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ANTIMICROBIAL INTERACTIONS DURING THE FERMENTATION OF CASSAVA DOUGH INTO AGBELIMA

BY

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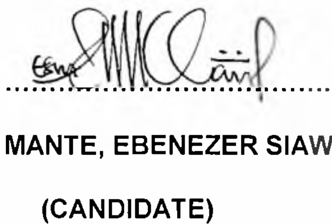
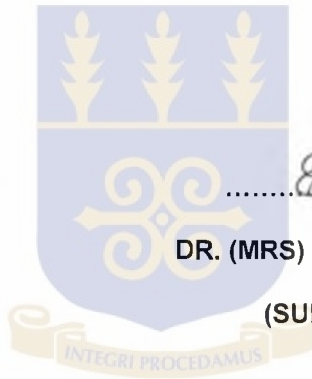
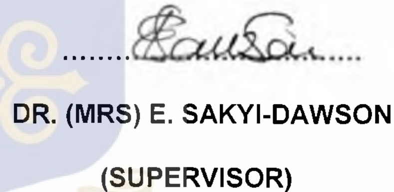
DECLARATION

I certify that this work was conducted by me, under supervision in the Department of Nutrition and Food Science, University of Ghana, Legon and the Food Research Institute (CSIR), Accra, Ghana.



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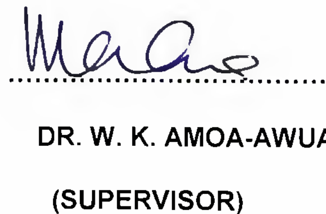

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DEDICATION

This thesis is written to the Glory of God, through Jesus Christ our Lord and dedicated to my wife, Lydia, without whose prayers, enthusiasm and encouragement, it would not have been completed.

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ABSTRACT

The microbial bioprocesses, which occur during the fermentation of cassava dough into agbelima cause considerable changes that affect its organoleptic properties, nutritional value and also enhances the microbial safety of the food.

Fermentation of cassava dough into agbelima involves activities of different groups of microorganisms, which result in the production of a sour dough with smooth texture.

The microorganisms include *Bacillus spp*. mainly *Bacillus subtilis*, Yeast-*Candida krusei, Candida tropicalis,Zygosaccharomyces spp*. and lactic acid bacteria mainly *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus brevis.*

The interactions within and between the different types of microorganisms were studied using the Agar Well Diffusion Assay method. Nearly all lactic acid bacteria isolated at the end of agbelima fermentation inhibited those, which were present at the start of fermentation. Three out of 10 isolates of *Bacillus spp*. tested were inhibited by all 20 isolates of lactic acid bacteria tested but these lactic acid bacteria inhibited none of the yeasts. Three out of 10 *Bacillus spp*., which was all *Bacillus subtilis*, inhibited growth of yeasts. Twelve isolates of *Lactobacillus plantarum* inhibited bacteriocin indicator organisms, namely *Lactobacillus sake* and *Listeria monocytogenes*. When supernatants of *L. plantarum* isolates were neutralized inhibitory effect of the isolates were lost on *Lactobacillus sake* and *Listeria monocytogenes* therefore none of the isolates studied tested positive for bacteriocin production.

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The growth patterns and acid production of some selected *Lactobacillus plantarum* isolates were also investigated using batch fermentation in a fermentor.

The growth pattern of *Lactobacillus plantarum* isolates (LAB 9, LAB 6, LP 11 and LP 3) corresponded with increases observed in titratable acidity and decreasing pH of the agbelima. Acid production was fastest in *Lactobacillus plantarum* isolate-LAB 9.

In investigating the microbiological safety of cassava dough-agbelima, pure cultures of enteric pathogens: *Salmonella typhimurium* 9, *Salmonella enteritidis* 226, *Vibrio cholerae* C-230, *Shigella dysenteriae* 2357T and *Escherichia coli* D2188, were inoculated into four cassava dough systems at a concentration of 10^6 -10^7 cfu per gram of cassava dough. When inoculated into spontaneously fermenting cassava dough there was a decrease in population of all the enteric pathogens from 10^7 to zero cfu/g after 24h of fermentation. When inoculated into 48h fermented cassava dough (pH 3.85) all the pathogens died after 4-8h. When inoculated into 48h fermented cassava dough, which had been cooked into a semi-stiff porridge the enteric pathogens disappeared between 4-8h. When inoculated into fermenting cassava dough of specific pHs between 4.40 and 3.81 depending on the pH of the dough the pathogens survived for between 2-5h . When the fermented cassava dough was neutralized the inoculated pathogens survived for 24h-48h.

The decrease in population of the pathogens in the different systems also corresponds with increases observed in titratable acidity and decreasing pH of

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the agbelima. This indicates that the acidic condition prevailing in the agbelima is effective in preventing the growth and survival of the pathogens used.

Microbial development and succession in the natural ecosystem of agbelima is therefore controlled by complex interactions, which are mainly determined by acid production.

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1. INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a crop that thrives in a variety of environmental conditions and is potentially one of the most efficient crops in terms of carbohydrate production and is now considered to be one of the most important food crops.

Cassava is currently the most widely cultivated root crop in Ghana with a total annual production of about 7.17 million metric tonnes contributing 22% of the Agricultural Gross Domestic Product, 19% of dietary energy intake and averaging 380 Kcal/ daily per person (Ministry of Agriculture, Ghana, 1998).

Three major factors limit utilization of cassava as food. These are the high perishability of cassava tubers which have a natural storage life of only about 2-3 days after uprooting, their low protein content of 1-3%, and the presence of the cyanogenic glucosides, linamarin and lotaustralin which impart toxicity to the tuber. The high perishability of cassava tubers, which is due to its rapid physiological deterioration, which occurs within 2-3d after harvest, is observed as a discolouration of the vascular tissues and storage parenchyma. This is accompanied by biochemical changes including changes in amounts and composition of membrane lipids, increases in phenylalanine, ethylene production, peroxidase and phenol oxidase

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activities and accumulation of phenolic compounds and ammonia lyase (Breeching *et al*. 1994).

Physiological deterioration is followed within 5-7days by microbial deterioration and root tissue softening caused by the activities of a complex of fungi including *Pythium, Mucor, Rhizopus* and *Penicillium* as well as some bacteria, *Bacillus* and *Xanthomonas spp*.(Booth, 1976).

Cassava is processed by a variety of methods all over Africa to convert the highly perishable rootcrop into shelf stable products. These methods include peeling, fermentation, grinding, drying, milling, roasting, steaming, pounding, sieving and mixing in cold or hot water. Specific combinations of these steps lead to a myriad of different cassava products appealing to a wide range of consumers and these include gari, attieke, kokonte and placali. Processing of cassava provides a means to overcome all the three main factors, which limit utilization of cassava as food.

The traditional processing of cassava into various fermented indigenous products is of crucial importance to the food supply system of Ghana as a result of the important role it plays in reducing the post-harvest loss of the highly perishable root crop.

The process of fermenting cassava is used in many parts of Africa to improve its preservation and sensory characteristics.

Fermentation can be described as a desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes. The microorganisms by virtue of their metabolic activities,

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contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture, shelf-life and safety.

One of the most important fermented cassava products in Ghana is Agbelima, which is consumed extensively in southern Ghana in combination with fermented maize dough. Agbelima is a moderately high moisture off-white fermented cassava meal prepared by fermented cassava mash in the presence of a traditional inoculum. Agbelima fermentation is reported to be both a souring and texture degrading process important to the development of a characteristic organoleptic quality (Sefa-Dedeh, 1995; Amoa-Awua and Jakobsen, 1995).

The microorganisms responsible for the fermentation of agbelima have been identified as *Bacillus sp. Lactic acid bacteria*, yeasts and moulds(Amoa-Awua, 1996). His work showed that during fermentation there is a decline in the population of *Bacillus* sp, Gram negative bacteria and some species of lactic acid bacteria whilst an increase in other species of lactic acid bacteria, such as *Lactobacillus plantarum*, *L. brevis*, *Leuconostoc mesenteriodes* occurs. This suggests the possibility of antimicrobial interactions between the various species of microorganisms present in the microflora of agbelima with lactic acid bacteria exhibiting antagonistic effect against the other microbial species.

Lactic acid fermentation of food is reported to contribute to the extension of the shelf-life and safety of food. Preservation of fermented foods by lactic acid bacteria is due primarily to sugar being converted to organic acids (lactic, acetic) causing a reduction of pH and removal of carbohydrates as

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nutrient sources In addition lactic acid bacteria produces substances including hydrogen peroxide, diacetyl, secondary reaction products and bacteriocins which have potential to inhibit a variety of other microorganisms (Daeschel, 1989 Gilliland 1989). Strains of *Lactobacillus plantarum* have been known to produce bacteriocins (Kanatani *et al*, 1994).

The mechanism involved in bringing about the reduction in population of some groups of microorganisms during agbelima fermentation needs to be established.

One of the main problems of traditional food processing in Ghana is poor hygiene and sanitation observed during processing. During handling and preparation, food contamination particularly by enteric pathogens may occur through polluted water, food handlers (eg, soiled hands), flies, pest, domestic animals and dirty utensils. It is observed that diarrhoeal diseases are a major health problem in these parts of the world and it is estimated that up to 70% of these diseases are of food-borne origin and the enteric pathogens of prime medical concern are *Salmonella, Shigella, Escherichia* and *Vibrio cholerae*. The lack of safe and hygienic practices during the processing of cassava dough into agbelima will therefore pose a health risk to consumers and the ability of these pathogens to survive in agbelima therefore need to be assessed.

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1.1 Objectives

1. To study antimicrobial interactions between three main groups of microorganism involved in cassava dough fermentation into agbelima: lactic acid bacteria, *Bacillus spp an*d yeasts.

2. To study the spectrum and mode of antimicrobial activity of *Lactobacillus plantarum* the dominant lactic acid bacteria.

3 To investigate the survival of enteric pathogens in agbelima during fermentation

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2.0 LITERATURE REVIEW

2.1 Fermentation of Food

Fermentation is one of the oldest and most important methods of preparing foods. In Ghana and most African countries fermenting food as part of traditional food processing is extensively practiced. Generally, fermented foods constitute a substantial part of the basic diet and are seen among other factors as means for introducing variety into the consumption of such staples as cassava, maize and fish.

Apart from the preservation effect, fermentation has been a means of improving sensory quality and acceptability of many raw materials to such an extent that several foods are preferred in a fermented state (eg, gari rather than unprocessed cassava tubers). Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes; particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavors, aromas and textures pleasant and attractive to the human consumer (Steinkraus, 1997).

Traditional fermentation of food in Ghana usually involves the use of mixed cultures in solid or semi-solid substrate fermentation. The mixed culture fermentation, that is, use of cultures from one or more microbial groups are prevalent because the traditional fermentations are mostly carried out

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spontaneously and the microbial populations usually occur as mixed cultures. The advantages of the spontaneous mixed culture solid substrate fermentations are that they rely on various microorganisms to produce different enzymes to breakdown the substrate and require minimal handling skills of the traditional processors (Amoa-Awua, 1996).

According to Hesseltine (1991), advantages of mixed culture fermentations over single pure culture fermentations are that product yield may be higher, growth rate of microorganisms may be higher, protection against contamination may be greater and remarkable stable association of microorganisms may occur permitting better utilization of substrate. Fermentation processes can also occur by the application of starter cultures such as yeast (*Saccharomyces cerevisiae*) in the brewing industry, lactic acid bacteria in the dairy industry and moulds in the fermentation of many oriental foods, for example, koji or soy sauce in Asia. The starter is normally a pure culture and is added to the substrate at a high concentration with the aim of a rapid and complete fermentation (Brock and Madigan, 1991). In fermentations that occur naturally, the microorganisms responsible are naturally occurring on the product undergoing the fermentation. The spontaneous fermentation of cereals is thus carried out by yeast and lactic acid bacteria originally present on the cereal (Akinrele, 1970). Therefore the microorganisms by virtue of their metabolic activities, contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture, shelf-life and safety.

Many fermented foods are known; some serve as main-cause meals, others are beverages and highly prized food condiments. Those which serve as

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main meals and beverages are usually derived from the fermentation of carbohydrate-rich raw materials, most of which have a low protein and vitamin content (Odunfa, 1985).

2.2 Cassava Dough-Agbelima

In Ghana and some West African countries such as Benin and Togo, one of the most important traditionally fermented cassava product is agbelima It is often included in local diets of people along the coastal and southern regions of Ghana. Agbelima is a moderately off-white fermented cassava meal which is produced by grating peeled washed cassava chunks together with a traditional cassava dough inoculum called “Kudeme” in a ratio of about 50:1, in a motor driven cassava grater. The milled cassava is packed into polythene sacks and left for 2 to 3 days to ferment whilst weights are placed on top of the sacks to partially dewater the mash during fermentation (Dovlo, 1975; Amoa-Awua and Jakobsen, 1995) (Fig. 1). Sefa-Dedeh (1989) found the moisture content of agbelima to decrease from 58% to between 54 and 52% and pH from 6.54 to about 4.02 during fermentation. The fermented meal agbelima is usually cooked and mixed with fermented maize in varying proportions into banku or akple, which are hardened porridges eaten with stew.

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Figure 1.

General flow diagram for the processing of cassava tubers into agbelima

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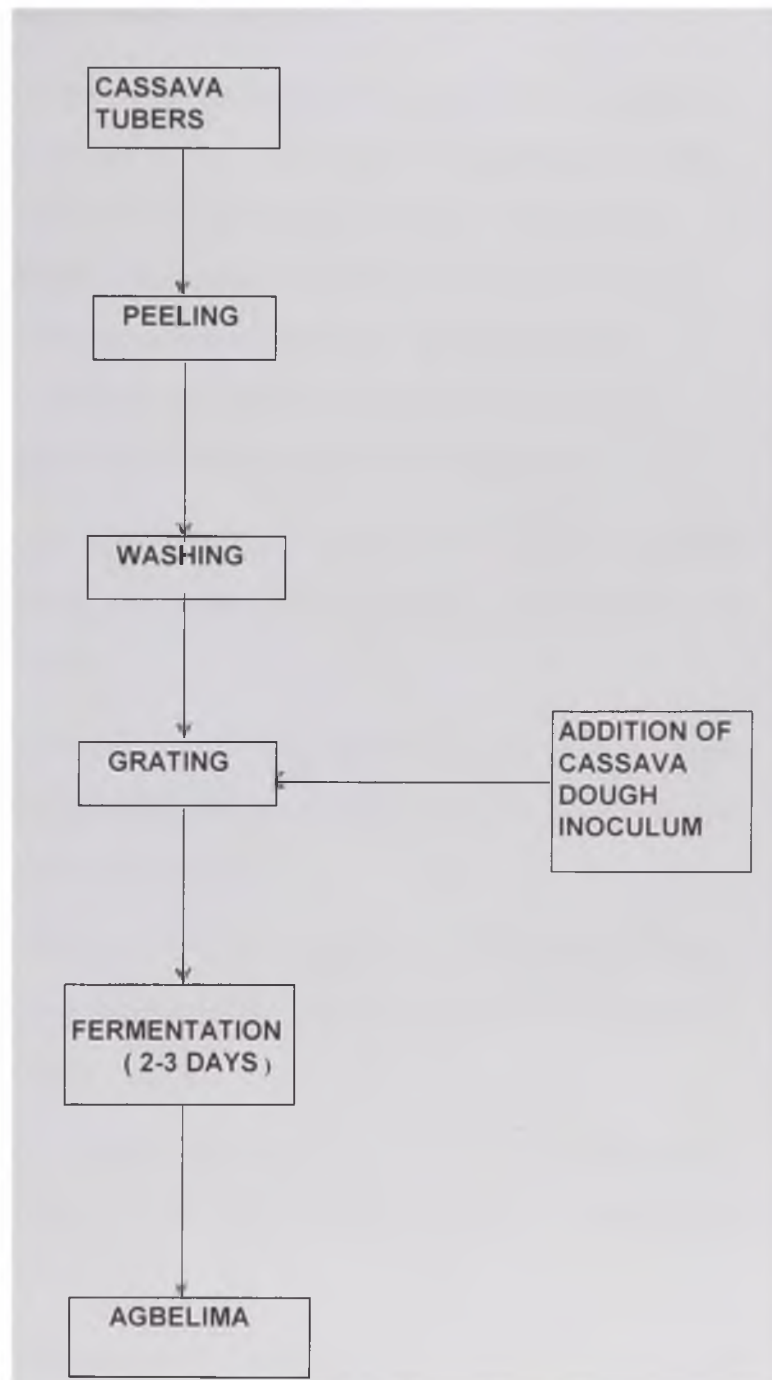

Source: Amoa-Awua *et al.* 1996

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2.5.1 Cassava Dough Inoculum - "Kudeme"

The cassava dough inoculum "Kudeme" is a very important material used to ferment cassava mash into good quality agbelima. According to traditional cassava processors, the sole aim of using inoculum to ferment cassava mash into agbelima is to breakdown the texture of the mash in order to obtain fermented dough with a smooth texture. Sefa-Dedeh (1995) suggests that the inoculum serves as a source of microorganisms and enzymes needed to cause both souring and texture modification.

Several different types of traditional cassava dough inocula are used to ferment agbelima and some of the frequently used methods are enumerated below:

1. Small chunks of peeled cassava are partially roasted over an open fire, wrapped in a piece of cloth, placed in a basket and left in a warm place for 2-4 days to ferment into inoculum.

2. Small chunks of peeled cassava are blanched by boiling for about 5 min, wrapped in a piece of cloth, placed in a basket and allowed to ferment in a warm place for about 2-4days.

3. Small chunks of peeled cassava are exposed to the sun for about 30min, wrapped in a piece of cloth, placed in a basket and left in a warm place to ferment for 2-4d.

4. Split peeled cassava tubers are partially sun-dried for about 8 hours and stuck under a thatch roof for 2-4 days to ferment.

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5. Small chunks of peeled cassava are roasted in a pan and stuck under a thatch roof for 2-4 days to ferment.

6. Small unpeeled cassava tubers are tightly packed into an earthenware pot and covered with plastic sheets. They are left in this state for about 7days.

7. Split cassava pieces are sun dried for 3-5 days and used as inoculum (Dovlo 1975; Dziedzoave, 1989; Budu, 1990; Sefa-Dedeh 1995; Amoa-Awua et al 1995).

In Cote d'Ivoire, a similar traditional inoculum is prepared by boiling cassava tubers for 15-20min, wrapping the boiled cassava with a plastic sheet, jute bag or plantain leaves and allowing to ferment for 3 days until the roots soften. The inoculum is used to ferment cassava dough during the preparation of indigenous products such as attiéké and placali in order to obtain a smooth textured dough with a characteristic flavour (Aboua, 1995).

2.2 .2 Microorganisms in Cassava fermentation

A survey of the modes of utilization of cassava in Africa revealed that nearly three out of four cassava based foods were fermented products (Westby, 1991). Nearly all fermentations of cassava rely on the fortuitous presence of microbes on the roots and/or in the water and on the prevailing favourable conditions for production of the desired product. In some instances, a small amount of a previous batch is kept and used to inoculate the next, but the

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fermentation is allowed to follow its natural course with little or no attempt to control it.

Westby (1991) has recognized two different types of cassava fermentation; an acid fermentation in which lactic acid bacteria are responsible for acidifying the product and a second type of fermentation which is characterized by the surface growth of moulds which are considered to be responsible for the fermentation. Westby and Twiddy (1991), have further classified acid fermentation of cassava into two;one in which cassava roots are grated and placed into sacks or baskets to ferment, such as in the processing of agbelima, gari, attiéké and placali and the other in which roots are soaked in water to ferment, such as in processing of fufu, lafun and chikwangue in West and Central Africa. They have further divided the second type of fermentation into two; those in which slow mould growth occurs as a result of slow drying times such as in kokonte and those in which mould growth is promoted by heaping cassava roots and covering them with leaves such as in udada production in Tanzania.

Meraz *et al* (1992) report of many different types of microorganisms which have been implicated by various workers in both spontaneous and controlled acidification of cassava including yeast and lactic acid bacteria like *Lactobacillus sp, Corynebacterium sp, Streptococcus sp,* and *Leuconostoc sp.* Ngaba and Lee (1979) found the acidic fermentation of cassava to follow a typical lactic acid fermentation in which a variety of microorganisms are present at the initial stages of fermentation in which other species are outgrown by the lactic acid bacteria as fermentation progresses. *Lactobacillus spp*. have been isolated as dominant organisms in acidic

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cassava fermentation (Okafor 1977; Ngaba and Lee 1979; Ejiofor and Okafor 1981).

Lactobacillus plantarum has been identified as a dominant *lactobacillus spp.* in cassava fermentation (Ngaba and Lee 1979; Okafor and Uzuegbe 1987; Gbedemah and Awafo 1990). *Leuconostoc spp.* and *Leuconostoc mesenteroides* have been isolated in significant numbers in acidic fermentation of cassava (Okafor 1977; Ejiofor and Okafor 1981; Okafor and Uzuegbe 1987). *Streptococcus spp, Streptococcus facium* and *Streptococcus faecalis* have been identified amongst the mixed flora of acidic cassava fermentation (Abe and Linsday 1978; Ngaba and Lee 1979; Okafor and Uzuegbe 1987). *Alcaligenes* have been isolated in significant numbers from fermenting cassava (Okafor 1977). *Bacillus spp.* has been isolated in low numbers from fermenting cassava pulp by Ejiofor and Okafor (1981). Essers (1995) isolated the fungi *Geotrichum candidum, Mucor racemosus, Neurospora sitophila, Rhizopus oryzae* and *R. stolonifer*, as well as *Bacillus sp* from farm heap-fermented cassava in Uganda.

Aidoo *et al.* (1982) isolated several species of moulds from dried fermented cassava chips, kokonte, with *Aspergillus* and *Penicillium sp* as the dorminant organisms. Yeast of the genus *Candida* has been isolated from fermenting cassava (Okafor, 1977; Ejiofor and Okafor, 1981 Oyewole and Odunfa, 1991).

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2.2.3 Fermentation of agbelima

The primary reason why traditional cassava processors use inoculum to ferment cassava during agbelima production is that the added inoculum has the ability to breakdown the texture of cassava dough yielding a smooth-textured dough. This claim by traditional cassava processors has been confirmed by Sefa-Dedeh (1989), who found that the particle sizes of market samples of agbelima produced with inoculum were finer than the particle sizes of laboratory samples of agbelima produced without inoculum.

A number of studies have reviewed the significant role microorganisms play in the fermentation of cassava dough into agbelima. In a study by Amoa-Awua *et al.* (1996), on the microflora of cassava dough, moulds were found to form a dominant part of the microbiota in one type of inoculum, the species being *Penicillium sclerotirum, P citrium, P nodulum, Geotrichum candidum* and a basidiomycete. The studies also showed that all the moulds were found to possess cellulase activity, which is responsible for the hydrolysis of cassava tuber cellulose during fermentation leading to a breakdown of the coarse texture of cassava dough.

Stutzenberger (1990) describes *Bacillus spp* as being ubiquitous and with an acknowledged ability to secrete a wide variety of depolymerizing enzymes suggesting that their primary ecological role is to degrade biomass polymers. This observation is indicative of the demonstrated ability of *Bacillus spp*. to disintegrate cassava tissue. Amoa-Awua *et al*. (1996), in an investigation examined *Bacillus spp* for the production of tissue degrading enzymes, polygalacturonase, pectin esterase, cellulase and amylase. All the

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Bacillus isolates exhibited cellulase activity and were also able to disintegrate cassava tissue. *Bacillus cereus, B. polymyxa* and *B. subtilis* exhibited polygalacturonase activity. The *Bacillus spp.* therefore through their cellulase activity apparently brought about the modification of cassava texture when they were introduced into agbelima fermentation through the use of inoculum. It was also shown that though *Bacillus spp.* could not play a direct role in the breakdown of cyanogenic glucosides, they contribute to cassava detoxification by their ability to breakdown cassava tissue. Such tissue degradation results in more intimate contact between endogenous cassava linamarase and the cyanogenic glucosides thus facilitating detoxification. *B. pumilus* and *B. amyloliquefaciens* have shown weak 2-glucosidase activity , an indication of linamarase activity.

Yeasts found in four types of traditional cassava dough inoculum used for agbelima fermentation included *Candida krusei*, *Candida tropicalis* and some strains of *Zygosaccharomyces spp.* showed weak cellulase activity and were able to break down cassava tissue on prolonged incubation. The yeasts therefore play a role in the breakdown of cassava tissue during agbelima fermentation but this is of secondary importance in comparison to the ability of *Bacillus spp* and moulds to hydrolyse cassava tissue. All yeasts produced polygalacturonase but none produced pectin esterase (Amoa-Awua, 1996). Analysis of the aroma components of ageblima showed that yeasts contributed to the development of the volatile aroma of the product (Amoa-Awua *et al.* 1996). Some of the compounds detected by GC-MS analysis such as ethyl acetate and the alcohols, 2-methyl-1-butanol and 3-

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methyl-1-butanol have been described by Hansen *et al.* (1989), as yeast fermentation products.

Lactic acid bacteria have been isolated in high numbers in fermenting cassava dough during agbelima production by Amoa-Awua *et al.* (1996) and this together with recorded titratable acidity determined as lactic acid, has demonstrated the acidification of agbelima to be lactic acid fermentation. Of the lactic acid bacteria identified in fermenting agbelima inoculated separately with four different types of traditional inoculum, the facultatively heterofermentative lactobacilli, *Lactobacillus plantarum* was identified as the dominant lactic acid bacteria, which accounted for about half of the lactic acid bacteria population. *Leuconostoc mesenteroides* and *Lactobacillus brevis* were the other species of lactic acid bacteria, which accounted for nearly the rest of the lactic acid bacteria population. *Streptococci* and other *cocci* belonging to the lactic acid bacteria group were found at much lower levels in the fermenting cassava dough and their numbers reduced drastically as fermentation proceeded (Amoa-Awua 1996; Amoa-Awua *et al*, 1996).

All lactic acid bacteria isolated from agbelima, *L. plantarum, L. brevis, L. fermentum*, *L. salivarius* and *Leuconostoc mesenteroides* were found to possess linamarase activity hence capable of directly breaking down the cyanogenic glucosides present in cassava. The sour taste of agbelima is due to the production of acids during fermentation. Since lactic acid bacteria produce the lactic and acetic acids present in agbelima, these organisms are responsible for the development of the dominant aroma of agbelima. In

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a product with similar characteristics, fermented maize meal, the major aroma components are reported to be lactic, acetic, butyric and propionic acids (Banigo and Muller 1972; Plahar and Leung 1982).

The presence of acetoin, 3-hydroxy-2-butanone in agbelima shows that lactic bacteria contribute to the development of the volatile aroma of agbelima (Amoa-Awua 1996; Amoa-Awua *et al.* 1996). The dominant lactic acid bacteria identified as being responsible for the souring of agbelima were the same as the species of lactic acid bacteria reported by Ngaba and Lee (1979) and Okafor and Uzuegbu (1987) to be responsible for the spontaneous anaerobic fermentation of cassava dough during acid production. (Fig. 2)

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Figure 2.

A diagrammatic summary of the microbiological and enzymatic activities which occur during the fermentation of cassava dough into agbelima

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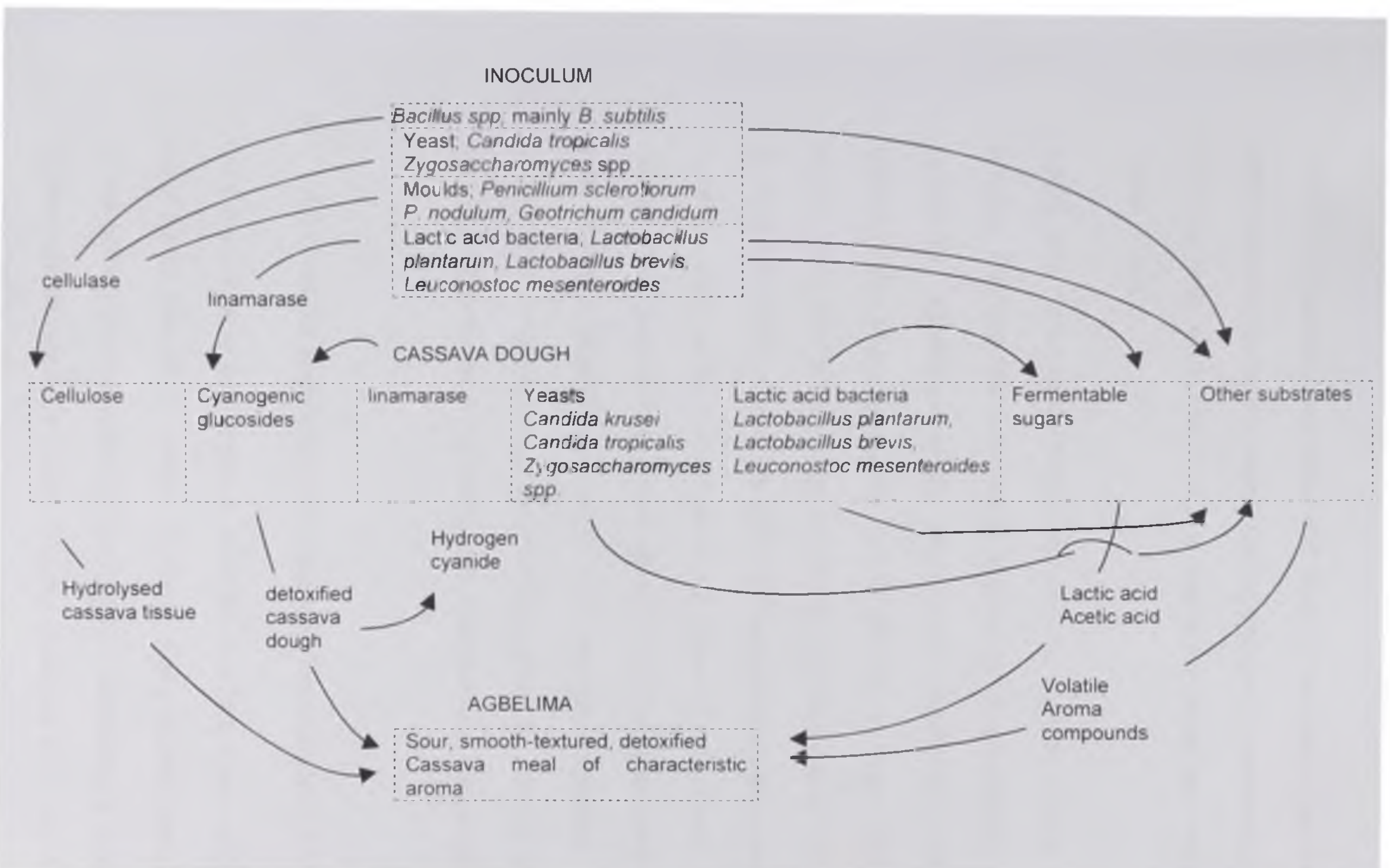

Source: Amoa-Awua *et al.*, 1996

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2.3 Lactic acid Fermentation

Among the bacteria associated with food fermentations, lactic acid bacteria are of predominant importance. Among the various fermentation processes in Africa, lactic acid fermentation is one of the oldest and most widespread (Dirar, 1992).

Lactic acid fermentation technology can be defined as the fermentation process involving the activities of a group of Gram-positive, non-sporing, non-motile, catalase-negative, non-aerobic organisms, which ferment carbohydrates to produce lactic acid as the sole or major organic acid. In Africa, lactic acid fermentation technology has developed indigenously for an extensive range of products from raw materials including cassava, maize, sorghum, milk, other cereals and root crops. These foods constitute a quarter of the human diet and are characterized by beneficial health effects and extended shelf-life compared with the raw materials. Lactic acid fermentation processes have survived throughout the centuries because of the following benefits of this technology (Hammes and Tichaczek, 1994):

(i) A household technology for improving food safety in Africa.

Diarrohoea due to poor hygienic condition has long been recognized as a major health hazard for infants in Africa. Many studies have shown that contamination of infant weaning food formulas constitutes a potential source of diarrhoeal diseases in African children (Rowland et al, 1978: Barrel and Rowland, 1979). The role of lactic acid bacteria in health and in disease

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control has been documented (Sandine, 1979). Yogurt and other milk ferments have been reported to be effective in the treatment of a variety of disorders including colitis, constipation and diarrhoea (Sanders,1993). The *in vitro* inhibition of growth of pathogenic microorganisms by lactic acid bacteria has encouraged investigations into its use as a prophylactic and therapeutic means of treating gastrointestinal and other diseases. *Lactobacillus acidophilus* has been found to be effective in the treatment of different types of diarrhoea in humans and chicks (Watkins and Miller, 1983). Acidophilus milk, which is yogurt produced by fermenting milk with *Lactobacillus acidophilus*, is now being used to treat *Escherichia coli, Salmonella and Shigella*-mediated diarrhoea and dysenteries in infants in some parts of Europe (Alms, 1983).

Other investigations, though few, have been carried out on some lactic acid fermented African foods which are used for feeding infants. Odugbemi *et al.* (1991) confirmed that enteropathogenic *Escherichia coli*, *Salmonella typhii* and *Salmonella paratyphii* were incapable of multiplying in "Ogi" a lactic fermented maize product used for feeding infants in West Africa.

In spite of the antimicrobial effects of the lactic acid bacteria from African fermented foods, the use of these organisms and their fermented products as probiotics is not common. The term 'probiotic' refers to a product containing mono or mixed cultures of live microorganisms which when applied to animal or man will improve the health status and or affect beneficially the host by improving its microbial balance.

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Olukoya *et al*, (1994) reported the isolation of a lactobacillus starter with anti-microbial activities against some diarrhoeagenic bacteria including *Salmonella, Shigella, Campylobacter, Aeromonas, Pleisiomonas*, enteropathogenic and *enteroxigenic Escherichia coli, Yersinia enterolytica* and *Vibrio cholerae*. As part of a programme to formulate foods to aid the control of diarrhoel diseases, an improved 'Ogi' named 'Dogik' was developed using this lactic starter.

(ii) Low-cost food processing method in Africa

Food preservation is a major problem contributing to the food crisis in Africa. The costs and infrastructural requirements of many advanced food preservation methods such as refrigerating, freezing, canning and irradiation which are common in industralized countries, greatly reduced their application in the developed world (Cooke et *al*, 1987). The inhibitory effects of the lactic acid bacteria, which are used in the fermentation production of many foods in Africa, have made this technology a low-cost means of preserving food in Africa. (Gibbs, 1987).

The preservative role of lactic acid fermentation technology has been confirmed in the following products:

The preservative role of lactic acid fermentation technology has been confirmed in some cereal products, including Nigerian "Ogi" and the Kenyan "Uji" (Akinrele, 1970; Mbugua, 1981).

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Dirar (1978) reported local findings that where lactic fermentation in Sudan is suppressed in very hot climates, coliform bacteria such as *Aerobacter aerogenes* and *Klebsiella pneumonia* develop in fermented milk yielding frothing products with low acidity. The growth of these spoilage organisms is however, not detected when milk products are produced through lactic fermentation processes.

(iii) Nutritional benefits.

Lactic acid fermentation technology has been found to be a very effective way by which Africans for centuries have improved their food raw materials to make them palatable and safe for consumption (Oyewole, 1997).

Lactic fermentation improves the nutritional status of food in the following basic ways:

Investigations by workers in Nigeria (Oyewole and Odunfa, 1991; Okafor and Ejiofor, 1985;Okafor and Ejiofor, 1986) and in France (Giraud *et al*, 1992; Giraud *et al*, 1993) have confirmed that certain strains of *Lactobacillus plantarum* and *Leuconostoc mesenteriodes* isolated from cassava have the ability to produce linamarase enzymes useful for hydrolyzing the potentially toxic components of cassava.

Available information shows that lactic acid bacteria have little or no effect on the viscosity of fermented porridge from sorghum. (Westby and Gallat, 1991) but effected an increase in the viscosity of fermented maize (Banigo and Muller, 1972; Adeyemi and Beckley, 1986). While microbial exoenzymes from the fermentation can have a thinning effect on the

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viscosity due to the medium toward the iso-electric point of porridge, proteins may induce a neutralizing effect on the viscosity (Lorri, 1993; Wanink *et al*, 1994).

Malnutrition is partly due to non-availability of food, low energy, nutrient density and low bioavailability of nutrients in the available foods (Ljungqvist *et al*, 1981). The presence of some anti-nutritional factors such as phytic acid, tannins and polyphenols in some cereals used as weaning foods is known to be responsible for the low availability of proteins (Maclean *et al*, 1980) and iron (Gilooly *et al*, 1984). Household lactic acid fermentation of cereals has been found to effectively reduce the amount of phytic acid, polyphenols and tannins and improved protein availability in sorghum and millet (Chavan *et al*; 1988; Khetarpaul and Chauhan, 1988),and minerals availability. (Khetarpaul and Chauhan, 1990) Lactic acid production of cereal based weaning foods in Africa has been found to improve the nutritional quality of these products by either decreasing the number of inhibitors or releasing the nutrients for absorption (Svanberg and Lorri, 1991).

2.4 *Lactobacillus plantarum*

Among the lactic acid bacteria, there has been much interest in *Lactobacillus plantarum* due to its potential application as starter culture for the fermentation of vegetables, meat and fish products (Mackay and Baldwin, 1990).

Lactobacillus plantarum is one of the widely used lactobacilli in food fermentation and several studies have identified them in significant numbers

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in several indigenous fermented African foods (Okafor 1977; Ngaba and Lee 1979; Oyewole and Odunfa 1990, Amoa-Awua *et al*; 1996).

The presence of *L. plantarum* has been reported in numerous traditional lactic fermented products that have been spontaneously processed (Johansson, 1995) and are also frequently used as starter cultures in the food and feed industry for the production of a wide variety of products (Mackay and Baldwin, 1990). Both traditional and non-traditional products in which *L. plantarum* has been isolated in high numbers or is used as starter cultures include cassava, ogi- a fermented maize product olives, legumes, cereals, sausages and sour dough (Gbedemah and Awafo ,1990; Vescovo *et al* 1993; Hansen and Hansen, 1994; Amoa -Awua *et al*, 1996).

Randler and Weiss (1986) suggested that the wide distribution of *L. plantarum* which has been isolated from most traditional habitats of lactobacilli such as vegetables and other plants materials, fish, meat and various indigenous foods as well as from human and animal mucous membranes, might partly be due to the fact that it is less fastidious than most other lactobacilli in its requirements of amino acids and vitamins. It is also able to ferment a broad spectrum of carbohydrates enabling it to grow in many different niches and is known for its extremely heterogeneous phenotype (Johansson, 1995).

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2.5 Antimicrobial components from Lactic acid bacteria

The antimicrobial activities of lactic acid bacteria have long been known and recognized as important in food fermentation, food preservation and intestinal ecology.

Lactic acid bacteria produce a variety of antagonistic factors that include metabolic end products, antibiotic-like substances and bactericidal products termed bacteriocins. The range of inhibitory activity of bacteriocins of lactic acid bacteria can be either narrow, inhibiting only those strains that are closely related to the producer organism or wide, inhibiting a diverse group of Gram-positive microorganisms.

2.5.1 Non - peptide inhibitors

Acetic and lactic acids are among the inhibitors produced by lactic acid bacteria. Acetic acid is generally a more effective inhibitor than lactic acid. (Gilliland, 1985; Doores, 1990).

Organic acids play a very important role. However, they work in concert with other inhibitors to preserve foods. Addition of lactic cultures (*Lactococcus, Lactobacillus, Leuconostoc and Pediococcus*) or their extracts to various food products including milk, meat, egg and seafood has been shown to increase shelf - life (Gilliland, and Ewell, 1983; Brenner and Statham,1983). Inhibition was attributed to a combination of organic acid, hydrogen peroxide and inhibitory substances (Gilliland, 1985).

Lactobacillus species also produce large amounts of hydrogen peroxide through pyruvate, L-lactase oxidase, NAD-independent D-lactate

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dehydrogenase, NADH oxidase (Daeschel, 1989). The compound accumulates because Lactobacillus does not produce catalase. Diacetyl (2,3-butanedione) is produced by the citrate-fermenting lactic acid bacteria, *Leuconostoc cremoris,* and *Lactobacillus lactis sp.*

The compound is synthesized from pyruvate. It produces a buttery flavour in fermented dairy products and to foods in which it is added as a flavour additive (Varnam and Sutherland, 1994).

Fermentation of sugars by heterofermentative lactic acid bacteria produces 1 mol (22.41 at STP) of carbon dioxide per mole of hexose dissimilated. This can contribute to the inhibition of other organisms in two ways.

Firstly, in conjunction with residual respiration of the food tissues, it will help establish anaerobic conditions that will preclude the growth of obligate aerobes such as moulds. Secondly, an increased partial pressure of carbon dioxide has its own specific antimicrobial activity. Microorganisms vary in their sensitivity to carbon dioxide; moulds and oxidative Gram-negative bacteria are most susceptible while lactobacilli and some yeast show high tolerance. The mechanism of inhibition is not well understood but is thought to involve a combination of decreased pH, inhibition of enzymic reactions by a mass action effect and interaction with the cell membrane to disrupt solute transport (ICMSF, 1980).

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2.5.2 Peptide/Protein inhibitors - Bacteriocins

Bacteriocins are proteins or proteins complexes with bacteriocidal activity directed against species that are usually closely related to the producer bacterium (Tagg *et al*, 1976; Klaenhammer, 1988). More recently bacteriocins have been further defined across Gram- positive and Gram-negative bacterial boundaries and bacteriocins represent a large class of heterogeneous bacteria antagonists which vary considerably in molecular

weight, biochemical properties, range of sensitive hosts and mode of action (Kleanhammer, 1993).

Bacteriocins of lactic acid bacteria are potent biopreservatives and the application of these in foods can be effective in controlling the incidence of food poisoning outbreaks (Daeschel, 1989; Abee *et al*, 1995). Several types of bacteriocins from food-associated lactic acid bacteria have been identified and characterized (Nettles and Barefoot, 1993).

In lactobacilli there are many reports of the production of bacteriocins such as Lactocin 27 from *Lactobacillus helveticus* LP27 (Upreti and Hinsdill, 1975), helventicin J from *L. helveticus* 481 (Joerger and Kleanhammer, 1989), Lactacin from *L. acidophilus* 88 (Muriana and Kleanhammer, 1987), Plantacin B from *L. plantarum* NCDO 1193 (West and Warner, 1988), Sakacin A from *L. sake* L6706 (Schillinger and Lucke, 1989), Brevicin 37 from *L. brevis* B 37 (Rammelberg and Radler, 1990). Lacticin A and B have also been produced by *Lactobacillus delbruecki subsp lactis* JCM1106 and JCM 1107 and JCM 1248 (Toba *et al*, 1991).

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Lactobacillus salivarius M7 produces the new broad-spectrum bacteriocin salivaricin B that inhibits the growth of *Listeria monocytogenes, Bacillus cereus, Brochothrix thermosphacta, Enterococcus faecalis* and many lactobacilli (Brink *et al*, 1994).

2.6 Lactic antagonisms

The widespread application of lactic acid bacteria in cultured/fermented foods, their recognition as safe food grade organisms and their presence as part of human intestinal flora have facilitated the application of their antimicrobial agents such as bacteriocins and acids in foods (Muriana and Spanier, 1993).

Antagonism may result from one or more factors becoming active during fermentation. Fermentations involving yeasts (alcoholic fermentations of beers and palmwine), moulds (eg tempe), Bacilli (eg alkalination as for Dawadawa) and especially lactic acid bacteria are generally considered as safe.

Adaptation and competitive behaviour of the typical organisms serve to some extent as safeguards against pathogens and spoilage organisms. Lactic acid production resulting in acidification to pH < 4.2 constitutes a major safety factor. However recent observations confirm that several other metabolites with antimicrobial properties such as acetic acid (from heterofermentative lactic acid bacteria), hydrogen peroxide, bacteriocins etc also contribute to the safety of lactic fermented foods (Holzapfel *et al*, 1995).

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Gram-negative bacteria including pathogens are particularly inhibited by organic acids, which on account of the typical dissociation constants show strong antagonistic effects in the undissociated form. Numerous reports confirm diarrhoeal diseases as the major cause of child morbidity in developing countries: the main aetiological agents are stated to be *Escherchia coli*,(ETEC, EHEC, and EPEC strains), *Salmonella, Shigella, Campylobacter, Vibrio cholerae* and rotavirus and are often the result of feacal contamination of foods(Black et al,1989; Motarjemi et al, 1993).

The suggestion of Metchnikoff (1907) at the beginning of this century that fermented foods such as yoghurt may counteract enteropathogens, has been confirmed also from traditional lactic fermented foods.

Several researchers have demonstrated the role of lactic acid bacteria in providing microbial safety in African fermented foods. *Salmonella typhimurium* was unable to survive in lactic acid fermented sorghum based porridge during storage at 30°C for 24h (Nout *et al*, 1987). Mensah *et al*, (1990) have demonstrated that lactic acid fermentation influenced the microbial safety of Ghanaian maize porridge if the pH of the ready-treat porridge is less than 4.5. In an investigation by Lorri and Svanberg (1991), they found a growth inhibition rate of 10^3 for the pathogens *Escherichia coli* (ETEC) and *Campylobacter jejuni* after 3h and *Shigella flexneri* and *Salmonella typhimurium* after 7h when the pathogens were inoculated into fermented cereal gruels in Tanzania.

In addition Svanberg et al (1992), found that a sustained inhibition of *Staphylococcus aureus* at pH<4.0 had been supported by an additional

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factor such as bacteriocins. Antimicrobial activities against bacteria causing diarrhoea have been related to the lactic acid bacteria involved in the fermentation of Uji. Mbugua and Njenga (1991) demonstrated that the numbers of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Shigella dysentriae* decreased when inoculated into fermenting Uji, a Kenyan fermented cereal porridge, during fermentation and storage.

In a study by Olsen et al (1995), a total of 241 lactic acid bacteria belonging to *Lactobacillus plantarum, Pendiococcus pentasaceus, Lactobacillus fermentum / reuteri*, and *Lactobacillus brevis* from various processing stages of maize dough fermentation were investigated. About half of the *L. plantarum* and practically all the *L. fermentum / reuteri* investigated were shown to inhibit other Gram-positive and Gram-negative bacteria, explaining the elimination of these organisms during the initial processing stages. The antimicrobial effect was explained by the combined effect of acids, compounds sensitive to proteolytic enzymes and other compounds with antimicrobial activity with the acid production being the most important factor. These apparent strong antimicrobial activities of the dominant lactic acid bacteria could be explained by the pronounced decrease of pH from 6.5 to 3.7 within 24h of fermentation (Halm *et al*, 1993) and specific effects of compounds like organic acids, hydrogen peroxide and bacteriocins during fermentation (Piard and Desmazeaud 1991; Piard and Desmazeaud, 1992).

In a study by Hutton *et al* (1991), a combination of *Pediococcus acidilactici* plus dextrose (known as the "Wisconsin process") was used in chicken

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salad to protect against the formation of botulinal toxin. At abuse temperatures, *Pediococcus acidilactici* ferments dextrose to produce lactic acid, which lowers the pH sufficiently to prevent formation of toxin. Tanaka *et al*, (1980) showed that this process was effective in reducing the risk of botulinal toxin formation in nitrite - reduced bacon.

Dahiya and Speck (1968) found that *Lactobacillus bulgaricus* and *Lactobacillus lactis* inhibited the growth of *S. aureus*. Inhibition was due partially to the presence of hydrogen peroxide. Storage at low temperature favoured hydrogen peroxide formation with the maximum at 5 ^{0}C and pH 7.0. Juffs and Babel (1975) evaluated commercial multistrain cultures containing *Lactococcus lactis* and *Leucouostoc cremoris* added to milk at 0.5%, which contained various psychrotropic microorganisms. Inhibition was demonstrated but the extent was dependent upon type of starter, initial member and type of psychrotroph, temperature and time. The antagonist was determined to be hydrogen peroxide formation.

Reiter and Harnulv (1984); Banks et al (1986) studied how hydrogen peroxide functions in the lactoperoxidase system of raw milk. In this system, lactoperoxidase catalyses the oxidation of thiocyanate by hydrogen peroxide producing hypothiocyanite ions, higher oxyacids and short-lived oxidation products (Gaya *et al*, 1991). Potential sources of hydrogen peroxide for the reaction are the lactic acid bacteria.

Increased attention is presently paid to bacteriocins from lactic acid bacteria and their possible role in food safety assurance. Olasupo (1996) attributed

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factors contributing to the increasing number of investigations on bacteriocins as follows:

1. Safe and efficacious use of nisin during the past 30 years.
2. Recent FDA approval of nisin as a “GRAS” substance in certain applications.
3. Consumer resistance to traditional chemical preservatives.
4. Concerns over the safety of existing food preservatives such as nitrates.
5. Realization that bacteriocingenicity is not a rare occurrence within the lactic acid bacteria.
6. Feasibility of using bacteriocin production and immunity as selectable genetic markers in starter culture bacteria.
7 Availability of molecular biology tools to transfer and sequence the genetic determinants and engineer genetic variants of bacteriocins.
8. Willingness of federal funding agencies, food commodity group and food processing corporations to fund basic and applied researchers.

The potential of a bacteriocin to be an effective biopreservation in foods depends upon its ability to inhibit a wide range of food-borne pathogenic and spoilage bacteria (Muriana and Luchavisky, 1993).

A disadvantage in the use of bacteriocins is that being peptides, they may be inactivated by proteolytic enzymes present in food substrates.

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Yet despite these obvious limitations some bacteriocinogenic lactic acid bacteria have been shown to effectively inhibit the growth of pathogens.

To improve the microbiological safety of African fermented foods, the use of bacteriocin- producing lactic acid bacteria in fermentation was suggested (Olukoya *et al*, 1993).

Literature information generally indicates a low frequency (from 0.6% to 22%) of bacteriocinogenic strains foods associated with lactic acid bacteria. Olasupo et al (1994) indicates the frequency of bacteriocinogenic lactic acid bacteria in African fermented foods within the same range.

In a study by Olukoya *et al* (1993), two hundred *lactobacullus* isolates obtained from selected African fermented foods were screened for ability to produce bacteriocins and a bacteriocin-producing strain, coded strain 012 was found to produce the most promising bacteriocin. The bacteriocin was heat stable at 121°C for 15mins, active at pH 2.0 to 10.0 and active against some food-borne pathogens including *Listeria sp*, enterotoxigenic *Escherichia coli* and *Vibrio cholerae.*

A cereal-based food (Ogi and its solid form agidi) was prepared using a bacteriocin producing *Lactobacillus* strain as the starter culture with the naturally fermented ogi serving as the control. The survival of an enterotoxigenic *Escherichia coli* was investigated in the two sets of ogi. After 6hours, the viable number of *E. coli* in locally fermented ogi was 2.54×10^6 cfu/ml whereas in ogi fermented with a bacteriocin producer it was reduced, to 0.5×10^2cfu/ml.

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Comparison of shell-life of agidi prepared from the naturally fermented ogi and with those fermented with a bacteriocin producing starter culture showed that the latter had a better shelf-life (Odunfa *et al*, 1996).

A bacteriocin-producing strain of *Enterococcus faecium* NAO1 Isolated from "Wara", a fermented cow milk product from West Africa, harboured plasmids of approximately 36.3 and 23.1 kb. These heat stable bacteriocins were inactivated by proteases and a-amylase and showed antimicrobial activity against some strains of *Listeria* and *Enterococcus faecalis* (Olasupo *et al*, 1995).

The growth and survival of *Staphylococcus aureus* and *Salmonella typhimurium* were investigated during the manufacturing and ripening of raw milk Montasio cheese. Initial inoculated in the cheese milk were about 10^5cfu/ml for *S. aureus* and 10^6 cfu/ml for *S. typtimurium*. Samples of curds and cheese were taken during manufacturing and storage and analyzed for pH and microbial populations. *S. aureus* increased slightlty in number during the early period of ripening and attained a population of about 10^6 cfu/ml during the remaining period of storage but persisted through 90 days. The addition of *Lactobacillus plantarum* culture produced a marked reduction in population of the test strains in 10 days of storage. Enterotoxin A was not detected in Montasio cheese even with an *S. aureus* population of 1.1×10^7 cfu/ml. The results obtained indicated that the compound excreted by *L. plantarum* was active against *S. aureus* and *S. typhimurium*

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and was ascribed to be acid production and bacteriocin production (Stecchini *et al*, 1991).

Currently there is interest in possible use of lactic acid bacteria as biocontrol agents to ensure safety of minimally processed refrigerated foods (MPR), which are not acidified. Lactic acid bacteria in general is much more resistant to low pH than are other bacteria and because of these attributes it is not surprising that lactic acid bacteria have been proposed for use as biocontrol agents in non-fermented foods including MPR fruit and vegetable products. In the event of prolonged storage or temperature abuse of MPR for the biocontrol culture (lactic acid bacteria) should grow and prevent the growth of pathogenic microorganisms by competitive inhibition (Gombas 1989: Holzapfel *et al*, 1995; Stiles, 1996). Vescovo *et al* (1996) isolated a series of psychrotrophic lactic acid bacteria strains from fresh vegetable salad ingredients. These lactic acid bacteria strains were screened to identify cultures producing bacteriocins or forming an inhibition zone in agar diffusion test, against a variety of Gram-negative and Gram-positive pathogens. The selected strains were then used in challenge studies in salad products with *A..hydrophilia, L. monocytogenes, Salmonella typhimurium and S. aureus.* While all of the pathogens were able to grow in the salad products (with an initial inoculum around 10^5 cfu/g) at 6 days, none were detected at 6 days when co-inoculated with lactic acid bacteria cultures.

An advantage of using biocontrol cultures is that the hurdles (including production of organic acids and bacteriocins) that inhibit growth of pathogens in a food product increase as conditions (eg storage time and

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temperature) become more favourable to growth of bacteria. Biocontrol cultures will likely be product specific as growth of bacteria in plant materials may be affected by the availability of nutrients and naturally present inhibitors.

2.7 Rapid identification of microorganisms - API systems -API 50 CHL

In this work API 50 CHL was used to identify the species of lactic acid bacteria.

The API 50 CHL is a microbial identification kit for Lactobacillus species. It is based on 49 carbohydrates assimilation tests such as Glycerol, D-arabinose, Erythritol, L-arabinose, Ribose, D-xylose,L-xylose, Adonitol, β-methyl-D-xyloside, Galactose, Glucose, Fructose, Mannose, Sorbose, Rhamnose, Dulcitol, Inositol, Mannitol, Sorbitol, α-methyl-D-mannoside, 2-methyl-D-glucoside, N-acetyl glucosamine, Amygdalin, Arbutin, Esculin, Salicin, Cellobiose, Maltose, Lactose, Melibiose, Sucrose, Trehalose, Inulin, Melezitose, Raffinose, Starch, Glycogen, Xylitol, Gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, Gluconate, 2-keto-gluconate and 5-keto-gluconate.

The 50 microtubes each contains an anaerobic zone (the tube portion) for the study of fermentation and an aerobic zone (the cupule portion) for the study of oxidation or assimilation. The first tube contains no substrate and is used as a negative control. The remaining tubes contain a defined amount of dehydrated substrate belonging to the carbohydrate family and its

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derivatives (heterosides, polyalcohols, uronic acids). These substrates may be metabolised by various biochemical pathways:

- Assimilation is indicated by growth of an organism in the cupule, when the substrate is the only source of carbon present.
- Oxidation is shown by a colour change in the cupule portion and is due to the aerobic production of acid detected by a pH indicator included in the chosen medium
- Fermentation is shown by a colour change in the tube portion and is due to the anaerobic production of acid detected by a pH indicator included in the chosen medium.

A suspension is made in the medium (API 50CHL medium) with the microorganism to be tested and each tube of the strip is inoculated. During incubation, carbohydrates are fermented to acids, which produce a decrease in the pH, detected by the colour change of the indicator. The results make up the biochemical profile of the strain and are used in its identification or typing.

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2.8 The Fermentor

In this study the growth kinetics and acid production by dominant cultures was studied in a fermentor, the Biostat B fementor (Aage Christensen A/S, Valby, Denmark).

The Biostat B fermentor is a compact, high quality laboratory fermentor system especially designed for small-scale fermentation. The Biostat B fermentor consists of the following main components: compact supply unit with integrated digital measurement and control unit and culture vessels of standard working volumes of 2 litres and 5 litres. The vessels are installed in a supporting frame for comfortable handling and transport (Biostat B User Manual, 1997). The supply unit contains the thermostat system and installations required for power supply, the supply of cooling water, pressurized air and waste water removal. The culture vessel of Biostat B is made of borosilicate glass and the standard model has a height/diameter ratio of about 2:1. It is heated via a double jacket. The top plate is connected to the upper-flanged ring of the glass vessel. It contains two parts with a diameter of 19mm, two parts with a diameter 12mm and eight parts with a diameter of 6mm. Four additional tubing connectors are welded into the top-plate. The culture vessel is completely equipped with all parts necessary for the operation. It has a stirrer shaft with two 6 bladed impellers, harvest pipe, gas supply pipe, temperature probe, level of anti-foam probe, pH and pO_2 electrode, exhaust cooler and 3 storage bottles for acid, alkali and anti-foam agent.

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2.9 Batch fermentation

Batch fermentation can be considered as a "closed system" At time T = 0, the sterilized nutrient solution in the fermentor is inoculated with microorganisms and incubation is allowed to proceed under optimal physiological conditions. In the course of the entire fermentation, nothing is added, though addition of oxygen (in the form of air), anti-foam agent and acid or base to control the pH are possible. The composition of the culture medium, the biomass concentration generally changes as a result of the metabolism of the cells. After the inoculation with microorganisms and cultivation under physiological conditions, four different phases of growth are observed: 1. Lag phase, 2. Log phase, 3. Stationary phase, 4. Death phase.

During the lag phase, the microorganism adapt to their new environment. Because of transfer to a new medium several parameters will probably alter the inoculum cells: change in pH value, increase in supply of nutrients, decrease of growth inhibitors. New transport system for nutrients must be induced within the cells. By the end of the lag phase the cells have adapted to the new conditions of growth and enter now the log phase. Growth of the cells mass can then be described quantitatively as an exponential growth per unit time for yeast and bacteria (Crueger and Crueger, 1990).Bacteria grow by division; cells increase in size and divide by binary fission. Most yeast reproduce by budding: a small protrusion (bud), which is formed on the side of a cell, grows and eventually separates. Both types of reproduction result in a doubling of the population at each division (generation). When cells double at regular intervals of time, they

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are said to grow exponentially. Under ideal conditions, doubling time varies considerably from the fastest growing yeast (1 to 2h) (Lewis and Young, 1995).

Although the cells alter the medium through uptake of substrate and excretion of metabolic products, the growth rate remains constant during the log phase. Growth rate is independent of substrate concentration as long as excess substrate is present (Crueger and Crueger, 1990). The stationary phase sets in when the most important substrates for microorganisms are used and/or when toxic substances, for example, high concentrations of ethanol have been formed. In the stationary phase the growth slows down or stops completely. The biomass increases only gradually or remains constant, although the composition of the cells may change. Due to lysis new substrates are released, which then may serve as energy source for the slow growth of the survivors (Crueger and Crueger, 1990). The death phase is characteristic as the energy reserves of the cells are exhausted. The length of time between the stationary phase and death phase is dependent on the organism and the process used. In commercial processes, the fermentation is usually interrupted at the end of the log phase or before the death phase begins.

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3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Production of inoculum for cassava fermentation

Peeled cassava tubers, weighing 2kg were cut into small chunks. They were roasted (not fully cooked) on a subdued open fire for 10min. and wrapped in a piece of cloth, which had previously been used to prepare inoculum. They were placed in a small basket and left in a warm place for 2 d to ferment. The fermented cassava was used as the inoculum.

3.1.2 Processing of cassava dough into agbelima

Fermented cassava dough (Agbelima) was produced by adding about 0.8kg of the inoculum to about 30kg of peeled washed cassava tubers and grated in a cassava grater with milled rasp teeth. The grated cassava mash was collected into aluminum bowls and carefully packed into polyethylene sacks. The sacks were placed on wooden boards and weights were put on top to slowly dewater the mash during fermentation. The bagged dough was left in the open for 2 d to ferment into cassava dough - agbelima.

3.2 Sampling procedure for inoculum and fermenting dough

Samples of 500-1000g of inoculum and dough were aseptically collected into stomacher bags (Seward Medical, London England) at 0 and 48h of fermentation and taken immediately to the laboratory for analysis. For sampling, the surface and inner portions of the inoculum, a sterile spoon was used to carefully remove the surface

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layers and fermenting cassava dough samples were taken from within the dough after the surface layers had been removed aseptically.

3.2.1 Enumeration of aerobic mesophiles, yeast and lactic acid bacteria

10g of sample were added to 90ml sterile diluent containing 0.1% NaCl with pH adjusted to 7.2 and homogenized in a stomacher (Stomacher 400, Struers) for 30s at normal speed.

From appropriate ten-fold serial dilutions, the population of aerobic mesophiles was enumerated on Plate Count Agar - PCA (Difco Laboratories, USA) incubated at 30°C for 3 d. Lactic acid bacteria were enumerated on DeMan, Rogosa, Sharpe - MRS agar (Merck, Darmstadt, Germany) incubated anaerobically in an anaerobic jar with anaerocult A (Merck) at 30°C for 5 d.

Yeast counts were enumerated on Malt Agar MA (Merck Darmstadt, Germany) containing 100mg chloramphenicol (Sigma, St Louis. MO, USA) and 50mg chlorotetracycline (Sigma, St Louis, MO, USA) per litre and incubated at 25°C for 9 d.

3.2.2 Isolation of colonies for identification

All colonies totaling about 20, from a sector of the highest dilution or appropriate plate were subcultured into the corresponding broth medium and streaked on the agar substrate.

Colonies from PCA were taken into nutrient broth, MRS plates into MRS broth and MA plates into MA broth. Continual streaking onto agar plates purified the cultures.

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3.2.3 Initial characterization of isolates

Pure isolates from PCA were subcultured in nutrient broth for 24h.Then a loop full was streaked onto nutrient agar. *Bacillus* sp were recognized from initial tests comprising colony and cell morphology, Gram reaction and catalase production.

Pure isolates from MRS were subcultured in MRS medium and examined for Gram reaction, catalase production colony and cell morphology.

Gram reaction was carried out according to a modification of the method of Lillie (1928); Parry *et al*, (1983). For catalase production, a loopful of culture was mixed into a drop of 3% hydrogen peroxide on a microscope slide and observed for the production of gas bubbles to indicate production of catalase.

3.2.4 Maintenance of pure isolates

Plate Count Agar isolates were maintained on Nutrient agar slants at 4°C.

Colonies isolated from MRS agar were preserved in MRS agar stabs in small bottles and stored at 4°C and transferred monthly.

Malt Agar isolates were maintained on MA slants and stored at 4°C.

3.2.5 Identification of lactic acid bacteria species

Cell and colony morphology of isolates from MRS were examined using a phase contrast microscope (Olympus BH-2).

The species of *Lactobacilli* were identified by assaying cultures in API 50 CH gallaries (Bio Mereux, SA, France) using API 50 CHL medium (Bio Mereux, SA).

Pure isolates were cultured on MRS agar medium and incubated anaerobically for 24h at 30°C.

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All colonies were picked up with sterile swab in order to make a heavy suspension of turbidity equivalent to Mcfarland standard 2. The suspension medium was sterile distilled water.

The strips of the API were prepared by distributing about 10ml of sterile distilled water into the honeycombed wells of the API kit and the strips placed in the incubation tray.

2ml of the lactic acid bacteria suspension was inoculated into the API 50 CHL medium aseptically and homogenized. This suspension was used to inoculate the strips, using a sterile pipette. The tubes were overlaid with sterile paraffin oil and incubated aerobically at 30°C for 48h.

All tests were read after both 24h and 48h incubation.

In a semi-quantitative way: 0 was assigned to negative reactions and 100 to positive reactions of maximum intensity. Values of 30, 40 50 or 60 were given to intermediate reactions (50, 60 and 70 being considered as positive).

The results were recorded on the result sheets, which form the biochemical profile of the microorganisms tested.

The biochemical profile was used to identify the microorganisms by comparison to profiles supplied by the API kit manufacturer.

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3.3 Antimicrobial studies

The inhibitory potential of lactic acid bacteria cultures and their supernatants were investigated using the Well Assay method as described by Schillinger and Lücke (1989) and Olsen *et al* (1995).

3.3.1 Preparation of wells (Agar well diffusion assay)

The appropriate agar was poured into petri dishes (4mm depth) and left to dry for between 1 to 2 days to solidify sufficiently in order to be able to make the wells. Circular wells, 8mm in diameter, were made using sterile cork borers.

3.3.2 Preparation of indicator lawns

Indicator lawns were made up of soft agar containing 0.7% agar. The indicator lawns were prepared by adding 0.25ml of 10^{-1} dilution from an overnight culture to 10ml of MRS for lactic acid bacteria, or NA for *Bacillus spp*. or MA for yeasts.

3.3.3 Lactic acid bacteria interactions

Lactic acid bacteria isolates were cultured in MRS broth at 30°C for 20-24h and 100µl of the lactic acid bacteria cultures transferred into the wells and left to diffuse into the agar for approximately 4-5h. The wells were then overlaid with about 10ml of MRS or Nutrient or Malt extract soft agar containing the indicator strains. The plates were incubated overnight at 37°C anaerobically for lactobacilli indicators and aerobically for *Bacillus spp* and yeasts.

Inhibitory reactions were described as clear zones around the wells.

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3.3.4 Test for bacteriocin using bacteriocin indicator organism

10ml MRS broths were inoculated with *Lactobacillus plantarum* isolates and were incubated at 37°C for 24h. 100µl of the *L. plantarum* cultures were transferred separately into the wells and overlaid with 10ml of MRS soft agar (0.7% agar) containing *Lactobacillus sake*. Inhibitory reactions were monitored.

3.3.5 Test for bacteriocin using culture supernatants digested with proteolytic enzymes

10ml of MRS broth was inoculated with *L. plantarum* isolates and incubated for 24h at 37°C. Bacterial cells were removed from the broth by centrifuging the culture at 3660rpm for 15mins at 4°C using a centrifuge (Mistral 3000i). The clear supernatant was transferred into sterile centrifuge tubes and filtered using 0.20µm sterile filters (Sartarorius Minisart, Gottingen, Germany) in order to remove any intact cells. The filtrates were designated as cell free extract.

100µl of the cell free extract were digested separately with Proteinase K and Pronase E at a concentration of 1mg/ml for 2h.

The supernatants with the proteinases were tested against indicator strains of *Lactobacillus sake* and *Listeria monocytogenes* using the Agar Well Diffusion assay method and incubated at 37°C for 24h.

3.3.6 Test for acid production as antimicrobial property

The cell free extracts of *L. plantarum* isolates were neutralized by adjusting the pH of the supernatants with 0.1M NaOH to pH 6.8 ± 0.1

The unneutralized and neutralized supernatants were sterilized by passage through 0.20µm sterile filter. The unneutralized and neutralized supernatants were tested

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separately against indicator strains of *Lactobacillus sake* and *Listeria monocytogenes* using the Agar Well Diffusion Assay method.

3.3.7 Test for Hydrogen peroxide as antimicrobial property

Catalase enzyme was sterilised by filtering through 0.20µm filter (Sartorius Minisart, Gottingen Germany) before use.

100: l of the supernatants of *L. plantarum* isolates were treated with the catalase for 2h at a final concentration of 1mg/ml. The supernatants with catalase were tested against indicator strains using the Agar Well difussion Assay method and incubated at 37°C for 24h.

3.4 Growth pattern and acid production by *L. plantarum* isolates

Lactobacillus plantarum cultures were activated by two successive transfers in 10ml MRS broth and incubated at 30°C for 24h. *L. plantarum* was inoculated into the fermentation medium to the concentration of 10^6 cfu/ml. The concentration of cells in the 24h cultures was determined by counting in the Thomas counting chamber using a microscope.The exact volume for inoculation was found by microscopic count of the 24h cultures. The volume of inoculum needed was determined by the following formula: Inoculation volume

$$\text{Inoculation volume} = \frac{\text{Constant x volume of fermentor}}{\text{Count of cells in 24h culture}}$$

3.4.1 Fermentation procedure in Fermentor

The kinetics for the production of acid by 4 different isolates of *L. plantarum* responsible for antimicrobial inhibitions were investigated in batch fermentation

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studies, carried out in a fermentor (Biostat B. B. Braun, Biotech International, Germany).

All fermentations were run at a temperature of 32°C and stirred at 50 rpm, under anaerobic conditions. The substrate used for the *L. plantarum* isolates was sterile agbelima irradiated using 15kG of ionizing radiation at Ghana Atomic Energy Commission, Kwabenya, Accra.

The pH of the medium was not controlled during the batch culture fermentation. Each fermentation was carried out for a minimum of 30h and samples of 20ml were taken at 2-3h intervals for analysis.

For each sample, the following analyses were carried out: pH, Titratable acidity and Plate count

3.4.2 pH determination

For each sample, 20g of cassava dough was mixed with 20ml of distilled water, stirred and pH measured using a pH meter (PHM 92, LAB pH meter).

3.4.3 Titratable acidity (TTA) determination

Ten ml of sample was mixed with distilled water and made up to 250ml.

100ml of the mixture was filtered and titrated against 0.1M NaOH using 1% phenolphthalein as indicator.

The titration was carried out in duplicate. The titratable acidity was expressed as percent lactic acid using the following formula:

$$\text{TTA (v/v)} = \frac{100\text{ml} \times \text{Titre} \times 0.009008 \times 250}{10\text{ml} \times 100\text{ml}}$$

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3.4.4 Enumeration of *L. plantarum*

Plate counts were made from ten-fold dilution in Salt Peptone solution (SPS).

For fermentation with *L. plantarum* pour plates of MRS agar were used. The plates were incubated anaerobically at 30°C for 72h, using anaerobic jars and the anaerobic system, Anaerocult A (Merck).

3.5 Survival of enteric pathogens in agbelima

The ability or inability of five different pathogens to survive in agbelima under different conditions was studied using five different systems.

The enteric pathogens used were:

Salmonella typhimurium 9

Salmonella enteritidis 226

Shigella dysenteriae 2357T

Escherichia coli D2188

Vibrio cholerae C-230

The five systems were:

1. Freshly prepared cassava dough which was inoculated with pathogens and spontaneously fermented for 48h
2. 48h fermented cassava dough (pH 3.85) which was inoculated with pathogens.
3. 48h fermented cassava dough, cooked into a semi-stiff porridge and inoculated with pathogens.
4. Fermenting cassava dough of specific pHs (between 4.40 and 3.81) inoculated with pathogens.
5. Cassava dough (agbelima) with neutral pH

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3.5.1 Cultivation of pathogens

Pure cultures of each pathogen were plated out on Nutrient Agar in duplicate and incubated at 37°C for 24h. 9ml of Salt Peptone solution SPS (Difco Laboratories, USA) was poured on one nutrient agar plate with visible growth and the entire growth on the plate was washed using the sterile inoculation needle to lightly remove the colonies. The cell suspension from the plate was withdrawn using sterile syringes and the suspension transferred to the second plate. This procedure yielded about 10^{10} cfu/ml of SPS.

Total viable counts were confirmed by plating tenfold serial dilution on Nutrient agar and incubated at 37°C for 24h.

3.5.2 Enumeration of enteric pathogens inoculated into agbelima

The pathogens were inoculated into the agbelima samples at a level of 10^{6}-10^{7} cfu/g of dough.

1kg of the agbelima was weighed and 1.5ml of cell suspension of the appropriate pathogen were added to attain a concentration of about 10^{6}-10^{7} cfu/g of dough.

The agbelima dough was stirred and thoroughly mixed. Samples were taken immediately after mixing (0hr) and after 4, 8, 24 and 48h and the population of surviving pathogens enumerated by spread plate on Statens Serum Institut Agar (SSI).

10g of each sample was weighed into stomacher bags, 90ml SPS added and homogenized in a stomacher for 30s at normal speed to obtain the 10^{-1} dilutions which were subsequently serially diluted using SPS.

0.1 ml of each serial dilution was inoculated onto Statens Serum Institut agar (SSI) by spread plate and incubated at 37°C for 24h. The count of each pathogen in colony

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forming units per gram was obtained by examining the plates under high magnification.

Salmonella typhimurium 9 appeared as black colonies, whilst *Salmonella enteritidis* 226 appeared as black colonies with cream outline. *Vibrio cholerae* C-230 appeared as cream colonies. *Escherichia coli* D2188 appeared as bright red colonies. *Shigella dysenteriae* 2357T appeared as dark reddish brown colonies.

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4. RESULTS AND DISCUSSIONS

4.1 The microbial population of traditional inoculum and fermenting cassava dough (agbelima)

The enumeration of the micropopulation of the roasted inoculum, 0hr and 48h fermenting cassava dough was done on Plate Count Agar (PCA), Malt Agar (MA) and DeMan, Rogosa Sharpe Agar (MRS).

Rods, often bearing phase bright terminal spores, dominated the aerobic mesophiles, which were isolated on the PCA plates. These were Gram-positive and catalase-positive and were identified as *Bacillus spp*. In addition to these there were also Gram-positive, catalase-negative cocci, which occurred, in single or short chains but their genus was not identified.

Biochemical tests for the identification of the *Bacillus spp*. were not carried out but their colony and cell morphologies were compared to known cultures previously isolated from agbelima (Amoa-Awua and Jakobsen 1995). The most frequently occurring species were found likely to be *Bacillus subtilis*.

On MRS agar, the dominant types of cultures found in both the inoculum and agbelima samples were a variety of short, long and mostly paired Gram-positive, catalase negative rods and cocci. These anaerobically cultured Gram-positive, catalase negative rods and cocci were non-sporing and non-motile. These isolates were tentatively identified as lactic acid bacteria.

The cell morphology of cultures, which were enumerated on Malt agar showed that they were yeasts and this, was confirmed by comparing their colony and cell morphology to known cultures which had previously been isolated from agbelima (Amoa-Awua, 1996).

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The population of the microflora of the fermenting cassava dough showed significant decreases for *Bacillus spp* and increases for lactic acid bacteria and yeasts during fermentation (Table 1).

The initial population of *Bacillus spp* of about 10^6 decreased to 10^4 cfu/g whilst lactic acid bacteria increased from 10^8 to 10^9 cfu/g and yeasts from 10^5 to 10^8 cfu/g.

Table 1 Microbial population in cfu/g of fermenting cassava dough (agbelima).

Microflora	Fermenting Cassava Dough (cfu/g)	
	0h	48h
Bacillus sp	1.1×10^6	9.1×10^4
Lactic acid bacteria	1.4×10^8	3.2×10^9
Yeasts	3.7×10^5	1.5×10^6

4.2 Identification of the lactic acid bacteria

Isolates from MRS considered to be lactic acid bacteria were examined using API 50CHL. The most dominant rods fermented glycerol, L-arabinose, ribose, galactose, D-glucose, D-frutose, D-mannose, mannitol, sorbitol, α-methyl-D-mannoside, N-acetyl glucosamine, amygdaline, β-gentiobiose,D-arabinose, gluconate and D-turnose. By comparing with data in tables provided in the API 50CHL kit these cultures were identified as *Lactobacillus plantarum*.

Other dominant species of lactic acid bacteria identified were *Lactobacillus fermentum* and *Lactobacillus brevis* (Table 2).

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Table 2 **Percentage of identified species of lactic acid bacteria, which fermented the various carbohydrates in the API 50CHL kit.**

Carbohydrate	***Lactobacillus plantarum***	***Lactobacillus fermentum***	***Lactobacillus brevis***
Glycerol	0	0	0
Erythritol	0	0	0
D-arabinose	0	0	0
L-arabinose	100	100	95
Ribose	100	100	100
D-xylose	100	100	0
L-xylose	0	5	0
Adonitol	0	0	0
β-methly-xyloside	0	0	0
Galactose	100	100	0
D-glucose	100	100	95
D-fructose	100	100	66
D-mannose	100	100	0
L-sorbose	5	0	0
Rhamnose	5	0	0
Dulcitol	0	0	0
Inositol	0	0	0
Mannitol	100	0	0
Sorbitol	100	0	0
α methyl-D-mannoside	0	0	0
α methyl-D-glucoside	100	0	0
N-acetyl glucosamine	100	0	0

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Carbohydrate(cont)	*Lactobacillus plantarum*	*Lactobacillus fermentum*	*Lactobacillus brevis*
Amygdaline	100	0	0
Arbutin	95	0	0
Esculin	100	100	0
Salicin	95	0	0
Cellobiose	100	0	0
Maltose	100	100	65
Lactose	100	100	0
Melibiose	100	95	0
Saccharose	100	100	0
Trehalose	100	100	0
Inulin	0	0	0
Melezitose	100	0	0
D-raffinose	95	100	0
Amidon	0	0	0
Glycogen	0	0	0
Xylitol	0	0	0
β-gentiobiose	65	0	0
D-turnose	0	0	0
D-lyxose	0	0	0
D-tagatose	0	0	0
D-fucose	0	0	0
L-fucose	0	0	0
D-arabitol	0	0	0
L-arabitol	0	0	0
Gluconate	10	5	10
2 - cetogluconate	0	0	0
5 - cetogluconate	0	0	0

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The results of the examination of the microbial population of the roasted inoculum and agbelima confirmed that *Bacillus* species, lactic acid bacteria and yeasts are all involved in the fermentation of agbelima. Previous work by Amoa-Awua (1996) has shown that the role of the *Bacillus* species is to produce cellulase enzymes, which hydrolyze the cellulose present in the cassava roots bringing about a breakdown in the texture of the cassava dough to produce a smooth-textured dough.

The lactic acid bacteria are responsible for the souring/acidification of the dough whilst the yeasts contribute to the production of aroma compounds and in some species also hydrolyze cassava texture. The dominant species of *Bacillus* reported was *Bacillus subtilis* and the most frequently isolated *Bacillus* species in this work was found likely to be *Bacillus subtilis*.

The dominating lactic acid bacteria found in the present work were *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus fermentum*. Amoa-Awua *et al* (1996) reported the dominance of *L. plantarum, L. brevis* and *Leuconostoc mesenteriodes* in spontaneously fermenting cassava dough and Ngaba and Lee (1979), *L. plantarum*, *Leuconostoc mesenteriodes* and *Streptococci sp.* in spontaneously fermenting cassava dough.

The dominance of *L. plantarum* in the souring of cassava found in the present work is in agreement with findings of other workers. *Leuconostoc mesenteriodes* though widely reported, was not isolated in this work and *L. fermentum* found in this work was only isolated in low numbers by Amoa-Awua *et al* (1996).

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4.3 Antimicrobial interactions

4.3.1 Antimicrobial interaction between isolates of lactic acid bacteria.

Screening of lactic acid bacteria using colony and cell morphologies made it possible to differentiate between lactic acid bacteria at the start and end of fermentation of cassava dough into agbelima.

Lactic acid bacteria which were present at the end of the 48h fermentation, were tested against lactic acid bacteria present at the start of fermentation (0h). Lactic acid bacteria isolated from fermentation after 48h were used as the test isolates and isolates from the start of fermentation were the indicator isolates.

Widespread inhibitory reactions were observed between lactic acid bacteria isolates at 0 and 48h of agbelima fermentation (Table 3). Nearly all lactic acid bacteria isolated at the end of agbelima fermentation inhibited those, which were present at the start of fermentation. This was not surprising because those isolated at the end of fermentation were cultures, which were able to survive microbial competition throughout the fermentation process. None of the test isolates inhibited LAB E_2 and six out of the 10 test isolates inhibited LAB E6. Two of the test isolates LAB 6 and LAB 9 showed very strong inhibition against three of the indicator strains namely LAB E3, LAB E4 and LAB E7 LAB 6 in addition strongly inhibited LAB E9 and LAB E10. These inhibitory reactions were seen as clear zones of about 5-10mm around the wells created.

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Table 3 The inhibitory effect within the lactic acid bacteria population of cassava dough (agbelima)

Test isolates (Lactic acid bacteria isolated at the end of fermentation)	Indicator isolates (Lactic acid bacteria isolated at the start of fermentation)									
	LAB E_1	LAB E_2	LAB E_3	LAB E_4	LAB E_5	LAB E_6	LAB E_7	LAB E_8	LAB E_9	LAB E_{10}
LAB 1	+	–	+	+	+	+	+	+	+	+
LAB 2	+	–	+	+	+	–	+	+	+	+
LAB 3	+	–	+	+	+	+	+	+	+	+
LAB 4	+	–	+	+	+	+	+	+	+	+
LAB 5	+	–	+	+	+	+	+	+	+	+
LAB 6	+	–	++	+	+	+	++	+	++	++
LAB 7	+	–	+	+	+	–	+	+	+	+
LAB 8	+	–	+	+	+	–	+	+	+	+
LAB 9	+	–	++	++	+	+	++	+	+	+
LAB 10	+	–	+	+	+	–	+	+	+	+

—: *No inhibition*
+: *>5mm inhibition zone*
++: *8-10mm inhibition zone (very strong inhibition)*

The results of the antimicrobial interactions between lactic acid bacteria from agbelima demonstrated occurrence of antimicrobial activity among closely related organisms. Lactic acid bacteria isolated after 48h of fermentation showed stronger inhibition against lactic acid bacteria from other stages of the fermentation. This is in agreement with a similar study by Olsen et al.(1995) on the antimicrobial activity of lactic acid bacteria from fermented maize (Kenkey). Lactic acid bacteria isolates from an advanced stage of the fermentation process showed strong inhibition against isolates from earlier processing stages. It was also

suggested that the inhibitory effect varied for isolates belonging to the same species or closely related group of lactic acid bacteria.

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4.3.2 Antimicrobial interactions between lactic acid bacteria and *Bacillus* species

All the 20 isolates of lactic acid bacteria (10 from 0h and 10 from 48h of fermentation were tested against 10 isolates of *Bacillus* spp for antimicrobial activity. In comparison to the interactions within the lactic acid bacteria, there was much less interaction between lactic acid bacteria and *Bacillus* isolates (Table 4).

Of the 10 *Bacillus* isolates, 3 were inhibited by almost all the 20 lactic acid bacteria isolates. 2 *Bacillus* isolates were not inhibited by any of the lactic acid bacteria test isolates. Only LAB 6, LAB 7, LAB E6 and LAB E7 inhibited *Bacillus* isolate B1. LAB 4 and LAB E4 inhibited bacillus isolate, B3, whilst LAB 2, LAB 10, LAB E2 and LAB E10 inhibited B4. LAB 9, LAB 10, LAB E9 and LAB E10 inhibited B7. LAB 9 and LAB E10 inhibited B10.

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Table 4 The inhibitory effects of lactic acid bacteria against *Bacillus* species

Test isolates (Lactic acid bacteria)	Indicator isolates (*Bacillus* species)									
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
LAB 1	–	–	–	–	+	–	–	–	+	–
LAB 2	–	–	–	+	+	+	–	–	–	–
LAB 3	–	–	–	–	+	+	–	–	–	–
LAB 4	–	–	+	–	–	+	–	–	+	–
LAB 5	–	–	–	–	+	+	–	–	+	–
LAB 6	+ +	–	–	–	+	+ +	–	–	+	–
LAB 7	+	–	–	–	+	+	–	–	+	–
LAB 8	–	–	–	–	+	+	–	–	+	–
LAB 9	–	–	–	–	+	+ +	+	–	+	+
LAB 10	–	–	–	+	–	+	+	–	+	–
LAB E_1	–	–	–	–	+	–	–	–	+	–
LAB E_2	–	–	–	+	+	+	–	–	–	–
LAB E_3	–	–	–	–	+	+	–	–	–	–
LAB E_4	–	–	+	–	–	+	–	–	+	–
LAB E_5	–	–	–	–	+	+	–	–	+	–
LAB E_6	+ +	–	–	–	+	+ +	–	–	+	–
LAB E_7	+	–	–	–	+	+	–	–	+	–
LAB E_8	–	–	–	–	+	+	–	–	+	–
LAB E_9	–	–	–	–	+	+ +	+	–	+	+
LAB E_{10}	–	–	–	+	–	+	+	–	+	–

—: *No inhibition*
+: *> 5mm inhibition zone*
++: *8-10mm inhibition zone (Very strong inhibition)*

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4.3.3 Antimicrobial interaction between lactic acid bacteria isolates and yeast.

All the 20 isolates of lactic acid bacteria (10 from 0h LAB E1-LAB E10 and 10 from 48h LAB1-LAB10 of fermentation) were tested against Yeast isolates from agbelima. None of the lactic acid bacteria isolates was able to inhibit any of the yeast (Table 5). This could be explained by the fact that in their natural environment lactic acid bacteria and yeasts live in a symbiotic association.

In some studies carried out yeasts often show a stimulating effect on the growth of lactic acid bacteria. Nout (1991) suggested that the development of lactic acid bacteria is stimulated by the presence of yeasts, which provides soluble nitrogen compounds and B-vitamins. According to Leroi and Pidoux (1993) yeasts metabolites, eg, CO_2, pyruvate, propionate acetate and succinate stimulate the growth as well as the lactic acid production of lactic acid bacteria in sugary kefir grains.

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Table 5 **The inhibitory effects of lactic acid bacteria against yeasts isolated from cassava dough (agbelima).**

Test isolates (Lactic acid bacteria)	Indicator isolates (Yeasts)		
	Y1	Y2	Y4
LAB I	–	–	–
LAB 2	–	–	–
LAB 3	–	–	–
LAB 4	–	–	–
LAB 5	–	–	–
LAB 6	–	–	–
LAB 7	–	–	–
LAB 8	–	–	–
LAB 9	–	–	–
LAB 10	–	–	–
LAB E_1	–	–	–
LAB E_2	–	–	–
LAB E_3	–	–	–
LAB E_4	–	–	–
LAB E_5	–	–	–
LAB E_6	–	–	–
LAB E_7	–	–	–
LAB E_8	–	–	–
LAB E_9	–	–	–
LAB E_{10}	–	–	–

–: *No inhibition*

The acidic environment created by lactobacilli is favourable for yeast growth, while the alcohol produced by the yeasts, the acids produced by the bacteria and the anaerobiosis induced by the fermentation, all contribute to the suppression of some

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other microorganisms present in the system. Furthermore, some yeasts species are able to resist the antibiotic substances produced by lactic acid bacteria. Those environmental conditions and the ecological system would lead to a natural selection of the resistant species of yeast. This explains why various fermenting systems involve some species rather than others (Boraam *et al*, 1993). In a study by Vollmar and Meuser(1992), they showed that yeasts multiplication was associated with an increase in acid formation of particular lactic acid bacteria for fermented products. Jerpersen *et al* (1994) confirmed that the presence of *Saccharomyces cerevisiae* and *Candida krusei* in maize dough stimulates the growth and acid production of lactic acid bacteria. More specifically Hounhouigan *et al* (1997), found that fast growth of *L. fermentum* was noticed when it was used in combination with *Candida krusei* and that *C. krusei* showed better growth performance than *Saccharomyces cerevisiae* when it was used in combination with any of the lactobacilli tested. It has also been observed that lactic acid bacteria that survive the fermentation processes usually do this in association with some yeast - *Saccharomyces sp* and *Candida sp* (Faid *et al*, 1993; Sanni, 1993; Oyewole and Aibor, 1992).

4.3.4 Antimicrobial interaction between *Bacillus* species and yeast isolates

Of the 10 *Bacillus* isolates tested against yeasts isolates isolated from agbelima, 3 isolates namely B1, B4 and B5; showed inhibitory reaction against the yeasts. However *Bacillus* sp, B6, inhibited only one yeast isolate, Y4 (Table 6).

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Table 6 **The inhibitory effects of *Bacillus* species against yeast isolated from cassava dough (agbelima)**

Test isolates (Bacillus species)	Indicator isolates (Yeasts)		
	Y1	Y2	Y4
B1	+	+	–
B2	–	–	–
B3	–	–	–
B4	+	+	+
B5	+	+	+
B6	–	–	+
B7	–	–	–
B8	–	–	–
B9	–	–	–
B10	–	–	–

—: *No inhibition zone*
+: *> 5mm-inhibition zone*

These inhibitions observed in the few instances suggest that those *Bacillus* isolates produced some substances, which prevented the growth of the yeast isolates. Fogarty et al, (1974) reported that *Bacillus* spp. represent one of the most important groups of bacteria which produce a range of industrially important enzymes as well as a number of cyclic or linear polypeptide antibiotics. Enzymes of commercial significance produced by the genus include amylases, proteases, 2-glucanases and isomerases. Other enzymes produced by the species include cellulases, hemicellulases, pectinases including polygalacturonase lyase, penicillinases including 2-lactanase and penicillin amidase, K-amylase, 2-amylase, amyloglucosidase, nucleases, cell wall lytic enzymes and acid, neutral and alkaline proteases,

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4.3.5 Antimicrobial interaction between *Lactobacillus plantarum* and other species of lactic acid bacteria .

The inhibitory effect of the dominant lactic acid bacteria isolated from agbelima, *Lactobacillus plantarum* was tested against other identified lactic acid bacteria isolated from agbelima; *L. fermentum* and *L. brevis.* All 10 isolates of *L. plantarum* showed inhibitory reaction against an isolate of *L. fermentum* and *L. brevis* with 4 isolates of *L. plantarum* showing strong antagonistic reaction against *L. fermentum* (Table 7). These inhibitory reactions were established as clear zones of about 8-10mm. However only one *L. plantarum* isolate out of the 10 strongly inhibited *L. brevis*.

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Table 7 **The inhibitory effect of *Lactobacillus plantarum* against other species of lactic acid bacteria isolated from cassava dough (agbelima).**

Test isolates (*L. plantarum*)	Indicator isolates	
	L. fermentum	***L. brevis***
L. plantarum LP 1	++	++
L. plantarum LP 2	+	–
L. plantarum LP 3	+	+
L. plantarum LP 5	+	–
L. plantarum LP 6	+	+
L. plantarum LP 11	++	+
L. plantarum B2 - 17	+	+
L. plantarum B2 - 14	+	+
L. plantarum B2 - 4	+	+
L. plantarum B0 - 1	+	+
L. plantarum LAB 6	++	++
L. plantarum LAB 9	++	++

—: *No inhibition*
+: *> 5mm inhibition zone*
++: *8-10mm inhibition zone*

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4.3.6 Antimicrobial interaction between *Lactobacillus plantarum* and *Bacillus* species

There was very little interaction between *L. plantarum* and *Bacillus* species. *L. plantarum* isolate LP 2, inhibited *Bacillus* isolates B3 and B5. *L. plantarum* LP 3 inhibited only *Bacillus* isolate B5. *L. plantarum* isolates LP11, LAB 6 and LAB 9 strongly inhibited *Bacillus* isolate B2. *L. plantarum* LAB 9 also inhibited Bacillus isolates B6, B7, B9 and B10 (Table 8).

Table 8 The inhibitory effect of *Lactobacillus plantarum* against *Bacillus* species all isolated from cassava dough - agbelima.

Test isolates	Indicator isolates									
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
L. plantarum LP 1	–	–	–	–	–	–	–	–	–	–
L. plantarum LP 2	–	–	+	–	+	–	–	–	–	–
L. plantarum LP 3	–	–	–	–	+	–	–	–	–	–
L. plantarum LP 5	–	–	–	–	–	–	–	–	–	–
L. plantarum LP 6	–	–	–	–	–	–	–	–	–	–
L. plantarum LP 11	–	++	–	–	+	+	–	–	–	+
L. plantarum B2-17	–	+	–	–	–	–	–	–	–	–
L. plantarum B2-14	–	–	–	–	–	–	–	–	–	–
L. plantarum R2-4	–	–	–	–	–	–	–	–	–	–
L. plantarum BO-1	–	–	–	–	–	–	–	–	–	–
L. plantarum LAB 6	–	++	–	–	–	+	–	–	–	–
L. plantarum LAB 9	–	++	–	–	–	+	+	–	+	+

–: *No inhibition zone*
+: *> 5mm inhibition zone*
++: *8-10mm inhibition zone*

From this study almost all the *L. plantarum* isolates used showed strong antimicrobial activity against lactic acid bacteria as well as some Bacillus strains. These

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antimicrobial effects explain the apparent reduction in numbers of some of the microflora during the fermentation process. The inhibitory effects of *L. plantarum* on other microorganisms during fermentation agree with a similar restriction of growth of aerobic organisms by *L. plantarum* in fermenting high moisture corn (Flores-Galarza *et al*, 1985). Inoculation of cooked beans for tempeh production (an Indonesian fermented product prepared by fermenting soybeans with *Rhizopus oligosporus*) with *L. plantarum* resulted in a lower pH, which controlled the proliferation of undesirable microorganisms (Ashenafiand Busse, 1991). In a similar study by Ashenafi and Busse, (1991), counts of *Bacillus cereus* reached ca 10^8 cfu/g within 40h in fermenting unacidified horsebean tempeh and resulted in complete spoilage of the product. Inoculation of these unacidified beans with *Lactobacillus plantarum* decreases the final count of *B. cereus* by 2 log units. Inoculation of acidified beans with *L. plantarum* resulted in markedly lower growth rate of *B. cereus*.

This present study is also in agreement with a study in which inoculation of unacidified horsebean, pea, chickenpea and soybean tempeh with *Lactobacillus plantarum* resulted in a complete inhibition of *Listeria monocytogenes* (Ashenafi, 1991).

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4.3.7 Antimicrobial interaction between *Lactobacillus plantarum* and yeast isolates.

The inhibitory effect of the dominant lactic acid bacteria isolated from agbelima, *Lactobacillus plantarum* was tested against yeast isolates. None of the twelve *L. plantarum* isolates exhibited any inhibitory activity against the three yeast isolates (Table 9).

This observation could also be possibly explained as a result of mutual associations, which exist between *L. plantarum* and the yeasts.

Table 9 The inhibitory effects of *L. plantarum* against yeasts isolated from cassava dough (agbelima).

Test isolates (*L. plantarum*)	Indicator isolates (Yeasts)		
	Y1	Y2	Y4
L. plantarum LP 1	–	–	–
L. plantarum LP 2	–	–	–
L. plantarum LP 3	–	–	–
L. plantarum LP 5	–	–	–
L. plantarum LP 6	–	–	–
L. plantarum LP 11	–	–	–
L. plantarum B2-17	–	–	–
L. plantarum B2-14	–	–	–
L. plantarum R2-4	–	–	–
L. plantarum BO-1	–	–	–
L. plantarum LAB 6	–	–	–
L. plantarum LAB 9	–	–	–

–: *No inhibition zone*

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4.3.8 Elucidation of inhibitory mechanism

The 10 selected isolates of *L. plantarum*, which were the dominant lactic acid bacteria at the end of agbelima fermentation, were tested against *Lactobacillus sake* and *Listeria monocytogenes*, which have been reported to be inhibited by *L. plantarum* bacteriocins. All the 10 *L. plantarum* isolates showed inhibitory reactions against *L. sake* and *Listeria monocytogenes*. Five *L. plantarum* isolates out of the 10 isolates showed very strong antagonistic activity against *L. sake*. Four *L. plantarum* isolates also indicated very strong inhibition against *Listeria monocytogenes* (Table 10). When *L. fermentum* isolates and *L. brevis* isolates were tested against *L.sake* and *Listeria monocytogenes* the inhibition was weak (Table 11).

Table 10 The inhibitory effects of *L. plantarum* against *L. sake* and *Listeria monocytogenes* (bacteriocin indicator strains)

Test isolates	Indicator isolates	
	L. sake	***Listeria monocytogenes***
L. plantarum LP 1	++	++
L. plantarum LP 2	+	+
L. plantarum LP 3	+	–
L. plantarum LP 5	+	+
L. plantarum LP 6	++	+
L. plantarum LP 11	++	++
L. plantarum B2-17	+	+
L. plantarum B2-14	+	+
L. plantarum R2-4	+	+
L. plantarum BO-1	+	+
L. plantarum LAB 6	++	++
L. plantarum LAB 9	++	++

+: *> 5mm inhibition zone*
++: *8-10mm inhibition zone*

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Table 11 **The inhibitory effects of *L. fermentum* and *L. brevis* against *L. sake* and *Listeria monocytogenes* (bacteriocin indicator strains)**

Test isolates	Indicator isolates	
	L. sake	***Listeria monocytogenes***
L. fermentum LAB E3	+w	–
L. fermentum LAB E4	–	–
L. fermentum LAB E5	–	+w
L. fermentum LAB E6	–	–
L. fermentum LAB E7	+w	+w
L. brevis LAB E10	–	+w

–: *No inhibition zone*
+*w* : *Very weak inhibition zone.*

The results of this study indicated the possible production of bacteriocin or other antimicrobial compounds, acids, hydrogen peroxide etc, effective against the indicator organisms.

The mechanism of inhibition by the *L. plantarum* isolates was examined using culture supernatants. The indicator bacteria used were Gram-positive, *L. sake* and Gram-negative, *Listeria monocytogenes*. All the supernatants of the *L. plantarum* used showed pronounced inhibition against both *L. sake* and *Listeria monocytogenes* the indicator bacteria. However after neutralization of the culture supernatants no such inhibitory reaction was observed for any of the cultures. This clearly demonstrated that the inhibitory action of *L. plantarum* isolates against the bacteriocin indicator strains was due to acids produced by *L. plantarum* rather than the production of bacteriocins(Table 12).

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Table 12 **The effect of pH neutralization on the inhibitory effect of supernatants of *L. plantarum* against *L. sake* and *Listeria monocytogenes***

Culture supernatant of *L. plantarum* isolates	***Bacteriocin indicator strains***	
Unneutralized culture supernatant	**Lactobacillus sake**	**Listeria monocytogenes**
L. plantarum LP1	+	+
L. plantarum LP 2	+	+
L. plantarum LP 3	+	+
L. plantarum LP 5	+	+
L. plantarum LP 6	+	+
L. plantarum LP 11	+	+
L. plantarum B2-17	+	+
L. plantarum B2-14	+	+
L. plantarum B0-1	+	+
L. plantarum LAB 6	+	+
L. plantarum LAB 9	+	+
Neutralized culture supernatant		
L. plantarum LP1	-	-
L. plantarum LP 2	-	-
L. plantarum LP 3	-	-
L. plantarum LP 5	-	-
L. plantarum LP 6	-	-
L. plantarum LP 11	-	-
L. plantarum B2-17	-	-
L. plantarum B2-14	-	-
L. plantarum B0-1	-	-
L. plantarum LAB 6	-	-
L. plantarum LAB 9	-	-

The addition of catalase and proteolytic enzymes did influence the inhibitory activity of the supernatants. These suggest that hydrogen peroxide and bacteriocins seem not to be involved.

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Thus for all the *L. plantarum* strains, the inhibitory effect was eliminated by pH neutralization. For all strains tested, the final pH of the MRS broth was in the range of 3.8-4.08.

From this present study, the mechanisms of these antimicrobial effects could be attributed to only acid production by the lactic acid bacteria. Studies have shown that acetic acid and lactic acid are among the inhibitors produced by lactic acid bacteria (Gilliland, 1985; Doores,1990) and these organic acids work in concert with other inhibitors of preserved foods. In a study by Amoa-Awua et al, (1996), titratable acidity expressed as lactic acid increased from 0.31-0.38 to 0.78-0.91% (w/w) indicating that fermentation of cassava dough is a process of acidification. Sefa-Dedeh (1989), also found out that the pH of agbelima decreased from 6.54 to about 4.02 during fermentation. Despite the effect of bacteriocins not being pronounced in this study, bacteriocin-producing lactobacilli have been isolated from fermented cassava product (fufu) and used to eliminate growth of some food-borne pathogens (Odunfa *et al*, 1996).

The present investigation on the antimicrobial interaction of the various microbial species has shown that there are strong antimicrobial activities determining the composition of the microflora during cassava dough fermentation into agbelima. Screening of isolates of lactic acid bacteria from agbelima demonstrated widespread occurrence of antimicrobial interaction against closely related organisms as well as against both, Gram-positive and Gram-negative bacteria.

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4.4.0 Growth pattern and acid production of *L. plantarum* isolates in batch fermentation studies

The growth pattern and rate of acid production of different isolates of *L. plantarum* in sterile cassava dough medium was investigated since observed antimicrobial properties of isolates were solely attributed to production of acid. Four strains were selected on the basis of their pronounced antimicrobial activity against indicator strains and inoculated into cassava medium in a fermentor at a. concentration of between 10^6 10^7 cfu/ml.

4.4.1 Growth patterns of *L. plantarum* isolates

All four isolates of *L. plantarum* (LAB 9, LAB 6, LP11 and LP 3) showed similar growth patterns in 10% cassava dough medium (Fig. 3). In all cases, lag phases of the different *L. plantarum* isolates were not pronounced. The exponential phases of the isolates were observed between 2-9h. The stationary phases started after about 9h except *L. plantarum* isolate (LAB 9) which started after 6h of fermentation. The growth pattern also showed the same concentration of cells of about 10^9 cfu/ml in the stationary phases for all the *L. plantarum* isolates.

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Figure 3.

Growth pattern of *Lactobacillus plantarum* isolates during batch fermentation of cassava dough (agbelima)

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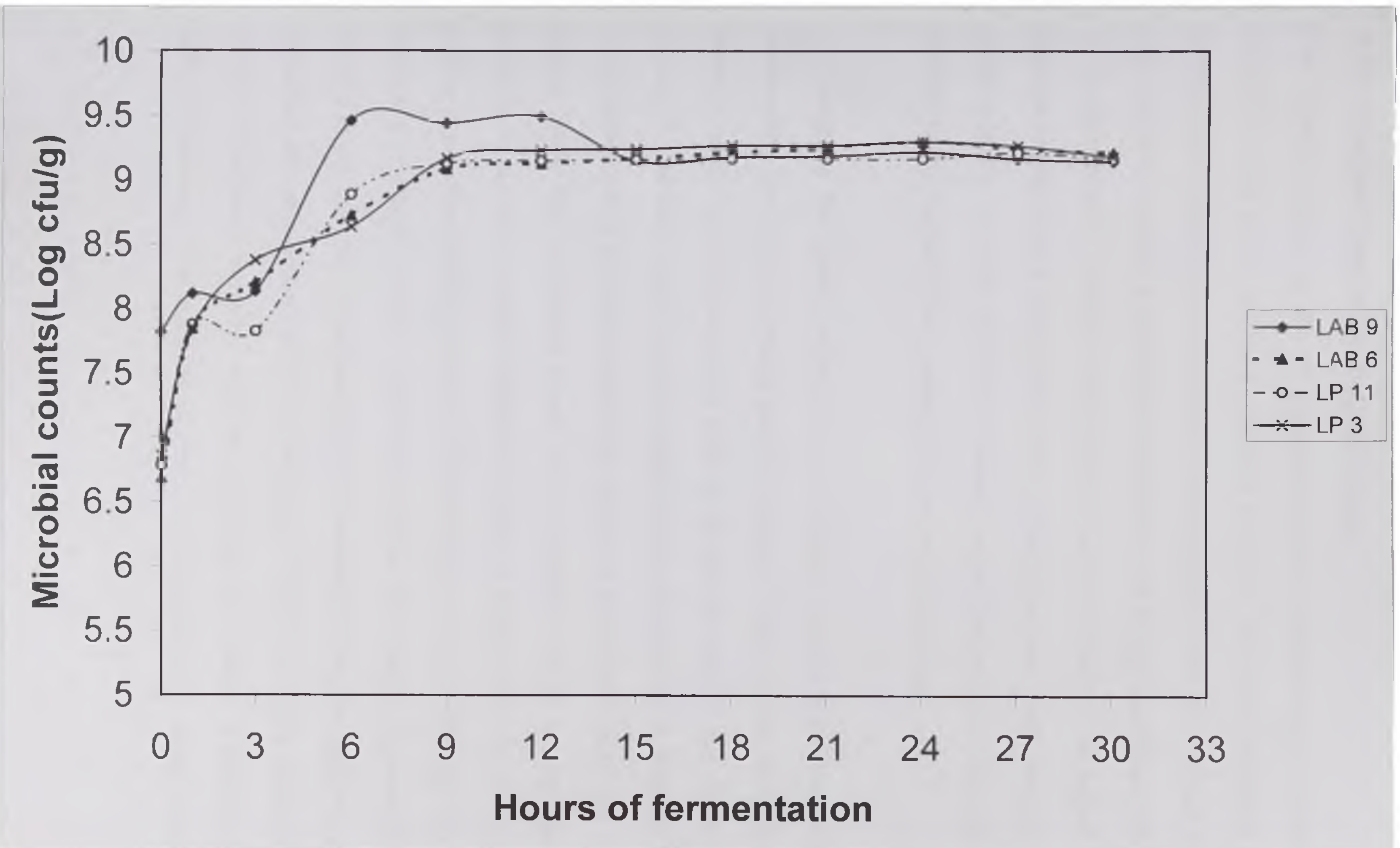

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4.4.2 Acid production of *L. plantarum* isolates

The growth patterns of the *L. plantarum* isolates corresponded with the acid production level of the isolates as depicted in Fig. 4. The acid productions of the isolates were measured in terms of the titratable acidity (TTA% v/v). All four isolates of *L. plantarum* showed a similar pattern of build up of acidity (decrease in pH) after 6h of fermentation. However acid production was observed to be fastest in *L. plantarum* isolate-LAB 9 whose antimicrobial potency has been demonstrated by the strong inhibition on other lactic acid bacteria, some *Bacillus* spp. and bacteriocin indicator microorganisms like *L. sake* and *Listeria monocytogenes*.

In considering the growth patterns of *L. plantarum* isolates, pH decreased with fermentation time whilst titratable acidity increased. The analyses with pH and titratable acidity were carried out in order to be able to describe the fermentation process. *L. plantarum* strains produce acids mainly lactic acid and the measure of pH and titratable acidity indicate therefore the growth of these microorganisms. In this present work, the exponential growth of *L. plantarum* isolate corresponded to decrease in pH and increase in titratable acidity. In most cases, the pronounced change in pH occurred after about 3h of fermentation. This was directly related to increase in numbers of the *L. plantarum* isolates. The trends observed for the changes in pH and total titratable acidity with fermentation time for agbelima are in agreement with observations made by Dziedzoave, (1996). The study observed that cassava fermentation proceeds with the production of a variety of organic acids, leading to increase in total acidity. During the course of fermentation some of these

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Figure 4.

Changes in acid production of *Lactobacillus plantarum* isolates during batch fermentation of cassava dough (agbelima)

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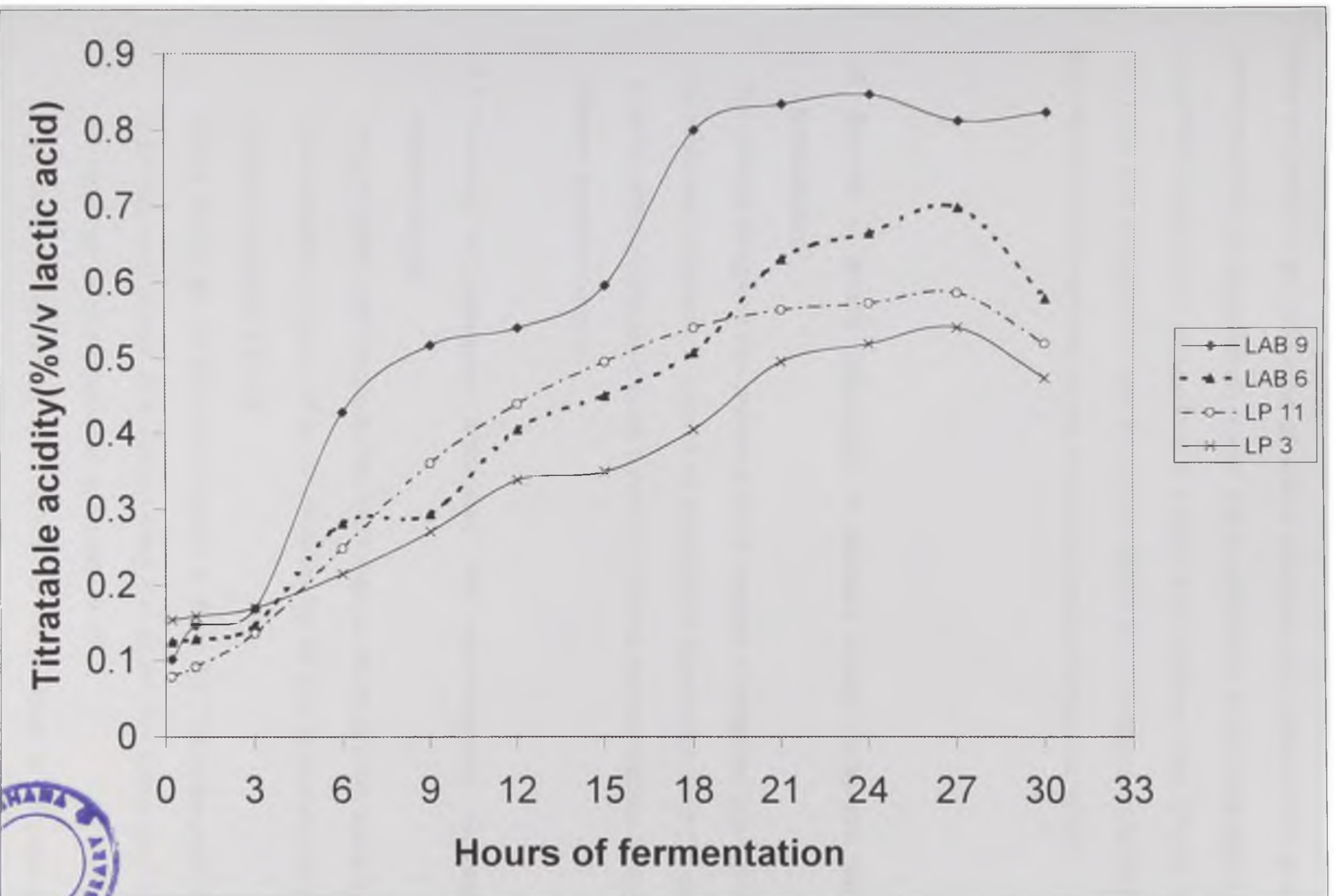

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acids are used for the production of various aldehydes and esters, which give the fermenting mash its characteristic aroma. The growth pattern in this study agrees with the growth kinetics of *L. plantarum* A 6 isolated from cassava roots (Giraud *et al*, 1991) and also in agreement with the growth pattern of *L. plantarum* NCIM 2084 in glucose medium to determine pH and antibacterial activity (Suma *et al*, 1998).

4.5 Survival of enteric pathogens in cassava dough (agbelima) during fermentation.

The general unhygienic environment in which cassava is processed into agbelima by traditional processors is a matter of public health concern therefore the extent to which different pathogens would survive in agbelima was investigated using four different agbelima systems.

4.5.1 Survival of pathogens inoculated into spontaneously fermenting cassava dough.

The pathogens were inoculated into fresh cassava dough of pH of about 6.0 at a concentration of about 10^6 to 10^7 cfu/g. During the 48h fermentation the pH dropped to between 3.8-4.15.

During the first 8h, the pH dropped rapidly to about 4.5. The subsequent drop in pH was much slower, till it reached about 4.15 at 24h. No further drop in pH was observed till fermentation was terminated at 48h.

A constant rate of decline was observed in the numbers of all pathogens inoculated into the cassava dough with the exception of *Salmonella*

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typhimurium 9 which showed a drop in the rate of decline after 24h. The change in the decline rate of *S. typhimurium* 9 coincided with the drop in the rate of acidification, that is, pH of the product.

Vibrio cholerae C-230 which seemed to be very sensitive declined at a rate of 1 log unit per hour and disappeared from the fermenting dough after 4h of inoculation.

The population of *Salmonella typhimurium* 9 was reduced by 4 log units in 8h to about 10^2 cfu/g and was not detected after 24h. *Shigella dysenteriae* 2357T and *Salmonella enteritidis* 226 showed a similar pattern of reduction in numbers and declined by 2 log units in 8h to about 10^4 cfu/g and decreased steadily till they were not detected after 24h of fermentation. *Escherichia coli* D2188 on the other hand showed a slight increase in the first 8h before it started declining in numbers and dropped to zero after 24h (Fig. 5).

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Figure 5.

Survival of enteric pathogens inoculated into spontaneously fermenting cassava dough (agbelima)

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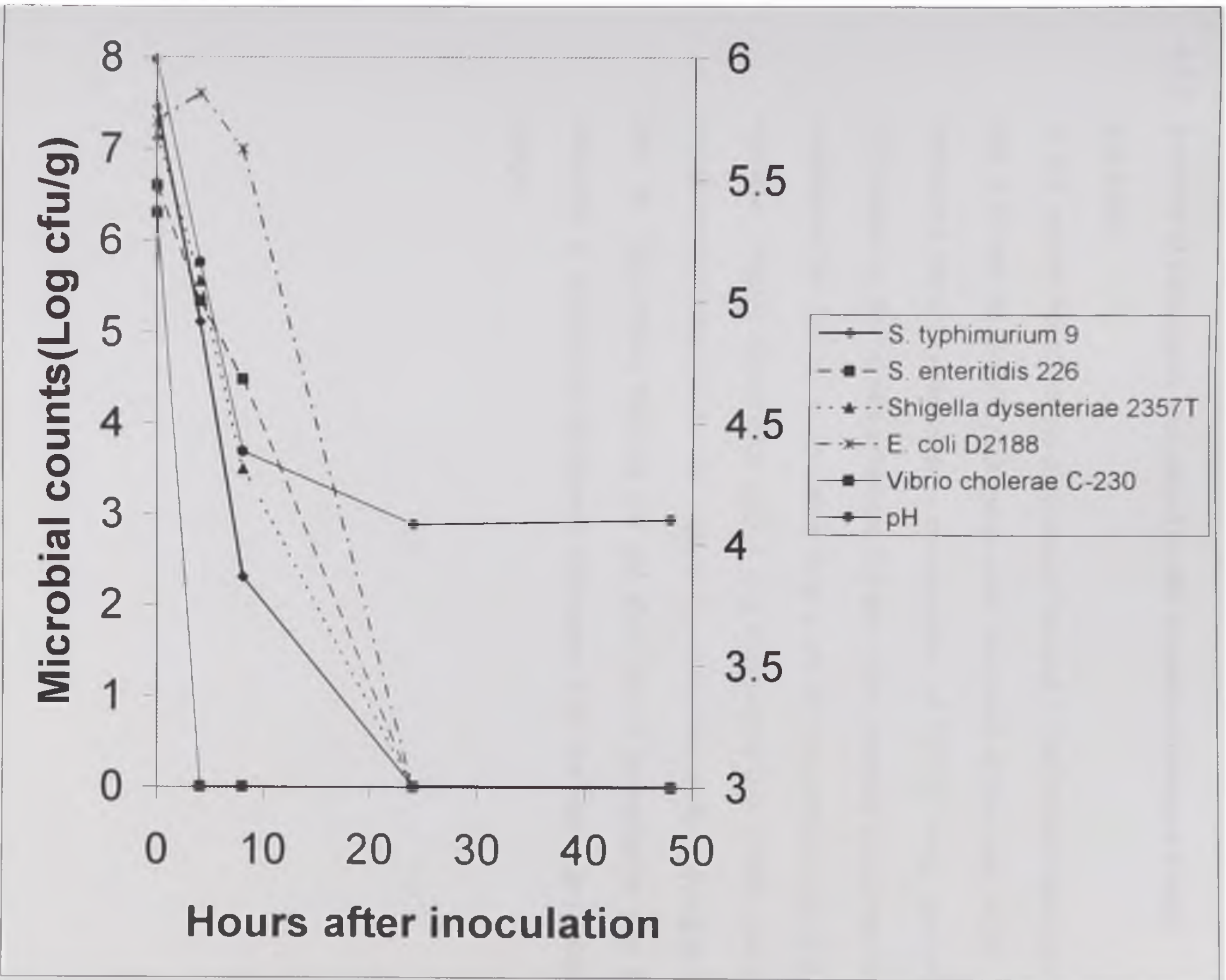

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4.5.2 Survival of pathogens inoculated in 48h fermented cassava dough (pH 3.85).

In this system the pH of the agbelima at the end of the fermentation process was 3.85 and the enteric pathogens were inoculated at this level of pH. The pathogens were inoculated at a concentration of 10^6-10^7 cfu/g. *Salmonella typhimurium* 9, *Salmonella enteritidis* 226 and *Vibrio cholerae* C-230 reduced in population from 10^6-10^5 to zero after 4h at a rate of 1 log unit per hour (Fig. 6). However *Shigella dysenteriae* 2357T and *Escherichia coli* D2188 had their population declining to about 10^3 cfu/g in 4h and subsequently dropping to zero after 8h. This shows that the low pH after 48h of fermentation was very influential in eliminating the enteric pathogens from the fermented cassava dough.

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Figure 6.

Survival of enteric pathogens inoculated into 48h fermented cassava dough (agbelima)

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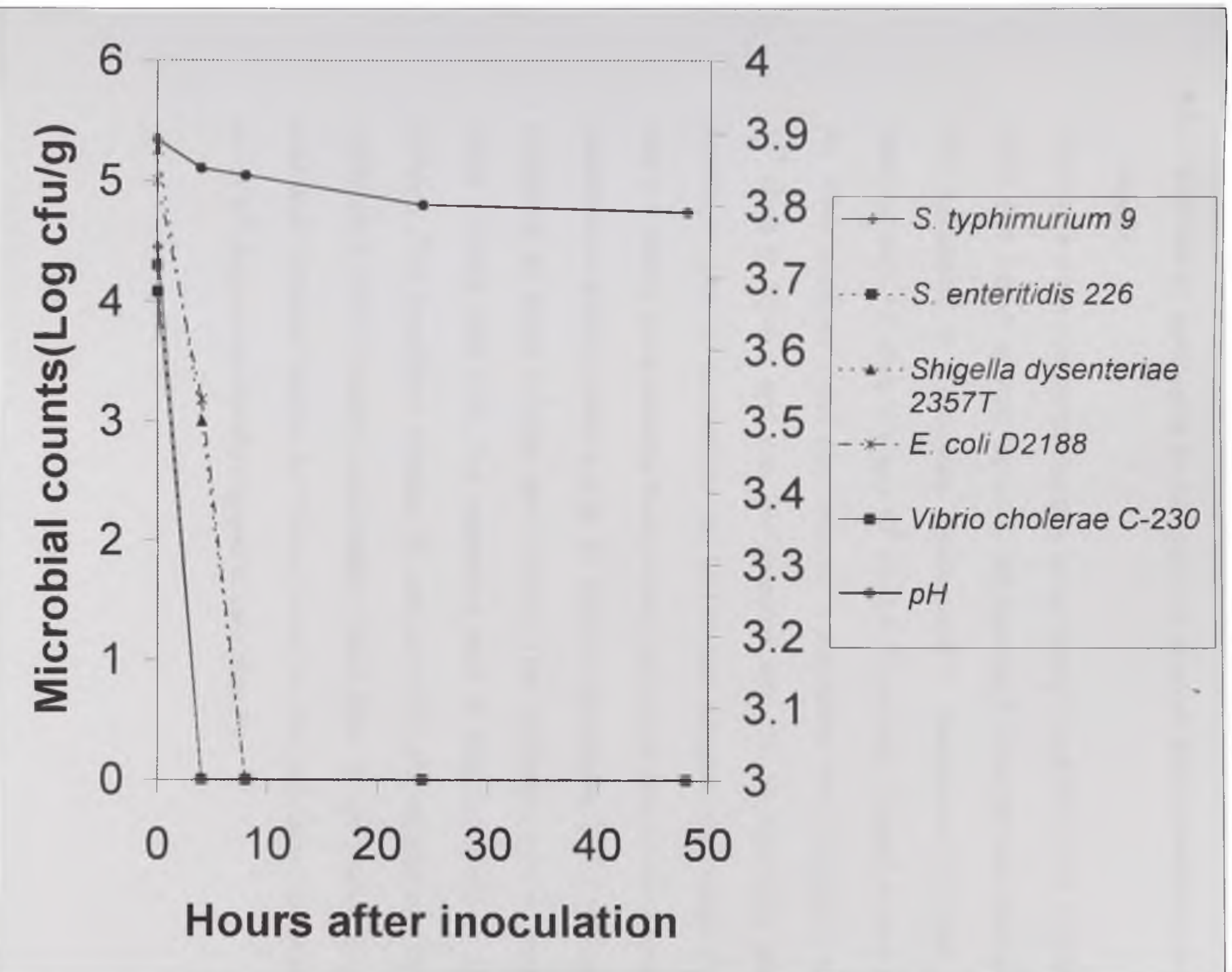

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4.5.3 Survival of pathogens inoculated into cooked 48h fermented cassava dough

Despite the 48h fermented cassava dough being mixed with some quantity of water and cooked to form the semi- stiff porridge the low pH was maintained. The population of *Salmonella typhimurium* 9, *Salmonella enteritidis* 226 declined from 10^6 cfu/g to about 10^5 cfu/g in 30min and dropped to zero after 1h. *Vibrio cholerae* C-230 also reduced in population from 10^6cfu/g to about 10^3 cfu/g in 30mins and was not detected after 1h. However the rate of population drop for *Escherichia coli* D2188 and *Shigella dysenteriae* 2357T was not drastic since possibly these enteric pathogens have some degree of resistance to acidic conditions (Fig. 7). *Shigella dysenteriae* 2357T reduced in population to about 10^5cfu/g after 30mins. The population was still high of about 10^4cfu/g after 1½h. The population after 4h was quite high of about 10^2cfu/g. The population dropped to zero after 8h. *Escherichia coli* D2188 exhibited a rather tolerant characteristic since after 2h of inoculation the population dropped slightly to 10^6cfu/g. After 4h the population declined to about 10^3cfu/g and eventually dropped to zero after 8h.

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Figure 7.

Survival of enteric pathogens inoculated into cooked fermented cassava dough (agbelima)

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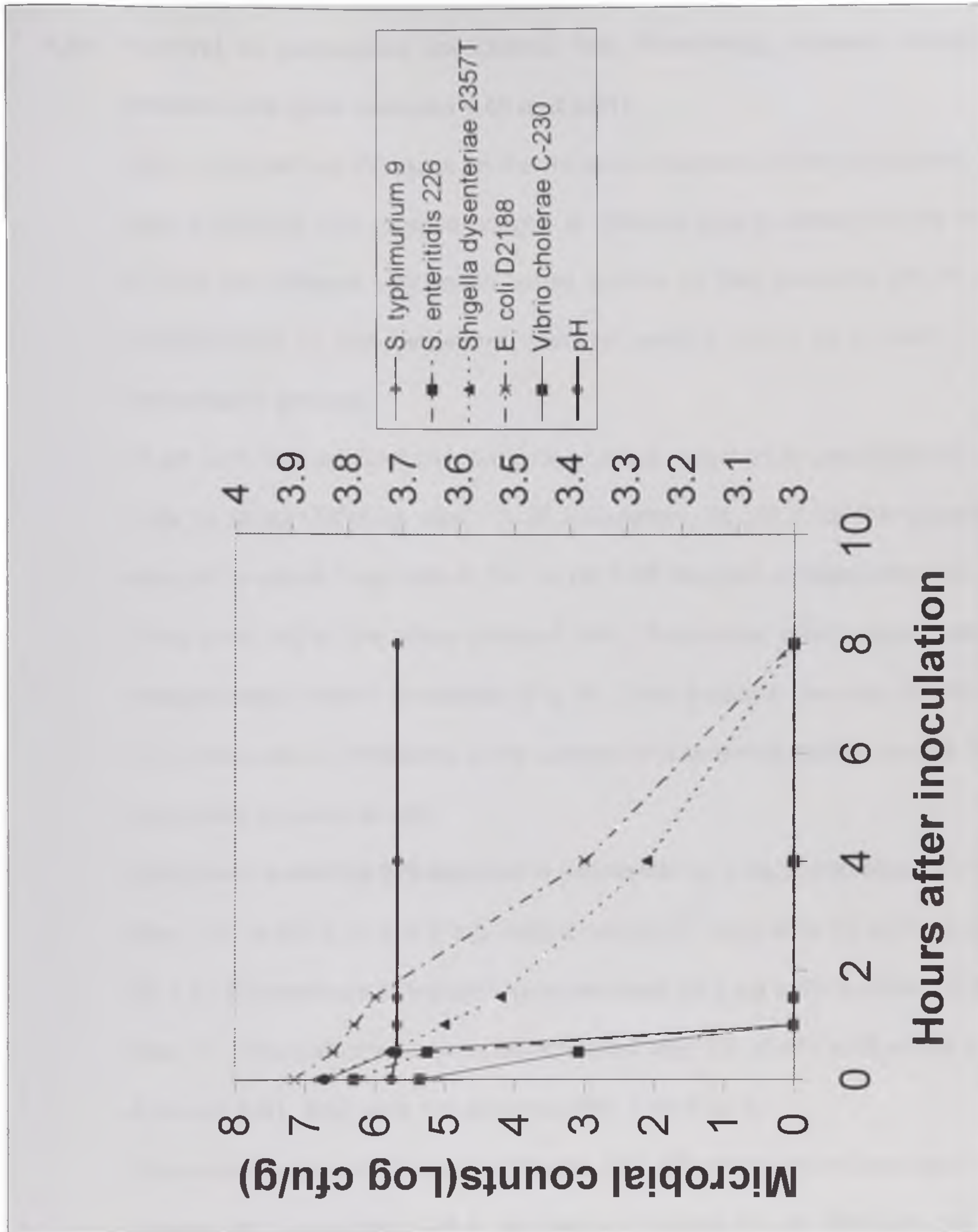

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4.5.4 Survival of pathogens inoculated into fermenting cassava dough at different pHs (pHs between 4.40 and 3.81)

Since initial pH had influence on the duration of survival of the pathogens, they were inoculated into cassava doughs at different pHs to determine the length of time the different pathogens would survive at that particular pH in case contamination of the fermented cassava product occur at a point in the fermentation process.

At pH 4.40 *Salmonella typhimurium* 9, showed reduction in population of 2 log units to about 10^4cfu/g after 1h of inoculation. At pH 4.08 the population dropped to about 3 log units in 1h. At pH 3.85 the drop in population was also 3 log units. At all the three different pHs, *Salmonella typhimurium* was not detected after 1½h of inoculation (Fig. 8). Thus between the pHs of 4.40 and 3.85 there was no difference in the survival of *Salmonella typhimurium* 9 in the fermenting cassava dough.

Salmonella enteritidis 226 dropped in population by 1 log unit to about 10^5cfu/g after 1½h at pH 4.38 and 2 log units to about 10^4 cfu/g after 1h at pH 4.09. At pH 3.81 the numbers of the pathogens reduced by 3 log units to about 10^3cfu/g after 1h. The pathogens were not detected after 2h at pH 4.38 whilst at pH 4.09 and 3.81; they were not detected after 1½h (Fig. 9).

Thus between the pH range of 4.38 and 3.81 differences were observed in the survival of *S. enteritidis* 226 in fermenting cassava dough. Between pH 3.81 and 4.09 *S. enteritidis* 226 died off within 1½h, however at pH 4.38 it survived for 30mins longer.

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Figure 8.

Survival of *Salmonella typhimurium* 9 inoculated into cassava dough dough at specific pHs

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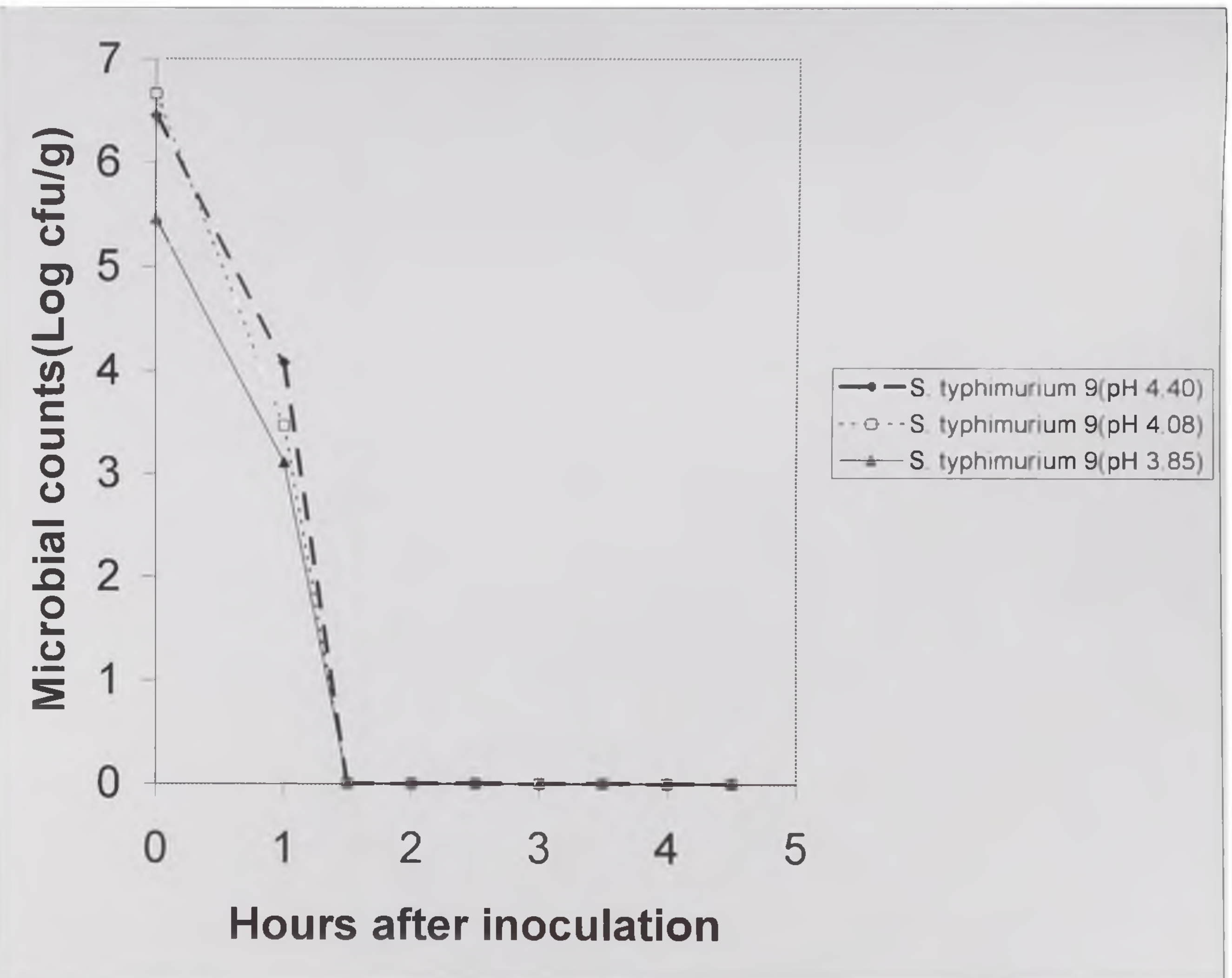

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Figure 9.

Survival of *Salmonella enteritidis* 226 inoculated into cassava dough at specific pHs

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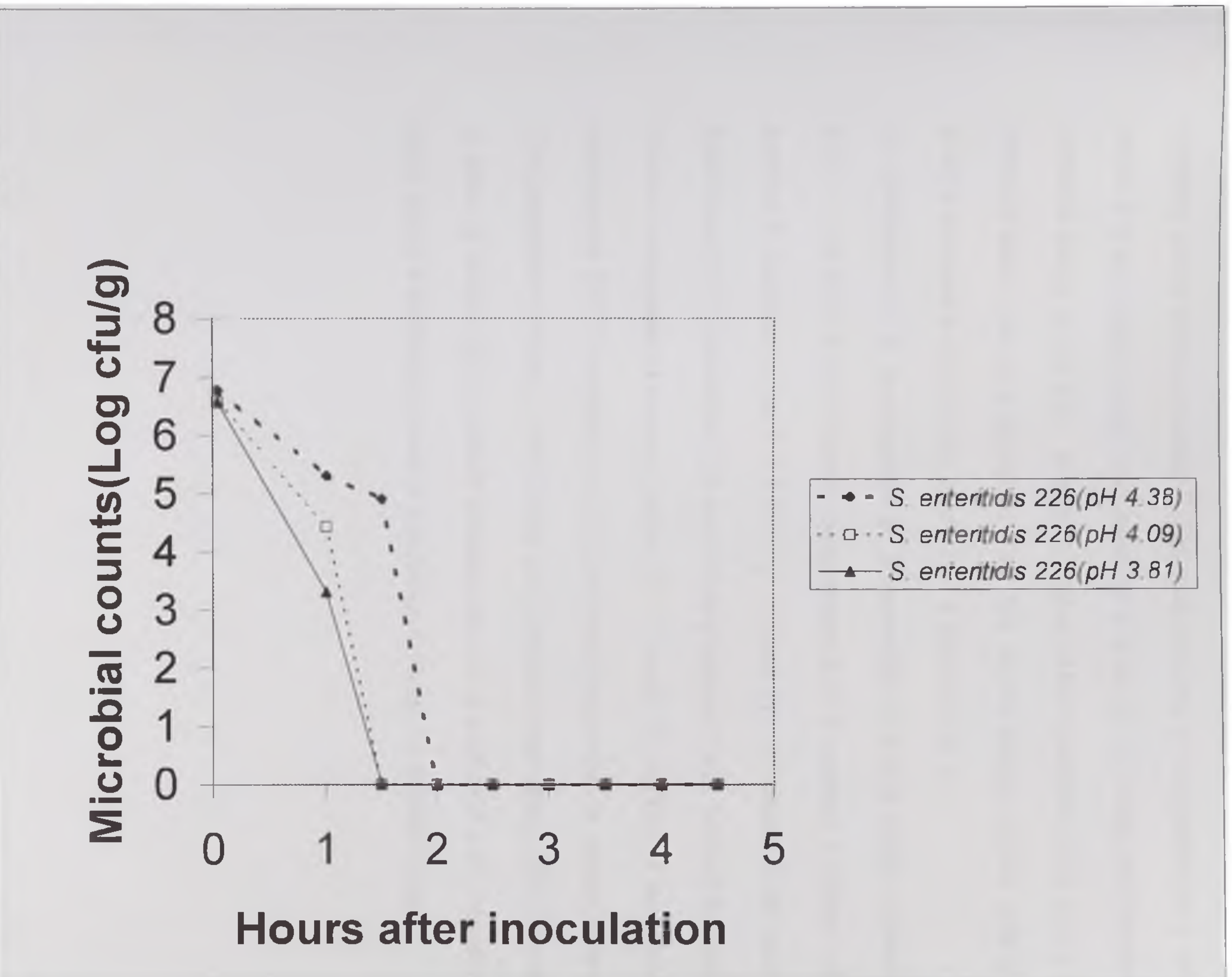

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Similarly whilst *Vibrio cholerae* C-230 was reduced in population at a rate of about 5-6 log units/h when inoculated at a level of 10^6 cfu/g into fermenting cassava dough of pH 3.81, at slightly higher pHs of between 4.09 and 4.36 a different death rate was observed (Fig. 10). At the slightly higher pHs (4.09-4.36) it survived for 1½h whilst at pH 3.81 it survived for 1h.

In comparison to *S. typhimurium* 9, *S. enteritidis* 226 and *Vibrio cholerae* C-230, *E. coli* D2188 and *Shigella dysenteriae* 2357T showed a higher rate of survival in cassava of pH 3.8-4.40 (Fig. 11 and 12). Whereas in all cases *S. typhimurium* 9, *S. enteritidis* 226 and *Vibrio cholerae* C-230 died off between 1-2h when inoculated at levels of about 10^5-10^7 cfu/g. *E. coli* D2188 and *Shigella dysenteriae* 2357T survived for 3½-4½h when they were no longer detected. The population of both *E. coli* D2188 and *Shigella dysenteriae* 2357T declined at rates of about 1½ log units/h between the pH of 4.08 and 4.37 However at pH of about 4.35 they survived in the cassava dough for 30mins longer.

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Figure 10.

Survival of *Vibrio cholerae* C-230 inoculated into cassava dough at specific pHs

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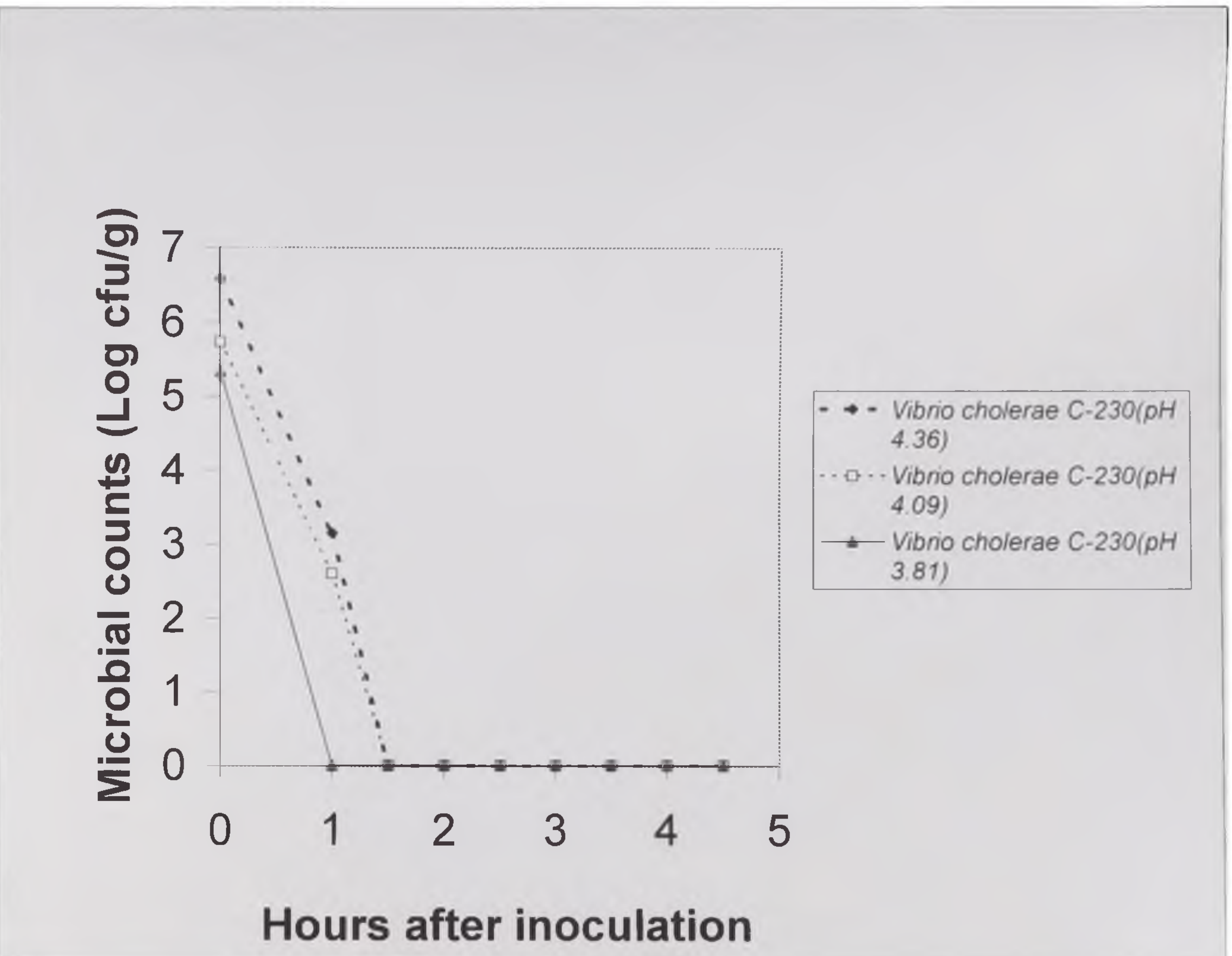

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Figure 11.

Survival of *Escherichia coli* D2188 inoculated into cassava dough at specific pHs

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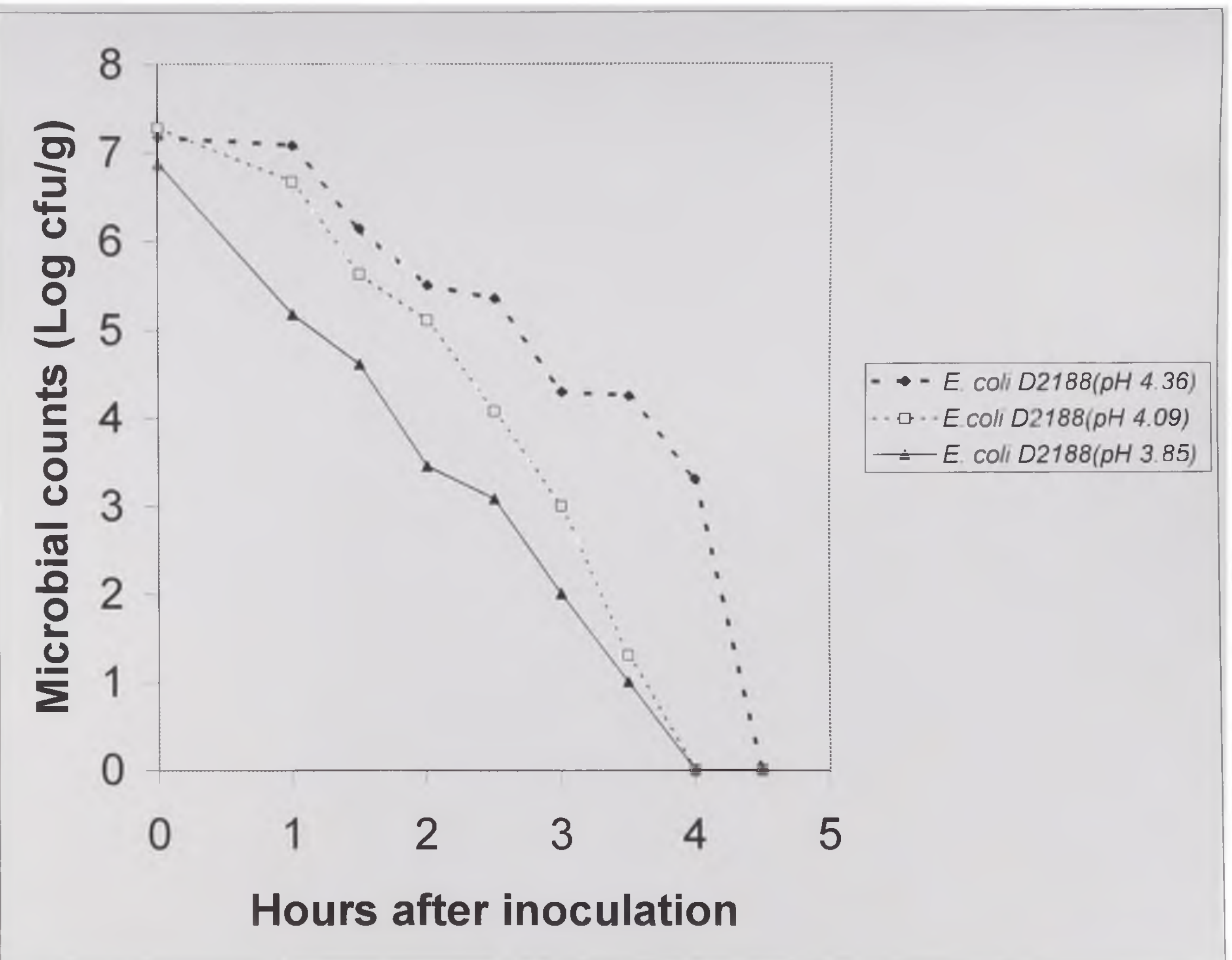

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Figure 12.

Survival of *Shigella dysenteriae* 2357T inoculated into cassava dough at specific pHs

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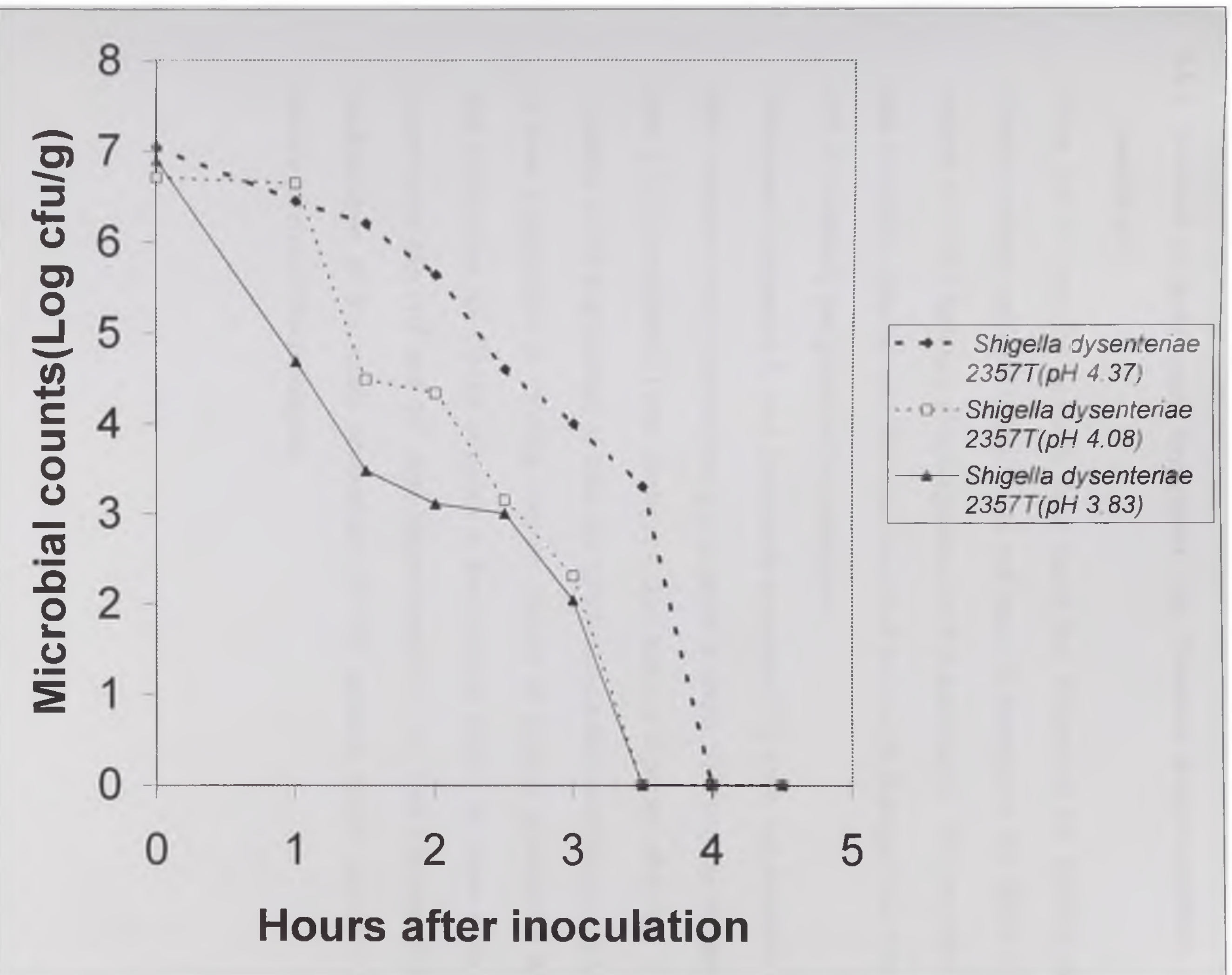

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4.5.5 Survival of pathogens inoculated into Cassava dough-agbelima at a neutral pH

Since low pH was the possible main factor that influenced the survival of the different enteric pathogens there was the need to investigate the effect of the neutral pH of the cassava dough-agbelima on the pathogens. The neutralization was a one-time process and therefore the pH of the dough changed over the next 48h of monitoring the growth of the pathogens.

Salmonella typimurium 9, and *Salmonella enteritidis* 226 were not detected after 48h. However it was observed that a population of about 10^4cfu/g was still present after 24h of inoculation. *Vibrio cholerae* C-230 was not detected after 24h. *Vibrio cholerae* C-230 that seemed to have low tolerance to acidic conditions were found to have a population of 10^4cfu/g after 8h. Growth of *Shigella dysenteriae* 2357T and *Escherchia coli* D2188 occurred in the cassava dough, as there were high counts after 48h (10^2 and 10^4 cfu/g respectively)(Fig. 13). This indicates that the neutralization of the acidic environment of the cassava dough (agbelima) did ensure survival of the pathogens.

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Figure 13.

Survival of enteric pathogens inoculated into cassava dough at neutral pH

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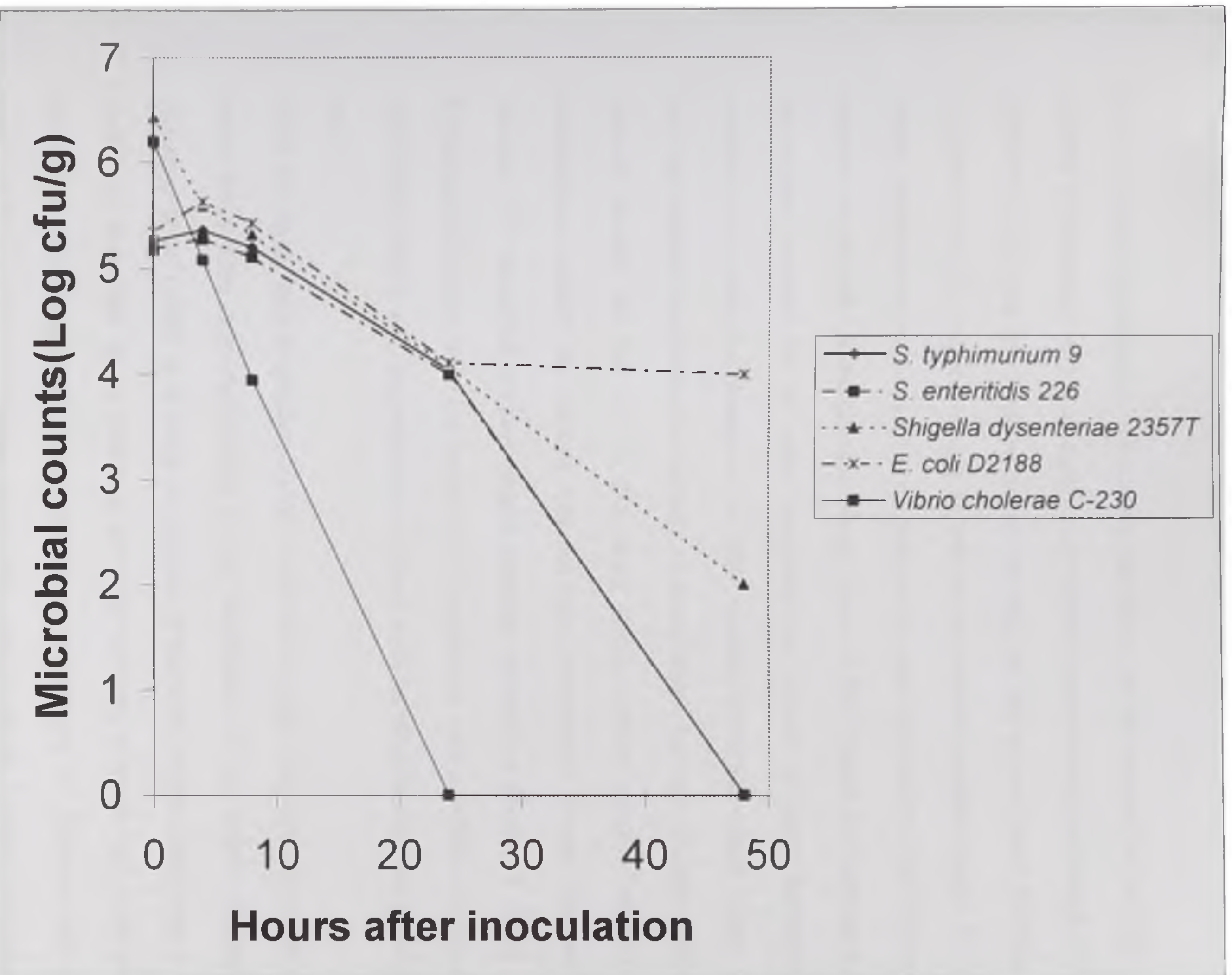

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In this present investigation there were variations in the survival of the various species of bacterial enteric pathogens in fermented cassava dough-agbelima. *Vibrio cholerae* C-230 was the most sensitive, as they did not survive after 30 mins of inoculation into the cassava dough as well as the cooked cassava dough. In most cases, Salmonella strains could not be detected 4h after inoculation in the fermented cassava dough and cooked cassava dough. Some of the *Shigella* and pathogenic *E. coli* strains survived for 24h after inoculation but showed a sharp decrease in numbers. In a study by Mensah et al,1990, cooking fermented maize dough into porridge reduced its antimicrobial activity on some enteric pathogens (*Stapylococcus aureus*) despite the low pH. In this study using cassava dough, reduction of antimicrobial property by cooking was not very pronounced. These observations showed that fermented cassava dough contains metabolites produced during the fermentation process which are bactericidal to organisms such as *Vibrio cholerae* and *Salmonella* strains and bacteriostatic to others such as *Shigella dysenteriae* and *E. coli*.

From this study acid production during fermentation might have greater effect, with other metabolites also contributing to the elimination of the enteric pathogens. Simango *et al*, (1992) in a study on survival of bacterial enteric pathogens in two traditional fermented foods (Mahewu and sour porridge) showed that none of the strains of *Aeronomas* and *Campylobacter* were detected in Mahewu and sour porridge 20mins after inoculation. *Salmonella* were not found 4h after inoculation in either fermented foods. A number of investigations on survival of enteric pathogens in fermented foods have shown that the sensitivity of pathogens to pH differs.

The pH requirements and tolerance of a number of pathogens have been investigated.

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Escherichia coli, *Shigellas* and *Salmonellas* could survive minimum pH of about 4.5 and maximum of about 8.0 and 9.0 (Jay, 1982). *Campylobacter jejuni* survives acidic pH of around 3.0 (Blaser *et al*, 1980; Gill and Harris, 1982). Experiment with yogurt showed *Campylobacter jejuni* was inhibited although the pH was between 4.2 and 5.3 (Cuk *et al*, 1987).

A few studies have shown *E. coli* is tolerant to acidic conditions in fermented foods where the organisms survived for at least 24h. In a study by Feresu and Nyati (1990), *E. coli* strains were detected 24h after inoculation in both traditional and commercial fermented milk but there was a decrease in numbers. In a study of a rice-based weaning food, in which *E. coli* ATCC 25922 was inoculated inhibition occurred only when a high initial level of lactic acid bacteria were present in comparison with a low level of the pathogen. The pathogen was unable to grow when added to the 24h prefermented weaning food (Yusof *et al*, 1993). Kingamkono *et al*, (1994), observed that enterotoxigenic *E. coli* ETEC 28662, reduced to undetectable limits after 32h in a cereal gruel containing lactic acid bacteria starter cultures.

From the studies on the survival of enteric pathogens in cassava dough-agbelima most of the pathogens investigated could not withstand the decrease in pH value of the fermenting medium.

Heterofermentative lactic acid bacteria can produce mixtures of ethanoic acid and lactic acid in some circumstances. Although they produce less total acidity than homofermenters, their early dominance in several natural vegetable fermentations could be important in the initial inhibition of other organisms and setting the fermentation on its subsequent course. To produce sufficient acid to

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achieve a useful inhibition of bacterial pathogens therefore requires a relatively large numbers of lactic acid bacteria to be present. In this study, microbial population of the lactic acid bacteria at 0h was 1.4×10^8 cfu/g, whilst at the end of fermentation, 48h, the population was 3.2×10^9 cfu/g. The relatively high count could be a great influence in inhibiting the pathogens.

This observation reflects two important properties of acids such as lactic and ethanoic acid:

i. They are weak, carboxylic acids which only partially dissociate in aqueous solution and

ii. In their undissociated form they have appreciable solubility, which allows them to diffuse freely through the bacterial plasma membrane into the cytoplasm.

According to Russel, 1992 in acidic food, the low pH increases the proportion of undissociated acid present. When the undissociated acid passes through the plasma membrane into the higher pH of the cytoplasm of the organism, it will dissociate, acidifying the cytoplasm and releasing the acid anion. This will have two effects on the organism. If it tries to maintain a constant intracellular pH, it will have to remove the protons using a proton translocating ATPase. This puts a metabolic burden on the cell, decreasing the cellular energy available to support growth functions. In addition, the cell will accumulate the acid anion which can disrupt intracellular process.

Thus, the antimicrobial potential of a weak organic acid will be determined in part by its pk_a, which describes how much undissociated, and therefore permeant, acid is present at a given pH. It will also be determined by the intrinsic toxicity of the

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acid anion, differences in which account for the differing antimicrobial effect of organic acids with similar pk_a values. It has been shown that the antimicrobial effect of mixtures of ethanoic and lactic acids exhibits a synergy which has been ascribed to lactic acid, the stronger acid, decreasing the pH and thus potentiating the effect of the ethanoic acid by increasing the proportion present in the undissociated form (Adams and Hall, 1988).

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5. CONCLUSION

The result of this investigation has confirmed that in fermentation of agbelima *Lactobacillus plantarum* is the dominant microorganism responsible for the souring of the dough. It was also isolated amongst other lactic acid bacteria and other microbial genera like *Bacillus spp.* and yeasts.

The present investigation of the natural ecosystem showed that microbial developments during agbelima fermentation are controlled by complex interactions of which acid production and pH have great influence in determining the final microflora of the fermented dough. Lactic acid bacteria isolated at the end of agbelima fermentation inhibited those, which were present at the start of fermentation. However these lactic acid bacteria together with those isolated at the start of fermentation inhibited only a few of the *Bacillus spp.* but were unable to inhibit any of the yeasts species isolated from agbelima. A few of the *Bacillus spp.* inhibited some of the yeast isolates. *Lactobacillus plantarum*, which was the dominant lactic acid bacteria isolated, showed strong inhibition against *L. fermentum* and *L. brevis*, which were also isolated from agbelima. A few of *L. plantarum* isolates showed antagonistic activity against the *Bacillus spp.* However there was no interaction between any of the *L. plantarum* isolates and the yeast isolates. Acid production was found to be the main factor contributing to the antagonistic activity of the lactic acid bacteria including the dominant lactic acid bacteria *L. plantarum*. Of four isolates of *L. plantarum* studied-LAB 9 showed the fastest rate of acid production during batch fermentation, and this corresponded with its strong antimicrobial activity demonstrated against other microbial isolates from

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agbelima. These interactions based primarily on acid production explain the microbial stability and safety of cassava-dough (agbelima).

The fermentation of cassava dough into agbelima, a typical lactic acid fermentation, inhibited growth and survival of a number of pathogenic bacteria. The rate and extent of inhibition differed with the various pathogens studied. *Salmonella typhimurium* 9, *Salmonella enteritidis* 226, *Vibrio cholerae* C-230, *Shigella dysenteriae* 2357T and *Escherichia coli* D2188 were all inhibited by different cassava dough systems to different degrees. *E. coli* D2188 and *Shigella dysenteriae* 2357Tshowed a higher rate of survival as compared to the other enteric pathogens. *Vibrio cholerae* C-230 was very sensitive and had a higher death rate. Thus cassava dough at various stages of fermentation develops strong antimicrobial effect against various pathogens and this can be exploited to provide safe weaning foods to combat diarrhoeal cases in children and in the development of starter culture for agbelima fermentation.

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RECOMMENDATIONS

1. Pure lactic acid bacteria isolated from agbelima should be screened for production of bacteriocins.
2. *Bacillus spp.* present in agbelima should be investigated for the production of antimicrobial compounds including Subtilin by *Bacillus subtilis*.
3. The effects of the antimicrobial properties of agbelima should be investigated with toxin producing pathogens.

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REFERENCES

Abe, M. O. and Lindsay, R. C., 1978. Evidence of lactic streptococcal role in Nigeria acidic cassava (*Manihot esculenta* Crantz) fermentations. J. Food Protect. 41:871-784

Abee, T, Krockel, L, and Hill, C., 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int. J. Food Microb. 28, 169-185.

Aboua, F., 1995. Optimization of Traditional fermentation of cassava. Trop. Sci. 35, 68-75

Adams, M. R. and Hall, C. J., 1988. Growth inhibition of Foodborne pathogens by lactic and acetic acids and their mixtures. Int. J. of Food Sc. and Technology 23, 287-292.

Adeyemi, I. A. and Beckley, O., 1986. Effect of period of maize fermentation and souring on chemical properties and amylograph pasting viscosity of Ogi. Cereal Science, 4, 353-360.

Aidoo, K. E., Henry, R. and Wood, J. B., 1982. Solid substrate fermentations. Adv. Applied Microbiol. 23, 201-237

Akinrele, L. A., 1970. Fermentation studies on Maize during their preparation of a traditional African starch-cake food. J. Sc. Of Agric. 21, 619-625.

Alms, L., 1983. The effect of *Lactobacillus acidophilus* administration upon survival of *Salmonella* in randomly selected human carriers. Progress in Food Nutritional Sciences, 7. 13-17.

University of Ghana http://ugspace.ug.edu.gh

Amoa-Awua, W. K. A., 1996. The Dominant Microflora and their role in Agbelima-Cassava dough fermentation. PhD thesis. Department of Nutrition and Food Science, University of Ghana, Legon.

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

Amoa-Awua, W. K. A., Appoh, F. and Jakobsen, M., 1996. Lactic acid fermantation of cassava into agbelima. Int. J. Food Microbiol. 31, 87-98.

Ashenafi, M. and Busse, M., 1991a. Microbial development during tempeh fermentation from various beans and effect of *Lactobacillus plantarum* on the natural microflora. Int. J. Food Sc. and Technology, 26, 501-506.

Ashenafi, M. and Busse, M., 1991b. Growth of *Bacillus cereus* in fermenting tempeh made from various beans and its inhibition by *Lactobacillus plantarum*. J. Applied Bacteriol. 70, 329-333.

Ashenafi, M., 1991. Growth of *Listeria monocytogenes* in fermenting tempeh made of various beans and its inhibition by *Lactobacillus plantarum*. Food Microbiol. 8, 303-310.

Banigo, E. O. I. and Muller, H. C., 1972. Manufacture of Ogi (a Nigerian fermented cereal porridge): comparative evaluation of corn, sorghum and millet. Canadian Journal of Food Science and Technology, 5, 217-221.

Banks, J. G., Board, R. G. and Sparks, N. H. C., 1986. Natural antimicrobial systems and their potential in food preservation of the future. Biotech. Appl. Biochem. 8, 103.

University of Ghana http://ugspace.ug.edu.gh

Barrel, R. A. E. and Rowland M. C. M., 1979. Infant food as a potential source of diarrhoea illness in rural West Africa. Tropical Medicine and Hygiene 73, 85-90.

Benson, H. J., 1990. Microbiological Applications: A laboratory Manual in General Microbiology. pp. 202-205. Wm.C. Brown Publishers. Dubuque. U.S.A.

Biostat B User Manual, 1997. Aage Christensen A/S, Valby Denmark.

Black, R. E., Lopez de Romana, G., Brown, K. H., Bravo, N., Bazaalar, O. G. and Kamashiro H. C., 1989. Incidence and etiology of infantile diarrhoea and major routes of transmission in Huascar, Peru. American Journal of Epidemiology. 129, 785-799.

Blaser, M. I., Hardesly, H. L., Powers, B. and Wang, W. L., 1980. Survival of *Campylobacter fetus subsp. jejuni* in biological milieu. Journal of Clinical Microbiol. 11, 309-313.

Booth, R. H., 1976. Storage of fresh cassava (*Manihot esculenta* Crantz)1. Post-harvest deterioration and its control. Experimental Agric., 12, 103-111.

Boraam,F, Faid, M., Larpent J.P., Breton, A. 1993. Lactic acid bacteria and yeast association with traditional Moroccan sour dough bread fermentation.Science des Aliments. An Intrenational Journal of Food Science and Technology,13, 501-509.

Breeching, H. A., Dodge, K. G., Moore, K. G., Phillips, H. and Wenham, J.E., 1994. Physiological deterioration in cassava: possibilities for control. Trop. Sci. 34, 335-343.

University of Ghana http://ugspace.ug.edu.gh

Brenner, H. A. and Statham, J. A., 1983. Spoilage of vacuum packed chilled-stored scallops with added *lactobacilli.* Food Technol. Aust. 35, 284.

Brink, B., Mireku, M., Vossen J. B., Leer, R. J. and Huis, J. H. J., 1994. Antimicrobial activity of *lactobacilli*; preliminary characterization and optimization of production of acidocin B, a novel bacteriocin produced by *Lactobacillus acidophilus* M46. J. Applied Bacteriology. 77: 2, 140- 148

Brock, T. D. and Madigan, M. T., 1991. Biology of microorganisms, Sixth Edition, Prentice Hall Inc. U.S.A.

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

Chavan, U. D., Chavan, J. K. and Kadam, S. S., 1988. Effect of fermentation on soluble proteins in vitro protein digestibility of sorghum, green gram and sorghum green gram blends. Journal of Food Science 53, 1574-1575.

Cooke, R. D., Twiddy, D. R. and Reilly, P J. A., 1987 Lactic acid fermentation as a low cost means of food preservation in Tropical countries. FEMS Microbiological Reviews 46, 369-379.

Crueger, W. and Crueger, A., 1990. Methods of fermentation. In: Biotechnology: A Textbook of Industrial Microbiology. Ed. Brock, T. D.; Sinauer Associates, Inc, Sunderland, MA, U. S. A. pp.64-69.

Cuk, Z., Annan-Prah, A., Jane, M. and Zaja-Saltler, J., 1987. Yogurt: an unlikely source of *Campylobacter jejuni/coli.* Journal of Applied Bacteriology. 63, 201-205.

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

University of Ghana http://ugspace.ug.edu.gh

Dahiya, R. S. and Speck, M. L., 1968. Hydrogen peroxide formation by *lactobacilli* and its effect on *Staphylococcus aureus*. J. Dairy Science. 51, 1568

Dirar, H. A., 1978. A microbiological study of Sudanese merissa brewing. Journal of Food Science, 43, 1683-1686.

Dirar, H. A., 1992. The indigenous fermented foods and beverages of Sudan. In Application of Biotechnology to food processing, pp. 23-40. Expert Group Meeting, Ibadan, Nigeria. UNIDO.

Doores, S., 1990. pH control agents and acidulants in food additives. pp 447-510. (A. L. Branen, P M. Davidson and S. Salminen. eds) Marcel Dekker, New York.

Dovlo, F E., 1975. Traditional methods of processing and preserving foods in Ghana. Food research Institute (CSIR), Ghana, report.

Dziedzoave, N. T., 1989. Some characteristics of market samples of agbelima and the effects of various traditional inoculants in fermenting cassava for agbelima. Food Research Institute (CSIR), Ghana, report.

Dzeidzoave, N. T., 1996. Quality control studies of Agbelima- Development of quality specifications and evaluation of cassava varieties for processing. M.Sc. thesis submitted to the Dept. of Biochemistry, University of Science and Technology, Kumasi, Ghana.

Ejiofor, M. A. N. and Okafor, N., 1981. Comparison of pressed and unpressed cassava pulp for gari making. In Tropical Root crops: Research strategies for the 1980's ed. Terry, E. R., Oduro, K. A. and Cavenness, F pp 154-158, Ottawa, Canada IDRC.

University of Ghana http://ugspace.ug.edu.gh

Essers, S. A. J. A., 1995. Removal of cyanogens from cassava. Studies on domestic sun drying and solid substrate fermentation in rural Africa. Ph.D. thesis, Wagningen Agricultural University, The Netherlands.

Faid, M., Fatima, Boraam, Achbab, A. and Larpent, J. P., 1993. Yeast-lactic acid bacteria interaction in Morrocan sour dough bread fermentation. Lebensmitte Wissenschaften Und Technologie, 26, 443-446.

Feresu, S. and Nyati, H., 1990. Fate of *Escherichia coli* strains in two fermented milk products. Journal of Applied Bacteriology, 69, 814-821.

Flores-Galarza, R. A., Glatz, B. A., Bern, C. J. and Van Fossen, L. D., 1985. Preservation of high moisture corn by microbial fermentation. Journal of Food Protection, 48, 407-411.

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

Gaya, P., Medina, M. and Nunez, M., 1991. Effect of the lactoperoxidase system on *Listeria monocytogenes* behaviour in raw milk at refrigeration temperatures. Appied Env. Microbiol., 57, 3355-3360.

Gbedemah, C. M. and Awafo, V., 1990. The effect of gamma radiation on the cellulolytic pectinolytic and amylolytic enzyme activity on some gari fermenting microorganisms. Nuclear and Related techniques in improvement of tradition fermentation processing of cassava. Int. Atomic Energy, Vienna.

Gibbs, P. A. 1987. Novel uses for lactic acid fermentation in food preservation. Journal of Applied Bacteriology. Symposium supplement, 51S-58S

Gill, C. O. and Harris, L. M., 1982. Survival and growth of *Campylobacter fetus subsp jejuni* on meats and cooked foods. Applied and Env. Microbiol. 44, 259-263.

University of Ghana http://ugspace.ug.edu.gh

Gilliland, S. E. and Ewell, H. R., 1983. Influence of combinations of *Lactobaccillus lactis* and potassium sorbate on growth of psychotrops in raw milk. J. Dairy Science, 66, 974.

Gilliland, S. E., 1985. Role of starter culture bacteria in food preservation. In bacterial starter cultures for food ed. Gilliland, S. E. pp175, Boca Roton: CRC Press.

Gillooly, M., Bothwell, T. H., Charlton, R. W., Torrance, T. D., Bezwoda, W. R., MacPhail, A. P., Derman, D. P., Novelli, L., Morral, D. and Mayet,F., 1984. Factors affecting the absorption of iron from cereals. British Journal of Nutrition, 51, 37-46.

Giraud, E., Gosselin, L. and Raimbault, M., 1992. Degradation of cassava linamarin by lactic acid bacteria. Biotechnology letters, 14, 593-598.

Giraud, E., Gosselin, L. and Raimbault, M., 1993. Production of *Lactobacillus plantarum* starter with linamarase and amylase activity for cassava fermentation. Journal of the Science of Food and Agriculture, 62, 77-82.

Gombas, D. E., 1989. Biological competition as a preserving mechanism. J. Food Safety, 10, 107-117.

Halm, M., Lillie, A., Sorensen, A. K. and Jakobsen, M., 1993. Microbiological and aromatic characteristics of fermented maize dough for kenkey production in Ghana. Int. J. Food Microbiol. 19, 135-143.

Hammes, W. P. and Tichaczek, P S., 1994. The potential of lactic acid bacteria for the production of safe and wholesome food. Zeitschrift-Fur Lebensmittel-Untersuchung- und Forschung, 198:3, 193-201.

University of Ghana http://ugspace.ug.edu.gh

Hammes, W. P. and Vogel, D., 1995. The genus Lactobacillus. In lactic acid bacteria Vol.2. The Genera of lactic acid bacteria ed. Wood, B. B> B. and Holzafel, W.H., pp19-54, Condon: Blackie Academic and Professional

Hansen, A., Lund, B. and Lewis, M. J., 1989. Flavour production and acidification of sourdough in relation to starter culture and fermentation temperature. Lebensm. Wiss. U. Technol. 22, 145-149.

Hansen, E. and Hansen, B. 1994. Influence of Wheat flour type on the production of flavours compounds in sour doughs. J. Cereal Science, 19-185-190.

Hesseltine, C. W., 1991. Mixed-cultures fermentations: an introduction to oriental food fermentations. In mixed cultures in Biotechnology, ed Zeikus, J. G. and Johnson, E. A., pp. 1-6, New York: Mc Graw-Hill.

Holzapfel, W. H., Geisen, R. and Schillinger, U., 195. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. of Food Microbiol. 24, 343-362.

Hounhouigan, D. J., Nout, M. J. R., Nago, C. M., Houben, J. H. and Rombouts F. M., 1997 Use of Starter cultures of *Lactobacilli* and yeast in fermentation of Mawe porridge. Proceedings on Third Seminar on African fermented foods. Ghana, Accra, July 1996.

Hutton, M. T., Chehak, P. A. and Hanlin, J. H., 1991. Inhibition of botulinum production by *Pediococcus acidilactici* in temperature abused refrigerated foods. J. Food safety, 11, 255-267.

ICMSF, 1980. Gases as preservatives. In Microbial Ecology of Foods, Vol. 1, pp 170-192, Academic Press, New York.

University of Ghana http://ugspace.ug.edu.gh

ICMSF, 1996. Microorganisms in Food. Volume 5: Microbiological specifications of Food pathogens. Blackie Academic and Professional. U. K.

Jay, J. M., 1982. Antimicrobial properties of Diacetyl. Applied Env. Microbiol., 44, 525.

Jellife, D. B., 1968. Infant Nutrition in the Tropics. World Health Organisation, Geneva, Switzerland.

Jerpersen, L., Halm, M., Kpodo, K. and Jakobsen, M., 1994. The significance of yeasts and moulds occurring in maize dough fermentation for Kenkey production. Int. J. Food Microbiol. 24, 239-248.

Joerger, M. and Kleanhammer, J. R., 1989. Characterization and purification of Helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481, J. Bacteriol. 167, 439-446.

Johansson, M. L., 1995. Systematics and Starter culture selection of *Lactobacillus* for human intestine and Nigerian Ogi with special reference to *Lactobacillus plantarum*. Ph.D Thesis, Lund University.

Juffs, H. S. and Babel, F J., 1975. Inhibition of psychotropic bacteria by lactic cultures in milk stored at low temperature. J. Dairy Science, 58, 1612-1619.

Kanatani, K. and Oshimura, M., 1994. Plasmid-associated bacteriocin production by a *Lactobacillus plantarum* strain. Biotech. Biochem. 58(11), 2084-2086.

Khetarpaul, N. and Chauhan, B. M., 1990. Effect of fermentation by pure cultures of yeasts and *Lactobacilli* on the available carbohydrate content of pearl millet. Tropical Science, 31, 131-139.

Kingamkono, R., Sjogren, E., Svanberg, U. and Kaijser, B., 1994. pH and acidity in lactic-fermenting cereal gruels: effects on viability of

University of Ghana http://ugspace.ug.edu.gh

enteropathogenic microorganisms. World Journal of microbiology and bacteriology, 10, 664-669.

Klaenhammer, T. R., 1988. Bacteriocins of lactic acid bacteria. Biochimie. 70: 337-349.

Klaenhammer, T. R.,1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev 12, 39-86.

Leroi, F and Pidoux, M., 1993. Characterization of interaction between *Lactobacillus hilgardii* and *Saccharomyces florenticus* isolated from sugary kefir grains. Journal of App. Bacteriol. 74, 54-60.

Lewis, M. J. and Young, T. W., 1995. Fermentation biochemistry. In: Brewing. ed: Lewis, M. J. and Young, T. W., Chapman and Hall, New York, U. S. A. pp173-190.

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

Ljungqvist, B., Mellander,O. and Svanberg, U., 1981. Dietary bulk as a limiting factor for nutrient intake in preschool children; a problem description. Journal of Tropical pediatrics, 27, 68.

Lorri, W. and Svanberg, U., 1991. The potential role of fermented cereal gruels in reduction of diarrhoea among young children. In preceedings of a regional workshop on traditional African foods – Quality and Nutrition workshop. International Foundation for Science. ed. Westby, A. and Reilly,P J. A., pp 33-38, Stockholm. IFS.

University of Ghana http://ugspace.ug.edu.gh

Lorri, W. S. M. and Svanberg, U., 1993. Lactic-fermented cereal gruels with improved in vitro protein digestibility. Int. Journal of Food Science and Nutrition, 44, 207-213.

Lorri, W. S. M., 1993. Nutritional and microbiological evaluation of fermented cereal weaning foods. Ph.D thesis. Chalmers University of Technology, Goteborg, Sweden.

Mackay, L.L. and Baldwin, K. A., 1990. Applications for Biotechnology, present and future improvement in lactic acid bacteria: A review. FEMS Microbiol. Rev. 87, 3-14.

Maclean, W. C., Lopez, R. G., Placko, R. D. and Graham, G. G., 1980. Protein quality and digestibility of sorghum in pre-school children: balance studies and plasma free amino acids. Journal of Nutrition, 111, 1928-1936.

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: Westby, A. and Reilly, P. J. A., Stockholm, IFS.

Mbugua, S. K., 1981. Microbiological and biochemical aspects of uji (an east African sour cereal porridge) fermentation and its enhancement through application of lactic acid bacteria. Ph.D thesis, Cornell University, pp 140.

Mensah, P P. A., Tomkins, A. M., Draser, B. S. and Harrison, J. T., 1990. Fermentation of cereals for reduction of contamination of weaning foods in Ghana. Lancet 336, 140-147.

Meraz, M., Shirai, K., Larral de, P. and Revah, S., 1992. Studies on bacterial acidification process of cassava (*Manihot esculenta* Crantz) J. Science Food Agric. 60, 457-463.

University of Ghana http://ugspace.ug.edu.gh

Metchnikoff, E., 1907. Lactic acid and inhibiting intestinal putrefaction. In Prolongation of Life, ed., P Chatmers Mitchell, pp 168-183, Heinemann, London.

Ministry of Agriculture, Ghana, 1998. Planning, policy, monitoring, evaluation and development.

Mortajemi, J., Kaferstein, F., Moy, G. and Quevedo, F., 1993. Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. Bulletin of the World Health Organization, 71, 79-92.

Muriana, P M. and Kleanhammer, T. R., 1987. Purification and partial characterization of lactacin F a bacteriocin produced by *Lactobacillus acidophilus* 11088. Appl. Env. Microbiol. 57, 114-121.

Muriana, P. M. and Luchansky, J. B. 1993. Biochemical methods for purification of bacteriocins In. Bacteriocins of lactic acid bacteria. eds. Hoover, D. G., Steenson, L. R. Academic Press, San Diego, pp 41-61.

Muriana, P M. and Spanier, A. M., 1993 Antimicrobial peptides and their relation to food quality, food flavour and safety: Molecular analysis and design: 203rd National Meeting of American Chemical Society, San Francisco, California, U.S.A. April 1992, 193. 303-321, ACS Symposium Series, 528.

Nettles, C. G. and Barefoot, S. F., 1993. Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. J. Food Prot. 56, 338-356.

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

University of Ghana http://ugspace.ug.edu.gh

Nout, M. J. R., 1991. Ecology of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. Int. J. Food Microbiol. 8, 351-361.

Nout, M. J. R., Hautvast, J. G. A. J., Van der Haar, F., Marks, W. E. W. and Rombarts, F. M., 1987. Energy, protein and microorganisms: the formulation and the microbiological stability of cereal- based composite weaning foods. In: Improving Young Child Feeding in Eastern and Southern Africa, pp 245-260. Household Level Food Technology, Proceedings of a workshop held in Nairobi, Kenya, 12-16 October 1987.

Odugbemi, T., Odujirin, H. T., Akintoye, C. O., Oyerinde, J. P. O. and Esumeh, F I., 1991. Study on the pH of Ogi, Nigerian fermented weaning food and its effect on enteropathogenic *Escherichia coli*, *Salmonella typhi* and *Salmonella paratyphii*. J. of Tropical Medicine and Hygiene, 94, 219-223.

Odunfa, S. A., 1985. African fermented foods. In: Microbiology of Fermented Foods. Vol. 2, ed, Wood, B. J. B. pp 155-199. Elsevier Science, London and New York.

Odunfa, S. A., Olasupo, N. A. and Olukoya, D. K., 1996. Potential of Bacteriocins in Food Safety in Lactic Fermented Cereal-Ogi. In Traditional fermented food processing in Africa. The Third Biennial Seminar on African Fermented Foods, Ghana, July 1996, pp 27-33.

Okafor, N. and Ejiofor, M. A. N., 1985. The linamarase of *Leuconostoc mesenteriodes* production, isolation and properties. J. of Food and Agric. 36, 669-678.

University of Ghana http://ugspace.ug.edu.gh

Okafor, N. and Ejiofor, M. A. N., 1986. The microbial breakdown of linamarin in fermenting pulp of cassava (*Manihot esculenta* Crantz). MIRCEN J. of Appl. Microbiol. and Biotechnology, 2, 327-338.

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

Okafor, N., 1977. Microorgaisms associated with cassava fermentation for gari production. J. of Appl. Bacteriol. 42, 279-284.

Olasupo, N. A., Olukoya, D. K. and Odunfa, S. A., 1994. Plasmid profiles of bacteriocin-producing lactobacillus isolated from African fermented foods. Folia Microbiology 39, 181-186.

Olasupo, N. A., Olukoya, D. K. and Odunfa, S. A., 1995. Studies on bacteriocingenic *Lactobacillus* isolates from selected Nigerian fermented foods. J. of Basic Microbiology, 35, 319-324.

Olsen, A., Halm, M. and Jakobsen, M. 1995. The antimicrobial activity of lactic acid bacteria from fermented maize (Kenkey) and their interactions during fermentation. J. of Appl. Bacteriology. 79, 506-512.

Olukoya, D. K., Ebigwe, S. I., Adebawo. O. O. and Osiyemi, F O., 1993. Plasmid profile and antibiotic susceptibility patterns of *Lactobacillus* isolated from fermented foods in Nigeria. Food Microbiology 10, 279-285.

Olukoya, D. K., Ebigwei, S. I., Olasupo, N. A. and Ogunjimi, A. A., 1994. Production of Dogik: an improved Ogi (Nigerian Fermented weaning food) with potential for use in diarrhoea control.

Olukoya, D. K., Tichaczek, P S., Butsch, A., Vogel, R. F and Hammes, W. P., 1993. Characterization of the Bacteriocins produced by *Lactobacillus pentosus*

University of Ghana http://ugspace.ug.edu.gh

DK 7 isolated from Ogi and *Lactobacillus plantarum* DK 9 from fufu. Chemische Micro. Technologie. Lenbensmittel 15, 65-69.

Olusupo, N. A., 1996. Characterization of *Lactobacillus* species useful in local food fermentation. Ph.D Thesis, University of Ibadan, Ibadan, Nigeria.

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

Oyewole, O. B. and Odunfa, S. A., 1990 Characterization and distribution of lactic acid bacteria in cassava fermentation during fufu production. J. Appl. Bacteriol. 68, 145-152.

Oyewole, O. B. and Odunfa, S. A., 1991. Characterization of *lactobacilli* in fermenting cassava and their evaluation as cassava starter cultures. In: Traditional African Foods- Quality and Nutrition. Ed. Westby, A. and Reilly, P J. A., pp 145-150, IFS, Sweden.

Parry, J. M., Turnbull, P C. B. and Gibson, J. R., 1983. A colour Atlas of *Bacillus* species. Ipswich: Wolfe Medical Publishing Ltd.

Piard, J. C. and Desmazeaud, M., 1991. Inhibiting factors produced by lactic acid bacteria. 1. Oxygen metabolites and catabolism end productions. Lait 71, 525-541.

Piard, J. C. and Desmazeaud, M., 1992. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. Lait 72, 113-143.

Plahar, W. A. and Leung, K., 1982. Effect of moisture content on the development of carboxylic acids in traditional maize dough fermentation. J. Sci. Agric. 33, 555-558.

University of Ghana http://ugspace.ug.edu.gh

Rammmelberg, M. and Radler, F., 1990. Antimicrobial polypeptides of *lactobacillus* species. Journal of Appl. Bacteriol. 69, 177-184.

Randler, O. and Weiss, N., 1986. Regular non-sporing Gram-positive rods. In Bergey's Manual of Systematic Bacteriology. Vol. 2, ed. P.H. A. Mair, N.S.Sharpe, and Holt, J. G. pp. 1208-1234, Baltimore: Williams and Wilkins.

Reiter, B. and Harnulv, B. G., 1984. Lactoperoxidase antibacterial system: natural occurrence, biological functions and practical applications. J. Food Prot. 47, 724-732.

Rowland, M. G. M., Barrell, R. A. E. and Whitehead, R. G. 1978. Bacterial contamination in traditional Gambian weaning foods. Lancet 1, 136-138.

Russel, J. B., 1992. Another explanation for the toxicity of fermentation acids at low pH; anion accumulation versus coupling. J. Appl. Bacteriol. 73, 363-370.

Sanders, M. E., 1993. Effect of consumption of lactic acid cultures on human health. In Advances in Food and Nutrition Research, ed, J.E. Kinsella, pp 67-130. Academic Press, New York.

Sandine, W. E., 1979. Roles of *Lactobacillus* in intestinal tract. J. Food Prot. 42, 259-262.

Sanni, A. I., 1993. The need for process of optimization of African fermented foods and beverages. Int. J. of Food Microbiol. 18, 85-95.

Schillinger, U. and Lucke, F. K., 1989. Antibacterial activity if *Lactobacillus sake* isolated from meat. Appl. Env. Microbiol. 55, 1901-1906.

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

University of Ghana http://ugspace.ug.edu.gh

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

Simango, C. and Rukure, G., 1992. Survival of bacterial enteric pathogens in traditional fermented foods. J. of Appl. Bacteriol. 73, 37-40.

Sneath, P H. A., Mair, N. S., Sharpe, M. E. and Holt, J. G., 1986. Bergey's manual of systematic bacteriology, Ninth edition, Williams and Wilkins. Baltimore, London, Los Angeles, Sydney.

Stecchini, M. L., Aquili, V., Sarais, J. and Pitotti, A., 1991. Inhibition of *Staphylococcus aureus* and *Salmonella typhimurium* in Montasio cheese by *Lactobacillus plantarum* culture. J. Food Safety, 34, 227-238.

Steinkraus, K. H. 1997. Classification of fermented foods: Worldwide review of household fermentation techniques. Food Control, 8, 311-317.

Steinkraus, K. H., 1996. Handbook of Indigenous fermented foods. Second edition. Marcel Dekker, New York.

Stiles, M. E., 1996. Biopreservatives by lactic acid bacteria. Antionne Van Leeuwenhoek, 70, 331-345.

Stutzenberger, F., 1990. Bacterial cellulases. In Microbial Enzymes and Biotechnology, 2nd edition, ed. Fogarty, W. M. and Kelly, C. T., pp 37-70, London, Elsevier Applied Science.

Suma, K., Misra, M. C. and Varadaraj, N., 1998. Plantaricin LP84, a broad-spectrum heat stable bacteriocin of *Lactobacillus plantarum* NCIM 2084 produced in a simple glucose broth medium. Int. J. of Food Microbiol. 40, 17-25.

University of Ghana http://ugspace.ug.edu.gh

Svanberg, U. and Lorri, W., 1991. Lactic fermentation of cereal-based weaning gruels and improved nutritional quality. In Traditional African Foods-Quality and Nutrition. ed. A. Westby and P J. A. Reilly, pp. 53-62, IFS. Sweden.

Svanberg, U., Sjogren, E., Lorri, W., Svennerholm, A. M. and Kaijser, B., 1992. Inhibited growth of common enteropathogenic bacteria in lactic-fermented cereal gruel. World Journal Food Microbiology and Biotechnology, 8, 601-606.

Tagg, J. R., Dajani, A. S. and Wannamaker, L. M., 1976. Bacteriocins of Gram-positive bacteria. Bacteriol. Rev., 40: 722.

Tanaka, N., Traisman, E., Lee, M. H., Cassens, R. G. and Foster, E. M. 1980. Inhibition of botulinum toxin formation in bacon by acid development. J. Food Prot. 43, 450-457.

Toba, T., Samant, S. K., Yoshioka, E. and Itoh, T., 1991. Reutericin 6, a new bacteriocin produced by *Lactobacillus reuteri* LA6. Letter in Applied Microbiology, 13, 281-286.

Upreti, G. C. and Hinsdill, R. D., 1975. Production and mode of action on Lactocin 27: Bacteriocin from homofermentative *Lactobacillus*. Antimicrob. Agents Chemother. 7: 145.

Varnam, A. H. and Sutherland, J. P., 1994. Milk and Milk Products: Technology, Chemistry and Microbiology, pp 451. Chapman and Hall, London.

Vescovo, M., Torriani, S., Dellaglio, F and Bottazzi, V., 1993. Basic characteristics, ecology and application of *Lactobacillus plantarum*: a review. Annals of Microbiology and Enzymology, 43, 261-284.

Vescovo, M., Torriani, S., Orsi, C. Macchiarlol, F and Scolari, G., 1996. Application of antimicrobial producing lactic acid bacteria to control pathogens in ready-to-use vegetables. J. Appl. Bacteriol. 81, 113-119.

University of Ghana http://ugspace.ug.edu.gh

Vollmar, A. and Meuser, F , 1992. Influence of starter cultures consisting of lactic acid bacteria and yeasts on the performance of a continuous sourdough fermenter. Cereal chemistry, 69, 20-27.

Wanink, J. F., Vliet, T. and Nout, M. J. R., 1994. Effect of roasting and fermentation on viscosity of cereal-legume based food formulas. Plant Foods for Human Nutrition, 46, 117-126.

Watkins, B. A. and Miller, B. F., 1983. Competitive gut exclusion of avaian pathogens by *Lactobacillus acidophilus* in gnoto-biotic chicks. Poultry Science 62, 1772-1779.

West, C. A. and Warner, P J., 1988. Plantacin B, a bacteriocin produced by *Lactobacillus plantarum* NCDO 1193. FEMS Microbiol. Lett. 49:163.

Westby, A. and Gallat, S., 1991. The effect of fermentation on viscosity of sorghum porridges. Tropical Science 31, 131-139

Westby, A. and Twiddy, R. D., 1991. Role of microorganisms in reduction of cyanide during traditional processing of African cassava products. In Proceedings of a regional Workshop on Traditional African Foods-Quality and Nutrition. Int. Foundation for Science, ed. Westby, A. and Reilly, P. J. A., pp 127-132, Stockholm: IFS

Yusof, R. M., Morgan, J. B. and Adams, M. R., 1993. Bacteriological safety of fermented weaning food containing L-lactate and nisin. J. Food Prot. 56, 414-417

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APPENDICES

Appendix 1

Growth patterns of different *L. plantarum* isolates during batch fermentation of cassava dough.

	Microbial count (Log cfu/g)			
Time	LAB 9	LAB 6	LP 11	LP 3
0	7.82	6.69	6.78	6.86
1	8.12	7.85	7.88	7.85
3	8.14	8.21	7.83	8.38
6	9.46	8.73	8.89	8.64
9	9.44	9.09	9.13	9.17
12	9.49	9.13	9.15	9.23
15	9.15	9.17	9.16	9.24
18	9.18	9.22	9.17	9.27
21	9.19	9.25	9.17	9.27
24	9.22	9.30	9.17	9.3
27	9.17	9.25	9.22	9.27
30	9.15	9.22	9.17	9.19

Appendix 2.

Changes in titratable acidity of different *L. plantarum* isolates during batch fermentation of cassava dough (agbelima)

	Titratable acidity (% v/v)			
Time	LAB 9	LAB 6	LP 11	LP 3
0	0.101	0.124	0.078	0.153
1	0.146	0.128	0.0908	0.158
3	0.168	0.146	0.135	0.169
6	0.428	0.281	0.248	0.214
9	0.517	0.293	0.36	0.27
12	0.54	0.405	0.439	0.338
15	0.596	0.45	0.459	0.349
18	0.799	0.507	0.54	0.405
21	0.833	0.63	0.563	0.495
24	0.845	0.664	0.572	0.518
27	0.811	0.698	0.585	0.54
30	0.822	0.578	0.518	0.473

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Appendix 3.

Survival of enteric pathogens inoculated into spontaneously fermenting cassava dough (agbelima)

		Microbial counts (Log cfu/g)				
Time	**pH**	***Salmonella typhimuriu m* 9**	***Salmonella enteritidis* 226**	***Shigella dysenteriae* 2357T**	***Escherichia coli* D2188**	***Vibrio cholerae* C-230**
0	5.99	7.45	6.6	7.15	7.3	6.3
4	5.16	5.11	5.34	5.56	7.6	0
8	4.38	2.3	4.48	3.49	7	0
24	4.08	0	0	0	0	0
48	4.10	0	0	0	0	0

Appendix 4.

Survival of enteric pathogens inoculated into 48h fermented cassava dough (agbelima)

		Microbial counts (Log cfu/g)				
Time	**pH**	***Salmonella typhimurium* 9**	***Salmonella enteritidis* 226**	***Shigella dysenteriae* 2357T**	***Escherichia coli* D2188**	***Vibrio cholerae* C-230**
0	3.89	4.45	4.30	5.26	5	4.08
4	3.85	0	0	3	3.18	0
8	3.84	0	0	0	0	0
24	3.8	0	0	0	0	0
48	3.79	0	0	0	0	0

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Appendix 5

Survival of enteric pathogens inoculated into cooked 48h fermented cassava dough (agbelima)

		Microbial counts (Log cfu/g)				
Time	pH	*Salmonella typhimurium* 9	*Salmonella enteritidis* 226	*Escherchia coli* D2188	*Shigella dysenteriae* 2357T	*Vibrio cholerae* C-230
0	3.72	6.7	6.31	6.80	7.15	5.36
0.5	3.71	5.78	5.25	508	6.6	3.08
1	3.71	0	0	5.0	6.3	0
1.5	3.71	0	0	4.2	6	0
4	3.71	0	0	2.1	3	0
8	3.71	0	0	0	0	0
24	3.71	0	0	0	0	0
48	3.71	0	0	0	0	0

Appendix 6.

Survival of enteric pathogens inoculated into cassava dough (agbelima) at neutral pH

	Microbial counts (Log cfu/g)				
Time	*Salmonella typhimurium* 9	*Salmonella enteritidis* 226	*Escherichia coli* D2188	*Shigella dysenteriae* 2357T	*Vibrio cholerae* C-230
0	5.26	5.18	5.34	6.43	6.2
4	5.36	5.28	5.63	5.59	5.08
8	5.2	5.11	5.43	5.32	3.95
24	4.04	4	4.11	4.08	0
48	0	0	4	2	0

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Appendix 7.

Survival of *Salmonella typhimurium* 9 inoculated into cassava dough at specific pHs

	Microbial counts (Log cfu/g) at different pHs		
Time	**pH 4.40**	**pH 4.08**	**pH 3.85**
0	6.447	6.662	5.46
1	4.079	3.47	3.11
1.5	0	0	0
2	0	0	0
2.5	0	0	0
3	0	0	0
3.5	0	0	0
4	0	0	0
4.5	0	0	0

Appendix 8.

Survival of *Salmonella enteritidis* 226 inoculated into cassava dough at specific pHs

	Microbial counts (Log cfu/g) at different pHs		
Time	**pH 4.38**	**pH 4.09**	**pH 3.81**
0	6.778	6.579	6.568
1	5.3	4.43	3.3
1.5	4.9	0	0
2	0	0	0
2.5	0	0	0
3	0	0	0
3.5	0	0	0
4	0	0	0
4.5	0	0	0

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Appendix 9.

Survival of *Escherichia coli* D2188 inoculated into cassava dough at specific pHs

	Microbial counts (Log cfu/g) at different pHs		
Time	pH 4.36	pH 4.09	pH 3.85
0	7.17	7.278	6.87
1	7.079	6.618	5.176
1.5	6.146	5.633	4.612
2	5.505	5.114	3.447
2.5	5.38	4.079	3.079
3	4.3	3	3
3.5	4.25	1.3	2
4	3.3	0	0
4.5	0	0	0

Appendix 10.

Survival of *Shigella dysenteriae* 2357T inoculated into cassava dough at specific pHs

	Microbial counts (Log cfu/g) at different pHs		
Time	pH 4.38	pH 4.09	pH 3.81
0	7.041	6.707	6.9
1	6.447	6.643	4.69
1.5	6.204	4.49	3.477
2	5.643	4.34	3.11
2.5	4.602	3.146	3
3	4	2.308	2.041
3.5	3.3	0	0
4	0	0	0
4.5	0	0	0

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Appendix 11

Survival of *Vibrio cholerae* C-230 inoculated into cassava dough (agbelima) at different pHs

	Microbial counts (Log cfu/g) at different pHs		
Time	**pH 4.36**	**pH 4.09**	**pH 3.81**
0	6.579	5.74	5.342
1	3.146	2.6	0
1.5	0	0	0
2	0	0	0
2.5	0	0	0
3	0	0	0
3.5	0	0	0
4	0	0	0
4.5	0	0	0