galactose, maltose, trehalose, cellobiose, xylose and not sucrose, lactose, melibiose

and raffinose. These assimilation and fermentation patterns are typical of *Candida tropicalis.* The observed morphological, cultural and sexual characteristics were also consistent with the profile of *Candida tropicalis.* The first types of yeasts were therefore identified as *Candida tropicalis.*

The second type of isolates assimilated glucose, sucrose, and raffinose and not galactose, maltose, lactose, trehalose, cellobiose, xylose and meliobiose. Isolates also fermented glucose, sucrose and cellobiose and raffinose and not galactose, maltose, lactose, trahelose, xylose and melibiose. These assimilation and fermentation patterns are typical of *Candida krusei.* The observed morphological, cultural and sexual characteristics were also consistent with the profile of *Candida krusei.* The second types of yeasts were therefore identified as *Candida krusei.*

The third type of yeasts isolated assimilated glucose, sucrose, galactose maltose, trehalose, melibiose, and raffinose and not lactose, cellobiose and xylose. Glucose, sucrose, galactose, maltose, trehelose, cellobiose, xylose, melibiose, and raffinose and not lactose were fermented. These assimilation and fermentation patterns are typical of *Zygosaccharomyces florentinus.* The observed morphological, cultural and sexual characteristics were also consistent with the profile of *Zygosaccharomyces florentinus.* The third types of yeasts were therefore identified as *Zygosaccharomyces florentinus*

Table 15 Assimilation of various Carbon Compounds by Yeasts Isolated during the Processing of *Akyeke*

ISOLATE GROUP	Assimilation of various Carbohydrates									
	Glucose	Sucrose	Galactose	Maltose	Lactose	Trahelose	Cellobiose	Xylose	Melobiose	Raffinose
Y_1	+	+	+	+	-	+	-	-	+	+
Y_2	+	+	-	-	-	-	-	-	-	+
Y_3	+	-	+	-	-	-	-	+	-	-
Y_4	+	-	+	+	-	+	-	+	-	-
Y_5	+	+	+	+	-	+	-	-	+	+
Y_6	+	+	+	+	-	+	-	-	+	+
Y_7	+	+	+	+	-	+	-	-	+	+
Y_8	+	-	+	+	-	+	-	+	-	-
Y_9	+	-	+	+	-	+	-	+	-	-

Table 16 Fermentation of various Carbon Compounds by Yeasts Isolated during the Processing of *Akyeke*

ISOLATE GROUP	Fermentation of various Carbohydrates									
	Glucose	Sucrose	Galactose	Maltose	Lactose	Trahelose	Cellobiose	Xylose	Melobiose	Raffinose
Y_1	+	+	+	+	-	+	+	+	+	+
Y_2	+	+	-	-	-	-	+	-	-	+
Y_3	+	-	-	-	-	-	+	+	-	-
Y_4	+	-	+	+	-	+	+	+	-	-
Y_5	+	+	+	+	-	+	+	+	+	+
Y_6	+	+	+	+	-	+	+	+	+	+
Y_7	+	+	+	+	-	+	+	+	+	+
Y_8	+	-	+	+	-	+	+	+	-	-
Y_9	+	-	+	+	-	+	+	+	-	-

Yeasts species identified: Y_1 *Zygosaccharomyces florentinus*
Y_2 *Candida krusei*
Y_3 *Geotrichum candidum*
Y_4 *Candida tropicalis*
Y_5 *Zygosaccharomyces florentinus*
Y_6 *Zygosaccharomyces florentinus*
Y_7 *Zygosaccharomyces florentinus*
Y_8 *Candida tropicalis*
Y_9 *Candida tropicalis*

The fourth isolate assimilated glucose, galactose and xylose and not sucrose, maltose, lactose, trehalose, cellibiose, melibiose and raffinose, glucose, xylose and cellobiose were fermented and not sucrose, galactose, maltose lactose, trehalose, melibiose and raffinose. These assimilation and fermentation patterns are typical of *Geotrichum candidum*. The observed morphological, cultural and sexual characteristics were also consistent with the profile of *Geotrichum candidum.* The fourth types of yeasts were therefore identified as *Geotrichum candidum.*

4.4.4 Composition and Population of Yeasts

The populations of all the yeasts isolates were high in the inoculum and tend to decrease along the *Akyeke* processing (Table 17 and 18). There was also no count of *Geotrichum candidum* in the wet and dried mash samples.

4.4 Acidity and pH Changes during *Akyeke* Processing

The titratable acidity, which is expressed as percentage lactic acid on dry weight basis and pH, were determined. All samples taken during the processing of cassava into *Akyeke* were acidic. The acidity of 0.33% and 0.38% was recorded in the inocula from Adjeza and laboratory samples respectively with corresponding pH of 4.18 and 4.47. Acidity in the laboratory samples however increased from 0.36% (0 hour) to 1.11% reflecting in pH

Table 17 The Population of various Species of Yeasts (cfu/g) present in *Akyeke* during Processing (Laboratory)

	Composition and Population of Yeasts			
	C. tropicalis	*C. krusei*	*Z. florentinus*	*G. candidum*
Fresh cassava	nd	nd	nd	nd
Inoculum	3.0×10^9	1.5×10^9	9.1×10^8	6.0×10^8
0 hour Fermentation	3.2×10^6	1.6×10^6	8.1×10^5	6.6×10^5
24 hour Fermentation	2.5×10^6	2.9×10^6	9.0×10^5	7.0×10^5
48 hour Fermentation	1.5×10^7	2.0×10^7	1.7×10^6	nd
72 hour Fermentation	4.5×10^7	3.5×10^7	4.0×10^6	nd
96 hour Fermentation	6.0×10^7	4.1×10^7	5.2×10^6	nd
120 hour Fermentation	6.9×10^7	5.6×10^7	7.5×10^6	nd
Wet cassava mash	4.3×10^5	2.9×10^5	1.2×10^6	nd
Dried cassava mash	1.4×10^6	5.6×10^5	9.1×10^5	nd

nd- not detected

Table 18 Various species of Yeasts present in *Akyeke* during processing (field samples)

	Samples taken during *Akyeke* preparation			
	Inoculum	Fermented cassava mash	Wet cassava mash	Dried cassava mash
Candida krusei	6.4×10^9	1.65×10^7	2.25×10^7	9.6×10^5
Candida tropicalis	1.92×10^{10}	1.6×10^7	2.0×10^7	8.4×10^5
Zygosacchromyces florentinus	3.2×10^9	9.4×10^6	7.5×10^6	6.0×10^5
Geotrichum candidum	3.2×10^9	4.7×10^6	nc	nc

nc represents no yeasts count

decreases from 5.15 to 3.21 at the end of 120 h fermentation time (Table 19). Similar observations were made by other investigators with a similar fermented cassava product *agbelima* (Adjei 1990; Budu 1990; Sefa Dedeh 1995 and Amoa-Awua *et al* 1996).

4.5 Breakdown of Cassava Tissue by Isolated Microorganisms

A sterile glass rod was used to prod the cassava slices on daily basis. Disintegrated cassava tissue felt soft when prodded with sterile glass rod and the intact cassava tissue remained firm. The following microflora were able to disintegrate sterile cassava tissues; *Bacillus subtilis, Candida tropicalis and Zygosaccharomyces florentinus.*

4.6 Detoxification of Cassava during Akyeke Preparation

The initial total cyanogens levels of cassavatubers were 69.3 and 110.3 mg CN equivalent kg^{-1}dry weight for the laboratory and field samples respectively and the residual levels in the *Akyeke* meal were 1.4 and 2.8 mg CN equivalent kg^{-1} dry weight for the laboratory and field samples respectively or 2% and 2.5% respectively of the initial level. The residual level of cyanohydrin and HCN in the *Akyeke* meal were 2.7 and 4.5 mg CN equivalent kg^{-1} dry weight for laboratory and field samples respectively (Tables 20 and 21).

Table 19 **The pH and titratable acidity build up determined as % dry weight and expressed as lactic and during fermentation of cassava into Akyeke**

	Index	Field Samples	Laboratory Samples
Inoculum	Acidity	0.38	0.33
	pH	4.18	4.47
Fermenting time			
0 hr	Acidity	5.50	0.36
	pH		5.15
24 hr	Acidity	nd	0.41
	pH	nd	4.81
48 hr	Acidity	nd	0.63
	pH	nd	4.60
72 hr	Acidity	nd	0.78
	pH	nd	4.25
96 hr	Acidity	Nd	0.90
	pH		3.61
120 hr	Acidity	1.67	1.11
	pH	4.01	3.23
Wet cassava mash	Acidity	0.63	0.10
	pH	4.12	4.06
Dried cassava mash	Acidity	0.14	0.11
	pH	4.20	4.50

nd not determined

Table 20 Cyanogen content (mg/kg) of samples taken in traditional Akyeke processing at Adjeza

Sample	Cyanogen potential[a]	Cyanogenic glucosides[b]	Cyanohydrin	Free cyanide[c]
Fresh cassava tuber	69.3	66.6	1.6	1.1
Fresh cassava grated with inoculum	67.1	42.2	21.9	3.0
Fermented cassava mash	28.2	0.0	23.4	4.8
Wet cassava mash	10.3	0.0	9.7	0.6
Dried cassava mash	6.6	0.0	6.4	0.2
Steamed meal	1.4	0.0	1.3	0.1

a– total cyanide content including cyanogenic glucosides, cyanohydrin and free cyanide
b- Linamarin and lotaustralin
c- present as hydrogen cyanide

Table 21 Cyanogen content(mg/kg) of samples taken during Akyeke processing in the laboratory

Sample	Cyanogen potential[a]	Cyanogenic glucosides[b]	Cyanohydrin	Free cyanide[c]
Fresh cassava tuber	110.3	105.8	2.9	1.6
Fresh cassava grated with inoculum	109.1	72.4	32.6	4.1
Fermented cassava mash	40.4	0.0	35.4	5.0
Wet cassava mash	20.6	0.0	19.8	0.8
Dried cassava mash	6.6	0.0	10.5	0.4
Steamed meal	2.8	0.0	2.7	0.1

a– total cyanide content including cyanogenic glucosides, cyanohydrin and free cyanide
b- Linamarin and lotaustralin
c- present as hydrogen cyanide

5.0 DISCUSSION

The processing of *Akyeke* is similar to that of *agbelima*. In both cases an inoculum is added to the peeled cassava before it is grated and allowed to ferment. The presence of the inoculum influences the fermentation. Several inocula are used to ferment *agbelima* and this includes the type used to ferment *Akeyke* in the Western Region. It is prepared by steeping chunks of cassava tubers in water for three days, followed by three days sun drying. It was therefore expected that the fermentation of *Akyeke* would bear close resemblance to the fermentation of *agbelima* with respect to the composition of the microflora and biochemical changes which occur during fermentation. *Akyeke* processors interviewed at Adjeza said that with the use of an inoculum, the product obtained after fermentation has a smooth texture and also a characteristic flavour. In the fermentation of *agbelima* work carried out by Sefa- Dede (1989) and Amoa-Awua (1996) has confirmed that the use of inoculum results in a breakdown of cassava texture to give a smooth dough. In La Cote d'Ivoire processors claim that without the use of inoculum for fermenting *Akyeke*, the product obtained has a rubbery texture and this can only be avoided by the use of inoculum (Amoa-Awua, Personal communication).

5.1 Modification of Cassava dough Texture

5.1.1 The Role of *Bacillus spp.* in *Akyeke* Fermentation

In the present work the population of aerobic mesophiles present in *Akyeke* inoculum and during fermentation was found to be dominated by *Bacillus spp.* Species identified were *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus* and *Bacillus pumilus*. *Bacillus subtilis* accounted for 50% of the total *Bacillus* population.

Corynebacterium spp. was also isolated and completely dominated the flora of aerobic mesophiles after 120 h of fermentation. The result of this work affirms earlier report that *Bacillus* spp. dominates fermentation of similar cassava product *agbelima* (Amoa-Awua and Jakobsen 1995). Okafor (1977) also identified *Corynebacterium spp* in another fermented cassava product *gari.*

When the *Bacillus* spp. were plated directly on sterile cassava pieces, they were able to disintegrate the texture after 24 h incubation. These results are in agreement with the findings of Amoa-Awua and Jakobsen (1995) who identified *Bacillus* spp. as being mainly responsible for the hydrolysis of cassava texture during *agbelima* fermentation through their cellulolytic activity.

5.1.2 Yeasts in Cassava Fermentation into *Akyeke*

The present work identifies *Candida tropicalis, Candida krusei, Zygosaccharamyces florentinus* and *Geotrichum candidum* as the main species of yeasts found in *Akyeke* inoculum and during the processing of *Akyeke*, with *Candida tropicalis* accounting for more than half of the total population of yeasts. The result of this work agrees with the findings of Amoa-Awua *et al* (1997) who reported *Candida tropicalis, Candida krusei, Zygosaccharamyces florentinus* and *Geotrichum candidum* to be involved in cassava fermentation.

When the yeasts species were plated directly on sterile cassava pieces, only *Candida tropicalis* and *Zygosaccharomyces florentinus* were able to disintegrate the texture after 48 h inoculation. This is also in agreement with the findings of Amoa-Awua *et al* (1997) who reported that *Candida tropicalis* and *Zygosaccharomyces spp.* play a

positive role in cassava fermentation by contributing to the modification of the texture of cassava dough during *agbelima* fermentation.

Yeasts population in this work was found to be at a level of 10^8-10^9 cfu/g in the inoculum and at the start of fermentation, 10^5-10^6 cfu/g and increased by ten fold at a level of 10^6-10^7 cfu/g during 120 h of fermentation. This is in agreement with earlier report by Amoa-Awua *et al* (1997) that the level of yeasts increased during cassava dough/agbelima fermentation. At the level of 10^6-10^7 cfu/g of yeasts found in *Akyeke* fermentation in the present work, the yeasts can be considered to play a role in fermentation of *Akyeke* even though only a ten-fold increase in population was observed.

5.2 Lactic Acid Fermentation of Akyeke

In addition to the breakdown of cassava texture during *Akyeke* fermentation, the present work has shown that lactic acid fermentation also occurs during the fermentation of cassava dough into *Akyeke*. The pH dropped from 5.15-5.50 to 3.23-4.01 for laboratory and field sample respectively and titratable acidity increased from 038%-0.32% to 1.67%-1.11% during 120 h fermentation. This is in agreement to Aboua (1999) survey in which consumer showed high preference of sour *Akyeke*.

Lactic acid bacteria isolated in both *Akyeke* inoculum and during fermentation were *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus fermentum, Leuconostoc mesenteroides* and *Lactobacillus salivarius*. The occurrence in large numbers of *Lactobacillus plantarum* during all the major stages of *Akyeke* processing indicated that it is the principal lactic acid bacteria responsible for the sour taste of the *Akyeke*.

The occurrence of *Leuconostoc mesenteroides* and *Lactobacillus brevis* in quite appreciable numbers together with large numbers of *Lactobacillus plantarum* in all the major stages during *Akyeke* processing also indicate their contribution in the acidification of *Akyeke* processing.

The result of the present work is also in agreement with the findings of Okafor and Uzuegbu (1987) who reported the dominant lactic acid bacteria in spontaneous fermenting cassava mash during *gari* processing to be *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. Ngaba and Lee (1979) reported the presence of *Lactobacillus plantarum*, *Leuconostoc mesenteroides,* and *Streptococcus spp* as the dominated lactic acid bacteria microflora in spontaneous fermenting cassava mash dough. Amoa-Awua *et al* (1996) identified *Lactobacillus plantarum, Lactobacillus brevis*, *Lactobacillus fermentum, Leuconostoc mesenteroides* and *Lactobacillus salivarius* in fermented cassava dough *agbelima* .

Lactobacillus plantarum, Lactobacillus brevis and *Leuconostoc mesenteroides* were found in all the major stages, in the inoculum and during *Akyeke* processing. *Lactobacillus plantarum* accounted for almost 50% of the entire lactic acid bacteria isolated. *Lactobacillus fermentum* were also found in the inoculum and during the entire fermentation but disappeared when water was added to fermented cassava to remove some amount of starch and colour. *Lactobacillus salivarius* also disappeared after 72 h of fermentation.

There was general increase in levels of the lactic acid bacteria from 10^6 - 10^8 cfu/g in the 0 h to 10^8 – 10^9 cfu/g at the end of fermentation. This level dropped in the next two stages (wet cassava mash and the dried cassava mash) in which water is added to decolour and remove some amount of starch from the fermented cassava mash. The reason could be that the water tend to wash away some of the lactic acid bacteria. There was a decrease in the lactic acid bacteria population during drying of the wet mash.

5.3 Detoxification of Cassava during Akyeke Preparation

The final total cyanide content after fermentation was between 28.2-40.4 mg HCN/kg and that of the final product *Akyeke* were between 1.4-2.8 mg HCN/kg. Compared to the acceptable level of HCN in *gari* 25-30 mg HCN/kg sample (Akinrele *et al* 1962; Wood 1965), cyanide level in *Akyeke* was lower than that of the acceptable in *gari.*

In the detoxification of *agbelima,* the ability of *Bacillus spp.* and some yeasts to hydrolyse cassava would have contributed to the reduction of total cyanide since it allowed greater contact between cyanogenic glucoside and the indigenous linamarase enzyme. This will be in addition to the disruption of cassava tissue during grating. Several workers have reported that lactic acid bacteria isolate from fermenting cassava dough possess linamarse activity. Some of these species including *Lactobacillus plantarum, Lactobacillus brevis*, *Lactobacillus fermentum, Leuconostoc mesenteroides* and *Lactobacillus salivarius* have been isolated in the present work and therefore could contribute to the detoxification of cassava dough during *Akyeke* fermentation.

6.0 CONCLUSIONS

The composition of microflora in *Akyeke* inoculum and during fermentation is made up of *Bacillus* spp., lactic acid bacteria and yeasts. The *Bacillus* spp. contributed to the breakdown of the cassava texture to give smooth textured dough. The microflora of aerobic mesophiles included *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Bacillus pumilus* and *Corybacterium spp.* and was dominated by *Bacillus subtilis*. Action of lactic acid bacteria resulted in the acidification of the product through mainly the production of lactic acid. The lactic acid bacteria population included *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus salivarius* and *Leuconostoc mesenteroides* with *Lactobacillus plantarium* being the dominant species. Yeasts species present were *Candida tropicalis, Candida krusei, Zygosaccharomyces florentinus* and *Geotrichum candidum*.

In the processing of *Akyeke,* these microflora *Bacillus subtilis*, *Candida tropicalis* and *Zygosaccharomyces florentinus* were able to break down or disintegrate cassava tissue when streaked directly on sterile cassava slices.

The substantial detoxification of cassava occurred during *Akyeke* processing and this could be attributed to the disruption of cells during grating. However, the role *Bacillus subtilis, Candida tropicalis* and *Zygosaccharomyces florentinus* played in the breakdown of the cassava tissue resulted in bringing about much more contact between endogenous and microbial linamarase and the cyanogenic glucoside.

The isolation of lactic acid bacteria in large numbers together with low acidity of the cassava meal during the fermentation of cassava into *Akyeke* demonstrates the fermentation process to be acidic.

7.0 REFRENCES

Aalbersberg, W.G.L. and Limalewa, L. 1991. Cyanide content of fresh and process Fijian cassava (*Manihot esculenta*) cultivars. *Trop.Sci.***31** 249-256.

Ababio, J. T. 1998. Factors affecting the development and production of Food grade Cassava Flour. MPhil. Thesis. Department of Nutrition and Food Science, University of Ghana.

Aboua, F. 1995. Optimization of traditional fermented food. Center Ivorien De Recherches Technology.

Adjei, M.D. 1990. Characterization of agbelima (cassava dough inoculum). BSc. Dissertation. Dept. of Nutrition and Food Science, University of Ghana.

Adegoke, G.O. and Babalola, 1988 Characteristics of microorganisms of importance in the fementation of *fufu* and *ogi,* two Nigerian foods. *Journal of applied Bacteriology.* **65** 449-453

Aidoo, A.E. 1992. Lesser known fermented foods. **In:** International meeting on cassava flour and starch, Jan. 11-15. Cali, Colombia.

Aidoo, A.E. 1986. Lesser-known fermented plant foods. *Tropical Sci.* **26** 249-258.

Akinrele, L.A. 1970 Fermentation studies on maze during the preparation of traditional African starch cake food. *Journal of the Science of Food and Agriculture* **21** 619-625.

Akinnele, I.A., Cook, A.S. and Holgate, R.A. 1962. The manufacturing of gari from cassava in Nigeria. 1st International congress of Food Science and Technology. London. Proceedings, Vol. **4** pg. 633-644.

Akintonwa, A. and Tunwashe, O. L. 1992 Fatal cyanide poisoning from cassava-based meal. Human and Experimental Toxicology, **11** 47-49.

Al-Hassan, R.M. 1989.Cassava in the economy of Ghana Collaborative Study of Cassava in Africa (COSCA) Working Paper Number **11** IITA

Amoa-Awua, W.K.A.,Frisvad, J.C., Sefa-Dedeh, S. and Jakobsen, M. 1997. The contribution of moulds and yeasts to the fermentation of *agbelima* cassava dough. *J. Applied Microbiology* **83** 288-296.

Amoa-Awua, W.K.A., Appoh, F. and Jakobsen m. 1996 Lactic acid fermentation into *agbelima. International Journal of Food Microbiology* **31** 87-98

Amoa-Awua, W.K.A. 1996. The dominant micro flora and their role in the fermentation of cassava dough agbelima fermentation. PhD thesis. University of Ghana.

Amoa-Awua, W.K.A. and Jakobsen, M. 1995. The role of Bacillus spp. In cassava fermentation. *J. Applied Bact.* **79** 250-256.

Antai, S.P. and Ibrahim, M.H. 1986 Microoganisms associated with African Locust bean (*Parkia filicoidea Welw)* fermentation for *dawadawa* production. *J. Applied Bact* **61 145-148.**

Anon, E. 1997. The World Cassava Economy: Recent trends and medium-term outlook. Chapter 3 and 4. FAO working notes, Workshop on Global Cassava Development Strategy, IFAD, Rome 10-11 June 1997.

Axelsson, T.L. 1993 Lactic acid bacteria: classification and physiology. **In** Lactic Acid Bacteria ed. Salminen, S. and von Wright, A. pp 1-64. New York: Marcel Dekker Inc.

Beeching, J.R., Dodge, K.G. Moore, K.G., Phillips, H.M. and Wenhan, J.E. 1994. Physiological deterioration in cassava: possibilities for control. *Trop. Sci.* **34** 335-343.

Berry, A. S. 1993. Socio-economic Aspects of Cassava cultivation and use in Africa; Implications for the Development of Appropriate Technology. Collaborative Study of Cassava in Africa (COSCA) Working paper Number **8** International Institute of Tropical Agriculture, Ibadan Nigeria.

Bokanga, M. 1995. Cassava fermentation and industrialization of cassava production. **In**: Akoroda, M.O.ed Root crops for food security in Africa, 22-28 November ISTRC-AB, CTA, IITA.

Bokanga, M.1992. Mechanisms of the elimination of cyanogens from cassava during traditional processing. **In**: International meeting on cassava flour and starch, Jan. 11-15. Cali, Colombia.

Booth, R.H. 1973. Storage of fresh cassava (*Mannihot esculenta)*. Post-harvest deterioration and its control. *Experiental Agric.*, **12** 103-111.

Brauman, A., Keleke, S. and Malong, M. 1994. Microbiological and biochemical aspect of cassava lactic fermentation in Central Africa. **In:** International meeting on cassava flour and starch, Jan. 11-15. Cali, Colombia.

Budu, A. S. 1990. Process and product characteristics of fermented cassava (*Manihot esculenta*) dough agbelima. M.Phil. Thesis. Nutrition and Food Science Department. University of Ghana.

Campbell-Platt 1994. *Fermented Foods of the World, A Dictionary and Guide.* London; Butterworths.

Chassy, B.M. and Murphy, C.M. 1993 Latococcus and Lactobacillus. **In** *Bacillus subtilis and Other Gram-Positive Bacteria, Biochemistry, Physiology and Molecular Genetics.* ed. Sonenshein, A.L., Hoch, J.A. and Losick, R.pp. *65-82*

Claus, D. and Berkerley, R.C.W. 1986 The genus *Bacillus.***In** *Bergy's Manual of Systematic Bacteriology,* Vol. 2 ed. Sneath, P.H.A. pp. 1105-1139. Baltimore: Williams and Wilkins.

Cock, J. H. 1982. Cassava: a basic energy source. *Tropic. Science*, **218** 755-762.

Collard, P. and Levi, S. 1959. A two-stage fermentation of cassava. *Nature* **18** 620 Collaborative Study of Cassava in Africa (COSCA) Working Paper Number **8** International Institute of Tropical Agriculture, Ibadan Nigeria.

Common African Pests and Diseases of Cassava, Yam, Sweet Potato and Cocoyam. Theberge, R. L. (ed.). International Institute of Tropical Agriculture. Ibadan, Nigeria 1985.

Cooke, R.D. 1978 Cassava: a major cyande containing food crop. **In** Cyanide in Biology ed. Vennesland, B., Conn, E.E., Knowles, C. J., Wstley, J. and Wissing, F. pp 92-114. London: Academic Press.

Conn, E.E. 1969 Cyanogenic glucosides: Their occurrence, biosynthesis and function. **In:** Nestle, B. and MacIntyre (ed), Chronic Cassava Toxicity. International Development Research Center Monograph (IDRC) OIOe, Ottawa, pp 43-48.

Daeschel, M.A. 1989 Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technol.* 43, 164

Dahnija, M. T., Akoroda, M.O., Alvarez, M.N., Kaindaneh, P.M., Ambe-Tumanteh, J., Okeke, J.E. and Jalloh, A. 1994. Development and Dissemination of Appropiate Root Crops Packages to Farmers in Africa. Invited paper: Ninth

symposium of the International Society for Tropical Root crops-Africa Branch held in Lilongwe, Malawi, 22-28 October 1995. International Institute of Tropical Agriculture, Ibandan Nigeria.

De bruijn, G.H. 1973 The importance of cassava in world food production. *Netherlands Journal of Agricultural Science* **37** 21-34.

Davidson, P.M. 1993 Antimicrobial components from lactic acid bacteria. **In** Lactic Acid Bacteria. ed. Salminen, S. and von Wright, A. pp. 127-159. New York: Marcel Dekker Inc.

Dosoo, E. and Amoa-Awua, W.K.A. 1992. Cassava in Ghana; its culture, and utilization, limitation, plans and perspectives. First Afro-Brazilian Seminar on Cassava. Maputo, Mozambique. July 27-31, 1992

Essers, S.A.J.A. 1995. Removal of cyanogens from cassava. Studies on domestic sundrying and solid subtrate fermentation in rural Africa. Ph.D Thesis. Wageningen Agriculcultural University, Netherlands.

Essers, S.A.J.A., Bosveld, M., van der Grift, R.M. abdVoragen, A.G.J. 1993 Studies on the quantification of specific cyangens in cassava products and introduction of a new chromogen. *J. Sci. Food Agric.* **63** 287-296

Eyeson, K. K. and Ankrah, E. K. 1975. *Composition of Foods Commonly used in Ghana.* Food Research Institute, Ghana.

FAO Processing of Cassava, Rome: Food and Agricultural Organization of United Nations. Agricultural Development Paper No. 31

Fogarty, W.M., Griffin, P.J. and Joyce, A.M. 1974. Enzymes of *Bacillus* species Part 1. Process Biochemistry. **9** 11-24

Fook-Min Youg, L. 1992. Future directions. **In:** The Cassava Biotechnology Network. Proceedings of the 2nd International Scientific Meeting. Working document No. 150. Bogor, Indonesia, 22-26 Augist 1992. Vol.II PG. 651-653.

Gilliland, S.E. 1985 Role of starter culture bacteria in food preservation. In *Bacterial Starter Cultures for food.* ed Gilliland, S.E. p. 175. Boca Raton: CRC Press.

Grace, M.R. 1977 Cassava processing. FAO Plant Production and Protection Series Number **3**, Rome.

Gotin, G., Reyes, A., Obando, G., Rojas, O., Farinet, J.L. and Alazard, D. 1994. Characteristicas Lidrodinamicas de soportes naturals potericialmeutes utilizables en biofiltraciou. **In:** International meeting on cassava flour and starch, Jan. 11-15. Cali, Colombia.

Hahn, S.K. 1992. An overview of African traditional cassava processing and utilization. *Outlook on Agriculture* **18** 110-118.

Hugh,R. andvLeison, E 1953 The taxonomic significance of fermentation versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol **66** 24-26.

International Fund for Agricultural Development (IFAD) 1996. Element for Global Cassava Development Strategy.Mimeo, IFAD, Rome, Italy.

Ingram, J.S. and Humpheries, J.R.O. 1972. Cassava storage: a review. *Trop. Sci* **14** 131-148.

Halm, M, Jespersen, L.,., Kpodo, K. and Jakobsen, M. 1993. The significance of yeasts and moulds occurring in maize dough fermentation for kenkey production. *Int. J. Food Microbiol.* **24** 239-248.

Kandler, O. and Weiss, N. 1986. Regular non-sporing Gram-positive rods. **In.** Bergey's Manual of Systematic Bacteriology, Vol. 2 ed. Sneath, P.H.A. pp 1208-1260. Baitimore: Williams and Wilkins.

Koch, L. 1992. 'Cassavaselectie,' Veenman and Zonen, Wagenigen.

Kreger-van Rij, N.J.W. 1984. *The Yeasts-A taxonomic study.* 3rd Edition. N.J.W. Kreger-van Rij (ED) Elsevier Science Publisher. Amsterdam.

Latham M. C. 1997. Human Nutrition in the Developing World. FAO Food and Nutrition Series Number **29**. Pg 484.

Lilie, R. D. 1928. The Gram stain. 1. A quick method for staining Gram positive organisms in the tissue. *Archives of Pathology* **5**, 828.

Lorri, W. and Svanberg. 1991. Thepotential role of fermented cereal gruel in reduction of diarrhoea among young children. **In** *Proceedings of a Rergional Workshop on Traditional African Foods- Quality and Nutrition.* International Foundation for Science. Ed. Westby, A. and Reilly. Pp 33-38. Stockholm:IFS

Madeley, J., 1993. Make way for super cassava. Series, **140** 5-6.

Mbugua, S.K. and Njenga, J. 1991 Antimicrobial properties of fermented *uji* as a weaning food. **In:** *Proceedings of a Regional Workshop on Traditional African Foods-Quality and Nutrition*. International Foundation for Science. ed. Wesby, A. and Reilly. Stockholm: IFS

.Mensah, P., Tomkins, A.m., Prasar, B.S. and Harrison, T.J. 1990 Antimicrobial effect of fermented Ghanaian maize dough. *Applied Bacteriology* **70** 203-210.

Ministry of Food and Agriculture (MOFA) 1999. Facts and Figures. August 1999.

Mkpong, O.E., Yan, H., Chism, G. and Sayre, R.T. 1991. Purification, characterization and localization of linamarase in cassava. *Plant Physiol.* **93** 176-181.

Molin, G., Jeppson, B., Johansson, M.L., Ahrne, S., Nobaek., S., Stahl, M. and Bengmark, S. 1993. Numerical taxonomy of Lactobacillus spp. associated to health and disease mucosa of human intestines. *J. Appl. Bacteriol.* **74** 314-323.

Neuenschwander,P. and Braima, D. J. 1998. Ecological sustainable Cassava Plant Protection in South America and Africa. Project Termination Report.

Ngaba, R.R. and Lee, J.S. 1979. Fermentation of cassava (*Manihot esculentum* Crantz). *J. Food Sci.* **144** 1570-1571

Nout, M.J.R. 1980 Micobiological aspects of the traditional manufacture of *Busaa,* a Kenyan opaque maize beer. *Chem. Mikrobiol. Technl. Lebensm.* **11** 51-55.

Nout, M.J.R.,Hautvast, J. G. A.J., Van derHaar, F., Marks, W. E. W. and Rombouts, F.M. 1987. Energyprotein and microoganisms; the formulation and microbial stability of cereal base composite weaning food. **In** *Improving Young Child Feeding in Eastern and Southern Africa. Household Level Food Technology.* Proceedings of a workshop, Nirobi, Kenya 12-16 October. Ottawa:IDRC.

Nweke, F. I. 1994. Cassava Distribution in Africa. COSCA Working paper Number **12**. International Institute of Tropical Agriculture. Ibadan Nigeria.

Nweke, F. I. 1996. Collaborative Study of Cassava in Africa (COSCA) Working paper number **14**. IITA.

O'Brien, M.G., Taylor, A.J. and Poulter, N.H. 1991. improved enzymic assay for cyanogoes in fresh and processed cassava. *J. Sci. Food Agric.* **26** (3) 277-289.

Odunfa, S.A., Adeleye, S., 1985. Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *J. Cereal Sci.* **3** 173-180

Molin, G., Jeppson, B., Johansson, M.L., Ahrne, S., Nobaek., S., Stahl, M. and Bengmark, S. 1993. Numerical taxonomy of Lactobacillus spp. associated to health and disease mucosa of human intestines. *J. Appl. Bacteriol.* **74** 314-323.

Neuenschwander,P. and Braima, D. J. 1998. Ecological sustainable Cassava Plant Protection in South America and Africa. Project Termination Report.

Ngaba, R.R. and Lee, J.S. 1979. Fermentation of cassava (*Manihot esculentum* Crantz). *J. Food Sci.* **144** 1570-1571

Nout, M.J.R. 1980 Micobiological aspects of the traditional manufacture of *Busaa,* a Kenyan opaque maize beer. *Chem. Mikrobiol. Technl. Lebensm.* **11** 51-55.

Nout, M.J.R.,Hautvast, J. G. A.J., Van derHaar, F., Marks, W. E. W. and Rombouts, F.M. 1987. Energyprotein and microoganisms; the formulation and microbial stability of cereal base composite weaning food. **In** *Improving Young Child Feeding in Eastern and Southern Africa. Household Level Food Technology.* Proceedings of a workshop, Nirobi, Kenya 12-16 October. Ottawa:IDRC.

Nweke, F. I. 1994. Cassava Distribution in Africa. COSCA Working paper Number **12**. International Institute of Tropical Agriculture. Ibadan Nigeria.

Nweke, F. I. 1996. Collaborative Study of Cassava in Africa (COSCA) Working paper number **14**. IITA.

O'Brien, M.G., Taylor, A.J. and Poulter, N.H. 1991. improved enzymic assay for cyanogoes in fresh and processed cassava. *J. Sci. Food Agric.* **26** (3) 277-289.

Odunfa, S.A., Adeleye, S., 1985. Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *J. Cereal Sci.* **3** 173-180

Odunfa, S.A. 1985 African fermented Foods. **In** *Microbiology of Fermented Foods.* Vol. 2, ed. Wood, B.J.B. London: Elsevier press.

Odunfa, S.A. 1981. Microoganisms associated with fermentation of Africa locust bean *Parkia filicoidea* during *iru* production. *J. Plant Food,* **3**, 245-250

Okafor, N., 1974. Microoganisms associated with cassava fermentation for garri production. *J. Appl. Bacteriol.* **41** 279-284.

Okafor, N., 1977b. Nigeria gari. Symposium on indigenous fermented foods. Bangkok, Thialand.

Okafor, N. and Uzuegabu, J.O. 1987. Studies on the contribution of microorganisms to the organoleptic properties of gari, a fermented food derived from cassava (*Manihot esculentum* Crantz). *J. Food Agric.* **2** 99-105

Okafor, N., Ijioma, B. and Oyolu, C. 1984. Studies on the microbiology of cassava for food production. *J. Appl. Bacteriol.*, **54**, 1-13

Osuntokum, B. O.1981. Cassava diet chronic cyanide intoxification and neuropathy in Nigeria Africans. *World Review of Nutrition and Dietetics*, **36** 141-173.

Oyewole, O.B. and Aibor, A.M. 1992. Fermentation of cassava with cowpea and soya bean for enriched *fufu*. *Trop. Sci* **33** 9-15.

Paresdes-Lopez 1992. Nutrition and Safety Considerations. **In:** The Cassava Biotechnology Network. Proceedings of the 2nd International Scientific Meeting. Working document No. 150. Bogor, Indonesia, 22-26 Augist 1992. Vol.II PG. 651-653.

Parry, J.M., Turnbull, P.C.B. and Gibson, J.R.. 1983 *A Colour Atlas of Bacillus Species.* Ipswich: Wolfe Medical.

Root and Tuber Improvement Programme (RTIP) FACTSHEET No. 1 OCTOBER 1998

Salovaara, H.. 1993. Lactic acid bacteria in cereal basid products. **In** : *Lactic Acid Bacteria.* ed. Salminen, S. and von Wright, A. pp. 237-294. New York: Marcel Dekker Inc.

Sharpe, E.M. 1979. Identification of lactic acid bacteria. **In:***identification Methods for Microbiologist*, 2nd ed. Appl. Microbacteriol. Technical. Series, No 14. ed Skinner, F.A. and Loveloak, D.W. pp 246-255. London: Academic press..

Sefa-Dedeh, S. 1995. Process and product characteristics of agbelima, a fermented cassava product. *Trop. Sci.* **35** 359-364.

Sefa-Dedeh, S. 1989 Effect of particle size on some physiochemical characteristics of *agbelima* (cassava dough) and corn dough. *Trop. Sci.* **28** 21-32.

Skinner, C.E. and Fletcher, D.W. 1960. *Bacteriological reviews* **24** 397

Sokari, T.G. 1992. Improving the nutritional quality of *ogo* and *gari*. **In:** International meeting on cassava flour and starch, Jan. 11-15. Cali, Colombia.

Steinkraus, K.H. 1997. Nigerian gari. **In**: Handbook of indigenous fermented foods. Marcel Dekker, New York. Pp 208-220

Steinkraus, K.H. 1991. African alkaline fermented foods and their relation to similar foods in other parts of the world. **In** :*Proceedings of a Regional Workshop on Traditional African Foods-QUALITY AND nutrition.* International Foundation for Science. ed. Wesby, A. and Reilly. Pp. 87-92. Stockholm: IFM

The'berge, R.L.ed. 1985. Common African Pests and Diseases of Cassava, Yam, Sweet Potato and Cocoyam. International Institute of Tropical Agriculture. Ibadan, Nigeria.

Tylleskar,T., Banea, M., Bikangi, N., Cooke, R., Poulter,N., and Rosling, H. 1992. Cassava cyanogens and konzo, an upper motor neuron disease found in Africa. *Lancet* **33** 208-211

Wood, T. 1965. The cyanogenic glucoside content of cassava and cassava products. *J. Sci. Food and Agric.* **17** 85-90.

Wosiacki, G., Kirchner, C.L. and Mends, M. 1994. Surface fermentation of *Trichosporon* sp. in KCN containing synthetic media. *Bacteriological reviews* **24** 397.