DEVELOPMENT OF STARTER CULTURE FOR THE FERMENTATION OF DEHULLED MAIZE INTO *NSIHO* (WHITE KENKEY)

BY

THEOPHILUS ANNAN
(10359128)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL FOOD SCIENCE DEGREE

JULY, 2013

DEDICATION

I dedicate this work to my lovely wife Mrs.Hellen Ama Annan, my wonderful daughters, Tamara Millicent Naa Adoley Annan and Theodora Naa Adorkor Annan and my siblings.

DECLARATION

I do hereby declare that this thesis is the result of my own research except for references to works of others that have been duly cited under the supervision of Prof. Tano-Debrah and Dr. Wisdom Amoa-Awua, and that this work either in whole or part has not been presented for another degree elsewhere.

....

THEOPHILUS ANNAN DATE

(STUDENT)

....

PROF. K. TANO-DEBRAH DATE

(SUPERVISOR)

....

Dr. W. AMOA-AWUA DATE

(SUPERVISOR)

ACKNOWLEDGEMENTS

This work was facilitated by financial support from the European Union under the project African Food Tradition rEvisited by Research (AFTER) KBBE-20009-2-3-02.

I wish to sincerely thank my supervisors, Professor K. Tano-Debrah, former Head of Department, Nutrition and Food Science, Legon and Dr. W. Amoa-Awua, Chief Research Scientist and former Head of Food Microbiology Division, Food Research Institute, Accra, for their constructive criticisms, guidance and mentorship which have brought this work to a successful completion.

I am grateful to Miss Angela Adam and Mr. Alexander Appiah for helping in preparation of microbiological media. My appreciation goes to Mr. Emmanuel Tetteh for making sure I always had sterile Petri dishes for my analyses. I am highly indebted to Miss Kukua Amo Thompson for her assistance and interest in this work. I am also thankful to Mr Kavi, Senior Librarian of Food Research Institute for giving me all the articles and publications I needed for this work. I would also thank Messr's Gariba and Safo for driving me to the field to collect my samples. A big thanks to all the AFTER team members for their support. Finally, I am most grateful to my wife for her support and encouragement.

I am above all most grateful to God almighty for giving me strength and wisdom to carry out this work.

ABSTRACT

Nsiho kenkey is a bland tasting stiff porridge produced from dehulled maize grains and consumed mainly in some parts of Southern and Eastern Regions of Ghana. This study was carried out to identify the microorganisms responsible for *nsiho* fermentation and to develop a starter culture for its controlled fermentation in order to improve the safety and quality of the product. A brief field study was carried out in two districts in the Eastern Region of Ghana to observe the processing operations in *nsiho* production and obtain samples for laboratory analysis. The population of aerobic mesophiles, lactic acid bacteria (LAB) and yeasts were enumerated on Plate Count Agar, de Man Rogosa Sharpe Agar and Oxytetracycline Glucose Yeast Extract Agar respectively. The species of the LAB and yeasts were tentatively identified by phenotypic characterization based mainly on their pattern of carbohydrate assimilation and fermentation. Lactic acid bacterria isolates were screened for rate of acidification, production of exopolysaccharides (EPS), amylase and protease activity as well as antimicrobial activity against some common enteric pathogens using the Agar Well Diffusion Assay. Starter culture was developed through combination of dominant strain of lactic acid bacteria and yeasts in production trails. Survival of four enteric pathogens (*Salmonella typhimurium,* NCTC 12023, *Staphylococcus aureus,* NCTC 657, *Vibrio cholerae* NCTC 11348 and *Escherichia coli.* NCTC 9001*)* were also studied during steeping enriched with the starter cultures. Mean pH values decreased from 5.99-3.58 units whilst Titratable acidity increased from 0.03-0.30 % during 48 h steeping of dehulled maize grains in *nsiho* prodcution. Similarly, pH values decreased from 5.94-3.50 units and Titratable acidity increased from 0.27-0.36 % of 12 h of subsequent dough fermentation. Lactic acid bacteria population increased from a concentration of 10^4 to10^8 cfu/ml during steeping and from 10^5 to10^8 cfu/g during the dough fermentation. Yeasts counts increased from 10^2 to 10^6 cfu/ml during steeping and from 10^3 to10^7 cfu/g during dough fermentation. The lactic acid bacteria responsible for *nsiho* fermentation were identified to be *Lactobacillus fermentum,* 47.1%, *Lactobacillus brevis,* 25%, *Lactobacillus plantarum,* 14.42%, *Pediococcus pentosaceus,* 8.65% and *Pediococcus acidilactici,* 4.8%. The dominant yeasts species were *Saccharyomyces cerevisiae,* 47.6%, *Candida krusei,* 29.1%, *Debaryomyces* spp., 15% and *Trichosporon* spp., 8.3%. Isolates of *Lactobacillus fermentum* exhibited a faster rate of acidification than the other LAB isolates tested. Most of the LAB isolates screened produced EPS, a few showed amylolytic activity whilst none exhibited protease activity. There was no interaction between the LAB isolates; however, a few of the LAB inhibited the growth of yeasts. The use of starter culture involving combination of LAB and yeasts isolates decreased the steeping time of the dehulled maize grains from 48 h to 24 h. The use of various combinations of LAB and yeasts as inoculum enrichment during fermentation trials produced kenkey acceptable to the taste panel, however, no significant differences ($p<0.05$) were recorded in taste, texture and odour when compared to the spontaneous fermented *nsiho*. In challenge testing during steeping of maize grains *Vibrio cholerae* could not be detected after 48 h whilst counts for *Salmonella typhimurium, Staphylococcus aureus* and *Escherichia coli* were reduced by 3 log cycles. Based on antimicrobial activity and technological properties exhibited by the isolates and the sensory quality of *nsiho*, the best starter organisms selected for *nsiho* production consisted of mixed cultures of *Lactobacillus fermentum, Lactobacillus brevis* and *Saccharomyces cerevisiae.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.1 Maize production and consumption

Maize (*Zea mays*) is a cereal grain which belongs to the grass family (Gramineae) and is ranked the third most abundant cereal crop produced worldwide. Maize is the principal cultivated cereal grown in Ghana, mainly in the middle-southern part (transitional and forest zones), with an estimated 15% grown in the northern regions of the country. Its gross production in 2009 was 1,620.000 metric tons with per capita consumption of 43.8 Kg/annum and net surplus of 115,750 metric tons (Ministry of Food and Agriculture, 2010).

As a cereal crop widely used in Ghana, maize contributes significantly to the total calorie of the Ghanaian diet, furnishing between 90-95% of the total calorie in the diet of the people in the coastal plains. However, the consumption of maize is less extensive in the northern part of the country where sorghum and millet are the main staples (Dovlo, 1970; Ijabadeniyi and Omoya, 2006).

1.2 Traditional Processing of Maize

The traditional processing of maize into various fermented and semi-fermented food products plays an important role in the food supply system of Ghana, and contributes to the curtailment of the post harvest losses and national food delivery (Sefa –Dedeh, 1993). The maize grains, by a variety of indigenous processes, is transformed into an intermediate or finished product with a stable shelf-life, more digestible, improved nutritive quality and desirable organoleptic properties. Processing may also include improvement of the protein quality of the maize product by fortification with legumes such as cowpeas and soybeans (Plahar and Leung 1983; Nche *et al*., 1994; Obiri-Danso *et al*., 1997; Plahar *et al*., 1997; Sefa-Dedeh *et al*., 2000).

Traditional maize processing methods involve combination of different unit processing operation including steeping, dehulling, roasting, milling, fermenting, *aflatalization*, moulding, cooking and packaging. These skills have been developed and handed down from one generation to the other. Processing maize into fermented dough is mainly by traditional methodologies and technologies which utilize local raw materials and local equipment which have strong links with rural traditional environment (Sefa-Dedeh, 1993; Afoakwa *et al*., 2004). Maize processing is one of the major rural /informal food industries in Ghana serving as a means of livelihood for a number of rural women who rely on family labour and engage in commercial activity as a household enterprise.

1.2.1 Processing of Maize into Kenkey

There are mainly two types of kenkey, the Ga kenkey locally called *Komi*, and the Fanti kenkey known as *Dokono*, and these differ in their organoleptic quality and the processing procedures (Halm *et al*., 2004). However, there is another type of kenkey, *Nsiho* or *Akporhi* which is produced from dehulled or polished maize, (referred to as white kenkey in the present work), that is widely consumed in parts of Eastern, Western, Central and Volta regions of Ghana. *Nsiho* production involves dehulling or polishing of maize grains which are then steeped for 48 h and milled into a moist meal. This is then kneaded with water into dough and left to ferment spontaneously for up to 24 h. The dough is pre cooked, moulded into balls, wrapped in corn husk and steamed for about 1-2 h.

Traditional processing of foods such as *nsiho* and other types of kenkey is an important socio-economic activity which serves as a livelihood for many families. However, many problems are encountered during the traditional processing of foods which need to be addressed to improve the activities of the traditional food processors.

Problems encountered during the traditional processing of foods in Ghana include, the time consuming nature of the process, lack of proper hygienic and sanitary practices, contamination of products, low production efficiency, uneconomic utilization of energy sources and unorganised and scattered nature of the areas of manufacture. Other problems include improper and ineffective organisation of production schedules, lack of proper recruitment and training of personnel, lack of proper specification of raw materials and rudimentary packaging of products compromising of good manufacturing practices (Lartey, 1975; Amoa-Awua *et al*., 2007). According to Halm *et al*., (2004), indigenous maize processing industries need the injection of suitable scientific and technological know-how in every facet of their operations. In addition key areas for ensuring compliance with Good Manufacturing Practice (GMP) have been identified by Amoa-Awua *et al*., 2007.

1.3 RATIONALE FOR THE STUDY

With increasing urbanisation, there is a gradual shift from a traditional based society to a modern society in several African countries. As a consequence some successful attempts have been made towards upgrading traditional processing technologies, including the production of Ga and Fante kenkey.

Also, a lot of literature has been published on kenkey produced from whole maize i.e *Ga* and *Fante* kenkey based on their in-depth scientific studies. These studies have formed the basis for continual attempts to improve and upgrade the production of quality *Ga* and *Fante* kenkey including the use of starter culture and implementation of quality assurance measures.

It is only currently that such detailed studies are being carried out on kenkey produced from dehulled or polished maize and which will form a basis for upgrading the potential of *nsiho*. It is in this regard that this work has been carried out to identify the dominant microorganisms involved in *nsiho* fermentation with a view to develop starter cultures for the production process.

The development of starter culture will optimise the process of *nsiho* fermentation and provide a means for controlling the quality of the product leading to stable organoleptic characteristics and also eliminate the quality risks inherent in traditional *nsiho* production. With the use of starter culture, it is expected that fermentation time will be significantly reduced and quality enhanced for mutual benefit of the producers and consumers in Ghana and the Diaspora.

1.4 MAIN OBJECTIVE

To develop a starter culture for the fermentation of dehulled maize grains into *nsiho*

1.4.1 SPECIFIC OBJECTIVES

- To isolate, characterise and identify the lactic acid bacteria and yeast responsible for the fermentation of dehulled maize into *nsiho*
- Determine the technological properties of the dominant lactic acid bacteria and yeasts involved in *nsiho* fermentation.
- To investigate the antimicrobial property of *nsiho* against common enteric pathogens during the steeping of dehulled maize grains
- Develop starter culture from lactic acid bacteria and yeast for control fermentation of dehulled maize in order to assure the quality of *nsiho*

2.0 LITERATURE REVIEW

2.1 Maize Grain

Maize (*Zea mays*) is a major staple crop in Ghana, and it is vital to the survival and food security of a large segment of the population, contributing over 55 % of the total energy intake (Sefa-Dedeh and Mensah, 1989). It is also an important component of poultry and livestock feed and to a lesser extent, a substitute in the brewing industry. Maize is an important commodity in West Africa sub-regional trade, particularly between Ghana, Burkina Faso, Mali, Togo and Niger through mainly informal trading.

Maize which is ranked the third most abundant cereal crop produced worldwide was originally cultivated in Central America as far back as 4,500 BC (Mc Gee, 1984). Maize was introduced to Ghana around the 15th century by the Portuguese (La Anyane, 1956). Nutritionally, it contains about 9.8 % protein, 4.9 % fat, 1.4 % ash, 2 % crude fibre, and 63.6 % carbohydrate (Alais and Linden, 1991). Like most cereals it is limiting in the amino acid lysine as well as tryptophan and contains anti-nutrients such as phytates.

2.2 Some Ghanaian Traditional Food Products Made From Maize

Maize utilization in Ghana is mainly in the form of whole maize dough, refined maize dough, and roasted corn or by cooking the whole maize. Whole grains may be processed into dry grits, or roasted into a meal or fermented for use in the making of maize beverages (Sefa-Dedeh, 1993). The bulk of the maize produced is consumed in the form of fermented products. Fermentation of maize in Ghana can be described as an indigenous technology that has been transferred from one generation to the other (Sefa-Dedeh, 1993). Many authors (Whitby 1968; Dovlo 1970; Sefa- Dedeh, 1993) have reviewed that maize is processed into different forms such as semi solid products like *banku*, kenkey and liquid products like *koko*. The production of *banku*, *fomfom* and *koko* will be described briefly but a more detailed description for *kenkey*; the principal product prepared from fermented whole dough and widely consumed in Ghana.

2.2.1 Fomfom

This product is popular in the Western region of Ghana. The indigenous procedure for processing maize into *fomfom* is illustrated in Fig 2.1. *Fomfom* is a stiff porridge similar to *kenkey* in consistency but made from dehulled maize. The maize is dehulled and milled. The milled dough is mixed with water and an inoculum of old dough is added to shorten fermentation period from 24 h to 6-9 h. The fermented dough is shaped into balls with holes made in the centre. The balls are cooked twice in boiling water. After each boiling, the balls are pounded in a mortar. After the second pounding, the mass is moulded into balls and packaged in banana leaves ready for consumption (Johnson and Halm, 1998).

2.2.2 Kokui (Ewe kenkey)

This maize meal is made by sifting to remove some of the bran. A portion of this is gelatinised (known as *aflata*) and mixed with the remaining ungelatinised portion. The mixture of *aflata* and the raw dough are left to ferment for about 8 h. Balls are made from this mixture and steamed

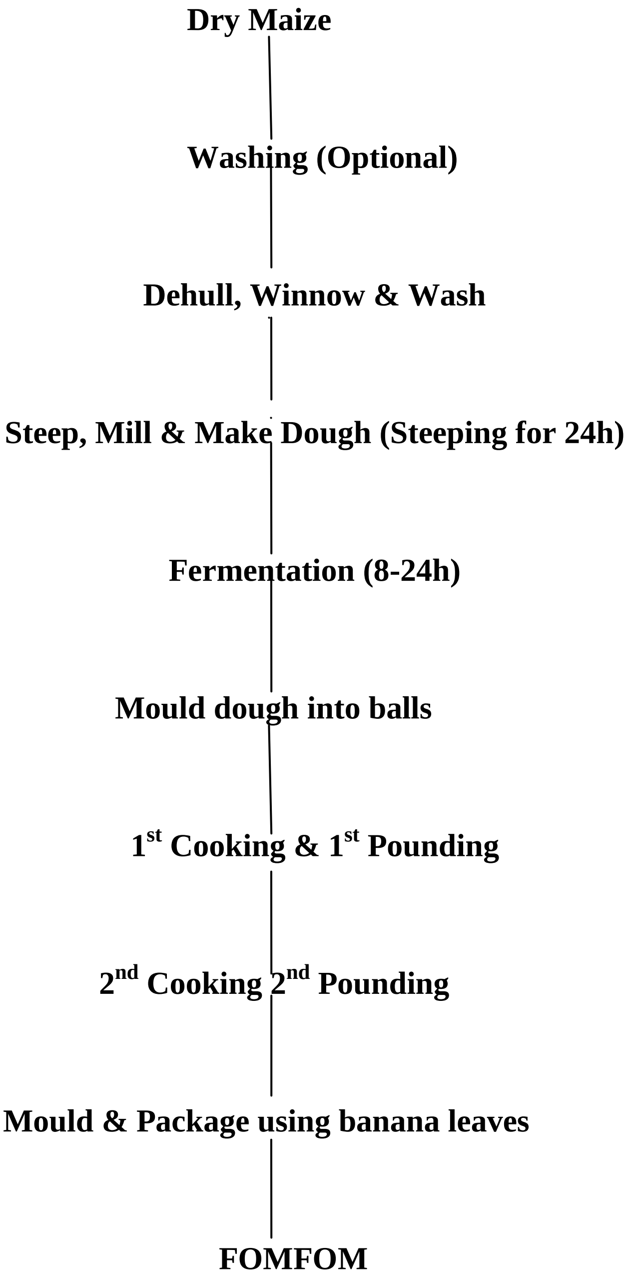


Fig 2.1: Production of Fomfom

Source: Johnson and Halm, (1998)

2.2.3 Akpiti (Ga), Bamfobisi (twi) or Amo kaklo (Ewe)

The preparation of this product, known by different names among the different ethnic groups is similar to *kokui* preparation except that sugar is added to the *aflata* /dough mixture and is left to ferment for 6-8 h. Balls are made from this mixture which are deep fried and served as snacks (Whitby 1968; Dovlo 1970).

2.2.4 Abolo

Depending on the ethnic group the maize is rinsed with water, ground and sifted to remove the bran. This is then reconstituted with water to form unfermented dough which is cooked to gelatinise (*aflta*). A mixture of cooked and uncooked dough is allowed to ferment for about 8 h and depending on which tribe; it is either cooked (*Ga*) or steamed (*Ewe*) (Whitby 1968; Dovlo 1970).

2.2.5 Banku

Banku is normally consumed along the coastal regions of Ghana. Fermented dough is made into slurry with water and stirred till cooked into a semi- solid consistency. This is then moulded into balls and eaten with sauce (Whitby, 1968, Owusu Ansah *et al*., 1980).

2.2.6. Koko

Koko is a thin gruel used by some as a weaning food for infants and consumed as breakfast porridge all over the country. It is made by adding a large volume of water to fermented whole maize dough to form slurry which is cooked by boiling into a thin gelatinous porridge. It can also be prepared by adding large quantity of water to milled steeped maize. The mixture is strained to get rid of chaff and the slurry left overnight to settle and ferment. The supernatant water is decanted and the slurry used to prepare *koko* (Halm *et al.*, 1996).

2.2.7 Kenkey

Kenkey is a principal and popular product prepared from fermented whole maize dough. It is a stiff gruel or dumpling from fermented maize dough which is wrapped in leaves and boiled. It is also a sour tasting food with moisture content between 62-68 %, pH of 3.7 and a shelf life of about 3-4 days with no refrigeration.

There are two main types of *kenkey*, the Ga *kenkey* locally called *komi* and the Fanti *kenkey* known as *dokono*. The difference lies in some processing aspect such as the packaging material for Ga *kenkey* which is maize husks, while that of Fanti *kenkey* is the plantain leaf. Salt is added to the Fanti kenkey. Several authors (Christain, 1966, 1970; Whitby, 1968; Dovlo, 1970; Nyarko-Mensah and Muller, 1972; Plahar and Leung, 1982; Sefa-Dedeh and Plange, 1989; Halm *et al*., 1993, 1996, and 2004) have described the various traditional methods of kenkey production. It involves various unit operations such as cleaning, steeping, milling and doughing, dough fermentation, *aflata* preparation, mixing of *aflata* and raw dough, moulding and packaging and cooking into *kenkey* (Fig 2.2).

2.2.7.1 Cleaning

The maize is cleaned by winnowing to remove chaff by stream of air, hand picking to remove stones, damaged grains, insects, and debris and maize cobs. Sedimentation is also used where the maize is poured into a big basin of clean water and stirred with a wooden ladle to allow mature and quality grains to settle and less dense immature grains, insect damaged and mouldy grains settle at the top. The latter is scooped off using baskets or sieve and discarded or used as animal feed (Lartey, 1967, Ofosu, 1971).

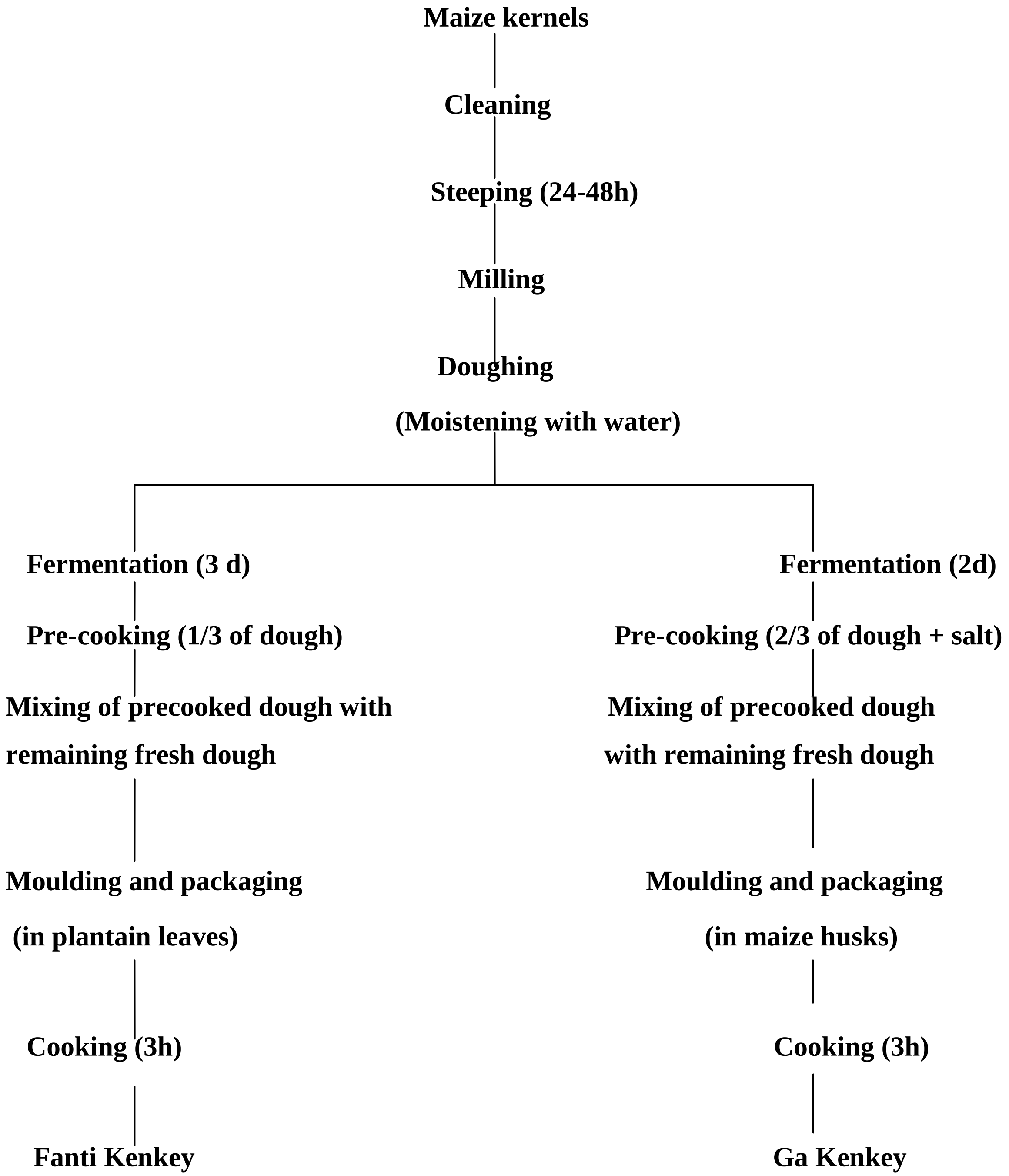


Fig. 2.2: Production of Ga and Fanti kenkey

Source: Halm *et al*., (1996)

2.2.7.2 Steeping

Cleaned maize grains are steeped in tap water in steeping tanks for one to three days depending on the moisture content, variety and hardness of the maize used. Steeping allows imbibition of water into the grains which facilitate increase of kernel size, facilitate size reduction and condition the grain for smooth milling (Akingbala *et al*., 1987; Nche *et al*., 1996). Lactic fermentation have been reported to begin during steeping and moulds present on the maize grains are inhibited and disappear after steeping (Sefa –Dedeh, 1993; Halm *et al*., 1993; Jespersen *et al*., 1994).

2.2.7.3 Nutritional Aspect of Kenkey

The nutritional value of the maize product kenkey is basically dependent on the variety of maize from which it is made and the processing technique applied in production. Processing methods such as soaking, packaging materials, cooking and fermentation may either reduce or increase one nutrient depending on the nature of the nutrient been considered (Ankrah, 1972). Calcium losses occur during kenkey production, phosphorus content, however, increases but due to anti-nutritive factors such as phytic acid which bound this mineral, there is a reduction in bio-availability of both phosphorus and calcium (Bediako-Amoa and Muller, 1976).

On a dry basis, the proximate composition of Ga *kenkey* is approximately 8.9-9.8 % protein, 1.3-3.2 % fat, 0.5-1.9 % ash, 10.6-78.6 mg/100g calcium, 202.4-213.8 mg/100g phosphorus, 6.5-12.6 mg/100g iron and 74.3-87.1 % total carbohydrates (Halm *et al*., 2004).

2.3 STARTER CULTURE

A starter culture may be described as a preparation containing high numbers of viable microorganisms, which may be added to accelerate the fermentation process and to bring about desirable changes in a food substrate. Being adapted to the substrate; a typical starter culture facilitates improved control of a fermentation process and predictability of its product (Holzapfel, 2007). Starter cultures are selected specifically for a substrate or raw material e.g. milk, meat, cereals, legumes, roots and tubers, etc. Microorganisms used in starter cultures include moulds, yeasts and bacteria.

2.3.1 Moulds

Moulds play a minor part in fermented foods of Africa. Foods such as *Miso* (prepared from rice and soy beans with *Aspergillus oryzae* and yeasts), Tempe (prepared from soy beans with *Rhizopus oligospos***)** and soy sauce or Shoyu (prepared from soy beans and wheat by a mixed-culture fermentation with *Asp. oryzae***,** yeasts and lactobacilli) are typical examples of traditional Asian foods produced on an industrial scale and well known worldwide (Ebine, 1989; Fukushima, 1989). In Europe, traditional mould ripened foods are mainly restricted to blue-mould *(Penicillium roqueforti)* and white-mould *(Penicillium camemberti)* cheeses and mould-ripened fermented sausages (Leistner, 1990).

2.3.2 Yeasts

Plant materials containing fermentable sugars provide suitable substrates for yeasts species of *Saccharomyces, Candida, Torula, Hansenula,* etc. These yeasts, especially *Saccharomyces,* are typically associated with spontaneous alcoholic fermentations such as African opaque beers and palm wine. Generally, these are mixed strain fermentations, as exemplified by the combined lactic and alcoholic fermentation of palm wines and most opaque cereal beers. For the industrial production of Western style beers and even traditional African beers, selected strains of *Saccharomyces cerevisiae* are used. Jespersen *et al*., (1994), isolated a mixed flora comprising *Candida, Saccharomyces, Trichosporon, Kluyveromyces, and Dabaryomyces* species from raw maize, during steeping and early phase of fermentation of maize dough fermentation for kenkey production.

2.3.3 Bacteria

Among the bacteria associated with food fermentation lactic acid bacteria (LAB) are of predominant importance. This is especially true for Africa, probably the continent with the largest spectrum and richest variety of lactic fermented foods. Lactic acid bacteria are basic starter cultures with widespread use in the dairy industry for cheese making, cultured butter milk, cottage cheese and cultured sour cream. It is also widely used in cereal fermentation in Africa (Jay, 1986; Holzapfel 2002). Therefore, identifying and providing a practical means of using appropriate starter culture is advantageous due to competitive role of microorganisms and their metabolites in preventing growth and metabolism of unwanted microorganisms.

2.4 Attributes of Starter Culture

Starter culture improved shelf-life; enhanced inhibition or elimination of foodborne pathogens; improved sensory quality (taste, aroma, visual appearance, texture, consistency); reduced preparation procedures (reduction of cooking times and lower energy consumption); improved nutritional value (‘upgrading’) by degradation of antinutrition factors. It also improves protein digestibility and bio-availability of micronutrients; biological enrichment, e.g. through the biosynthesis of vitamins, essential amino acids and proteins, improved toxicological safety by degradation (detoxification) of toxic components such as linamarin in cassava; and probable degradation of mycotoxins (Holzapfel, 1997; 2002).

2.4.1 Desirable Properties of Starter Culture

A starter culture is used to improve a fermentation process, such as lactic, alcoholic or alkaline fermentation. The use of ‘back-slopping’to start a new batch resembles the principle of starter cultures in an empirical sense.

The primary consideration before introducing starter cultures for traditional food fermentations is to significantly contribute to an improvement of processing conditions and product quality with respect to:

- rapid or accelerated acidification or alcohol production,
- an improved and more predictable fermentation process,
- desirable sensory attributes,
- improved safety and a reduction of hygienic risks.

According to Holzapfel (1997), traditional procedures, back-slopping, adaptation and the particular environment have contributed to the selection of a microbial population typical of a particular fermentation process. This has therefore necessitated for the study of interactions between single and mixed strain cultures from traditional products, both under defined conditions. Food substrates, with respect to: competitive behaviour, viability and survival, antagonism against pathogens and spoilage microbes, rates of acid or alcohol production, sensory changes, and, also taking into account the type of primary metabolites formed (e.g. lactic isomers), degradation of antinutrition factors.

Table 2.1: Mixed starter cultures applied in the production of traditional acid- and acid-alcoholic fermented gruels

Product	Country	Raw materials	Inoculum (starter)	Storage conditions	Microorganisms
Mawe (sourdough)	Benin	maize	previous batch	active fermentation	heterofermented lactobacilli and yeasts
Mahewu (wheat flour)	Southern Africa	maize	small portion of whole- lactobacilli (yeasts)	(?)	mainly heterofermented
Kocho (flour)	Ethiopia	*ensete* (false banana)	fermenting kocho	pit	LAB, yeasts
Pito	Ghana,	sorghum	“Inoculation belt”	drying	Lactobacilli, yeast

Source: Holzapfel (2000).

2.5 Starter Cultures in Africa Cereal Fermentation

The basis of the development of starter cultures is the ability to control the specific microorganisms or the succession of microorganisms that dominate the microflora of foods (Caplice and Fitzgerald, 1999). Adaptation to the substrate allows a typical starter culture to gain improved control of a fermentation process, particularly the initial phase, and the predictability of fermentation products (Holzapfel, 1997). Identifying and providing a practical means of using starter cultures is advantageous due to the competitive role of microorganisms and their metabolites in preventing growth and metabolism of unwanted microorganisms. A strong starter may reduce fermentation times, minimize dry matter losses, avoid contamination with pathogenic and toxigenic bacteria and moulds, and reduce the risk of incidental micro-flora causing off-flavours in foods (Haard, 1999). The challenge remains however, to improve fermentations without losing other desirable traits or introducing accidentally, undesirable characteristics.

Strains of lactic acid bacteria and yeasts have been successfully used as starter cultures in a number of indigenous fermented foods. The following describes the use of starter cultures in selected African foods.

2.5.1 Use of Starter Cultures in Maize Fermentation

Using combined inocula of *Saccharomyces cerevisiae* and *Candida kefyr*, Nyarko and Danso (1991), found that the acceptability of fermented maize dough was significantly improved over the non- inoculated naturally fermented maize dough. Nche *et al*., (1994) were able to accelerate the fermentation of maize dough for kenkey production from two days to 24 h using a back-slopping inoculum. Although a pH drop from 5.65 to 3.79 was achieved within 24 h, sensory evaluation indicated poor acceptability of the textural characteristics of the dough for kenkey production due to the omission of the soaking step practised traditionally. Maize dough prepared from the accelerated fermented dry milled flour had inferior gelatinisation and pasting properties compared to the traditionally prepared dough. Soaking or steeping of cereal grains in water prior to wet milling, softens the grain endosperm. The penetration of water into the interior of the kernels during steeping requires hours in an ambient temperature and simultaneous fermentation occurs by fermenting microorganisms, which originate from the surface of the kernels or other sources such as the steeping vessel (Odunfa, 1985). The resulting starchy material from wet grinding carries the sour flavour, which has become an essential and desired element in local foods cooked from the fermented slurry or dough (Campbell-Platt, 1987).

Use of six strains of *L. fermentum* and one strain of yeast, *S. cerevisiae*, showed that the typical final pH of 3.7 could be attained within 24 h of dough fermentation instead of 48 h as observed with spontaneous dough fermentations (Halm *et al*, 1996). The organoleptic acceptability of kenkey and koko, the most popular traditional products, prepared from maize dough fermented with starter cultures was in most cases not significantly different from those prepared from traditional spontaneously fermented dough. Poorer textural characteristics of kenkey produced from 24 h fermented dough with starter cultures contributed to the unacceptability of this product by a trained panel of judges. Halm *et al*, (1996), also suggested that other enzymatic and biochemical changes may be important in the spontaneous fermentation process that contributes to the development of texture. The sour porridge, koko was however acceptable when prepared from dough fermented with starter culture for 24 or 48 h. The authors attributed this to the less important role texture may have played in the acceptability of koko, it being a thin slurry, in relation to the stiff dumpling-like texture of kenkey.

2.5.2 Use of Starter Cultures in *Ogi* Preparation

Akinrele (1970) showed that 'Ogi' produced from a mixed culture of *Lactobacillus* and *Acetobacter* enriched the nutrient quality by increasing the concentrations of riboflavin and niacin above that found in both the unfermented grain and the 'Ogi' produced by traditional spontaneous fermentation. A mixed culture inoculum of *L. plantarum*, *Lactococcus lactis* and *Saccharomyces rouxii* was developed for cultured 'Ogi' production, which resulted in increased rate of souring of the dough (Banigo *et al*., 1974). Sanni *et al*., (1994), found higher levels of ethanol in spontaneously fermented Nigerian 'Ogi' as compared to samples using inocula of lactic acid bacteria. Using fifty mutants from *L. plantarum* and seven mutants from yeast strains selected from cultures capable of over producing lysine, twelve- and three-fold increases, respectively, in lysine production were observed in 'Ogi' (Odunfa *et al*., 1994). Starter culture strains of lactobacilli isolated from local fermented foods with strong antibacterial activity were used in the production of ‘Dogik’, an improved ‘Ogi’ with potentials for use in diarrhoea control (Olukoya *et al*., 1994). ‘Ogi’ prepared by fermentation with *L. plantarum* as starter culture decreased the pH from 5.9 to 3.4 within 12 h compared to 2-3 days required in the normal traditional process (Sanni *et al*., 1994). High increases in levels of lysine and methionine were also observed in ‘Ogi’ prepared from dehulled maize grains inoculated with mixed starter cultures of *Saccharomyces cerevisiae* and *Lactobacillus brevis* (Teniola and Odunfa, 2001). The best tasting ‘Ogi’ comparable to traditionally fermented ‘Ogi’ was achieved with single starter cultures of *L. brevis*.

2.5.3 Use of Starter Cultures in *Uji* Preparation

The ability of pure lactic cultures isolated from naturally fermenting ‘Uji’ mash and pure cultures of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Lactobacillus delbruecki* to ferment ‘Uji’ was investigated by Mbugua and Ledford, (1984). They found that most bacterial strains failed to successfully ferment sterile or heat-treated ‘Uji’ slurries as evidenced by poor acid formation. They attributed this to the absence of symbiotic relationships in sterile media, usually present in mixed bacterial populations, as well as the destruction of thermolabile factors and changes in the isolated organisms during the sub-culturing process. ‘Uji’, fermented by mixed native ‘Uji’ bacteria was more organoleptically acceptable than isolated starter culture of *L. bulgaricus* or *S. thermophilus*. In another study, Masha *et al*. (1998) compared in laboratory trials the fermentation of ‘Uji’ using a starter culture of lactic acid bacteria *(L. plantarum, L. brevis, L. buchneri, L. paracasei* and *Pediococcus pentosaceus*), using the method of backslopping (inoculum from a previous fermentation) and by spontaneous means at 30ºC. They found that the lowest pH of 3.5 was attained with the lactic acid bacteria starter culture fermentation while viscosity of

'Uji' was only marginally affected by method of fermentation. The aroma profile of 'Uji' fermented with lactic acid bacteria was characterised by high concentrations of acids (hexanoic, octanoic and nonanoic) and some alcohols (1-propanol, 1-hexanol, 1- nonanol and 2- undecenol), spontaneously fermented samples were shown to have high concentrations of esters (ethyl butanoate, hexyl acetate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate and ethyl nonanoate) other alcohols (ethanol, 1- butanol, 3-methyl- 1-butanol and 2-methyl-1-propanol) and acids (acetic and heptanoic acid), while the backslopping method of fermentation was characterised as having low concentrations of all volatiles identified. Unfermented 'Uji' was characterised as having mainly high levels of aldehydes (pentanal, hexanal, heptanal, nonanal, (E)-2-heptenal and (E)-2-octenal) and other compounds (2-heptanone, 2-pentyl furan, 1-octen-3-ol and isopropyl alcohol).

2.5.4 Use of Starter Cultures in the Production of *Mawe*

Single starter cultures of *L. fermentum* and *L. brevis* were found to be effective in fermenting sterile 'Mawe' suspensions to produce porridge with similar acidity levels as the naturally fermented ' Mawe' (Hounhouigan *et al*., 1999). Starter cultures of only the yeasts, *C. krusei* and *S. cerevisiae* produced 'Mawe' with high pH (5.6 and 5.5 respectively) and low titratable acids expressed as percentage lactic acid (0.05 and 0.06, respectively). Results of sensory evaluation showed that the porridges produced with 'Mawe' fermented with starter cultures had less flavour than the traditional commercially produced 'Mawe' porridge.

2.5.5 Use of Starter Culture in *Mahewu* Production

The traditional spontaneously produced ' Mahewu' is considered undesirable because it involves a long fermentation time (about 36 h), proceeds too irregularly, permitting the development of undesirable bacteria resulting in undesirable off-flavours from secondary fermentations (van der Merwe *et al*., 1964). For these reasons considerable research has been devoted over the years to the use of starter cultures to produce 'Mahewu' of consistently high quality and in relatively shorter times of 8 to 12 h (Schweigart and Fellingham, 1963, van der Merwe *et al*., 1964, Schweigart, 1970, van Noort and Spence, 1976, Hesseltine, 1979). The most satisfactory acid producing starter culture was found to be *L. delbruecki* (van der Merwe *et al*., 1964). Freeze or spray-dried ' Mahewu' cultures consisting mainly of *L. delbruecki* could be used effectively as starter culture for bulk fermentations, however, a lag phase of 8 h in contrast to 3 h with the use of fresh starter cultures was observed (Schweigart, 1971).

Table 2.2: Traditional Lactic Acid Cereal- Based Fermented Foods and Beverages

Product	Country	Cereal	Nature of Fermented Product	Product Use
Banku	Ghana	Maize, Cassava	Dough	Cooked Dough
Ben-saalga, koko	Burkina Faso, Ghana	Pearl millet	Slurry	Gruel
Bushera	Uganda	Sorghum, millet	Slurry	Beverage
Gowé (Sifanu)	Benin	Sorghum	Cooked slurry	Beverage
Hussuwa	Sudan	Sorghum	Dough	Dough-like food
Injera	Ethiopia	Tef, sorghum, corn, finger millet, barley	Batter	Flat bread
Mahewu	South Africa, Zimbabwe	Maize	Slurry	Beverage
Mawé	Benin,Togo	Maize	Dough	Basis for ready-to-serve foods
Ogi	West Africa	Maize,millet,sorghum	Slurry	Basis for ready-to-serve foods
Poto poto	Congo	Maize	Slurry	Gruel
Togwa	East Africa	Maize	Cooked slurry	Beverage

Source: (Adams, 1998, Blandino *et al*., 2003)

2.6 The Role of Lactic Acid Bacteria in Fermentation

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

Lactic acid bacteria are the most widespread of desirable microorganisms in food fermentation. They are found in fermented cereal products, milk, cheese and fermented meats (Campbell-Platt, 1987). Lactic acid bacteria convert the available carbohydrate to organic acids and lower the pH of food. These acids as well as other flavour compounds which include diacetyl, acetaldehyde and acetoin contribute to the desired taste and flavour of food (Pederson, 1971).The low pH that is created also makes the food unfavourable for the growth of spoilage and pathogenic bacteria (Adams and Moss, 1995).

Lactic fermentation can be divided into two broad categories distinguishable by the products formed from glucose (Vandamme *et al*., Stiles and Holzapfel, 1997). These are referred to as homofermentation and heterofermentation.

Homofermenters convert glucose 1,6-diphosphate using Embden Meyerhof (EM) pathway (Dirar and Collins, 1972). The enzyme aldolase cleaves fructose 1,6 diphosphate between C_3 and C_4 to give the phosphate esters dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate. The end product of this fermentation pathway is lactic acid (de Vries and Stouthamer, 1968), (Figure 2.3).

The overall lactic acid fermentation pathway can be expressed as follows:

1 Glucose + $2NAD^+$ + 2ADA +2Pi → 2Pyruvate + 2NADH + $2H^+$ +$2ATP^+$

Pyruvate +NADH +H^+ ↔ Lactate +NAD^+

In heterolactic acid fermentation (Figure 2.3), the pentose phosphate pathway is used instead of the EM pathway of glycolysis. This type of fermentation produces ethanol and carbon dioxide in addition to lactic acid in the molar ratio 1:1:1.

The ethanol and the CO_2 come from the glycolytic portion of the pathway. There two possible ways by which ethanol is formed, (Holzapfel and Wood, 1995). Acetaldehyde formed by the cleavage of pyruvate by pyruvate decarboxylase is reduced in the presence of alcohol dehydrogenase to form ethanol. Ethanol can also be formed by a combination of acetyl coA reduction to acetaldehyde followed by reduction of acetaldehyde by reduction of ethanol dehydrogenase.

The overall reaction for the heterofermentation reaction can be expressed as follows:

Glucose +ADP +Pi → Lactic acid + Ethanol +CO_2 + ATP

2.6.1 Microorganisms Involved in the Lactic Acid Fermentation

The lactic acid bacteria are rod shaped or spherical microorganisms. Their names derives from the fact that ATP is synthesised through fermentation of carbohydrates, which yield lactic acid as a major and sometimes the sole end –product (Vandamme *et al*., Stiles and Holzapfel, 1997).

The taxonomy of lactic acid bacteria is in a state of flux but the principal genera are *Lactobacillus, Pediococcus, Steptococcus, Leuconostoc, Carnobacterium, Enterococcus,* *Lactococcus, lactospaera, Oenococcus, Weissella, tetragenococcus* and *Vagococcus* (Adams and Nicolaides, 1997; Axelsson, 2004).

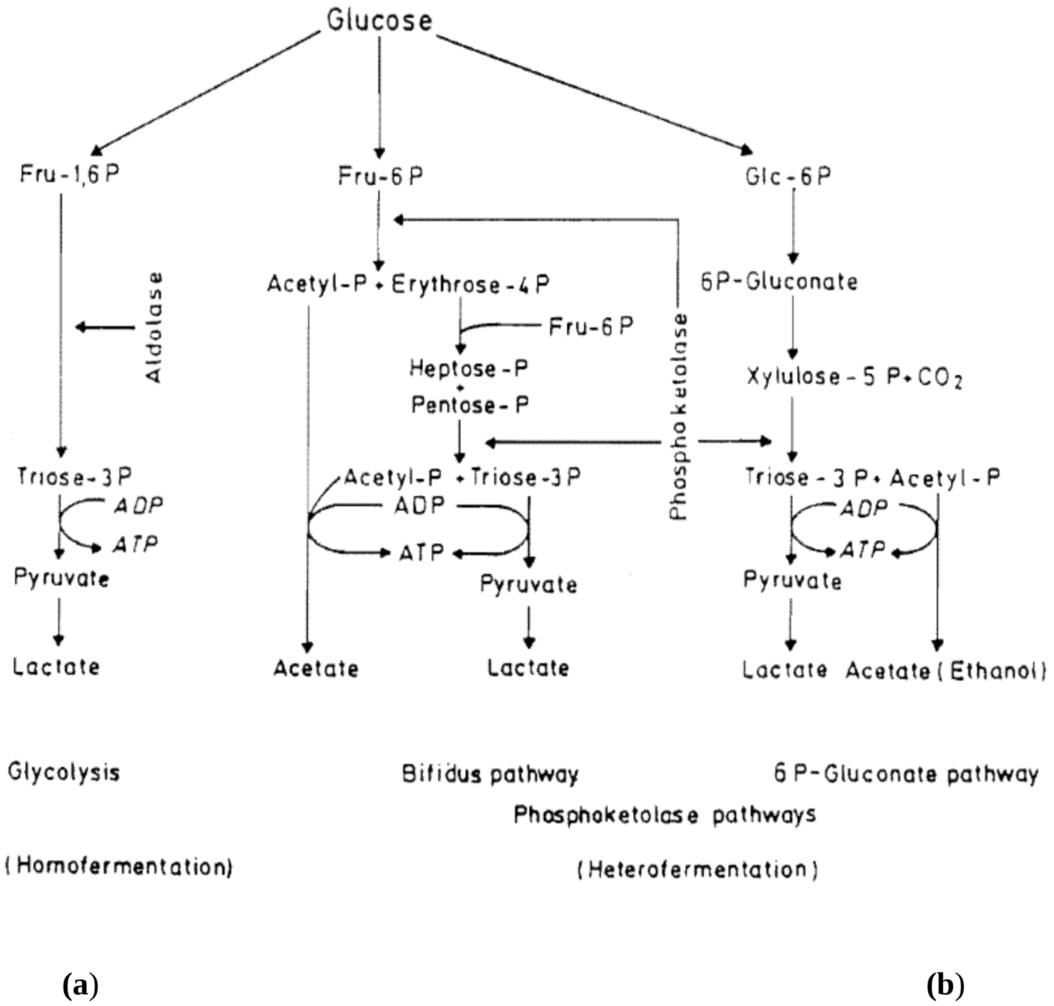


Fig. 2.3: The path way for glucose dissimilation by homofermentative and heterofermentative bacteria (Kandler, 1983)

Lactobacilli are Gram positive, catalase negative rods that often occur in chains. They can also be coccoid. They grow poorly in air but better under reduced oxygen tension. Although those in foods are typically microaerophilic, many true anaerobics exist. They are chemoorganotrophic and require complex media. Their metabolism is fermentative and at least one of the carbon end products is lactate. Their optimum growth is 30-40 ^{o}C (Axelsson, 1993).

All members of the genera *Pediococcus* and *Streptococcus* are homofermentative, Gram positive, catalase negatative cocci. Pediococcus divide in two planes at right angles to produce tetrads of cells but sometimes only pairs of cells are seen. Single cell are rare and chains are not formed. They are facultative anaerobes although some strains are inhibited on incubation in air. They are chemoorganotrophic and require nutritionally rich media and a fermentable carbohydrate (mainly mono- and diasaccharides). Glucose is fermented with the production of acid but no gas. The major product is lactate. The optimum growth temperature is 25-40 ^{o}C.

Streptococci divide in one plane to produce chains (Hardie, 1986). The cells are spherical or ovoid occurring in chains or pairs. They are facultatively anaerobic chemo-organotrophs which require nutritionally rich media for growth and sometimes 5 % carbon dioxide. Growth is generally restricted to 25- 45 ^{o}C (optimum 37 ^{o}C).

Leuconostoc are Gram positive, catalase negative cocci that are heterofermentative. The cells are spherical or somewhat longer than broad when in or pairs. Sometimes short rods with rounded ends and occurs in long chains. They grow slowly, producing small colonies that may be slimy on media containing sucrose. They are facultative anaerobes and chemo-organotrophic with obligate requirements for a fermentable carbohydrate as well as nutritionally rich medium. Glucose is fermented with the production of D (-) lactate, ethanol and gas. The optimum growth temperature is 20-30 ^{o}C (Garvie, 1986).

Carnobacteria are Gram positive, catalase negative, straight slender rods. They occur singly or pairs and sometimes in short chains. They may or not be motile. They are non sporing chemoorganotroghs that are heterofermentative. They produce mainly L (+) lactate from glucose and gas is produced by some species. They grow at 0 ^{o}C but not at 45 ^{o}C (optimum 30 ^{o}C). They differ from Lactobacilli in being unable to grow on acetate medium.

Enterococci were once a sub-group of the genus *Streptococcus.* The cells are spherical or ovoid and occur in pairs or short chains. They are sometimes mobile by means of scanty flagella. They lack obvious capsules, are facultative anaerobic and are chemoorganotrophs with fermentative metabolism. A wide range of carbohydrates, including lactose are fermented with the production of mainly L (+) lactic acid. Gas is not produced. They are gram positive and grow at 10 ^{o}C and 45 ^{o}C with an optimum of 37 ^{o}C (Devriese *et al.*, 1991).

Lactococci are Gram positive, catalase negative, non motile ovoid cells that occur singly, in pairs or in chains. Endospores are formed. They are non motile and without capsules. They are facultative anaerobes which can grow at 10 ^{o}C but not 45 ^{o}C with optimum temperature of 30 ^{o}C. They are chemoorganotrophs with fermentative metabolism. A number of carbohydrates are fermented with the production of lactic acid but no gas (Schleifer *et al.*, 1985).

Vagococci are Gram positive, non-sporing, spheres, ovals or short rods which occur singly, in pairs or in short chains, some motile by peritrichous flagella. They are catalase negative and chemoorganotrophic with a fermentative metabolism. They produce acid but not gas from a number of carbohydrates. Glucose fermentation yields mainly L (+) lactate. They can grow at 10 ^{o}C but not at 45 ^{o}C with optimum growth at 25-35 ^{o}C.

Lactic acid bacteria differ with respect to the isomers of lactic acid that they produce. This is determined by the specificity of the lactic dehydrogenases which mediate pyruvate reduction. Some species contain only D-lactic dehydrogenase and hence form the L-isomer. Some species contain two lactic dehydrogenase of differing stereospecificity and form racemic lactic acid (Vandamme *et al*., 1996; Stiles and Holzapfel, 1997).

2.7 Methods used for the identification of lactic acid bacteria

2.7.1Conventional Taxonomy

Lactic acid bacteria have been traditionally grouped and identified based on their biochemical, physiological and morphological tests (Sharpe, 1979). Bottazzi (1988) classified LAB on six physiological tests which included production of gas from glucose, hydrolysis of arginine, growth and survival at 15, 45 48, 60 and 65 ^{o}C and tolerance of 4, 6, and 8 % NaCl. The use of features such as the isomer of lactic acid produced, the comparative DNA base composition and cell wall composition have been included in LAB identification schemes (Sharpe, 1979; Kandler and Weiss, 1986). All the above features have been combined in a number of studies for the isolation and identification of LAB from different environment (Garvie, 1984; Hastings and Holzapfel, 1987; Schillinger and Lucke, 1987). The use of rapid identification systems, such as the API (API systems S.A., La Balme Les Grotte, Montalieu, France) can examine isolates using different carbohydrate fermentation characteristics.

2.7.2 Numerical Taxonomy

The application of numerical taxonomy as a technique to characterise bacteria is well established and allows the stimulation examination of large numbers of phenotypic characterisation test in an objective manner (Dykes, 1991). This is achieved by coding the results of individual characteristics tests as either binary or numerical values, the combination of which represents specific profiles characteristics of a particular strain. The principle of numerical taxonomy has been applied to lactic acid bacteria from a number of sources including processed meats (Hastings and Holzapfel, 1987); wine (Dicks and Van Vuuren, 1988); Scotch whisky distilleries (Priest and Pleasant, 1988). These methods have proved to be useful in the taxonomic status of isolates.

2.7.3 DNA Based Techniques

The analysis of the diversity of natural microbial populations relied on direct extraction, purification and sequencing of 5S rRNA molecules from enrionmental samples (Theron and Cloete, 2000). Determination of DNA base composition of LAB by electrophoresis is a well known technique (Garvie, 1984). This technique has limited use in bacterial taxonomy since the base composition of two biological unrelated organisms as determined by these methods may be the same. However, examination of bacteria at the molecular level by DNA homology technique is regarded as one of the most reliable indicators of natural relationships between different strains (Dykes, 1991). This technique has widely been used for LAB taxonomy. It lead to the creation of new genera such as *Lactococus* and *Carnobacteria* (Collins *et al*., 1987).

DGGE is an electrophoretic separation method based on differences in melting behaviour of double stranded DNA fragment (Fisher *et al.,* 2003). DGGE exploits the fact that identical DNA molecules, which differ by only one nucleotide within a low melting domain, will have different melting temperatures. When separated by electrophoresis through a gradient of increasing chemical denaturant (usually from amide and urea), the mobility of the molecule is retarded at the concentration at which the DNA strands of low melt domain dissociate (Webster *et al.,* 2002). The branched structure of the single stranded moiety of the molecule becomes entangled in the gel matrix and no further movement occurs. Complete strand separation is prevented by the presence of a high melting domain, which is usually artificially created at one end of the molecule by incorporation of a GC clamp. This is accomplished during PCR amplification using a PCR primer with a 5'tail consisting of a sequence of 40 GC.

2.8 Anti-microbial Substances Associated with Lactic Acid Bacteria

2.8.1 Organic Acids and Low pH

Lactic acid fermentation is characterised by the accumulation of organic acids, primarily lactic and acetic acids, and the accompanying reduction in pH (Berry *et al.*, 1990). Acid production is an efficient tool for inhibiting pathogenic and spoilage bacteria since organic acids produced during fermentation has broad antibacterial activities (Piard and Desmazeaud, 1991; Cherrington *et al.*, 1991). Lactic acid, generated in situ, is traditionally used for improving food safety and shelf-life (Adams and Hall, 1998). Levels and proportions of organic acids produced depend on the species of the micro organism involved, the chemical composition of the culture environment and the physical conditions encountered during fermentation (Fields *et al.*, 1981; Lindgren and Dobrogosz, 1990; Sanni, 1993).

The preservative action of acids may be partly due to the depression of internal (cytoplasmic) pH (Russel, 1992). Undissociated acid molecules are lipophilic and pass readily through the plasma membrane by diffusion. In the cytoplasm acid molecules dissociate into charged anions and protons (Salmond *et al.*, 1984). These cannot pass across the lipid bilayer and accumulate in the cytoplasm, thus reducing the pH. The acidified cytoplasm in turn inhibits metabolism, in particular the enzymes of metabolism (Krebs e*t al*, 1983; Stratford and Anslow, 1998).

A principal target for many antimicrobial compounds is the plasma membrane and an alternative mechanism which may explain how acids act as preservatives is that they eliminate the proton motive gradient (Eklund, 1985). The selective permeability of the plasma membrane to protons allows the cells to create a p H gradient and an electrical potential which together form the proton motive force (Eklund, 1989). In the chemiosmotic theory, the energy contained in the proton motive force is then used to drive the uptake of essential nutrients such as amino acids (Bracey *et al.*, 1998). The accumulation of charged acid particles in the cytoplasm disrupts the proton motive force and prevents uptake of amino acids (Freese *et al.*, 1973).

2.8.2 Bacteriocins

Lactic acid bacteria are well- known for their production of antimicrobial proteins of peptides collectively known as bacteriocins (Hugas 1998). These protein complexes (protein aggregates, lipocarbohydrate proteins, glycoproteins) are active against Gram positive bacteria and normally known to display a narrow range of inhibitory activity that affects closely related species within *Lactobacillaceae* (Klaenhammer, 1998). Bacteriocins have been isolated from fermented milk and dairy products (Litopoulou-Tzanetaki, 2000) and *mahewu* (Holzapfel, 1995).

The ability of many bacteriocins to inhibit some food-borne pathogens makes them attractive as potential food preservation agents. The best characterised bacteriocin produced by lactic acid bacteria is nisin. Nisin is produced by *Lactococcus lactis subsp. Lactis* and has been available commercially in concentrated form since 1959 (Daeschel, 1989). Most micro-organisms require an intact plasma membrane. Nisin is strongly attracted to phospholipids in bacterial and liposomal membranes. Cationic nisin molecules initially interact by electrostatic attractions with anionic membrane phospholipids. They reorient themselves in the membrane such that they form non-selective pores (Sahl *et al.*,1995). The net result is that nisin makes the cytoplasmic membrane permeable which causes the release of accumulated amino acids from the cells as well as membrane vesicles of sensitive bacteria by leakage (Hurst, 1981; Kalchayanaad *et al.*, 1994).

Nisin exhibits broad spectrum inhibitory activity against Gram positive bacteria, including spore-forming bacteria (Klaenhammer, 1998). It inactivates thermophilic spoilage microorganisms in canned goods (Stevens *et. al.*, 1992; Maisnier-Patin *et al*., 1992). Nisin and pediocin, a bacteriocin produced by *Pediococcus* species, have been shown to be effective in controlling *Listeria monocytogenes* in white pickled cheese, skim milk, yoghurt and other foods (Montville and Winkowki 1997).

2.8.3 Hydrogen Peroxide

The antimicrobial effect of hydrogen peroxide is well documented, and it is produced by a wide range of lactic acid bacteria which do not produce catalase but possess flavoprotein oxidases which react with dioxygen (Condon 1987; Earnshaw 1992; De Vuyst and Vandamme 1994b). The use of oxygen as an electron acceptor is favoured as an additional energy generated (Lindgren and Dobrogosz 1990). Because lactobacilli are catalases negative, H_2O_2 can accumulate in the cells and effect antimicrobial activity (Kandler and Weiss 1986). The bacteriocidal effect is attributed to its oxidizing effect on the bacteria cell, and to the destruction of basic molecular structure of the cell protein (Lindgren and Dobrogosz 1990). H_2O_2 in some cases can be precursor for the production of potent agent such as superoxide and hydroxyl radicals (Condon 1987).The lactoperoxidase system is an antimicrobial system that occurs in milk oxidised by hydrogen peroxide to produce antimicrobial hypothiocynate and thiocyanous acid. This system has been used successfully in extending the shelf life of raw milk and cottage cheese (Earnshaw and Banks 1989).

2.8.4 Carbon Dioxide

Carbon dioxide is produced by heterolactic fermentation and contributes to an anaerobic environment that is toxic to most aerobic food microorganisms. Also, carbon dioxide in itself has an antimicrobial activity because it creates partial pressure (Lindgren and Dodrogosz, 1990). Carbon dioxide is believed to accumulate in the lipid bilayer due to inhibition of enzymatic decarboxylations causing disfunction of membrane permeability.

2.8.5 Diacetyl

Aside from being an important flavour and aroma contributor to fermented products and especially in butter, diacetyl is an antimicrobial agent (Marshall 1987). This compound is formed by the citrate fermentation via pyruvate and further metabolized anaerobically and aerobically to diacetyl and acetoin (De Vuyst and Vandamme 1994). Diacetyl is an antimicrobial agent needed in high amounts to be effective against only Gram negative bacteria and yeasts .Inhibitory activity is by interference with arginine utilization, which is achieved by a reaction with the arginine-binding protein of the susceptible organism (Jay and Rivers, 1984).

Jay (1982) reported that yeast and Gram negative bacteria were more sensitive than the non-lactic acid bacteria and Gram positive bacteria to diacetyl. Lactic acid bacteria and the clostridia seemed to be sensitive to its inhibitory activity.

Table 2.3: Metabolic products of lactic acid bacteria which exhibit antimicrobial properties

Product	Main target organisms
Organic acids	
Lactic acid	Putrefactive and Gram- negative bacteria, some fungi
Acetic acid	Putrefactive bacteria, clostridia, some yeasts and fungi
Hydrogen peroxide	Pathogens and spoilage organisms in protein- rich foods
Low- molecular metabolites	
Reuterin (3-OH-propionaldehyde)	Wide spectrum of bacteria, moulds and yeasts
Diacetyl	Gram- negative bacteria
Fatty acids	Different bacteria
Bacteriocins	
Nisin	Some LAB and Gram-positive bacteria
Other	Gram- positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

Source: Holzapfel *et al*., (1995)

2.9 Lactic Acid as Probiotics

Probiotics are viable microbial food supplement containing single or mixed cultures of live microorganisms that beneficially affects the host (human or animal) by enhancing or maintaining a desirable microbial balance in the gut leading to improving the health status of the host (Salminen *et al*., 1998). They act by reducing harmful enteric microorganisms to maintain a healthy gut micro flora for potential health benefit. Metchnikoff (1908) observed that feeding of probiotic cultures prevented or treated disease. Probiotic cultures are consumed in foods or capsules or facilitated by ingestion prebiotics (compounds that enhance the proliferation of beneficial indigenous bacteria), Okamura *et al.*,(1986) demonstrated with tissue culture infection assay that administration of *Bifidobacterium infantis* prohibited invasion and intracellular multiplication of *S. flexneri*. Fuller (1992) observed that probiotic cultures have inhibitive or antagonistic effects against almost all foodborne pathogens including *Salmonella, Shigella, E. coli, Campylobacter, Clostridium, Yersinia, Vibro and Candida.* Saavedra *et al* (1994) showed that supplementing infant formula with *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduced the incidence of acute diarrhoea and rotavirus shedding infants. Bernet *et al.,* (1994) observed that consumption of a greater number of lactobacilli provided increased protection against cell association by enterotoxigenic and enteropathogenic *E. coli, S. typhimurium and Y. pseudotuberculosis.*

Intestinally derived lactobacilli and bifidobacterium predominate as probiotics (Hughes and Hoover, 1991) and act by establishing themselves in the human gastrointestinal tract in proportionally high numbers, thus protecting the gut against invasive pathogenic agents that cause foodborne intestinal infection like diarrhoea. Hence regular consumption of foods containing probiotics has a strong potential to help maintain beneficial and stable intestinal microflora that promotes intestinal health especially in compromised or under developed gut flora such as the elderly, infants and patients treated with antibiotics or chemotherapy.

2.10 Prebiotics

Prebiotics are non-digestible food ingredients that have a beneficial effect on the host by selectively stimulating growth of health-promoting bacteria (Desai *et al*., 2004). They are assimilated by beneficial bacteria such as *Bifidobacteria* hence improving their growth activity leading to an enhanced intestinal balance. For a food to qualify as a prebiotic, it has to be non-digestible by human enzymes; has to undergo selective fermentation by potentially beneficial bacteria in the colon; should cause an alteration in the composition of the colonic microbiota towards a healthier composition and all these changes should lead to a beneficial health to the host (Pariyaporn *et al*., 2003). Examples of prebiotic substrates include inulin, lactulose, fructo-oligosaccharides, sugar alcohols such as lactitol and xylitol (Salminen *et al*., 1998). Most of these are obtained from natural sources or synthesized naturally from sucrose (Roberfroid, 1998).

2.11 Role of Yeasts in Maize Fermentation

Yeasts are unicellular fungi as compared to moulds which are multicellular. They differ from bacteria by their larger cell size and their oval, elongated, elliptical or spherical cell shape. By Kurtzman (1994) definition, yeasts represent a unique group of fungi characterised by vegetative growth that is predominantly unicellular and by the formation of sexual state which are not enclosed in fruiting bodies. Yeasts have been traditionally classified for several years based on their physiological, morphological and biochemical criteria. These conventional methods are found to be laborious and time consuming as well as being unreliable in their ability to differentiate at species level (Casey *et al*., 1990). To alleviate the problems associated with the classical methods, DNA- based methods have been develop in an attempt to simplify the identification of yeasts isolates. Nucleic acid techniques such as DNA and PCR are sensitive and have the advantage of not been influenced by the environmental conditions (Kurtzman 1994).

Yeasts are known to produce a wide range of compounds such as organic acids, esters, alcohols, aldehydes, lactones and terpenes (Stam *et al*., 1998). Several authors (Obiri-Danso, 1994; Hayford and Jespersen, 1999; Hayford and Jakobsen, 1999; Jespersen, 2002) have found *Candida krusei* and *Saccharomyces cerevisiae* as the dominant yeast during steeping and maize dough fermentation. Jespersen *et al*, (1994), found a mixed flora comprising *Candida*, *Saccharomyces, Trichoporon, Kluveromyces* and *Debaryomyces species* from raw maize during steeping and the early stages of fermentation. The presence of large numbers of yeasts (*Candida krusei* and *Saccharomyces cerevisiae)* in maize dough fermentation either in pure cultures or combination have been found to influence the organoleptic and structural quality of maize dough (Nyarko and Obiri-Danso, 1992; Jespersen *et al*., 1994).

2.11.1 Saccharomyces cerevisiae

The yeast *S. cerevisiae* belongs to the genus *Saccharomyces* which is one of the largest genus under the phylum Ascomycota. It is perhaps the most useful yeast, which has been very instrumental in baking and brewing since ancient times. The cells of *S. cerevisiae* are round to ovoid, with a diameter of 5–10 micrometres and reproduce vegetatively by multilateral budding. All strains of *S. cerevisiae* can grow aerobically on sugars such as glucose, maltose, and trehalose but not on lactose and cellobiose. (Beneke and Stevenson 1987; Kurtzman and Fell, 1998).

Early classification allocated to genus *Saccharomyces,* a large number of species which constituted a somewhat heterogenous mixture. In current classification system baker’s yeast, brewer’s yeasts, wine yeasts, champagne yeasts are all considered to be strains of *Saccharomyces cerevisiae* (Kurtzman and Fell, 1998).

2.11.2 Candida krusei

Candida krusei belongs to the genus Candida, a member of the group of imperfect yeasts (anarmorphic) of the phylum Ascomycota. Vegetative reproduction is by multilateral budding and the cells occur singly, budding and in chains. The cells are ellipsoidal and elongate, and pseudohyphae and septate hyphae may be present (Kurtzman and Fell, 1998). In the new edition of "The Yeast" *Candida krusei* is discussed under *Issatchenkia orientalis* and this is because *Candida krusei* is considered to represent the anamorphic form of this species, as the type strains of both species including other isolates showed significant (93 % - 100 %) DNA base sequence complementary. The assimilation pattern of *Candida krusei* is quite homogenous and there is no difference between the two pathogenic organisms and organisms from natural fermentation of maize dough. *Candida krusei* is not easily differentiated from other phenotypically similar species such as *Candida valida* (Kreger van Rij, 1984; Kurtzman and Fell, 1998).

Many authors (Halm *et al*., 1993; Jespersen *et al*; 1994; Hayford and Jakobsen 1999) have found *Candida krusei* as dominant yeast in many African fermented cereals such as maize and sorghum, root crops like cassava (Amoa-Awua *et al*., 1997; Oyewole, 1990) and alcoholic beverages such as palm wine in Ghana, sorghum beer in West Africa (Nout 1980, Konlani *et al*., 1996a). This yeast is able to survive in acid fermented products due to its acid resistance nature (Spicer and Schroder, 1980).

2.12 Technological Properties of Lactic Acid Bacteria

2.12.1 Exopolysaccharides

Lactic acid bacteria strains have been documented to produce expolysacharides (EPSs) that have received a lot of attention due to their contribution to improvement of texture and viscosity of fermented food products (Patricia *et al*., 2002; Savadogo *et al*., 2004). The lactic acid bacteria produce EPS probably as a protective function in their natural environment such as against desiccation, phagocytosis, phage attack, osmotic stress, antibiotics or toxic compounds (Patricia *et al*., 2002). The EPS may have a role in cell recognition, adhesion to surfaces and formation of biofilms that facilitate colonisation to various ecosystems. This is a beneficial attribute for probiotics in their endeavor to colonize the GIT. Health benefits have been attributed to some exopolysaccharides. They have been reported to possess anti-tumor, anti-ulcer, immunomodulating and cholesterol lowering effects (De Vuyst and Degeest, 1999). Kazitawa and Ittoh (1992) reported an increase in B-cell dependent mitogenic activity induced by slime material products from *L. lactis subsp. cremoris*; water soluble EPS from kefir grains were shown to retard tumor. Kalui *et al*., (2009), reported the production of EPS ranging from 298.53 to 431 mg/l by *L. plantarum* and *L. rhamnosus* isolates from *ikii*, a spontaneously fermented maize porridge. Spontaneously fermented products therefore have potential and it is therefore necessary to assess them for production of EPS with an aim of tapping into the health benefits associated with EPS.

2.12.2 Starch Hydrolysis

Blandino *et al*., (2003) indicated that the level of carbohydrate, some non digestible and oligosaccharides decrease during cereal fermentation. Amylolytic lactic acid bacteria have been isolated from cereal fermentation in tropical climates (Ga”nzle *et al*., 2008, Sanni *et al*., 2002). Many authors have indicated the presence of lactic acid bacteria in some African fermented products. Olasupo *et al*., (1996) isolated amylolytic lactic acid bacteria from Ghanaian kenkey (fermented maize dough) and nono (Nigeria). Agati *et al*., (1998), found amylolytic *L plantarium* strains from retted cassava in Nigeria and Congo respectively, while amylolytic *L. fermentum* (strains Ogi E1 and Mw2) were isolated from *mawe* and *ogi* in Benin. Hounhouigan *et al*., (1993b) reported some amylolytic lactic acid bacteria in *mawe* from Benin whiles Johansson *et al*., also indicated that amylolytic lactic acid bacteria accounted for 14 % of the total lactic acid bacteria isolated from Nigerian *ogi*.

2.12.3 Proteolysis

Protein degradation during cereal fermentation is among the key phenomena that affect the overall quality of product (Ga¨nzle *et al*., 2008). Overall, LAB plays a minor role in protein hydrolysis (Wieser *et al*., 2008). Most cereal-associated LAB do not possess extracellular proteinase activity and prefer peptides uptake rather than amino acids transport (Thiele *et al*., 2003). Thus, primary proteolysis is exerted by the cereal endogenous enzymes, which are activated by the low pH. Further hydrolysis of peptides into amino acids is then exerted by intracellular peptidases of LAB in a strain-specific manner: the type and amount of released amino acids depend on the fermenting strain (Di Cagno *et al*., 2002). Though LAB do not influence the overall amount of hydrolysed proteins in respect to aseptically acidified cereal doughs, they affect the pattern of hydrolysed products, increasing the amount of dipeptides and amino acids (Di Cagno *et al*., 2002; Thiele *et al*., 2003). Studies conducted by Nanson and Field (1984), showed that during the fermentation of cornmeal, the concentrations of available lysine, methionine and tryptophan increased. In another study by Hamad and Field (1979), fermentation was observed to significantly improve the protein quality and the level of lysine in maize, millet and sorghum. Also, the tryptophan content of *ogi*, a millet based porridge increased with fermentation (Mckay and Baldwin, 1990)**.**

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in two towns in the Asuogyaman District in the Eastern Region of Ghana, namely, Anum and South Senchi where dehulled kenkey is widely consumed.

3.2 Study Design

A brief field study involving informal interaction and administering of semi structured questionnaires to producers, consumers and vendors was carried out. The study also involved observation of the processing procedures and two experienced processors were selected from each area for collection of samples.

3.2.1 Sample Collection and Preparation

Samples were aseptically collected from four processors at the various stages of processing maize into n*siho*. The samples collected were dehulled maize, steep water at interval of 0, 24, and 48 h of steeping and fermenting dough at 0, 4, 8 and 12 h of dough fermentation. Samples were transported in an ice chest with ice packs to the CSIR-Food Research Institute's microbiology laboratory in Accra for microbiological and chemical analyses.

3.3 Chemical Analyses

3.3.1 Determination of pH

The pH of steep water was determined directly using a pH meter (Radiometer pHM 92. Radiometer Analytical A/S, Bagsvaerd, Denmark) after calibration using standard buffers, and fermenting dough was determined after blending with distilled water in a ratio of 1:1.

3.3.2 Determination of Titratable Acidity

The titratable acidity was determined by the method described by Amoa-Awua *et al*., (2006). For each sample (steep water and fermenting dough) 10 ml or 10 g of sample was made up to 200 ml with distilled water and 80 ml titrated against 0.1 m NaOH using 1 % freshly prepared phenolphthalein as indicator. One millilitre of 0.1 N NaOH was taken as equivalent to 9.008×10^{-3} g lactic acid.

3.4 Microbiological Analyses

3.4.1 Enumeration of Microorganisms

3.4.1.1 Homogenization and Serial Dilution

For all solid samples, ten grams (10 g)were added to 90.0 ml sterile Salt Peptone Solution (SPS) containing 0.1 % peptone and 0.8 % NaCl, with pH adjusted to 7.2 and homogenized in a stomacher (Lad Blender, Model 4001, Seward Medical, England), for 30 s at normal speed. From appropriate ten-fold dilutions 1 ml aliquots of each dilution was directly inoculated into sterile Petri dish plates and the appropriate media added for enumeration and isolation of microorganisms. All analyses were done in duplicate.

3.4.1.2 Enumeration of Aerobic Mesophiles

Aerobic mesophiles were enumerated by the pour plate method on Plate Count Agar medium (Oxiod CM325; Oxoid Ltd., Basingstoke, Hampshire, UK). Plates were incubated at 30°C for 72h in accordance with the Nordic Committee on Foods Analysis Method (NMKL. No. 86, 2006).

3.4.1.3 Enumeration of Lactic Acid Bacteria

Lactic acid bacteria were enumerated by the pour plate method on deMan, Rogosa and Sharpe (MRS, Oxoid CM361) agar (De Man *et al.*, 1960), pH 6.2 to which has been added 0.1 % cycloheximide supplement to inhibit yeast growth and incubated anaerobically in anaerobic jar with anaerocult A at 30 °C for 5 d.

3.4.1.4 Enumeration of Yeasts

Yeasts and moulds were enumerated by the pour plate method on Oxytetracycline-Glucose Yeast Extract Agar (OGYEA), (Oxoid CM545; Oxoid Ltd., Basingstoke, Hampshire, UK) to which OGYEA supplement was added to suppress bacteria growth. The pH was adjusted to 7.0 and incubated at 25 °C for 3-5 d in accordance with ISO 7954 (1987).

3.4.2 Isolation of Lactic Acid Bacteria

About 20 colonies of lactic acid bacteria were selected from a segment of the highest dilution or suitable MRS agar plate. The colonies were sub-cultured in MRS broth medium and streaked repeatedly on MRS agar until pure colonies were obtained.

3.4.3 Characterisation of Isolates

3.4.3.1 Microscopic Examination

Cell shape and arrangements were determined by phase contrast microscopy and the wet mount technique. A drop of sterile distilled water was placed on a clean slide and a small amount of the pure culture emulsified in it. A cover slip was placed on it and examined under the microscope using the X40 magnification and oil immersion using the X100.

3.4.3.2 Gram's Reaction

Gram's reaction was determined using 3 % freshly prepared potassium hydroxide solution as described by Gregersen (1978). The tip of cover slip was used to pick a pure colony of LAB onto a clean microscopic slide and a drop of 3 % KOH was placed onto the colony on the slide. The colony was mixed thoroughly with the solution using the cover slip and drawn for the production of slime. Formation of a slime indicated Gram negative reaction and non-slimy reaction indicated Gram positive reaction.

3.4.3.3 Catalase Reaction

A drop of 3 % freshly prepared hydrogen peroxide solution was placed on a clean glass slide and a single colony of the pure culture picked and emulsified in the drop of hydrogen peroxide. This was then observed for bubbles or effervescence resulting from the liberation of free oxygen as gas bubbles. This indicated the presence of catalase in the culture and vice versa.

3.4.3.4 Oxidase Test

Oxidase test was done using Identification Sticks (Oxoid Ltd., Basingstoke, Hampshire, UK). The oxidase sticks were smeared on pure colonies and observe for colour change. Purple colouration indicated positive results.

3.4.3.5 Growth at Different Temperatures

Two tubes containing MRS broth (Oxoid CM359) were inoculated with pure colony mass of the test organism and incubated at 10 °C and 45 °C respectively for 72-96 h. Growths were determined by visual turbidity after the incubation period. This was repeated for all the isolates.

3.4.3.6 Gas Production from Glucose

The ability to ferment and produce gas from glucose was assessed in MRS basal medium to which glucose had been added. The medium was composed of peptone 10 g, yeast extract 5 g, tween 80 1 ml, di-potassium hydrogen phosphate 2 g, sodium acetate 5 g, tri-ammonium citrate 2 g, $MgSO_4.7H_2O$ 0.2g, $MnSO_4$. $4H_2O$ 0.05g, 1 litre distilled water, pH 6.5, thus without glucose and meat extract. The basal medium was dispensed in 5 ml amounts into testtubes containing inverted Durham tubes and sterilized by autoclaving at 121 °C for 15 minutes. The glucose was prepared as 10 % solution and sterilized by filtration and added aseptically to the basal medium to give a final concentration of 2 % (de Man *et al*., 1960). The inoculated tubes were examined for the production of gas after 3 days of incubation.

3.4.3.7 Salt Tolerance Test

Salt tolerance test was done using MRS broth (Oxoid CM359) containing 6.5 % and 18 % (w/v) NaCl with incubation period of 5 days at 30 °C. Growths were determined by visual turbidity after the incubation period.

3.4.3.8 Growth at Different pH

Growth in MRS broth (Oxoid CM359) with pH adjusted to 4.4 using concentrated HCl and 9.6 using 0.1 N NaOH were determined by visual turbidity after 72 h of incubation at 30 °C.

3.4.4 Identification of Lactic Acid Bacteria by Carbohydrate Fermentation Profiles

Isolates were tentatively identified by determining their pattern of carbohydrate fermentation using the API 50 CH kit (BioMérieux, Marcy-l'Etoile, France) and comparing them to the API database.

3.4.5 Isolation of Yeast

All colonies totalling about15 from a segment of the highest dilution or suitable OGYEA plate were selected and examined by microscopy, purified by successive sub culturing in Malt Extract Broth (Oxoid CM57) and streaked on OGYEA (Oxoid CM545) pH 7.0 until pure colonies were obtained.

3.4.5.1 Macroscopic and Microscopic Examination

Colonies on solid media were examined macroscopically for colonial morphology. Characteristics described included colour, surface, size, edge, and elevation. Cultures were also microscopically observed as wet mounts for cellular morphology.

3.4.6 Identification of Yeasts Isolates using the ID 32 C Kit

Yeast isolates were grown on OGYEA (Oxoid CM0545) and incubated at 25 °C for 48 h. About 3 pure colonies of yeast on each agar plate were suspended in an ampule of API C medium (BioMérieux, Marcy-l'Etoile, France) and 135 µl used to inoculate the ID 32 C strips that consisted of 32 copules, each containing a dehydrated carbohydrate substrate. These were then covered with the incubation lids and incubated at 30 °C for 48 h. At the end of the incubation period, isolates were identified by determining their pattern of fermentation and assimilation of various carbohydrates in ID 32 C galleries.

3.5 Technological Properties of Identified Lactic Acid Bacteria

3.5.1 Rate of Acidification of Maize Grains and Dough by LAB

Duplicate trial fermentations were carried out as inocula enrichment with seven dominant LAB cultures identified earlier during steeping of dehulled maize and dough fermentation (*L. fermentum*1, *L. fermentum* 2, *L. brevis* 1, *L. brevis* 2, *L. plantarum*, *P. acidilactici* and *P. pentosaceous*). For the preparation of the dehulled maize grains; 300 g of washed dehulled maize were distributed into 1litre conical flask and 450 ml (1:1.5w/v) of sterile water were used to steep the grains. The steeped grains were milled, mixed with water and kneaded into dough. The lactic acid bacteria cultures used as inocula enrichment was prepared from a 16 h culture incubated at 37 ºC and 100 µl of the culture was transferred into sterile SPS and diluted to a concentration of about 10^6 cfu/ml. This was checked by microscopic counting using a Thomas counting chamber and by plating out on MRS agar. Seven different batches of dehulled maize and dough were steeped and fermented respectively, and to each batch, one isolate was added as inoculum enrichment. The mixture was shaken to obtain uniform distribution, and left at room temperature to ferment for 12 h. One batch of each dehulled maize and dough was not inoculated and used as control (spontaneous fermentation). Every 4 h, 10 ml of steep water and 10 g of dough were aseptically collected for determination of pH and titratable acidity at 0-4 h, 4-8 h and 8-12 h and 0 h, 4 h, 8 h and 12 h respectively.

3.5.2 Production of Exopolysaccharides (EPS) by LAB Isolates

Screening of isolates for EPSs production was carried out according to Guiraud (1998). Isolates cultured on MRS agar were streaked onto LTV agar [0.5 % (w/v) tryptone (Difco), 1 % (w/v) meat extract (Fluka, Biochemika, Chemie GmbH, Buchs, Switzerland), 0.65 % (w/v)

NaCl (Sigma), 0.8 % (w/v) potassium nitrate (Merck, KgaA), 0.8 % (w/v) sucrose (PA Panreac Guimica SA, Barcelona, Espana), 0.1 % (v/v) Tween 80 (Merck), 1.7 % (w/v) agar (Sigma), pH 7.1±0.2] and incubated at 30 ºC for 48 h. The colonies were tested for slime formation using the inoculated loop method (Knoshaug *et al*., 2000). Isolates were considered positive for slime production if the length of slime was above 1.5 mm. Positive isolates were confirmed using MRS- Sucrose Broth without glucose and peptone as described by Pidoux *et al*., (1990) [1 % (w/v) meat extract, 0.5 % (w/v) yeast extract (Fluka, Biochemika), 5 % (w/v) sucrose (PA Panreac Guimica), 0.2 % (w/v) $K_2HPO_4.3H_2O$ (Merck), 0.5 % (w/v) sodium acetate trihydrate (Merck), 0.2 % (w/v) triammonium citrate anhydrous (Fluka, Biochemika), 0.02 % (w/v) $MgSO_4.7H_2O$ (Merck), 0.005 % (w/v) manganese (II) sulphate monohydrate (Merck), 0.1 % (v/v) Tween 80, pH 5.0 ± 0.2)]. The isolates were cultured in MRS- sucrose broth and incubated at 30 ºC for 24 h. A volume of 1.5 ml of the 24 h culture was centrifuged at 4000 *g* for 10 min (4 ºC) and 1 ml of the supernatant put in a glass tube and an equal volume of 95 % ethanol added. In the presence of EPSs, an opaque link is formed at the interface. The positive isolates were noted according to the intensity of the opaque link.

3.5.3 Tests for Amylase Secretion by LAB Isolates

Purified bacterial cultures were streaked on Nutrient Agar (Oxiod CM3; Oxoid Ltd., Basingstoke, Hampshire, UK) containing 2 % soluble starch (with pH adjusted to 7.2) and incubated in an anaerobic jar at 30 °C for 3 days. After incubation, the plates were flooded with iodine solution. Production of amylase was indicated by the formation of a clear zone around the colonies with the remaining parts of the plates staining blue-black as described by Almeida *et al*., (2007). The extent of secretion was quantified by measuring the width of the clear zone around the colony.

3.5.4 Test for Protease Secretion by LAB Isolates

Purified bacteria cultures were streaked on Plate Count Agar (Oxiod CM325; Oxoid Ltd., Basingstoke, Hampshire, UK) supplemented with 0.5 % casein. The plates were incubated at 30 ^{o}C for 3 days and then flooded with 1M HCl. Protease positive was indicated by a clear zone around the colonies as described by Almeida *et al*., (2007).

3.5.5 Antimicrobial Studies

The inhibitory potential of lactic acid bacteria cultures was investigated using the Agar Well Diffusion method as described by Schillinger and Lücke (1989) and Olsen *et al*., (1995). The MRS agar was poured into Petri dishes and allowed to solidify at room temperature. Circular wells were made in the agar using a sterile cork borer (no 4). The cultures of lactic acid bacteria isolated at different stages of steeping and dough fermentation were cultured in MRS broth (Oxoid CM359) at 30 °C for 24 h. A volume of 0.1 ml of each test culture was transferred into a different well and left to diffuse into the agar for approximately 4-5 h. The plate was then overlaid with 10 ml soft Nutrient Agar (Oxoid CM3) containing 0.25m1 of 10^{-1} dilution of an overnight culture of the indicator pathogens: four tested strains and one negative control, (*Salmonella typhimurium*, NCTC 12023, *E.coli*, NCTC 9001, *Vibro cholerae*, NCTC 11348 and *Staphylococcus aureus*, NCTC 657) obtained from Food Research Institute were used, and incubated at 37 °C for 24 h followed by examination for inhibitory reactions as described by Mante *et al*., (2003).

3.6 Development of Starter Culture

3.6.1 Dehulled Maize

Dehulled maize was purchased from a local producer on the open market at Anum, in the Eastern region. The grains were stored in a jute sack at room temperature until ready for use.

3.6.2 Starter Cultures

Two cultures of lactic acid bacteria *(L. fermentum*, and *L.brevis;* AD.4/12 and AD.12/8 respectively) and two yeast cultures (*C. krusei* and *S. cerevisiae;* AD8/10 and SS.48/5 respectively) isolated earlier from n*siho* fermentation were used. The cultures were stored in 50 % glycerol at – 20 ^{o}C.

3.6.2.1 Preparation of Inoculum

Stock cultures of *L. fermentum*, and *L. brevis* (lactic acid bacteria) and *C. krusei* and *S. cerevisiae*. (yeasts) isolated earlier in the present work were sub cultured in MRS Broth, (MRS, Oxoid CM361) and Malt Extract Broth (Oxoid CM57) respectively and incubated at 30^{o}C for 24 h. Dilutions were made by transferring 0.1 ml of the sub- cultures into 9 ml growth media and incubated for 24 h at 30 ^{o}C. After incubation, l ml from each culture was transferred into 100 ml of growth medium and incubated at 30 ^{o}C for 24 h. The cells were then harvested by the following procedure:

1. Nine hundred microlitres of distilled water were added to 100 µl of culture to obtain 1:10 dilution

2. The concentration of cells in each dilution was determined by counting under the microscope using the Thomas counting chamber. The concentration of cells was determined

by multiplying the cell count in the 16 chambers by the dilution and by a factor of 10^4: (microscopic count x dilution factor x 10^4).

3. Concentration of 10^7 cells /ml was obtained for LAB and 10^6 cells/ml were obtained for the yeast.

4. The cultures for inoculation were then centrifuged at 4000 rpm for 15 minutes, the supernatant discarded and the cultures re-suspended in 4 ml sterile distilled water and centrifuged again.

5. The supernatants were again discarded and the washed cultures reconstituted in sterile distilled water and used to inoculate the steeped maize and the dough.

3.6.3 InoculationTrials

Fermentation experiments were conducted in duplicate and results represent duplicate measurement. Starter cultures, *L. fermentum*, and *L. brevis* and *C. krusei* and *S. cerevisiae* were used.

3.6.3.1 Fermentation with Single Starter Culture

For each of the fermentation trails 2.5 kg of dehulled maize kernels were steeped in 5.0 litre water in 10 L capacity plastic bowl. Either 10^7cfu/ml of lactic acid bacteria or 10^6 cfu/ml of yeast was inoculated into the steep water or dough as single starter culture (*L. fermentum*, *L.brevis*, *C. krusei* and *S. cerevisiae*). The dehulled maize grains were left to steep at ambient temperature (28-30 ^{o}C) for 48 h and sampled at 0 h, 24 h, and 48 h for determination of pH, titratable acidity and microbiological analysis. After steeping, the grains were recovered, milled and kneaded into dough. The dough was allowed to ferment and sampled at 0, 4, 8 and 12 h for determination of pH, titratable acidity and microbiological analysis. Four batches of steep water and dough were inoculated and one batch was not inoculated and served as control.

3.6.3.2 Fermentation with Combined Starter Culture

Dehulled grains of 2.5 kg batches were steeped in 5.0 L water (1:2 w/v) in 10 L capacity plastic bowl. Eight separate batches were prepared by adding to the steep water and dough, cultures of *L. fermentum*, *L.brevis*, *C. krusei* and *S. cerevisiae* in the following combinations: Con (control/spontaneous): no starter culture; *CK*: S. *cerevisiae* + *C. Krusei; FK: L. fermentum* + *C. Krusei; FC: L. fermentum* + S. *cerevisiae, FCK: L. fermentum* + S. *cerevisiae* + *C. Krusei; FBKC: L. fermentum* + *L.brevis* + *C. krusei* + *S. cerevisiae; FBK: L. fermentum*, *L. brevis*, *C. krusei* and CBK: *S. cerevisiae* + *L. brevis* + *C. Krusei*. Yeasts cultures were inoculated to attain concentrations of 10^6 cells/ml while LAB was inoculated to attain concentrations of 10^7 cells/ml. Samples of steep water and fermenting dough were collected for analyses as described above.

3.7 Survival of Enteric Pathogens during Steeping of Dehulled Maize

The ability of different enteric pathogens to survive in steeping water during steeping of dehulled maize grains was studied by the method described by Mante *et al*., (2003). The enteric pathogens used were certified cultures of *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholerae*, all obtained from the Microbiology laboratory, Food Research Institute, Ghana. A pure culture of each pathogen in Nutrient Broth at a concentration of 10^6cfu/ml was inoculated into fermenting maize during steeping. 10 ml was collected at 12 h and the population of surviving pathogens enumerated by spread plate incubated at 37 °C. *Salmonella typhimurium* was enumerated on XLD medium (Oxoid, CM469), *Escherichia coli* were enumerated on VRBA (Oxoid, CM107), *Staphylococcus aureus* on BP medium (Oxoid) and *Vibrio cholerae* on TCBS Cholera medium (Oxoid, CM333).

3.8 Preparation of *Nsiho* in the Laboratory for Sensory Evaluation

Steeped maize fermented with added starter cultures were milled into a smooth paste using the disc attrition mill. 500 g of the milled maize was mixed with 500 ml of water into slurry. The slurry was poured into boiling (500 ml) water in a pot on an electric stove and pre-cooked by stirring for 15 min into a thick paste known as '*ohu or aflata*'. The *ohu* was moulded into balls of about 100 g each. Each ball was wrapped with a cleaned maize husk, packed into an electric steamer and steamed for 30 min.

3.8.1 Sensory Analysis

The kenkey samples prepared with different starter cultures were evaluated by a sensory panel for acceptability based on texture, aroma (odour), taste, and overall acceptability. The sensory panel consisted of twenty untrained panellist selected from the CSIR- Food Research Institute who were familiar with the product. The coded samples were assessed on a nine-point hedonic scale where 1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7= Like moderately, 8 = Like very much and 9 = like extremely (Appendix 1). Data obtained was analysed using the mean and standard deviation to assess the level of consumer acceptability of the product as well as analysis of variance (ANOVA) to establish significant difference among the various treatment. Multivariate data analysis (Principal Component Analysis, PCA was carried out on mean values with Latentix Version 2.0 (Latent 5, Copenhagen, Denmark). Each variable was meancentered and scaled (multiplied by the inverse of the standard deviation) to give it the same variance. PCA was used to visualize the inter-sample and inter-variable (sensory attribute intensities) relationships.

4.0 RESULTS

4.1 Field Study

The field study revealed that only women were involved in the traditional production of white kenkey. These women had little or no formal education, and were engaged in the traditional processing as a family trade or business in which skills had been handed from one generation to another. The production is therefore carried out at the family level involving about three to four women on a small scale and is artisanal in nature. Production of white kenkey was the main source of employment in these families and it was reported to be a profitable business. At the sites visited, the women produced a minimum of 5 kg and a maximum of 10 kg of white kenkey per batch.

The main raw material for white kenkey production is the dehulled maize, corn husk and table salt. All these materials are purchased locally. The procedures used to produce *nsiho* are shown in Figure 4.1 and appendix 3. Processing involves winnowing and sorting of the maize to remove dust, chaff and stones. The cleaned maize is dehulled in a disk attrition mill and steeped in water for 48 h. After steeping, the grains are washed and milled into a smooth meal. Two different procedures were observed for further processing after production of the smooth meal and this depended on the locality. At Senchi all the milled meal is pre-cooked for about 30-60 minutes into a thick gelatinous paste (*ohu*). The *ohu* is moulded into balls and wrapped in a clean corn husk. The balls are packed into a pot containing small amount of water lined with sticks and corn husk and steamed for 1-2 h.

In the other processing procedure which was observed at Anum, the milled meal is kneaded into stiff dough and fermented spontaneously for 6-12 h. After fermentation a two-third portion of the dough is pre-cooked for about 30-60 minutes into *ohu*. The hot *ohu* is mixed with the remaining one-third uncooked dough as done for Ga and Fante kenkey. The mixture formed is moulded into balls and wrapped in clean corn husks. The balls are packed into perforated pan and placed over a pot of boiling water and steamed for 1 to 2 h.

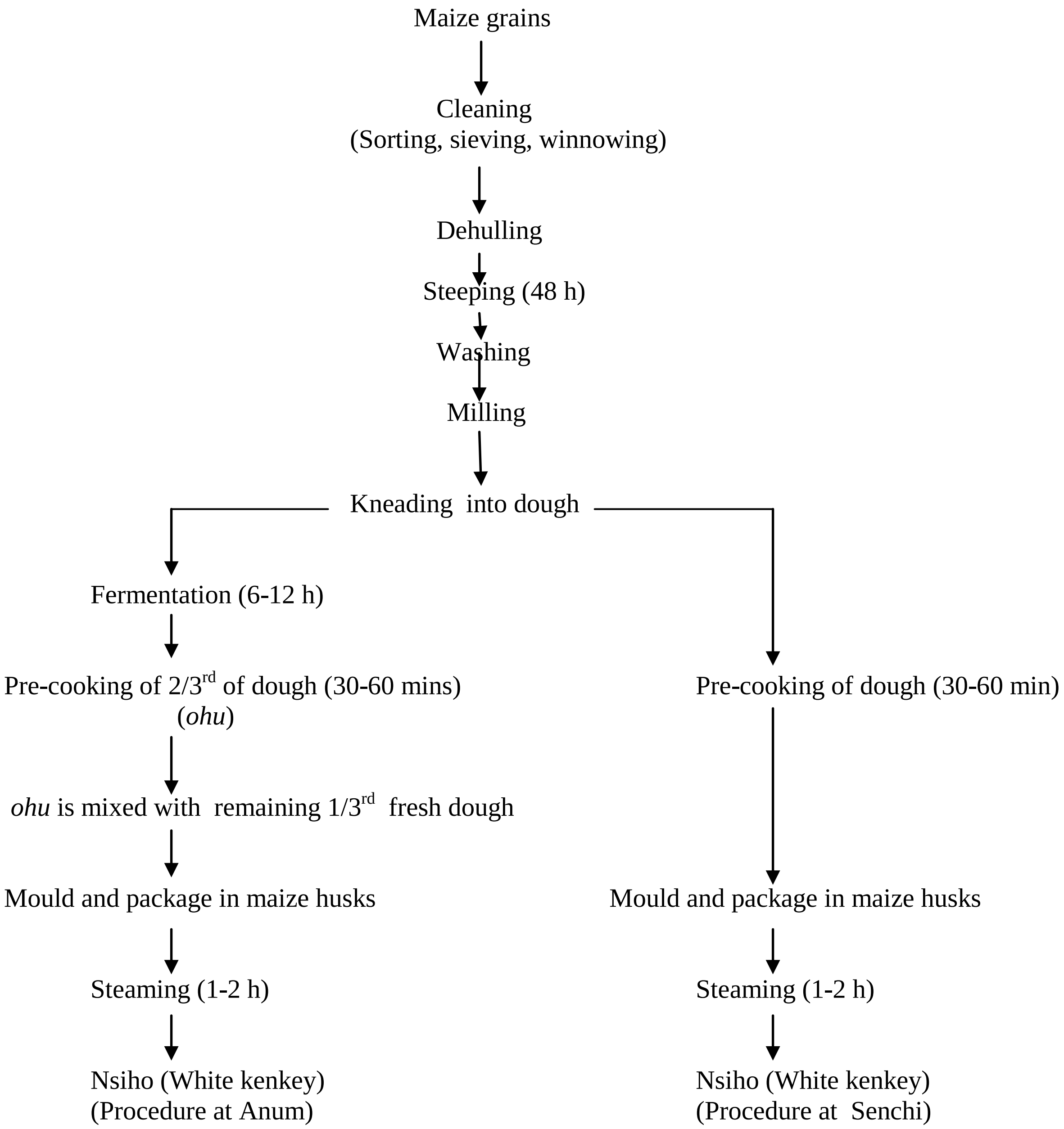


Fig. 4.1. Flow diagram of the production of Nsiho (white-kenkey).

4.2 Chemical Analyses of Maize during Steeping and Dough Fermentation

The pH and titratable acidity (expressed as percentage lactic acid) from all four production sites during steeping and dough fermentation are shown in (Tables 4.1 and 4.2). Mean pH values during steeping decreased from between 6.05 and 5.93 at the start of steeping to between 3.59 and 3.55 at the end of steeping. During dough fermentation, pH decreased from an initial range of 6.02- 5.80 for freshly prepared dough to a range of 3.52-3.46 (Table 4.1). The most drastic drop in pH was achieved during the first day of fermentation.

The titratable acidity (TTA) values during steeping and dough fermentation process are shown in Table 4.2. The TTA percentages obtained during steeping ranged from 0.02-0.03 % from the start of steeping to 0.27-0.32 % after 48 h of steeping. Similar results were observed for dough fermentation with TTA increasing from between 0.25 and 0.27 % at the start of fermentation to between 0.35 and 0.38 % at the end of fermentation. Titratable acidity increased in samples from all four production sites with a corresponding decrease in pH (Table 4.1 and 4.2).

Table 4.1 Mean pH values during the fermentation of dehulled maize into *nsiho*

	Mean pH values			
Sample	Processor 1	Processor 2	Processor 3	Processor 4
Steep water				
0 h	5.98± 0.05	6.00± 0.03	6.05± 0.03	5.93± 0.01
24 h	4.01± 0.03	4.00± 0.01	3.81± 0.10	3.90± 0.01
48 h	3.59± 0.04	3.59± 0.03	3.57± 0.03	3.55± 0.02
Fermenting dough				
0 h	5.98± 0.02	6.02± 0.01	5.98± 0.01	5.80± 0.02
4 h	5.44± 0.01	5.51± 0.01	5.51± 0.06	5.46± 0.01
8 h	3.55± 0.01	3.58± 0.03	3.76± 0.03	3.57± 0.04
12 h	3.51± 0.02	3.52± 0.04	3.49± 0.01	3.46± 0.01

Table 4. 2 Mean Titratable acidity (%) changes during the fermentation of dehulled maize into *nsiho*

	Mean Titratable values			
Sample	Processor 1	Processor 2	Processor 3	Processor 4
Steep water				
0 h	0.02± 0.01	0.03± 0.01	0.03± 0.01	0.03± 0.01
24 h	0.25± 0.01	0.25± 0.01	0.27± 0.01	0.28± 0.01
48 h	0.27± 0.01	0.29± 0.01	0.30± 0.01	0.32± 0.01
Fermenting dough				
0 h	0.27± 0.03	0.25± 0.02	0.26± 0.01	0.27± 0.04
4 h	0.28± 0.01	0.26± 0.03	0.28± 0.01	0.28± 0.03
8 h	0.31± 0.03	0.29± 0.03	0.31± 0.02	0.32± 0.05
12 h	0.36± 0.01	0.35± 0.04	0.35± 0.01	0.38± 0.01

4.3 Changes in Microbial Population during Steeping and Dough Fermentation of *Nsiho*

4.3.1 Population of Aerobic Mesophiles

The population of aerobic mesophiles during steeping and dough fermentation from the four production sites during the production of *nsiho* is shown in Table 4.3. At the start of steeping the average microbial load was about 10^5 cfu/ml which increased drastically to a level of 10^8 cfu/ml after 24 h. The same level was maintained till the end of steeping at 48 h. For the dough fermentation the microbial population increased steadily over the 12 h of fermentation from the mean concentration of 10^6 to 10^8 cfu/g (Table 4.3). The microbial population consisted of Gram positive catalase-negative rods and cocci, Gram positive catalase positive cocci and Gram negative bacteria.

Table 4.3 Mean (cfu/ml or g) mesophilic counts during the fermentation of dehulled maize into nsiho

	Mean mesophilic counts			
Sample	Processor 1	Processor 2	Processor 3	Processor 4
Steep water				
0 h	$(3.4 \pm 1.6)10^5$	$(4.5 \pm 0.3)10^4$	$(1.1 \pm 0.2)\ 10^6$	$(2.0 \pm 1.2)\ 10^6$
24 h	$(5.5 \pm 2.4)10^8$	$(4.6 \pm 1.7)10^8$	$(1.0 \pm 0.2)\ 10^8$	$(3.0 \pm 0.4)\ 10^8$
48 h	$(6.1 \pm 1.3)10^8$	$(4.8 \pm 1.6)\ 10^8$	$(3.9 \pm 0.9)\ 10^8$	$(9.1 \pm 0.4)\ 10^8$
Fermenting dough				
0 h	$(1.7 \pm 0.6)\ 10^6$	$(3.0 \pm 1.4)\ 10^6$	$(5.1 \pm 0.4)\ 10^7$	$(2.2 \pm 1.5)\ 10^6$
4 h	$(7.6 \pm 0.8)\ 10^6$	$(7.6 \pm 0.8)\ 10^6$	$(1.1 \pm 0.2)\ 10^8$	$(2.2 \pm 0.6)\ 10^7$
8 h	$(2.1 \pm 0.6)\ 10^7$	$(7.8 \pm 0.7)\ 10^7$	$(8.6 \pm 0.4)\ 10^8$	$(4.8 \pm 0.6)\ 10^8$
12 h	$(4.9 \pm 0.9)\ 10^8$	$(5.6 \pm 0.5)\ 10^8$	$(1.0 \pm 0.5)\ 10^9$	$(8.7 \pm 1.0)\ 10^8$

4.3.2 Population of Lactic Acid Bacteria

Isolates on MRS which were Gram positive catalase negative rods, coccobacilli or cocci were considered to be lactic acid becteria. They were mainly rods, oxidase negative and non-sporing. The counts ranged from a level of 10^3cfu/ml and 10^5cfu/ml at the beginning of steeping to 10^6-10^8 cfu/ml after 24 h. The counts obtained at three production sites reduced from 10^8 to 10^7 cfu/ml after 48 h. A similar trend was observed in the dough fermentation with an increase from between 10^5-10^6 cfu/ml to 10^8cfu/ml at the end of fermentation (Table 4.4).

Table 4. 4 Mean LAB counts during the fermentation of dehulled maize into *nsiho*

	Mean LAB counts			
Sample	Processor 1	Processor 2	Processor 3	Processor 4
Steep water				
0 h	(1.1± 0.1) 10^5	(5.1± 0.6) 10^4	(7.2± 0.4) 10^4	(1.7± 0.8) 10^3
24 h	(5.4± 1.0) 10^8	(2.2± 0.4) 10^8	(2.8± 1.3) 10^8	(7.7± 0.7) 10^6
48 h	(1.8± 0.4) 10^7	(4.1± 0.8) 10^7	(8.8± 0.9) 10^7	(3.2± 0.9) 10^8
Fermenting dough				
0 h	(1.0± 0.1) 10^5	(2.31± 1.3) 10^6	(2.8± 1.2) 10^6	(2.1± 1.1) 10^6
4 h	(6.7± 0.6) 10^6	(3.1± 0.9) 10^6	(3.2± 2.0) 10^7	(2.5± 1.3) 10^7
8 h	(3.0± 0.6) 10^7	(1.6± 0.1) 10^7	(2.1± 1.5) 10^9	(5.0± 0.9) 10^7
12 h	(6.3± 1.2) 10^8	(5.1± 1.5) 10^8	(2.4± 0.7) 10^9	(2.6± 2.1) 10^8

4.3.3 Population of Yeasts

Initial counts of yeasts at the start of steeping at all production sites were at a concentration 10^2 cfu/ml and increased to 10^4-10^6 cfu/ml after 48 h of steeping. Likewise, during dough fermentation yeast counts increased gradually from between 10^3-10^4 cfu/g to 10^6-10^7 cfu/g after 12 h (Table 4.5).

Table 4.5 Mean yeast counts during the fermentation of dehulled maize into *nsiho*

	Mean Yeast counts			
Sample	Processor 1	Processor 2	Processor 3	Processor 4
Steep water				
0 h	$(4.1\pm0.5)10^2$	$(2.8\pm 0.6)\ 10^2$	$(1.6\pm 0.8)\ 10^2$	$(7.5\pm 0.6)\ 10^2$
24 h	$(4.9\pm 0.3)\ 10^4$	$(6.9\pm 0.4)\ 10^4$	$(1.9\pm 1.1)\ 10^5$	$(9.7\pm 0.8)\ 10^4$
48 h	$(1.8\pm 0.5)\ 10^5$	$(9.8\pm 0.1)\ 10^4$	$(9.1\pm 0.4)\ 10^5$	$(1.9\pm 0.9)\ 10^6$
Fermenting dough				
0 h	$(5.7\pm 0.8)\ 10^3$	$(5.9\pm 0.1)\ 10^4$	$(7.8\pm 1.1)\ 10^4$	$(9.1\pm 0.7)\ 10^3$
4 h	$(2.5\pm 0.6)\ 10^5$	$(4.9\pm 0.5)\ 10^5$	$(1.8\pm 0.1)\ 10^5$	$(5.9\pm 0.5)\ 10^4$
8 h	$(6.0\pm 0.3)\ 10^5$	$(1.0\pm 0.1)\ 10^6$	$(1.0\pm 0.4)\ 10^6$	$(4.0\pm 0.6)\ 10^5$
12 h	$(1.8\pm 0.7)\ 10^7$	$(7.8\pm 0.6)\ 10^7$	$(2.7\pm 0.6)\ 10^6$	$(7.8\pm 0.5)\ 10^6$

4.4 Characterisation and Identification of Lactic acid Bacteria

A total number of 208 LAB colonies were isolated from steeped water and dough fermentation. They were all Gram positive catalase negative rods, cocobacilli or lactococci with rods being dominant. These were considered to be lactobacillus spp. Most of the isolates were found to be heterofermentative by their ability to produce CO_2 from glucose and grew at pH 4.4 and 9.6 and 45 ^{o}C but not at 10 ^{o}C nor in 18 % NaCl concentration (Table 4.6).

The most dominant strains were heterofermentative lactobacilli and utilised galactose, D-glucose, D-fructose, D-mannose, ribose, melibiose, saccharose, gluconate and maltose. The rest are D-raffinose, 5-ketogluconate, D-xylose, lactose, cellobiose, esculin, trehalose, β-gentobiose, salin, amygdalin, l-arabinose, galactose and mannitol in the API 50 CHL galleries (Appendix 2) and were identified as *Lactobacillus fermentum.*

The second most dominant species were very short rods or cocobacilli and grew at pH 4.4 and 9.6 and at 45 ^{o}C but not at 10 ^{o}C and 18 % NaCl (Table 6) and were able to ferment L-arabionose, ribose, D-xylose, galactose, D-glucose, D-frucrose, amygdaline, maltose, melibiose, saccharose, gluconate and 2 keto-gluconate but did not utilized Glycerol, erythritol, sorbose, rhamnose and were identified as *Lacobacillus brevis.*

The third most dominant species which were rods were identified as *Lactobaccillus plantarum* and grew at pH 4.4 and 9.6, but not in 6.5 % and 18 % NaCl. They were able to ferment-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, D-turanose, mannitol, esculin, salicin, sorbitol, maltose, lactose, cellobiose and Gluconate.

Other species identified based on carbohydrate fermentation were *Pediococcus pentosaceus* 8.65 % and fermented L-arabinose, ribose, galactose, D-xylose, D- fructose, D-glucose, D-manose, salicin, cellobiose, esculin, lactose, mellibiose, saccharose and β-gentobiose and lastly *Pediococcus acidilactici* 4.81 % which fermented ribose, D-xylose, L-xylose, D-fructose, D-glucose, D-manose but not mellibiose and sacchrarose.

Table 4.6 Biochemical characteristics of lactic bacteria isolated from steeping water and fermenting dough

Groups	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Cell form	rods	rods	cocci	rods	rods	rods	cocci
Cellular arrangement	singles /pairs	pairs	tetrad	pairs	singles /pairs	pairs	pairs
Gram reaction	+	+	+	+	+	+	+
Catalase reaction	–	–	–	–	–	–	–
Oxidase reaction	–	–	–	–	–	–	–
Anaerobic growth	+	+	+	+	+	+	+
CO_2 from glucose	+	+	–	+	+	+	–
Growth at 10^{o}C	–	–	+	–	–	–	–
Growth at 45^{o}C	+	+	+	–	–	+	+
Growth in 6.5% NaCl	–	+	+	–	–	–	+
Growth in 18% NaCl	–	–	+	–	–	–	+
Growth at pH4.4	+	+	–	+	+	+	+
Growth at pH 9.6	+	+	+	+	+	+	+
Isolate identified	*L. fermetum* 1	*L. fermentum* 2	*P. pentotaseus*	*L. brevis* 1	*L. plantarum*	*L. Brevis* 2	*P. acidilactici*
Isolate source	**AD**.4/ 12	**SS**.24/ 8	**AS**48/ 4	**AD**.12/ 8	**SD**.8/ 13	**AD**.0/ 14	**AS**.48/ 9

A=Anum, **S**=South Senchi, D=Dough, S=Steep water, 4-14=isolate number

4.5 Characterisation and Identification of Yeasts Population

A total of 185 yeast colonies were isolated from steep water and fermenting dough from the four production sites. Initial characterisation was based on colony and cell morphology and by fermentation of sugars in API ID 32C gallaries. The most dominant yeasts (47.6 %) were isolated from all the processing stages and utilized galactose, glucose, sucrose, raffinose, maltose, DL-lactate, trehalose, α-metyl-D-glucoside, melibiose but could not assimilate lactose and were identified as *Saccharomyces cerevisiae*. The second dominant yeast (29.1 %) isolated utilized glucose, N-acetyl- glucosamide and DL-lactate out of the 32 carbohydrate tested in the ID 32 galleries and was identified as *Candida krusei*. The third yeasts isolates utilized D-melizitose, D-melibiose D-glucose and were identified as *Debaryomyces* spp. and constituted 15 % whilst the last group *Trichosporon* spp. 8.3 % utilzed D-melibiose D-glucose, mannose, maltose, etc. *Debaryomyces* spp. and *Trichosporon* spp mainly occurred during the initial stages of dehulled maize grains steeping.

4.6 Technological properties of Lactic acid Bacteria Isolates

4.6.1 Rate of Acidification by Lactic Acid Bacteria Isolates

pH and titratable acidity were used to evaluate rate of acidification during steeping and dough fermentation. Figures 4 a-c show the rate of acidification during steeping. At 0-4 h the rate of acidification ranged from 0.01 to 0.03 unit with *L. fermentum* 2 showing the lowest rate of acidification (Fig.4.a). At 4-8 h fermentation (Fig 4.b), change in pH ranged from between 0.32 to 0.84 units and *L. fermentum* 2 and *L. brevis* 1 recorded the highest rate of acidification with *P. pentosaceus* being the least. At 8-12 h of steeping all the isolates had increased the rate of acidification by more than 1 unit with *P. pentosaceus and P. acidilactici* showing the highest pH change (Fig 4.c). Rate of acidification was faster during the fourth to the eighth hour, but was the least from the zero to fourth hour during the fermentations. The Titratable acidity expressed as percentage lactic acid, increased in both steeping water and fermenting dough (Table 4.7 & 4.8).

Figures 4 d-e show changes in the rate of acidification for the fermenting dough. Rate of acidification increased by 1.15 to 1.20 units within the first four (0-4) hours: with *L. fermentum* 2 showing the fastest rate (Fig 4.d). Rate of acidification was faster during the fourth to the eighth hours of fermentation and was the least in the eighth to twelveth hour. Figure 4 e shows *L. fermentum* 1 and *L. brevis* 1 as the isolates with the highest rate of acidification.

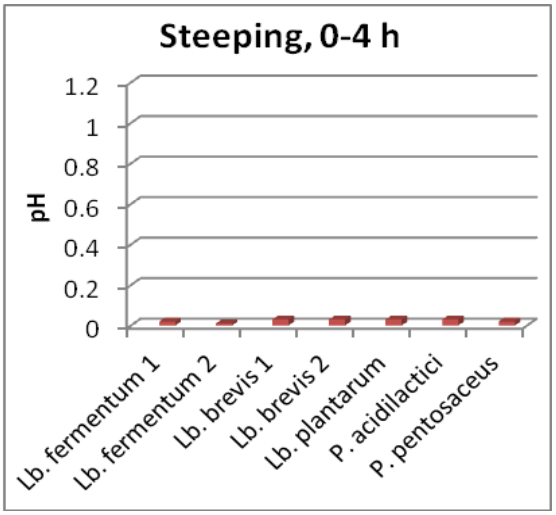


Fig. 4.2 a

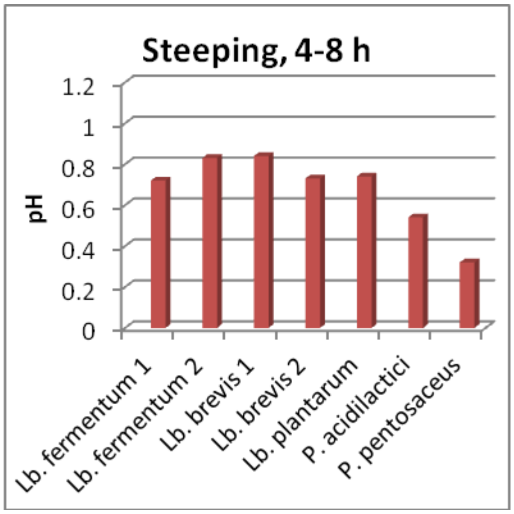


Fig. 4.2 b

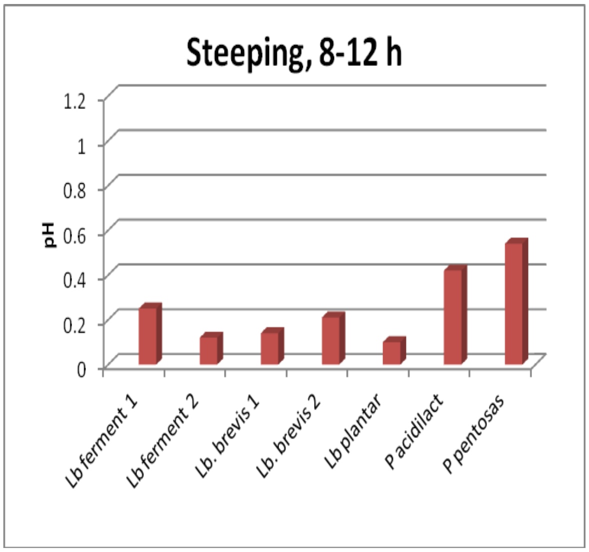


Fig. 4. 2 c

Fig 4.2 a-c Rates of acidification of steeped dehulled maize by lactic acid bacteria isolates

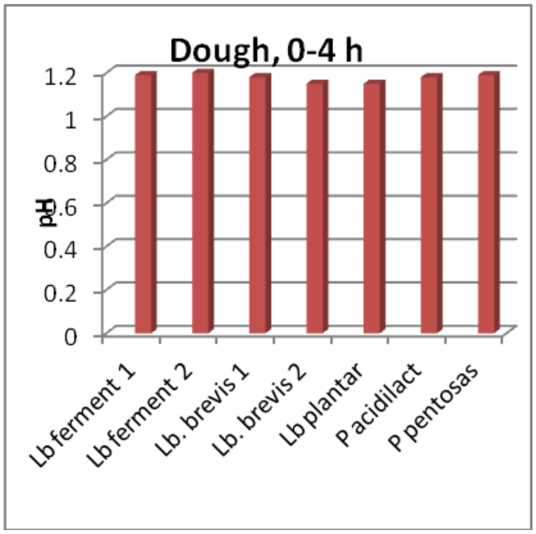


Fig. 4.2 d

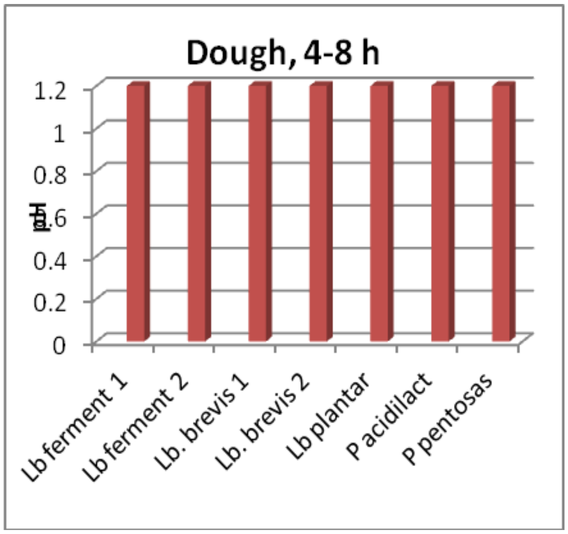


Fig. 4.2 e

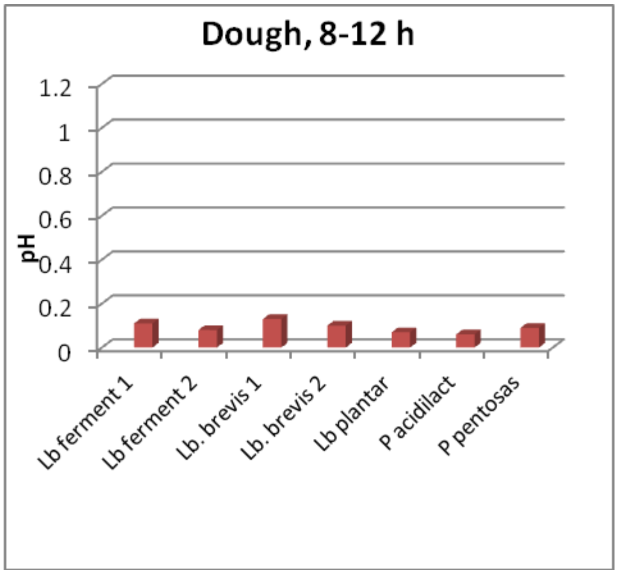


Fig. 4.2 f

Fig 4.2 d-f Rates of acidification of fermenting dough by lactic acid bacteria isolates

Table 4.7 Changes in Titratable acidity during acidification of steeping water by lactic acid bacteria

	Time of steeping			
Isolates	0 h	4 h	8 h	12 h
L. fermentum 1	0.02± 0.01 a	0.17± 0.02a	0.24± 0.01a	0.32± 0.03a
L. fermentum 2	0.02± 0.01 a	0.15± 0.01a	0.22± 0.02a	0.30± 0.01a
L .brevis 1	0.03± 0.01 a	0.12± 0.01b	0.16± 0.01b	0.31 ±0.01a
L .brevis 2	0.02± 0.01 a	0.10± 0.01b	0.17± 0.02b	0.30± 0.02 a
L .plantarum	0.02± 0.01 a	0.14± 0.01a	0.18± 0.01b	0.29± 0.02a
P. acidilactici	0.01± 0.01 a	0.15± 0.01a	0.16± 0.03b	0.25± 0.02b
P. pentosaceus	0.01± 0.01 a	0.16± 0.02a	0.19± 0.01b	0.22± 0..03b

Means with same letters in a column are not significant different (p<0.05)

Table 4.8 Changes in Titratable acidity during acidification of fermenting dough by lactic acid bacteria

Time of fermenting dough				
Isolates	0 h	4 h	8 h	12 h
L. fermentum 1	0.02± 0.01a	0.19± 0.01a	0.24± 0.01a	0.36± 0.01a
L. fermentum 2	0.02± 0.01a	0.18± 0.01a	0.27± 0.01b	0.32± 0.01b
L. brevis 1	0.02± 0.01a	0.18± 0.02a	0.22± 0.01a	0.33 ±0.01b
L. brevis 2	0.02± 0.01a	0.17± 0.01a	0.21± 0.01a	0.29± 0.03 c
L. plantarum	0.02± 0.01a	0.20± 0.01a	0.25± 0.01a	0.29± 0.03c
P. acidilactici	0.02± 0.01a	0.18± 0.01a	0.23± 0.01a	0.31± 0.01b
P. pentosaceus	0.03± 0.01a	0.11± 0.01b	0.19± 0.01c	0.25± 0.01d

Means with same letters in a column are not significant different (p<0.05)

4.6.2 Production of Exopolysaccharides (EPS) by Lactic Acid Bacteria Isolates

A total 108 lactic acid bacteria were screened for production of exopolysaccaride. The isolates produced varied degrees of EPS ranging from 1 mm to 4 mm of slime. About 26 % comprising of *L. brevis, P.acidilactici P. pentosaceus, L. plantarum* produced slime of less than 2 mm in length, whiles about 37 % comprising of *L. fermentum1, L. fermentum 2* produced slime exceeding 2 mm in length (Table 4.9).

4.6.3 Amylase Secretion by Lactic Acid Bacteria Isolates

The lactic acid bacteria isolates were compared for their ability to secrete amylase by growing them on modified Nutrient agar containing 2 % starch. A total of 144 isolates comprising 43 *L. fermentum*, 34 *L. brevis*, 22 *L. plantarum*, 13 *P. acidilactici* and 8 *P. pentosaceu*s were screened. Out of these isolates 18.24 % of *L. fermentum* produced clear zones ranging from 1mm to 2 mm, 10.12 % of *L .brevis* produced clear zones, 12.78 % of *L. plantarum* produced clear zones and 8.28 % of *P. pentosaceus* also demostrated amylase secretion. None of the *P. acidilactici* demonstrated amylolytic activity (Table 4.9).

4.6.4 Protease Secretion by Lactic Acid Bacteria Isolates

Out of the total of 108 lactic acid bacteria isolates tested for protease activity, none of them isolates secreted protease (Table 4.9).

Table 4.9 Amylase, protease and Exopolysaccharides (EPS) secretion by lactic acid bacteria isolated during maize and dough fermentation.

LAB isolates	Amylase	Protease	EPS
L. fermentum 1	+	-	++
L. fermentum 2	+	-	++
L.brevis 1	-	-	+
L.brevis 2	-	-	+
L.plantarum	+	-	+
P.acidilactici	-	-	+
P.pentosaceus	+	-	+

-: no inhibition zone, +: 1-2mm inhibition zone, ++: 3-4mm inhibition zone, +++:5+mm inhibition zone

4.6.5 Antimicrobial Activity of Lactic Acid Bacteria against Some Common Pathogens

Lactobacillus fermentum showed a strong antimicrobial activity against all the pathogens tested (*Salmonella typhimurium*, NCTC 12023, *E.coli*, NCTC 9001, *Vibro cholerae*, NCTC 11348 and *Staphylococcus aureus*, NCTC 657). The strongest inhibition were aginst *Vibro cholerae* and *Staphylococcus aureus* with inhibition zones exceeding 5 mm while *Salmonella typhimuruim* and *E. coli* showed inhibition zones of less than 3 mm (Table 4.10). *P. acidilactici* showed a weak inhibition against all the pathogens tested whilst *P. pentosaceus* was only able to inhibit *Staphylococcus aureus* weakly. *L. plantarum* showed a fair inhibition against *Staphylococcus aureus* and weak inhibition towards *Vibro cholerae, Salmonella typhimurium* and *E.coli.* Only few of the isolates prevented the growth of *E. coli*

Table 4.10 Antimicrobial activity of lactic acid bacteria against pathogens indicator-strains

Tested isolates	**Indicator strains**			
	E.coli	*Staphylococcus aureus*	*Salmonella typhimurium*	*Vibrio cholera*
L. fermentum 1	++	+++	++	+++
L.fermentum 2	+	++	++	++
L.brevis 1	-	+	-	++
L.brevis 2	-	-	-	++
L.plantarum	+	++	+	+
P.acidilactici	-	+	+	-
P.pentosaceus	-	+	-	-

-: no inhibition zone, +: 1-2mm inhibition zone, ++: 3-4mm inhibition zone, +++:5mm inhibition zone

4.6.6 Antimicrobial Interaction between Lactic Acid Bacteria and Yeasts Isolates

There was no microbial interaction between any of the lactic acid bacteria and *Saccharomyces cerevisiae* or *Candida krusei.* However, *L. fermentum* 1, *L. brevis 1&2 and L. plantarum* showed antagonism against *Debaryomyces* spp and *Trichosporon* spp with clear zones ranging from 2-3 mm. *P. acidilactici* and *P. pentosaceus* showed weak inhibition against only *Debaryomyces* with inhibition zone of 2 mm (Table 4.11). *Debaryomyces* and *Trichosporon* spp were found only in the steeping water.

Table 4.11Antimicrobial interaction between lactic acid bacteria and yeasts isolates

	Indicator strains (Yeasts)			
Test isolates (LAB)	*S.cerevisiae*	*C.krusei*	*Dabaromyces* species	*Trichosporon* species
L. fermentum 1	-	-	++	++
L. fermentum 2	-	-	++	++
L.brevis 1	-	-	+	++
L.brevis 2	-	-	+	+
L. plantarum	-	-	+	+
P.acidilactici	-	-	+	+
P.pentosaceus	-	-	+	+

-: no inhibition zone, +: 1-2mm inhibition zone ++: 3-4mm inhibition zone +++:5+mm inhibition zone

4.6.7 Antimicrobial Interaction between Lactic Acid Bacteria Isolates

There was no interaction between the lactic acid bacteria isolates except for *L. fermenutm*1 and *L. plantarum* which showed weak inhibition towards *P. acidilactici* (Table 4. 12).

Table 4.12 Antimicrobial interaction between lactic acid bacteria isolates

	Indicator strains						
Test isolates	*L. fermentum1*	*L. fermentum 2*	*L. brevis 1*	*L. brevis 2*	*L. plantarum*	*P. acidilactici*	*P. pentosaceus*
L. fermentum 1	NA	-	-	-	-	+	-
L. fermentum 2	-	NA	-	-	-	-	-
L. brevis 1	-	-	NA	-	-	-	-
L. brevis 2	-	-	-	NA	+	-	-
L. plantarum	-	-	-	-	NA	+	-
P. acidilactici	-	-	-	-	+	NA	-
P. pentosaceus	-	-	-	-	-	-	NA

-: no inhibition zone, +: 1-2mm inhibition zone, ++: 3-4mm inhibition zone, +++:5+mm inhibition zone NA: No interaction

4.7 Starter Culture Trials

4.7.1 Changes in Microbial Population

Changes in the population of lactic acid bacteria and yeasts following the addition of different starter cultures are shown Table 4.13. Isolates used for the starter culture trials were *L. fermentum* and *L. brevis (*LAB) and *S. cerevisiae* and *C. krusei* (Yeasts). Following the addition of the LAB isolates i.e *L. fermentum* and *L. brevis*, high counts of lactic acid bacteria were recorded throughout compared to the spontaneous fermentation or addition of yeasts. Addition of yeast as starter cultures did not significantly affect the LAB counts compared to the spontaneous fermentation. The population of lactic acid bacteria reached a final count of 10^9 cfu/ml in fermentation with *L. fermentum* and *L. brevis*, but 10^7 cfu/ml in the fermentation with yeast and also in the spontaneous steeping of the dehulled maize grains (Table 4.13).Yeast counts in fermentations with added *S. cerevisiae* and *C. krusei* increased in both cases from initial levels of 10^5 cfu/ml to 10^7 cfu/ml after 24 h of steeping whilst maximum counts were 10^4cfu/ml and 10^5 cfu/ml in spontaneous steeping and with added *L. brevis* or *L. fermentum* starter culture respectively.

Dough fermentation to which *L. fermentum* and *L. brevis* had been added as starter culture recorded higher LAB counts compared to the other fermentations. Also, after 8 h, fermentation to which *L. fermentum* and *L. brevis* had been added as starter culture attained the same level of 10^8 cfu/g LAB as in 12 h spontaneous in fermented dough. Addition of *C.krusei* and *S. cerevisiae* starter cultures recorded higher yeast counts to a level of 10^8 cfu/g at the end of fermentation as compared to the other fermentations (Table 4.13).

Table 4.13 Mean microbial counts (cfu/ml and cfu/g) for fermentation trials carried out with starter culture

		Types of fermentation				
Sample	Fermentation time (h)	spontaneous	*L. fermentum*	*L. brevis*	*C. krusei*	*S. cerevisiae*
Steep water						
LAB	0 h	$(4.9\pm2.6)10^{4\,a}$	$(2.0\pm0.8)10^{7b}$	$(5.3\pm2.8)10^{7\,b}$	$(5.5\pm1.4)10^{4\,a}$	$(5.5\pm0.9)10^{4\,a}$
	24 h	$(4.8\pm1.8)10^{7\,a}$	$(3.3\pm1.3)10^{8\,b}$	$(2.4\pm1.6)10^{8\,b}$	$(6.7\pm1.8)10^{7\,a}$	$(6.4\pm1.5)10^{7\,a}$
	48 h	$(7.7\pm1.4)10^{7\,a}$	$(4.3\pm1.5)10^{9\,b}$	$(2.1\pm0.2)10^{9\,b}$	$(4.9\pm0.6)10^{7\,a}$	$(8.3\pm2.1)10^{7\,a}$
Yeast	0 h	$(5.3\pm1.0)10^{2\,a}$	$(3.1\pm0.3)10^{2\,a}$	$(6.8\pm0.4)10^{2\,a}$	$(4.1\pm1.7)10^{5\,b}$	$(3.8\pm1.6)10^{5\,b}$
	24 h	$(5.3\pm1.7)10^{4\,a}$	$(1.3\pm0.1)10^{5\,b}$	$(5.6\pm0.5)10^{4\,a}$	$(3.6\pm0.4)10^{7\,c}$	$(4.1\pm0.7)10^{7\,c}$
	48 h	$(1.5\pm0.5)10^{5\,a}$	$(4.5\pm0.7)10^{5\,a}$	$(2.8\pm1.3)10^{5\,a}$	$(5.3\pm2.3)10^{7\,b}$	$(8.3\pm1.6)10^{7\,b}$
Fermenting dough						
LAB	0 h	$(5.5\pm2.6)10^{5\,a}$	$(3.0\pm0.9)10^{7\,b}$	$(2.0\pm4.0)10^{7\,b}$	$(3.0\pm4.0)10^{6\,c}$	$(2.0\pm3.0)10^{6\,c}$
	4 h	$(4.8\pm0.5)10^{6\,a}$	$(8.0\pm0.8)10^{7\,b}$	$(5.6\pm0.7)10^{7\,b}$	$(8.5\pm1.0)10^{6\,a}$	$(6.4\pm1.0)10^{6\,a}$
	8 h	$(8.0\pm0.2)10^{6\,a}$	$(3.0\pm0.7)10^{8\,b}$	$(5.8\pm2.2)10^{8\,b}$	$(6.9\pm2.7)10^{7\,c}$	$(6.1\pm1.8)10^{7\,c}$
	12 h	$(2.2\pm0.4)10^{8\,a}$	$(1.5\pm0.6)10^{9\,b}$	$(1.7\pm0.8)10^{9\,b}$	$(5.8\pm1.9)10^{8\,c}$	$(4.8\pm0.9)10^{8\,c}$
Yeast	0 h	$(8.7\pm1.8)10^{3\,a}$	$(1.0\pm0.4)10^{4\,a}$	$(3.6\pm0.4)10^{4\,b}$	$(3.3\pm0.5)10^{5\,c}$	$(2.7\pm1.4)10^{5\,c}$
	4 h	$(1.0\pm0.3)10^{5\,a}$	$(7.6\pm0.8)10^{4\,a}$	$(5.2\pm1.3)10^{4\,a}$	$(5.7\pm0.4)10^{5\,b}$	$(4.4\pm1.4)10^{6\,c}$
	8 h	$(6.8\pm2.2)10^{5\,a}$	$(4.2\pm1.7)10^{5\,a}$	$(8.4\pm1.2)10^{5\,a}$	$(5.9\pm1.5)10^{7\,b}$	$(5.3\pm1.2)10^{7\,b}$
	12 h	$(1.3\pm0.6)10^{7\,a}$	$(4.9\pm0.8)10^{7\,a}$	$(4.1\pm1.2)10^{7\,a}$	$(1.3\pm0.6)10^{8\,b}$	$(1.6\pm0.6)10^{8\,b}$

Values are means of determinations from two separate fermentation trails. Means with same letter in a row are not significantly different ($p<0.05$)

4.7.2 Acidification of Steep Maize and Fermenting Dough in Fermentation Trials with Starter Culture

Table 4.14 shows pH of steep water and fermenting dough to which single starter cultures were added. The pH recorded for the spontaneous (control) fermentation dropped from 6.00 at start of steeping to 3.52 after 48 h of steeping. The pH values for steeping with starter cultures ranged from initial levels of 6.02 -5.96 to 3.56 -3.49 at the end of steeping. Among the starter cultures, the highest drop was recorded for *L. fermentum* with 3.49 and the least for *C. krusei* being the least 3.56 (Table 4.14). Dough fermentation showed similar results with the starter cultures reducing the pH from 6.03-5.99 at the start of fermentation to 3.44-3.38 at the end of fermentation (Table 4.14).

Titartable acidity increased in all fermentations as expected. The TTA values recorded during stepping ranged from 0.03-0.04 at the start of fermentation to 0.30- 0.38 after 48 h fermentation. Also, during dough fermentation a value of 0.37-0.53 was attained at the end of fermentation. The concentration of lactic acid was highest in both steeping water and dough fermented with *L. fermentum* and lowest with spontaneous fermentation (Table 4.14).

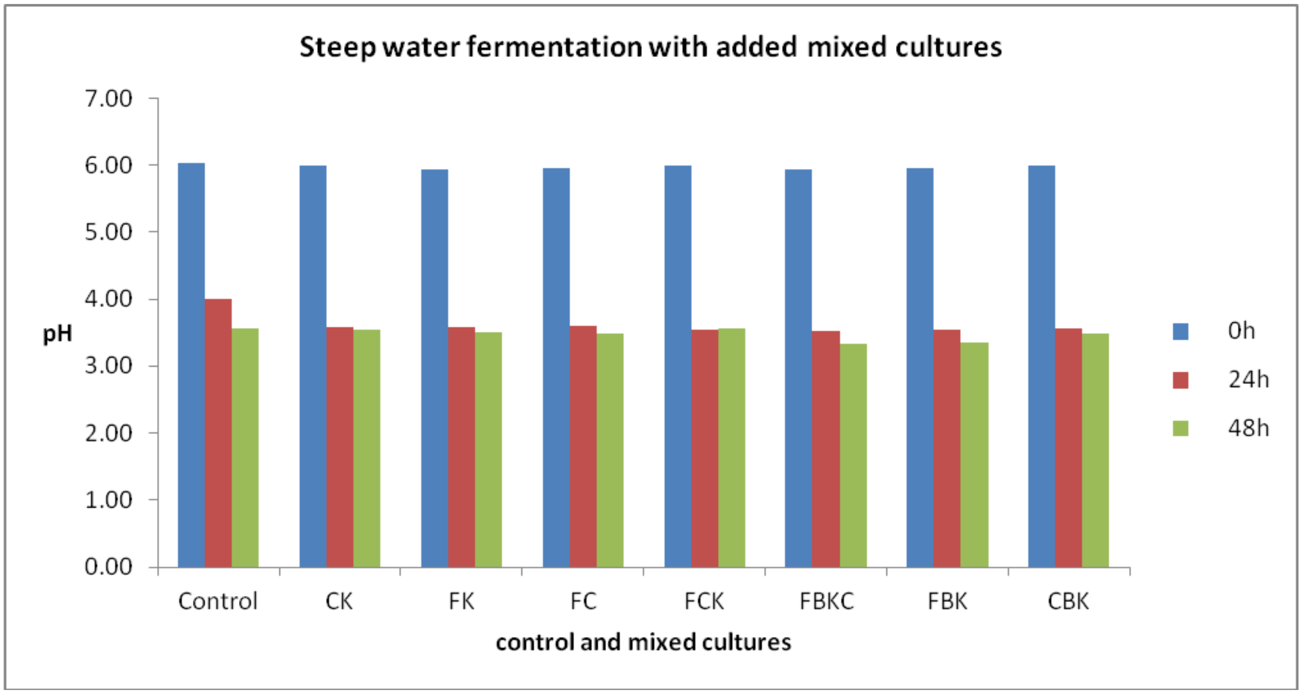
Table 4.14 Mean pH and Titratable acidity values for fermentation trials with starter culture

		Types of fermentation				
Sample	Fermentation time (h)	Control	*L. fermentum*	*L. brevis*	*C.krusei*	*S. cerevisiae*
Steep water						
pH	0 h	6.00± 0.03	5.96± 0.01	5.96± 0.02	6.00 ±0.01	6.02 ±0.01
	24 h	4.02± 0.01	3.63± 0.01	3.68 ±0.02	3.71 ±0.05	3.70 ±0.02
	48 h	3.62 ±0.01	3.49 ±0.01	3.50 ±0.01	3.56 ±0.02	3.55± 0.02
Titratable acidity	0 h	0.03± 0.01	0.04 ±0.01	0.04± 0.02	0.04± 0.01	0.03 ±0.01
	24 h	0.26 ±0.01	0.31 ±0.02	0.29 ±0.01	0.28 ±0.02	0.28 ±0.01
	48 h	0.30 ±0.01	0.38± 0.01	0.35 ±0.01	0.35 ±0.02	0.34 ±0.01
Fermenting dough						
pH	0 h	6.02± 0.01	5.99± 0.03	6.02± 0.01	6.03 ±0.01	6.03± 0.01
	4 h	5.46 ±0.01	5.28 ±0.02	5.32 ±0.01	5.40 ±0.03	5.41 ±0.01
	8 h	3.72 ±0.05	3.46 ±0.04	3.53 ±0.05	3.50 ±0.01	3.49 ±0.01
	12 h	3.53 ±0.03	3.38 ±0.01	3.41 ±0.02	3.41 ±0.01	3.44 ±0.01
Titratable acidity	0 h	0.26± 0.01	0.27± 0.01	0.27± 0.01	0.27± 0.02	0.25± 0.01
	4 h	0.28 ±0.02	0.32 ±0.01	0.32 ±0.01	0.30 ±0.01	0.28 ±0.01
	8 h	0.32 ±0.01	0.38 ±0.01	0.37 ±0.01	0.34 ±0.01	0.33 ±0.01
	12 h	0.37 ±0.01	0.49 ±0.02	0.47 ±0.03	0.47 ±0.01	0.46 ±0.02

4.7.3 pH Changes during Steeping and Dough Fermentation Inoculated with Combinations of Starter Cultures

The pH changes during *nsiho* fermentation and with combinations of starter cultures during steeping fermentation and dough are shown in figures 4.3 and 4.4. Addition of any of LAB and yeasts starter cultures produced lower pH values both during steeping and dough fermentation in comparison to the spontaneous fermentation. The lowest pH values at all stages of fermentation were recorded when all four starter cultures were used in combination, i.e *L. fermentum* + *L. brevis C. krusei*+ *S. crervisiae*. At 24 h of steeping all fermentations using starter cultures had the pH value recorded for the spoataneous fermentation at the end of steeping i.e 48 h. Generally, the more cultures that were combined in starter culture mix ie 2, 3, or 4 different cultures, the faster and lower the drop in pH observed. Using a combination *L. fermentum* + *L. brevis* + *C. krusei* for steeping reduced the pH more than using a combination of *S. crervisiae* + *L. brevis* + *C. krusei* or *L. fermentum* + *S. crervisiae* + *C. krusei*. The same trend was observed during dough fermentation. The pH ranged from between 5.98-5.93 at the start of fermentation to between 3.44- 3.06 at the end of fermentation, while combination with *L. fermentum* + *L. brevis* + *C. krusei*+ *S. crervisiae* ranged from 5.93 to 3.06 after 12 h (Fig4.4).

Fig 4.3 Changes in pH during steeping of dehulled maize grains using different starter cultures



Control (spontaneous): no starter culture added

CK: *S. crervisiae* + *C. krusei*

FK*: L. fermentum* + *C. krusei*

FC: L. fermentum + *S. crervisiae*

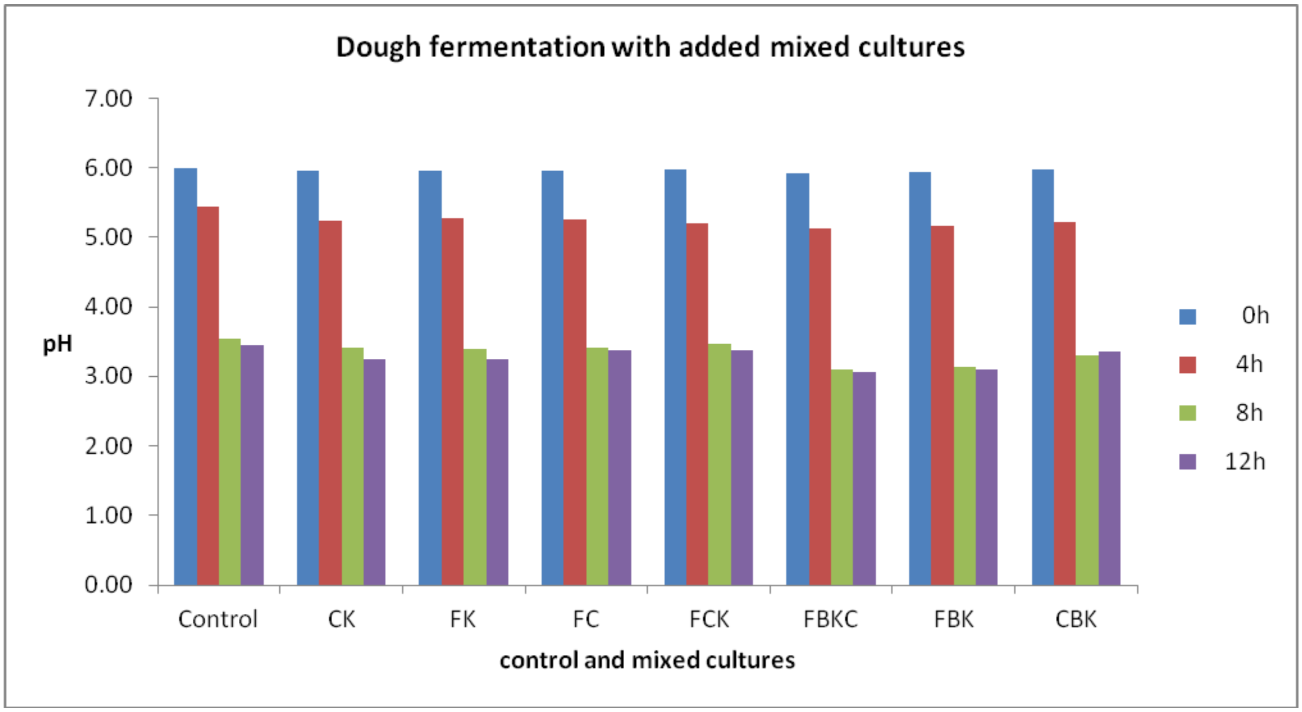
FCK*: L. fermentum* + *S. crervisiae* +*C. krusei*

FBKC*: L. fermentum* + *L. brevis* + *C. krusei*+ *S. crervisiae*

FBK*: L. fermentum* + *L. brevis* + *C. krusei*

CBK: *S. crervisiae* + *L. brevis* + *C. krusei*

Fig 4.4 Changes in pH during dough fermentation using different starter cultures



Control (spontaneous): no starter culture added

CK: *S. crervisiae + C. krusei*

FK*: L. fermentum + C. krusei*

FC: L. fermentum + S. crervisiae

FCK*: L. fermentum + S. crervisiae +C. krusei*

FBKC*: L. fermentum + L. brevis C. krusei+ S. crervisiae*

FBK*: L. fermentum + L. brevis + C. krusei*

CBK: *S. crervisiae + L. brevis + C. krusei*

4.7.4 Changes in Titratable acidity during Steeping and Dough Fermentation Inoculated with Different Combinations of Starter Cultures

The mean total acidity during the different types of fermentations is shown in Table 4.15. The highest value of Titratable acidity was produced by combination of all four starter cultures, with spontaneous fermentation yielding the least in both steep water and dough during the fermentation periods (Table 4.15). The combination of lactic acid bacteria and yeast produced more lactic acid than only yeast combinations. With the three combinations, *L. fermentum* + *L. brevis* + *C. krusei* combination produced more lactic acid after 48 h of steeping while *L. fermentum* + *S. crervisiae* + *C. krusei* produced a higher value TTA after 12 h of dough fermentation (Table 4.15).

Table 4.15 Percentage Titratable acidity values during steeping and dough fermentation of *nsiho* using different starter cultures

	Types of fermentation							
Fermentation time (h)	Control/spontaneous	CK	FK	FC	FCK	FBK	CBK	FBKC
Steep water								
0 h	0.03± 0.00a	0.04± 0.01a	0.04± 0.01a	0.05± 0.01a	0.04 ±0.01a	0.05± 0.01a	0.04± 0.01a	0.06± 0.01b
24 h	0.25 ±0.01a	0.28 ±0.02a	0.31 ±0.01b	0.33 ±0.02b	0.30 ±0.02b	0.30 ±0.01b	0.29 ±0.01b	0.35± 0.03c
48 h	0.28± 0.01a	0.37 ±0.01b	0.39 ±0.03b	0.38 ±0.01b	0.39 ±0.04b	0.40 ±0.02c	0.37 ±0.01c	0.42± 0.02c
Fermenting dough								
0 h	0.25± 0.01a	0.25± 0.00a	0.26± 0.01a	0.27± 0.02a	0.28± 0.01a	0.27± 0.01a	0.26± 0.02a	0.30± 0.01a
4 h	0.27 ±0.01a	0.30 ±0.01b	0.33 ±0.02b	0.31 ±0.01b	0.32 ±0.01b	0.32 ±0.01b	0.33 ±0.01b	0.38 ±0.01c
8 h	0.32 ±0.02a	0.35 ±0.01a	0.39 ±0.01b	0.37 ±0.01b	0.41 ±0.33b	0.42 ±0.01b	0.43 ±0.02b	0.47 ±0.01c
12 h	0.40 ±0.02a	0.49 ±0.01b	0.53 ±0.02b	0.50 ±0.04c	0.58 ±0.01c	0.57 ±0.02c	0.56 ±0.03c	0.61 ±0.02d

Means with same letters in a row are not significant different ($p<0.05$)

4.7.5 Microbial Population during Steeping using Different Starter Cultures

The microbial population during the steeping of dehulled maize grains using different starter cultures is shown in Table 4.16. With addition of any of the lactic acid bacteria the total LAB counts obtained at the end of 48 h steeping was at a concentration of 10^9 cfu/ml compared to 10^8 cfu/ml for spontaneous fermentation during steeping. Using a combination of *L. fermentum* + *L. brevis C. krusei*+ *S. crervisiae* gave the highest LAB counts during steeping (Table 4.16). Sponatneous fermentation recorded the least LAB counts of 10^8 cfu/ml at the end of steeping and was significant different from the use of any of the starter cultures. Among the three mix starter, *L. fermentum* + *L. brevis*+ *C. krusei* showed higher counts than *L. fermentum* + *S. crervisiae*+ *C. krusei* and *S. crervisiae* + *L. brevis* +*C. krusei* ; also, in the two mix starter *S. crervisiae* + *C. krusei* produced lower counts as compared to *L. fermentum* + *C. krusei* and *L. fermentum* + *S. crervisiae* (Table 4.16). With yeasts counts, combinations with the two yeasts produced higher yeasts counts of 10^8 cfu/ml after 48 h of steeping, whiles using a single yeast gave a count of 10^7 cfu/ml compared to 10^5 cfu/ml for steeping without any starter culture.

Table 4.16 Mean microbial counts (cfu/ml) during the steeping of dehulled maize grains using starter culture

		Types of fermentation							
Sample	Fermentation time	Control/ spontaneous	CK	FK	FC	FCK	FBK	CBK	FBKC
Steep water									
LAB	0 h	$(5.5\pm0.4)10^{4a}$	$(6.9\pm0.7)10^{5a}$	$(3.4\pm0.6)10^{7b}$	$(2.1\pm0.6)10^{7b}$	$(4.5\pm0.2)10^{7b}$	$(6.2\pm0.2)10^{7b}$	$(3.2\pm1.8)10^{7b}$	$(6.0\pm1.3)10^{7a}$
	24 h	$(5.4\pm2.1)10^{7a}$	$(1.5\pm0.3)10^{8a}$	$(5.6\pm0.9)10^{8b}$	$(7.4\pm2.1)10^{8b}$	$(4.8\pm0.9)10^{8b}$	$(7.5\pm0.4)10^{8b}$	$(4.6\pm1.4)10^{8b}$	$(8.6\pm1.6)10^{7c}$
	48 h	$(5.4\pm1.6)10^{8a}$	$(1.1\pm0.4)10^{9b}$	$(6.0\pm0.9)10^{9b}$	$(4.7\pm1.1)10^{9b}$	$(5.8\pm2.4)10^{9b}$	$(6.1\pm1.4)10^{9b}$	$(1.6\pm0.7)10^{9a}$	$(9.0\pm0.6)10^{9b}$
Yeast	0 h	$(5.7\pm1.5)10^{3a}$	$(4.3\pm0.3)10^{7b}$	$(5.4\pm1.3)10^{6c}$	$(3.9\pm0.6)10^{6c}$	$(7.5\pm2.1)10^{7b}$	$(6.2\pm2.1)10^{6c}$	$(5.2\pm1.9)10^{7b}$	$(7.4\pm1.3)10^{7b}$
	24 h	$(8.6\pm1.6)10^{4a}$	$(4.8\pm1.1)10^{7b}$	$(2.9\pm0.2)10^{7b}$	$(3.4\pm0.6)10^{7b}$	$(7.7\pm1.3)10^{7b}$	$(3.1\pm0.4)10^{7b}$	$(8.1\pm0.7)10^{7b}$	$(7.8\pm0.7)10^{7b}$
	48 h	$(6.4\pm1.3)10^{5a}$	$(8.7\pm2.1)10^{7b}$	$(4.6\pm1.9)10^{7b}$	$(3.3\pm0.8)10^{7b}$	$(9.4\pm1.4)10^{7c}$	$(7.7\pm0.7)10^{7b}$	$(2.0\pm1.5)10^{8c}$	$(2.6\pm1.4)10^{8c}$

Means with same letters in a row are not significant different ($p<0.05$)

CON: control /spontaneous fermentation (without starter culture), CK: *S. cerevisiae +C. krusei*, FC: *L.fermentum+ S. cerevisiae*, FK: *L.fermentum+ C. krusei*, BK: *L .brevis+ C. krusei*, FCK: *L.fermentum+ S. cerevisiae+ C. krusei*, FBK: *L.fermentum+ L. brevis+ C. krusei*, FBKC: *L.fermentum+ L. brevis+ C. krusei+ S. cerevisiae*

4.7.6 Microbial Counts during *Nsiho* Dough Fermentation using Different Starter Culture

The microbial population during *nsiho* dough fermentation using different starter cultures are shown in Table 4.17. Using different combinations of culture which included a LAB resulted in hundred fold increase in the population of LAB at the start of dough fermentation compared to the spontaneous fermentation. By the end of fermentation the difference in population of LAB between these fermentations and that of spontaneous fermentation were reduced to ten fold; 10^9 cfu/g compared to10^8 cfu/g. The use of yeasts did not result in a higher LAB population during dough fermentation compared to spontaneous fermentation. All combinations of starter cultures used contained yeasts and these resulted in higher yeasts couts in all fermentations compared to the spontaneous fermentation. Yeast counts for all fermentation containing yeast starter culture reached an initial level of 10^6 cfu/g at the start of fermentation with spontaneous fermentation reaching a value of 10^3 cfu/g (Table 4.17).

Table 4.17 Mean microbial counts (cfu/g) during *nsiho* dough fermentation using different starter cultures

		Type of fermentation							
Sample	Fermentation time	Control/ spontaneous	CK	FK	FC	FCK	FBK	CBK	FBKC
Fermenting dough									
LAB	0 h	$(1.7\pm0.2)10^{5a}$	$(3.6\pm0.2)10^{5a}$	$(2.0\pm0.3)10^{7b}$	$(5.0\pm0.5)10^{7b}$	$(3.0\pm1.7)10^{7b}$	$(7.0\pm0.7)10^{7b}$	$(4.0\pm1.5)10^{7b}$	$(9.0\pm0.7)10^{7b}$
	4 h	$(4.3\pm1.3)10^{6a}$	$(3.4\pm2.1)10^{6a}$	$(7.6\pm1.7)10^{7b}$	$(3.4\pm0.4)10^{8c}$	$(7.0\pm1.1)10^{7b}$	$(2.2\pm1.6)10^{8b}$	$(9.2\pm2.6)10^{7b}$	$(8.0\pm0.8)10^{8c}$
	8 h	$(6.1\pm0.9)10^{7a}$	$(2.5\pm1.7)10^{7a}$	$(3.3\pm1.6)10^{8b}$	$(4.5\pm0.3)10^{8b}$	$(6.4\pm2.8)10^{8b}$	$(7.8\pm1.6)10^{8b}$	$(3.6\pm0.9)10^{8b}$	$(6.2\pm2.1)10^{9c}$
	12 h	$(4.4\pm1.7)10^{8a}$	$(7.4\pm1.9)10^{8a}$	$(2.5\pm1.1)10^{9b}$	$(1.8\pm0.9)10^{9b}$	$(4.2\pm0.2)10^{9b}$	$(3.6\pm2.4)10^{9b}$	$(2.8\pm0.9)10^{9b}$	$(8.9\pm0.6)10^{9b}$
Yeast	0 h	$(6.7\pm0.7)10^{3a}$	$(2.2\pm0.9)10^{6b}$	$(3.8\pm2.2)10^{6b}$	$(4.7\pm0.7)10^{6b}$	$(5.6\pm2.4)10^{6b}$	$(2.1\pm1.2)10^{6b}$	$(1.0\pm0.8)10^{7b}$	$(8.3\pm0.8)10^{6b}$
	4 h	$(2.1\pm1.0)10^{4a}$	$(6.2\pm4.2)10^{6b}$	$(7.2\pm1.3)10^{6b}$	$(8.7\pm0.7)10^{6b}$	$(6.1\pm1.9)10^{7c}$	$(7.2\pm0.8)10^{6b}$	$(5.1\pm1.1)10^{7c}$	$(3.3\pm2.1)10^{7c}$
	8 h	$(1.8\pm0.8)10^{5a}$	$(7.9\pm2.1)10^{7b}$	$(8.6\pm1.1)10^{6b}$	$(1.2\pm0.5)10^{7b}$	$(3.0\pm1.9)10^{8b}$	$(6.7\pm0.7)10^{7b}$	$(8.2\pm0.7)10^{7b}$	$(3.2\pm0.3)10^{8c}$
	12 h	$(8.5\pm0.5)10^{6a}$	$(3.3\pm0.6)10^{8b}$	$(8.6\pm1.6)10^{7c}$	$(9.3\pm1.2)10^{7c}$	$(5.5\pm1.3)10^{8b}$	$(2.3\pm2.0)10^{8b}$	$(6.2\pm0.8)10^{8b}$	$(5.5\pm1.3)10^{8b}$

Means with same letters in a row are not significant different ($p<0.05$)

CON: control/spontaneous fermentation (without starter culture), CK: *S. cerevisiae* +*C. krusei*, FC: *L. fermentum+ S. cerevisiae,* FK: *L. fermentum+ C. krusei*, BK: *L .brevis+ C. krusei*, FCK: : *L.fermentum+ S. cerevisiae+ C. krusei*, FBK: *L. fermentum+ L. brevis+ C. krusei*, FBKC: *L. fermentum+ L. brevis+ C. krusei+ S. cerevisiae*

4.8 Survival of Enteric Pathogens during the Steeping of Dehulled Maize Grains using Different Starter Cultures

The survival of four enteric pathogens during the steeping of dehulled maize grains using different starter cultures are shown in Table 4.18. The pathogens were inoculated into steep water at a concentration of 10^6 cfu/ml. The microbial numbers of *Vibro cholerae* reduced steadily during steeping and was not detected after 48 h in any of the steep water including that of the spontaneous fermentation as control. There was also a significant reduction in microbial population of *Salmonella typhimurium, E.coli* and *Staphylococcus aureus* during steeping in all instances, however, these pathogens were not completely eliminated after 48 h of steeping. Apart from the use of starter culture composed of only yeasts, there was generally a ten fold reduction in the counts of pathogens at the end of steeping compared to spontaneous steeping. The only exception to this was the use of *Lactobacillus fermentum* and *Candida krusei* as a combination against *E. coli* and *Staphylococcus aureus* in which case the same concentration was recorded as in that of the spontaneous fermentation.

Table 4.18 Count for survival of enteric pathogens inoculated into spontaneous and mixed culture fermentation of steeped dehulled maize

	Types of fermentation							
Time (h)	Spontaneous	CK	FK	FC	FCK	CBK	FBK	FBCK
Vibro cholera								
0 h	$7.4x10^5$	$4.7x10^5$	$8.0x10^5$	$6.6x10^4$	$7.5x10^5$	$8.3x10^4$	$6.7x10^5$	$5.7x10^5$
12 h	$5.8x10^4$	$7.3x10^4$	$6.8x10^4$	$5.8x10^3$	$6.9x10^3$	$7.9x10^4$	$3.9x10^4$	$6.8x10^4$
24 h	$8.1x10^3$	$6.7x10^3$	$2.2x10^3$	$3.5x10^3$	$1.7x10^3$	$8.9x10^3$	$8.4x10^3$	$7.5x10^3$
36 h	$7.8x10^2$	$6.3x10^1$	$4.7x10^2$	$9.7x10^1$	$8.8x10^2$	$4.9x10^2$	$5.8x10^2$	$8.4x10^1$
48 h	nd	nd	nd	nd	nd	nd	nd	nd
Salmonella typhimurium								
0 h	$8.4x10^5$	$6.8x10^5$	$6.9x10^5$	$3.7x10^5$	$5.7x10^5$	$6.3x10^5$	$5.5x10^5$	$4.8x10^5$
12 h	$7.5x10^5$	$7.1x10^4$	$6.3x10^5$	$4.6x10^5$	$5.4x10^4$	$8.3x10^4$	$5.8x10^4$	$7.2x10^4$
24 h	$5.7x10^4$	$5.5x10^4$	$7.7x10^3$	$6.2x10^3$	$7.6x10^3$	$8.2x10^4$	$6.6x10^3$	$7.3x10^3$
36 h	$2.6x10^4$	$5.4x10^3$	$6.3x10^3$	$7.0x10^3$	$6.3x10^3$	$9.3x10^2$	$5.7x10^3$	$4.0x10^3$
48 h	$2.3x10^3$	$1.2x10^3$	$3.6x10^2$	$5.1x10^2$	$8.8x10^2$	$7.3x10^2$	$6.5x10^2$	$4.9x10^2$

nd: not detected

Cont.

				Types of fermentation				
Time (h)	Spontaneous	CK	FK	FC	FCK	CBK	FBK	FBCK
E. coli								
0 h	9.4×10^5	5.5×10^5	4.2×10^5	7.6×10^4	7.4×10^5	7.9×10^5	7.8×10^5	6.6×10^5
12 h	6.5×10^5	6.4×10^5	7.8×10^4	7.2×10^4	7.8×10^4	7.5×10^5	8.2×10^4	7.9×10^4
24 h	7.7×10^4	7.8×10^4	9.1×10^3	8.5×10^3	2.7×10^4	9.5×10^3	8.1×10^3	8.5×10^3
36 h	4.6×10^4	3.3×10^4	8.3×10^3	9.6×10^2	9.6×10^3	7.8×10^3	4.9×10^2	9.1×10^2
48 h	8.8×10^3	6.4×10^3	4.3×10^3	8.9×10^2	5.5×10^2	6.6×10^2	3.5×10^2	2.9×10^2
Staphylococcus aureus								
0 h	6.3×10^5	7.2×10^5	4.8×10^5	5.9×10^5	7.6×10^5	7.0×10^5	5.6×10^5	5.9×10^5
12 h	7.6×10^4	9.2×10^4	7.1×10^5	3.9×10^5	6.2×10^4	5.3×10^4	6.5×10^4	8.3×10^4
24 h	6.7×10^3	7.5×10^4	7.7×10^3	6.2×10^3	7.6×10^3	8.2×10^4	6.6×10^3	7.3×10^3
36 h	7.9×10^3	6.2×10^4	6.3×10^3	7.0×10^3	6.3×10^3	7.3×10^2	5.7×10^3	4.0×10^3
48 h	7.3×10^3	2.2×10^3	2.9×10^3	5.7×10^2	8.8×10^2	4.3×10^2	6.4×10^2	4.6×10^2

4.9 Sensory Quality of *Nsiho Produced* Using Starter Cultures

The sensory scores on a 9 point hedonic scale for *nsiho* prepared by spontaneous or through the use of starter culture are shown in Table 4.19. Apart from texture, the sensory score for every attribute i.e aroma/ odour, taste and overall acceptability was between 6.0 to 7.0 which correspond to like slightly and to like moderately for every sample assessed including the control/spontaneous sample. The score for texture of the *L. brevis* and the combined yeast sample were just above scale 7 with the others being in the range of 6.0 to 7.0

A Principal Component Analysis bi-plot (PCA) used to visualise the inter sample and sensory attributes relationship is shown in Figure 4.5. From the plot samples, *S. cerevisiae* + *C. krusei*, *L. fermentum* + *C. krusei*, and *L. brevis* + *C. krusei* are closely correlated in terms of aroma.

Table 4.19 Sensory scores for *nsiho* prepared by spontaneous fermentation or through the use of a starter culture

Attribute	Type of fermentation									
	Con	Lb	Lf	CK	FC	FK	BK	FCK	FBK	FBKC
Aroma	6.23±0.18	6.63±0.04	6.65±0.21	6.80±0.35	6.38±0.18	6.73±0.25	6.58±0.39	6.28±0.11	6.00±0.07	6.18±0.18
Taste	6.40±0.00	6.65±0.42	6.28±0.60	6.53±0.04	6.50±0.28	6.53±0.11	6.43±0.11	6.28±0.18	6.65±0.57	6.35±0.49
Texture	6.33±0.11	7.08±0.32	6.25±0.42	7.09±0.30	6.78±0.04	6.63±0.11	6.75±0.14	6.40±0.07	6.83±0.46	6.73±0.25
Overall Acceptability	6.30±0.07	6.58±0.67	6.25±0.35	6.13±0.53	6.33±0.46	6.23 ±0.53	6.30±0.14	6.00±0.00	6.73±0.67	6.28±0.18

Values are mean of 20 panellists on a nine point hedonic scale, 1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7= Like moderately, 8 = Like very much and 9 = Like extremely

CON: control /spontaneous fermentation (without starter culture), Lb: *L .brevis,* Lf: *L. fermentum,* CK: *S. cerevisiae +C. krusei,* FC: *L. fermentum+S. cerevisiae,* FK: *L. fermentum + C. krusei,* BK: *L .brevis + C. krusei,* FCK: *L. fermentum + S. cerevisiae + C. krusei,* FBK: *L. fermentum + L. brevis + C. krusei*

FBKC: *L. fermentum + L. brevi s+ C. krusei + S. cerevisiae*

No significant difference between fermentation types (p < 0.05)

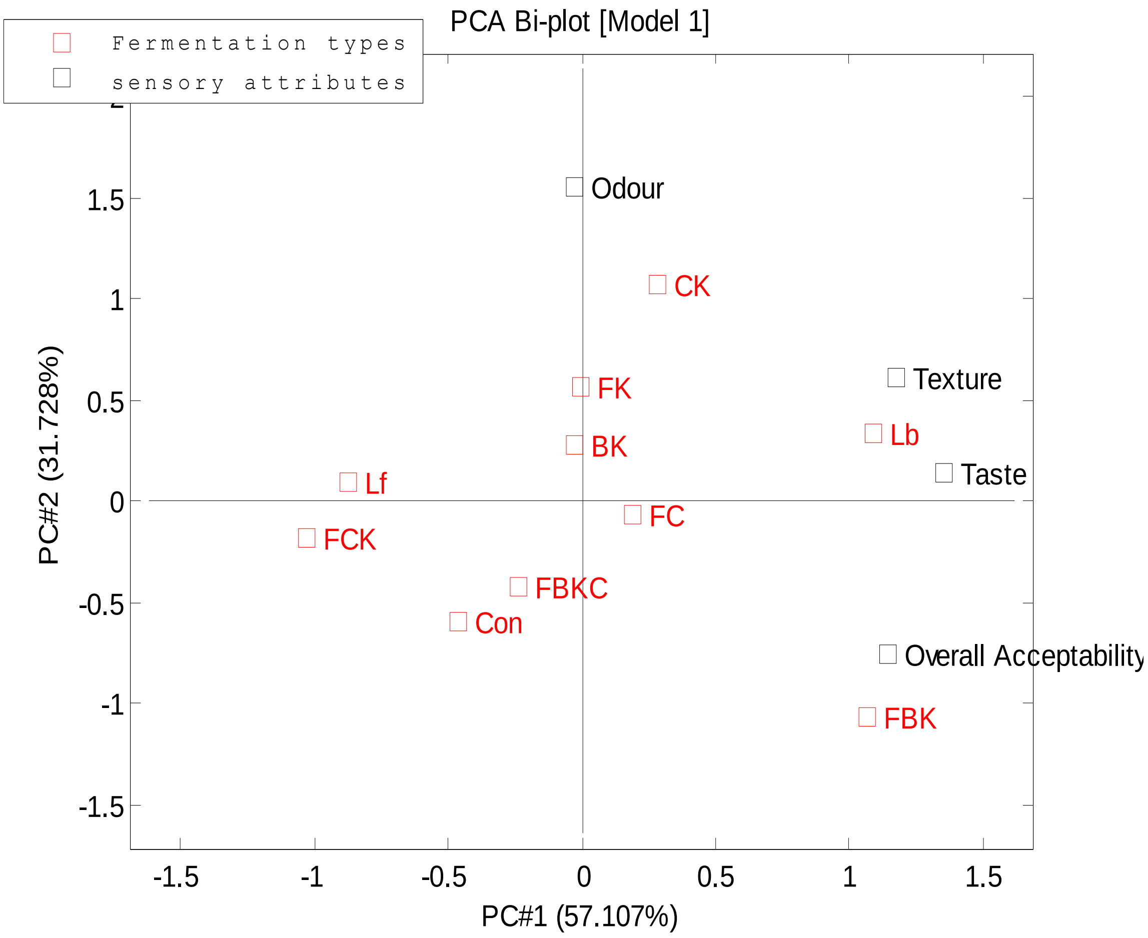


Fig 4.5 Principal Component Analysis Bi plot

5.0 DISCUSSION

5.1 Changes in Acidity during Spontaneous Fermentation

Results of acidity in the present study indicate a steady increase in titratable acidity with a corresponding decrease in pH during steeping of maize grains and dough fermentation. This observation could be attributable to the increased growth of lactic acid bacteria responsible for the fermentation of the *nsiho* which had produced more lactic acid and likely utilisation of free sugars by yeast and lactic acid bacteria (Efiuvwevwere and Akona, 1995).

Similar results have been reported by many authors in the area of maize steeping and dough fermentation (Plahar and Leung, 1982; Jespersen*et al*., 1994; Halm *et al*., 1996; Kalui *et al*; 2009). Wakil and Daodu (2011); Olukoya *et al*., (1993); Odunfa and Adeyele, (1985), have reported the dominance of lactic acid bacteria during spontaneous fermentation of cereals. Wakil and Daodu (2011) observed a decrease in pH from 5.65 to 3.5 units within 24 h of fermenting with a corresponding increase in titratable acidity from 0.2065 to 0.2650 after 48 h. The cause of the increase in acidity and consequent drop in pH during fermentation of cereals is as a result of production of lactic acid by fermentative organisms.

5.2 Role of Lactic Acid Bacteria in *Nsiho* Fermentation

The present study showed a drastic increase in the population of lactic acid bacteria between 10^3 and 10^4cfu/ml to 10^8 cfu/ml within 24 h of steeping of dehulled maize grains and from 10^5 cfu/g to 10^8 cfu/g within 12 h of dehulled dough fermentation. This confirms that lactic acid fermentation occurs during steeping of dehulled maize grains and dough fermentation. The role of lactic acid fermentation in food processing is to effect preservation by converting sugars to organic acids thus causing a reduction in pH; by removing carbohydrates as nutrient source and by producing antimicrobial compounds such as hydrogen peroxide, bacteriocin and other secondary metabolites. Whilst, preservation is important, lactic acid bacteria are currently often used to provide diversity in food supply by altering flavour, texture, and appearance of raw commodities in desirable way (Chassy and Morphy 1993; Davidson 1993; Salovara1993).

The dominant lactic acid bacteria identified in the present work to be responsible for *nsiho* fermentation was *Lactobacillus fermentum* which formed about half of the lactic acid population isolated from the steep water and fermented dough. This result is in agreement with the work of Halm *et al*., (1993), who found fermentation of fermented whole maize meal during production of *Ga* and *Fante* kenkey to be dominated by a group of obligatory heterofermentative lactobacilli consistent with *Lactobacillus fermentum* and *Lactobacillis reuteri* in their pattern of carbohydrate fermentation. Hayford *et al.*, (1999) later confirmed the dominant species to be *Lactobacillus fermentum* using molecular characterization. It is therefore not surprising that *Lactobacillus fermentum* has been found to be responsible for the fermentation of maize into *nsiho* which is also a type of kenkey produced from dehulled maize grains in the present work. Removing the testa and the germ of the maize therefore does not affect the species of LAB which dominates its fermentation during steeping and dough fermentation.

In Benin, Hounhouigan (1993a) had also reported *Lactobacillu*s *fermentum* as the dominant lactic acid bacteria responsible for the fermentation of maize into *mawe* which also involves fermentation of partially dehulled maize grains.

In the present study *Lactobacillus plantarum* and *Lactobacillus brevis* were also isolated in high numbers during maize steeping and dough fermentation. The presence of *Lactobacillus plantarum* in maize dough fermentation has been reported. Nche *et al*., (1994), identified *Lactobacillus plantarum*, *Lactobacillus brevis, Lactobacillus confuses* and *Pediococcus* species as the main lactic acid bacteria present in fermenting maize and maize cowpea dough. Olasupo *et al*., (1997) in their studies on selected African fermented foods obtained 48 lactobacillus isolates from kenkey which they identified as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus delbruckii* and *Lactobacillus acidophilus*. Olsen *et al*., (1995) found *Lactobacillus plantarum* at the initial stage of kenkey fermentation where it dominated the heterofermentative lactic acid bacteria present.

Studies on other types of fermented products reported that lactic acid bacteria were the dominant microorganisms involved in the fermentation of *gari* and *kenkey*, with *Lactobacillus plantarum* been identified most often (Amoa-Awua *et al*., 1996, Kostinek *et al*., 2005).

Sawadogo-Lingani *et al*., (2007) reported *Lactobacillus fermentum* as the dominant lactic acid bacteria responsible for souring of *dolo*, an alcoholic beverage.

Lei and Jacobson (2004), isolated *W. confuses and Lactobacillus fermentum* as the dominant lactic acid bacteria for the fermentation of millet into *koko* from five production sites in Northern Ghana.

5.3 Role of Yeasts in *Nsiho* Fermentation

Saccharomyces cerevisiae and *Candida krusei* have been identified in the present work as the dominant yeasts involved in fermentation of dehulled maize during *nsiho* production. *Debaryomyces* and *Trichosporon* species were also identified at the initial stages of steeping.

Halm *et al*., (1993) had previously reported *Candida* and *Saccharomyces* species as the most frequent isolated yeast during maize steeping and dough fermentation. These species were later confirmed by using molecular methods to be *Saccharomyces cerevisiae* and *Candida krusei* respectively (Hayford and Jesperson, 1999). Obiri-Danso (1994) reported *S. cerevisiae* and *C. krusei* as the yeasts species during fermentation of maize dough in kenkey production. Jesperson *et al*., (1994) isolated *S. cerevisiae* and *C. krusei* as the dominant yeast in maize dough fermentation and suggested that since yeast are known to produce a wide range of aromatic compounds including organic acids, esters, aldehydes, alcohols, lactones and terpenes, they are likely to influence the organoleptic and structural quality of fermented maize dough. Hamad *et al*., (1992) found that fermented sorghum dough with high numbers of *C. krusei* had a more pleasant aroma than dough with less yeast.

5.4 Antimicrobial Activity of Lactic Acid Bacteria against Common Enteric Pathogens

In the present study *L. fermentum* was shown to exhibit a strong antimicrobial activity against *Staphylococcus aureus* and *Vibrio cholerae*. A similar result was observed in *dolo* production by Sawadogo-Lingani *et al*., (2008) who reported a high level of antimicrobial activity by *L. fermentum* against *Staphylococcus aureus* but weak activity against *E. coli* and *Listeria innocua*.

Other investigations have also reported antimicrobial activity of lactic acid bacteria isolated from African fermented foods against some common enteric pathogens (Mensah *et al*., 1991; Olsen *et al*., 1995). Savadogo *et al*., (2004) reported the production of a bacteriocin by *L. fermentum* strain isolated from *dolo* in Burkina –Faso. Kostinek *et al*., (2005) reported high production of hydrogen peroxide and a bacteriocin by a heterofermentative strain of lactic acid bacteria isolated from fermented cassava.

5.5 Microbial Interactions during *Nsiho* Fermentation

There were very limited antimicrobial interactions observed beween the different species of micoorganisms isolated from *nsiho.* There were no interactions between the different species of lactic acid bacteria at the different stages of steeping and dough fermentation of the dehulled maize grains as reported for whole maize grains by Olsen *et al.*, (1995). Olsen *et al.*, (1995), again reported inhibition of lactic acid bacteria cultures isolated at the early stages of fermentation during the production of kenkey from whole maize by lactic acid bacteria isolates from the advanced stages of fermentation. The lack of antagonism observed in the present work between the different lactic acid bacteria could be due to the fact that they were not specifically selected from different stages of fermentation.

Lactic acid bacteria to lactic acid bacteria interaction can be typified by antagonism where bacteriocin produced by one species or strain inhibits or eliminates another species. Interaction between *L. fermentum* and *L. plantarum* against *P. acidilactici*, could be due to microbial succession that takes place during maize fermentation which leads to selection of stable flora (Halm *et al*., 1993; Hayford *et al*., 1999). Lactic acid bacteria that persist to the advanced stages of fermentation showed inhibition against isolates from early stages of fermentation (Olsen *et al*., 1995).

There were also no antimicrobial interactions observed between the different species of lactic acid bacteria isolates and the yeasts; *Saccharomyces cerevisiae* and C*andida krusei* in this present study.

However, the different species of lactic acid bacteria isolated from *nsiho* exhibited some degree of antagonism against *Debaryomyces* and *Trichosporon* species. These two isolates had been isolated at the start of steeping. Elimination of yeast species by lactic acid bacteria could be due to antimicrobial substances produced by the lactic acid bacteria species (Adams and Nicolaides, 1997). The present results are in agreement with investigation by Jesperson *et al*., (1994) who reported a mixed flora comprising of *Candida*, *Saccharomyces*, *Trichosporon* and *Kluveromyces* from raw maize during steeping and early stages of kenkkey fermentation. They indicated that a natural selection was observed during steeping and maize dough fermentation with *Candida krusei* and *Saccharomyces cerevisiae* becoming the dominant species at the end of fermentation. The dominance of *Candida krusei* and *Saccharomyces cerevisiae* have been confirmed by Nyarko and Obiri-Danso, (1992); Obiri-Danso (1994).

Many authors have reported the co-existence of yeast and lactic acid bacteria in different African fermented foods (Jesperson *et al*., 1994; Hounhouigan *et al*., 1998; Oyewole and Odunfa 1990; Jesperson 2003; Nout 1980; Halm *et al*., 1993; Iwuoha and Eke 1996). Other studies described the association as symbiotic (Wood and Hodges 1985; Saunders *et al*., 1972; Gobbeti *et al*., 1994). They indicated that yeast selectively utilized the suitable amino acids after proteolytic activity of *L. brevis* subsp. *linneri* CBI resulting in an enhanced yeast growth. Halm *et al*., (2003) also indicated that the lactic acid produced by lactic acid bacteria creates an acidic environment suitable for yeast growth. Gobbeti *et al*., (1994) explained that growth of lactic acid bacteria could occur in the presence of high inoculum of yeast which results in an increased amino acid synthesis and subsequent excretion by yeast cell autolysis creating a source of free amino acid that satisfies lactic acid requirement. Leroi and Peduox, (1993) showed that carbon dioxide; pyruvate, propionate, acetate and succinate produced by yeast were responsible for the stimulation of lactic acid bacteria.

5.6 Technological Properties

The different isolates of lactic acid bacteria from *nsiho* displayed different rates of acidification during steeping and dough fermentation. Overall, *Lactobacillus fermentum* exhibited the fastest rate of acidification in maize slurry which gives it an advantage over the other species in selecting for a starter culture. The decreasing of pH favours the growth of yeast and characteristic during the spontaneous fermentation of cereal based products (Sawadogo-Lingani *et al*., 2007, Sulma *et al*., 1991; Hounhouigan *et al* 1996; Lei and Jacobson, 2004).

The ability of the isolates to secret amylase was examined on modified NA containing 2% starch. Isolates of *L. fermentum, L. plantarum* and *L. brevis* demostrated amylase activity. Many authors have indicated the presence of amylolytic lactic acid bacteria from cereal fermented products. Olasupo *et al*., (1996) isolated amylolytic lactic acid bacteria from Ghanaian kenkey (fermented maize dough) and nono (Nigeria). Agati *et al*., (1998), found amylolytic *L. plantarum* strains from retted cassava in Nigeria and Congo respectively, while amylolytic *L. fermentum* (strains Ogi E1 and Mw2) were isolated from *mawe* and *ogi* in Benin. Hounhouigan *et al*., (1993b) reported some amylolytic lactic acid bacteria in *mawe* from Benin whiles Johansson *et al*., (1995) also indicated that amylolytic lactic acid bacteria accounted for 14% of the total lactic acid bacteria isolated from Nigerian *ogi.*

None of the lactic acid bacteria isolates showed any protease activity. According to Wieser *et al*., (2008), lactic acid bacteria play a minor role in protein hydrolysis.

Most of the lactic acid bacteria isolates produced exopolysaccharides which could contribute to improvement of texture. According to Patricia *et al*., (2002) and Savadogo *et al*., (2004) production of microbial exopolysaccharides contribute to the improvement of texture and viscosity of fermented food products. The formation of exopolysaccharides by lactic acid bacteria may function in foods as viscosifying agents, stabilizers, emulsifiers, gelling agents and water binding agents (Van-der Berg *et al*., 1995; De-Vuyst *et al*., 2001), and may play an important role in the rheological behaviour and texture of the product (Sikena and Oba, 1998; De-Vuyst and Diggest, 1999). Kalui *et al.*, (2009), assessed production of exopolysaccharides and amounts ranging from 298.53 to 431 mg/l were produced by *L. plantarum* and *L. rhamnosus* isolate from ikii, a spontaneously fermented maize porridge.

5.7 Selection of Starter Cultures

One of the major aims of isolating starter cultures for use in the production of fermented foods is to ensure consistency and to preserve the unique flavour, aroma and texture attributes of the products (Annan *et al*., 2003; Glover *et al*., 2009). In this present work there was a drastic increase in acidity development with the inoculation of starter cultures to the already existing microflora during steeping and dough fermentation. The decline in levels of pH with corresponding rise in amounts of titratable acids have been similarly reported by many authors (Plahar and Leung, 1982; Jesperson *et al*., 1994; Halm *et al*., 1996; Masha *et al*., 1998; Nche *et al*., 1994; Sanni *et al*., 1994., Hounhouigan *et al*., 1996). Fermentations involving starter cultures of lactic acid bacteria have typically been characterised by drastic drop in pH. Masha *et al*., (1998), reported a decrease in pH from over 5.0 in the unfermented sample to final pH levels of 3.5 in *Uji,* a maize and millet gruel, fermented with pure cultures of lactic acid bacteria and 4.1 in spontaneous fermented *Uji.* Halm *et al*., (1996), using six strains of *L. fermentum* and one strain of *S. cerevisiae,* as starter culture in undehulled maize dough fermentation, observed a rapid decrease in pH from over 5.0 at the start of steeping maize kernels to values of 3.65 to 3.81 within 24 h of dough fermentation as compared to 3.90 in spontaneous fermented dough. In the present study, however, the rate of acidification was more drastic than those obtained from the undehulled maize within 24 h period. Akinrele, (1970) showed that 'Ogi' produced from a mixed culture of *Lactobacillus* and *Acetobacter* enriched the nutrient quality by increasing the concentrations of riboflavin and niacin above that found in both the unfermented grain and the 'Ogi' produced by traditional spontaneous fermentation. A mixed culture inoculum of *L. plantarum*, *Lactococcus lactis* and *Saccharomyces rouxii* was developed for cultured 'Ogi' production, which resulted in increased rate of souring of the dough (Banigo *et al*., 1974). In another study, Masha *et al*. (1998) compared in laboratory trials the fermentation of 'Uji' using a starter culture of lactic acid bacteria *(L. plantarum, L. brevis, L. buchneri, L. paracasei* and *Pediococcus pentosaceus*), employing the method of backslopping (inoculum from a previous fermentation) and by spontaneous means at 30ºC. They found that the lowest pH of 3.5 was attained with the lactic acid bacteria starter culture fermentation. Double-strain starter culture combinations of lactic acid bacteria and yeasts have been reported in several studies on sourdough to produce more aroma compounds and in many cases to improve flavour than when used individually (Martinez-Anaya *et al*., 1990; Hansen and Hansen, 1994; Meignen *et al*., 2001). In the present study, the levels of counts of added starter cultures were more than that of spontaneous fermentation. The observed significant increase in cell numbers is advantageous in providing enough enzymes to hydrolyse the polysaccharides, hence, the production of more lactic acid and subsequent reduction in pH.

5.8 Survival of Enteric Pathogens during Maize Steeping Inoculated with Starter Culture

Lactic acid bacteria and yeast are often inhibitory to other microorganisms and this is the reason of their ability to improve the quality and safety of fermented foods. The general factors that contribute to the inhibiting properties include low pH, production of organic acids, hydrogen peroxide, antimicrobial substances, as well as oxidation-reduction potential (Mbugua and Njenga, 1992; Kingamko *et al*., 1994; Tanasupawat and Komagata, 1995; Sawadogo-Lingani *et al.*, 2010). In the present study four enteric pathogens (*Vibrio cholerae, Salmonella typhimurium, Escherichia coli and Staphylococcus aureus*) were inoculated into steeped maize containing different combinations of starter cultures. Generally, the concentrations of the pathogens were reduced during the steeping process with *Vibrio cholerae* completely eliminated after 48 h. The reduction and elimination of the pathogens could be due to the fact that, during maize kernel fermentation there was a drop in pH and production of other metabolites. Similar findings have been confirmed by other researchers of the inability of enteric pathogens to survive in acidifying fermenting foods.

A study by Lorri and Svanberg, (1994), indicated that, a group of children fed with lactic acid fermented gruel had a mean number of 2.1 diarrhoea episodes compared to 3.5 for the group fed with unfermented gruel. Similarly, Adams, (1990) suggested that lactic acid bacteria are inhibitory to many other microorganisms when they are cultured together, and this is the basis of the extended shelf life and improved microbiological safety of lactic-fermented foods. Lactobacillus species can produce a variety of metabolites, including lactic and acetic acids which lower pH, that are inhibitory to competing bacteria, including *Vibrio cholera, Salmonella typhimurium, Escherichia coli and Staphylococcus aureus* (Breidt and Fleming, 1997). Mensah *et al.*, (1990), in their study of maize dough weaning foods prepared by mothers in a Ghanaian village found that the extent of contamination was higher in unfermented dough (5.0 log cfu/g) than in fermented dough (4.0 log cfu/g). They also indicated that inoculation of maize dough and porridge with *Shigella flexneri* and enterotoxinogenic *Escherichia coli* (ETEC) showed that if the dough was fermented, it inhibited the inoculated bacteria to a greater extent. Even after cooking, the porridge from fermented maize dough showed some inhibition of the inoculated bacteria (Mensah *et al.*, 1991). Effect of lactic fermentation on survival of enteropathogens in cereal gruels, commonly used as weaning foods, were investigated by Kingamkono *et al.*, (1996) who added 28 enteropathogens to cereal gruels prepared from low tannin sorghum and inoculated them with a lactic acid starter culture. After 6 h of fermentation, *Campylobacter* strains were not detectable and after 12 h, *Salmonella, Shigella* and *Staphylococcus* strains were also not detectable. Antimicrobial activity of

241 lactic acid bacteria belonging to *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *L. fermentum/reuteri* and *L. brevis* isolated from various processing stages of maize (corn) dough fermentation (for kenkey) were shown to inhibit other Gram-positive and Gram-negative bacteria (Olsen *et al.*, 1995). In this present study, the drastic reduction of the enteric pathogens was similar to work done by Annan-Prah and Agyeman (1997) who showed that the populations of enterotoxigenic *Staphylococcus aureus* J955 and 101685, *Staphylococcus aureus* and *Klebsiella pneumonia* were reduced by 1-3 log units in Ga kenkey and kenkey water.

6.0 CONCLUSION

The results of this study have confirmed the fermentation of *nsiho* to be lactic acid fermentation. This occurs during both steeping of dehulled maize grains and dough fermentation where reduction in pH and corresponding increases in titratable acidity are observed. There is close similarity between the fermentation of whole maize grains during the production of Ga and Fanti kenkey and the fermentation of dehulled maize during the production of *nsiho*. The lactic acid fermentation that occurs in both instances involves *Lactobacillus fermentum* as the dominant lactic acid bacteria and also includes *Pediococcus pentosaceus* and *Pediococcus acidilactici*. In *nsiho* fermentation, however, *Lactobacillus plantarum* was found to represent a substantial part of the lactic acid bacteria population but has rarely been reported in whole maize fermentation. *Lactobacillus brevis* was also found to play an important role in the fermentation of the dehulled maize grains. Yeasts are also involved in these fermentations and *Saccharomyces cerevisiae* and *Candida krusei* dominate the yeasts biota in both whole maize and dehulled maize fermentation.

The different lactic acid bacteria species from *nsiho* demonstrated different capacities to acidify dehulled maize grains. Overall, *Lactobacillus fermentum* and *Lactobacillus brevis* demonstrated the fastest rate of acidification and this occurred during 0 h to 8th hours of steeping and 4th to the 8th hours of dough fermentation.

All isolates of lactic acid bacteria produced exopolysaccharide, a few showed amylolytic activity whilst none showed protease activity. Through the use of lactic acid bacteria and yeasts as starter culture, a more rapid reduction in pH was achieved both during steeping and dough fermentation. However, no significant differences were assessed in the colour, texture, aroma, taste and overall acceptability of *nsiho* through the use of the starter cultures as inocula enrichment.

Antimicrobial activity against four enteric pathogens, *Salmonella typhimurium*, *Staphylococcus aureus*, *Vibrio cholerae* and *Escherichia coli* was demonstrated during steeping of dehulled maize grains and was enhanced when lactic acid bacteria isolates were included as starter culture. *Vibrio cholerae* was completely eliminated while the others were drastically reduced to a level of 10^2 cfu/ml after 48 h of steeping.

Based on antimicrobial activity and technological properties exhibited by the isolates and the sensory quality of *nsiho*, suitable starter culture selected for *nsiho* production consisted of *Lactobacillus fermentum, Lactobacillus brevis* and *Saccharomyces cerevisiae.*

7.0 RECOMENDATIONS

1. Molecular methods should be carried out to confirm species of the dominant lactic acid bacteria and yeasts responsible for *nsiho* fermentation.

2. The use of the starter cultures for the dough fermentation should be studied.

3. The aroma profile of *nsiho* produced using the different starter cultures as well as during spontaneous fermentation should be studied.

REFERENCES

Adams, M.R. 1998. Fermented weaning foods. In: J.B Wood (Ed), Microbiology of fermented foods. London Blackie Academic. pp.790-811.

Adams, M. R. and Nicolaides, L. 1997. Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control*, 8: 227- 239.

Adams M.R. 1990. Topical aspects of fermented foods. *Trends in Food Science & Technology* 1: 140-144.

Adams, M. R. and Hall, C. J. 1988. Growth inhibition of food-borne pathogens by lactic and acetic acids and their mixtures. *International Journal of Food Science Technology*, 23:287–292.

Adams, M.R. and Moss, M.O. 1995. Fermented and Microbial foods. In: Food Microbiology, The Society of Chemistry. pp. 255-263

Afoakwa, E.O, Sefa-Dedeh, S. and Sakyi-Dawson, E. 2004. Effects of cowpea fortification, dehydration method and storage time on some quality characteristics of maize-based traditional weaning foods. *African Journal of Food Agriculture Nutrition and Development*, 4**(**1): 67-72.

Agati, V., Guyot, J.P., Morlon-Guyot, J., Talamond, P., Hounhouigan, D.J. 1998.Isolation and characterization of new amylolytic strains of Lactobacillus fermentum from fermented maize doughs (mawé and ogi) from Benin. *Journal of Applied Microbiology*, 85**:** 512-520

Akingbala, J.O., Onochie, E.U., Adeyemi, I. A and Oguntimein, G. B. 1987. Steeping of whole and dry-milled maize kernels in ogi preparation. *Journal of Food Processing and Preservation*. pp.1-11

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

Alais, C. And Linden, G. 1991. Food Biochemistry. Ellis Horwood Ltd., New York.

Almeida, E.G., Caio, C.T.C. and Rachid, R.F.S., 2007. Microbial population present in fermented beverage *cauim* produced by Brazillian Amerindians. *International Journal of Food Microbiology,* 120: 146-151.

Amoa-Awua, W.K.A., Appoh, F.W., Jakobsen, M. 199. Lactic acid fermentation of cassava dough into agbelima. *International Journal of Food Microbiology,* 31: 87-98.

Amoa-Awua, W. K., Ngunjiri, P., Anlobe, J., Kpodo, K., Halm, M., Hayford, A. E., Jakobsen, M. 2007. The effect of applying GMP and HACCP to traditional food processing at a semi-commercial *kenkey* production plant in Ghana. *Food Control,* 18: 1449–1457.

Amoa-Awua, W.K.A., Appoh, F., Jakobsen, M., 1996. Lactic acid fermentation of cassava into agbelima. *International Journal Food Microbiol*. 31: 87– 98.

Amoa-Awua, W.K.A., Frisvad, L.M., Sefa-Dedeh, S. and Jakobsen, M.1997. Contribution of moulds and yeasts to the fermentation of cassava dough. *Journal of Applied Microbiology,* 83: 288-296.

Ankrah, E.K. 1972. Riboflavin content of some fermented foods of Ghana. *Ghana Journal of Agricultural Science,* 5: 96-98.

Annan NT, Poll L, Sefa-Dedeh S, Plahar WA, Jakobsen M, 2003. Influence of starter culture combinations of *Lactobacillus fermentum, Saccharomyces cerevisiae* and *Candida krusei* on aroma in Ghanaian maize dough fermentation. *European Food Research Technol*ogy, 216: 377-384.

Annan-Prah, A. and Agyeman, J.A. 1997. Nutrient content and survival of selected pathogenic bacteria in kenkey used as a weaning food in Ghana. Acta Tropica, 65: 33-42.

Axelsson, L.T. 1993. Lactic acid bacteria: classification and physiology. In lactic acid Bacteria ed. Salminen, S. and von Wright, A., New York; Marcel Dekker Inc. pp. 237-294.

Banigo, E.O.I., de Man, J.M. and Duitschaever, C.L. 1974. Utilisation of high-lysine corn for the manufacture of ogi, using a new improved processing system. *Cereal Chemistry*, 51: 559-573.

Bediako-Amoa, B. and Muller, H.G. 1976. Studies on kenkey with particular reference to calcium and phytic acid. *Cereal Chemistry,* 53:365-375.

Beneke, E.S. and Stevenson, K.E. 1987. Classification of food and beverages. In *Food and Beverage Mycology* 2nd ed Beuchat L.R. an AVI Book. New York. pp. 1-50.

Bernet, M.F., Brassart, D., Neeser, J.R. and Servin, A.L. 1994. Lactobacillus acidophilus LA1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. *Gut*, 35: 483- 489.

Berry, E. D., Liewen, M. B., Mandigo, R. W., and Hutkins, R. W. 1990. Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus* during the manufacture of fermented semidry sausage. *Journal of Food Prot.* 53:194.

Blandino, A., Al-Asseri, M.E., Pandiella, S. S., Cantero, D., and Webb, C. 2003. Cereal based fermented foods and beverages. *Food Research International,* 36: 527-543.

Bottazzi, V. 1998. An introduction to rod-shaped lactic acid bacteria. *Biochimie,* 70:303-315

Bracey, D., Holyoak, C.D., Coote, P.J. 1998. Comparison of the inhibitory effect of sorbic acid and amphotericin B on *Saccharomyces cerevisiae:* is growth inhibition dependent on reduced intracellular Ph? *Journal Applied Microbiology***,** 85: 1056-1066.

Breidt F & Fleming HP (1997): Using lactic acid bacteria to improve the safety of minimally prcssed fruits and vegetables. *Food Technology*, 51: 44-46, 48: 51

Campbell-Platt, G. 1987. Fermented foods of the world.A Dictionary and Guide. Butterworths, London. pp. 291.

Caplice, E. and Fitzgerald, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology,* 50: 131-149.

Casey, G.P., Pringle, A.T. and Erdmann, P.A. 1990.Evaluation of recent techniques used to identify individual strains of *Saccharomyces* yeasts. *Journal of the American Society of Brewing Chemists,* 48: 100-106.

Chassy, B.M. and Murphy, C.M. 1993. Lactococcus and Lactobacillus. In *Bacillus subtilis* and other Gram-Positive Bacteria, Biochemistry, Physiology and Molecular genetics. Ed Sonenshein, A.L. Hoch, J.A. and Losick, R. pp. 65-82.

Cherrington, C.A., Hinton, M., Mead, G.C. and Chopra, I. 1991. Organic acids: Chemistry, antibacterial activity and practical applications. *Advanced Microbiology Phys.* 32: 87-108

Christian, W.F.K. 1970. Lactic acid bacteria in fermenting maize dough. *Ghana Journal of Science,* 10: 22-28**.**

Christian, W.F.K. 1966. Fermented foods in Ghana. A paper presented at a symposium on Food Science and Technology, Accra, Ghana.

Codon, S. 1987. Responses of lactic acid bacteria to oxygen.*FEMS Microbiology Reviews,* 46: 269-280.

Collins, M.D., Farrow, J.A., Philips, B.A., Ferusus, S and Jones, D. 1987. Classification of lactobacillus divergence, lactobacillus piscicola and some catalase negative asporogenous rod shape bacteria from poultry in a new genus, carnobacterium. *International Journal System Bacteriology*, 37: 310-316.

Daeschel, M.A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technology*, 43:164-170.

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

De Man, J.C., Rogosa, R. and Sharpe, M.E. 1960.A medium for the cultivation of lactic acid bacteria. In: Lactic acid bacteria (Eds.) Salminen, S. and von Wright, A. New York: marcel Dekker. pp. 129-159.

Desai, A.R., Powell, I.B., and Shah, N.P. 2004. Survival and activity of probiotic lactobacilli in skim milk containing prebiotics, *Journal of Food Science*, 69: M57–M60.

Devriese, L.A., Collin M.D. and Wirth, R. 1991. The genus *Entecoccus*. In: A. Balows *et al.*, (Eds.), The prokaryotes. Springer- Verlag, New York, USA, pp 1465-1481.

De Vries, W. and Southamer, A. H. 1968. Fermentation of glucose, lactose, galactose, manitol and xylose by bifidobacteria. *Journal of Bacteriology*, 96**:** *472-478.*

De Vuyst, L. and Degeest, B. 1999.Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology*, 23:153-177.

De Vuyst, L., De Vin, F., Vaningelgem, F. and Degeest, B. 2001. Recent development in the biosynthesis and application of heteropolysaccharides from lactic acid bacteria. *International Dairy Journal*, 11: 687-707.

De Vuyst, L. and Vandamme, E.J. 1994. Antimicrobial potential of lactic acid bacteria. In: *Bacteriocin of lactic acid bacteria: microbiology, genetics and applications*. (Eds) L. De Vuyst and E.J Vandamme. London: Blackie Academic and Professional. Pp. 91-142.

Di Cagno, R., De Angelis, M., Lavermicocca, P., De vincenci,M., Giovannini, C., Faccia, and M., Gobbetti, M. 2002. Proteolysis by sourdough lactic acid bacteria: effect on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. *Applied and Environmental Microbiology,* 68: 623-633.

Dicks, L.M.T. and Van Vuuren, H. J.J. 1988. Identification and physiological characteristics of heterofermentive strains of *Lactobacillus* from South Africa red wines. *Journal of Applied Bacteriology*, 64: 505-513.

Dovlo, F. 1970. Maize in the Ghanaian diet.Food Research Institute Bulletin. Food Research Institute, CSIR, Accra, Ghana.

Dykes, G.A. 1991. Characterization of lactic acid bacteria associated with the spoilage of vacuum packaged Vienna sausages. M.Sc. (Microbiology) thesis, Faculty of Science, University of Witwatersrand, Johannesburg, South Africa.

Earnshaw, R.G. 1992. The antimicrobial action of lactic acid bacteria and natural food preservation systems. In *Lactic Acid Bacteria in Health and Diseases*. ed. Wood, B.J.B. Blackie Academic and Professional. London. pp. 211-261.

Earnshaw, R.G. and Banks, J.G.1989. A note on the inhibition of *Listeria monocytogenes* NCTC 11994 in Milk by an activated lactoperoxidase system. *Letters in Applied Microbiology,* 8: 203-205.

Ebine, H. 1989. lndustrialisation of Japanese Miso fermentation. In *Industrialisation of Indigenous Fermented Foods,* (Eds.) K. H. Steinkraus, Marcel Dekker, New York. pp. 89-126.

Efiuvwevwere B.J.O., Akona O. 1995. The microbiology of *kununzaki*, a cereal beverage from northern Nigeria during the fermentation (production) process. *World Journal Microbiology Biotechnology*, 11:491–493.

Fields, M.L., Hamad, A.M. and Smith, D.K. 1981. Natural lactic acid fermentation of corn meal. *Journal of Food Science,* 46: 900-902.

Eklund, T. 1989. Organic acids and esters. In: Mechanisms of action of food preservation procedures, pp. 161-200. Ed. Gould, G.W. *Elsevier Applied Science*, London.

Eklund, T. 1985. The effect of sorbic acid and esters of parahydroxybenzoic acid on the proton motive force in *Escherichia coili* membrane vescicles. *Journal of General Micrbiol*ogy, 131: 73-76.

Fischer, S.E., Miguel, M.J., Mori, G.B. 2003. Effect of root exudates on the exopolysaccharide composition and the lipopolysaccharide of *Aspergillum brasilense* Cd under saline stress. *Elsevier Science B.V.* 219: 53-62.

Freese, E., Sheu, C.W. and Galliers, E. 1973. Function of lipophillic acids as antimicrobial food additives. *Nature,* 241: 321-325.

Fukushima, D. 1989. Industrialisation of fermented soy sauce production centering around Japanese Shoyu. In *Industrialisationof Indigenous Fermented Foods,*ed. K. H. Steinkraus, Marcel Dekker, New York.pp. l-88.

Fuller, R. 1992. Problems and prospects.In *Probiotics, The scientific basis* (*E*ds.), R. Fuller. Chapman and Hall, London. pp. 377-386.

Ga"nzel, M.G., Lopenen, J., Gobbetti, M. 2008. Proteolysis in sourdough fermentation: mechanism and potential for improved bread quality. *Trends in Food Science and Technology*.

Garvie, E.I. 1984. Separation of Species of the Genus *Leuconostoc* and Differentiation of the *Leuconostoc's* from Other Lactic Acid Bacteria, In: Methods in Microbiology vol. 16. Ed. Bergen, T., Academic Press, London. pp. 147-177.

Garvie, E.I. 1986.Genus *Leuconostoc In* P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.), Bergey's Manual of Systematic Bacteriology. Vol. 2, Williams, Wilkings, Baltimore. pp. 1071-1075.

Glover, R.K., Sawadogo-Lingani, H., Diawara, B., Jespersen, L. and Jacobsen M. 2009. Utilization of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* as starter culture in the production of dolo. *Applied Bioscience* 22: 1312-1319.

Gobbetti M, Corsetti A, Rossi J, 1994. The sourdough microflora. Interactions between lactic acid bacteria and yeasts: metabolism of amino acids. *World Journal Microbiol. Biotechnology*, 10: 275- 279.

Gregersen, T. 1978. Rapid methods for distinction of Gram positive bacteria. European Journal of Applied Microbiology and Biotechnology, 5: 123-127.

Guiraud, J.P. 1998. *Microbiologie alimentaire*. pp. 292-293. Paris, France: Ed. Dunod.

Haard, N.F. 1999. Cereals: rational for fermentation. Fermented Cereals – A global Perspective, 24, FAO *Agricultural Service Bulletin*, vol. 138, FAO, Rom. pp. 1-30.

Halm, M., Amoa-Awua, W. K. A., and Jakobsen, M. 2004. *Kenkey*: An African Fermented Maize Product. In: *Handbook of Food and Beverage Fermentation Technology*. Eds Hui, Y. H., Meunier- Goddik L., Hansen, A.S., Josephsen, J., Nip, W-K., Stanfield, P.S., Toldra, F. and Marcel Dekker, Inc., New York, USA. pp. 799–816.

Halm, M., Lillie, A., Sorensen, A.K. and Jakobsen, M. 1993. Microbiological and aromatic characteristics of fermented maize dough for kenkey production in Ghana. *International Journal of Food Microbiology*, 19: 135-144.

Halm, M., Osei-Yaw, A., Hayford, K.A., Kpodo, K.A., Amoa-Awua, W.K.A. 1996. Experiences with the use of a starter culture in the fermentation of maize for *kenkey* production in Ghana. *World Journal of Microbiology and Biotechnology,* 12: 531-536.

Hamad, A.M. and Fields, M.L. 1979. Evaluation of protein quality and available lysine of germinated and ungerminated cereals. *Journal of Food Science,* 44: 456-459.

Hamad S.H., Bocker G., Vogel R.F. and Hammes W.P. 1992. Microbiological and chemical analysis of fermented sorghum dough for kisra prodcution. *Applied Microbiol Biotechnology.* 37: 728-731.

Hansen B. and Hansen A, 1994. Volatile compounds in wheat sourdoughs produced by lactic acid bacteria and sourdough yeasts. Z. L. Unters. *Forsch*. 198: 202-209.

Hardie, J.M. 1986. Other Streptococci. In: Bergey's Manual of Systematic Bacteriology. Vol. 2, Williams, Wilkings, Baltimore, pp. 1086-1071.

Hastings, J.W. and Holzapfel, W.H. 1987. Conventional taxonomy of lactobacilli surviving radurization of meat. *Journal of Applied Bacteriology,* 62: 209-216.

Hayford, A.E, and Jakobsen, M. Characterization of *Candida krusei* strains from spontaneous fermented maize dough by profiles of assimilation, chromosome profile, polymerase chain reaction and restriction endonucleases analysis. *Journal of Applied Bacteriology*. 87: 29-40.

Hayford, A.E. and Jespersen, L. 1999. Characterization of *Saccharomyces cerevisiae* strains from spontaneously fermented maize dough by profiles of assimilation, chromosome polymorphism, PCR and *MAL* genotyping. *Journal of Applied Microbiol,* 86: 284-294.

Hayford, A.E., Petersen, A., Vogensen, F.K. and Jakobsen, M. 1999.Use of conserved randomly amplified polymorphic DNA (RAPD) fragments and RAPD patterns of characterization of *Lactobacillus fermentum* in Ghanaian fermented maize dough. *Applied and Environmental Microbiology*, 65: 3213-3221.

Hesseltine, C.W. 1979. Some important fermented foods of Mid-Asia, the Middle East, and Africa. *Journal of the American Oil Chemists Society*, 56: 367-374.

Holzapfel, W.H. 1997. Use of starter cultures in fermentation on a household scale. *Food Control*, 8: 241-258.

Holzapfel, W.H. 2002. Appropraite starter culture technologies for small –scale fermentation in developing countries. *International Journal of Food Microbiology*, 75: 197-212.

Holzapfel, W.H., Geisen, R. and Schillinger, U. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiol*, 24: 343-362.

Holzapfel, W.H. and Wood, B.J.B. 1995. Lactic acid bacteria in contemporary perspective. In: B.J.B Wood & W.H. Holzapfel (Eds.), The genera of lactic acid bacteria Vol. 2, Blackie Academic & Professional , London , UK. pp.1-6.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H and Rombouts, F.M. 1993a. Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of *mawe,* a fermented maize dough from Benin. *International Journal of Food Science and Technology*. 18: 279-287.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H and Rombouts, F.M. 1993b. Composition and microbiological and physical attributes of *mawe*, a fermented maize dough from Benin. *International Journal of Food Science and Technology*. 28: 513-517.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H. and Rombouts, F.M. 1999. Use of starter cultures of lactobacilli and yeast in the fermentation of Mawé, an African maize product. *Tropical Science* 39: 220-226.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H. and Rombouts, F.M. 1996. Use of starter culture of Lactobacilli and yeast in the fermentation of *mawe* porridge. *In: Traditionai Food Processing in Africa, 3rd Biennial seminar on African Fermented Foods*. July, 1996, pp. 43-53. Ed. Halm, M, and Jacobsen, M.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H. and Rombouts, F.M. 1998. Use of starter culture of Lactobacilli and Yeasts in the fermentation of *mawe* porridge. *In: Traditional Food Processing in Africa, 3rd Biennial Seminar on African Fermented Foods*. July 1996. ed Halm, M and Jacobsen, M. pp43-52.

Hugas, M. 1998. Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products, *Meat Sci*., 49: S139–S150.

Hughes, D.B. and Hoover, D.G. 1991. Bifidobacteria: Their potential for use in American dairy products. *Food Technology*, 45 (4): 74-83.

Hurst, A. 1981. *Nisin. Advanced. Appied. Microbiol.* 27:85–123.

Ijabadeniyi, A.O and Omoya, F.O. 2006. Safety of Small –Scale Food Fermentations in Developing Countries.Department of Microbiology, Federal University of Technology, Akure, Nigeria. pp. 1833-1845.

Iwuoha, C.I. and Eke, O.S. 1996. Nigerian indigenous fermented foods: Their traditional process operation, inherent problems, improvement and current status. *Food research International*, 29: 527-540.

Jay, J.M. 1982. Antimicrobial properties of diacetyl. *Applied Environmental Microbiology*, 44: 525-532.

Jay, J.M. 1986. “Modern Food Microbiology”, 3rded. Van Nostrand Reinhold Co., New York. pp. 278.

Jay, J.M. and Rivers, G.M. 1984. Antimicrobial activity of some food flavouring compounds. *Journal of Food Safety*, 6: 129-139.

Jespersen, L. 2002. Occurrence and taxonomic characteristics of strains of Saccharomyces cerevisiae predominant in African fermented foods and beverages. *FEMS Yeast Research*, 1525: 1- 10.

Jespersen, L., Halm, M., Kpodo, K. and Jakobsen, M. 1994. Significance of yeast and moulds occurring in maize dough fermentation for kenkey production. *International Journal of Food Microbiology,* 24: 239-248.

Jesperson, L. 2003. Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African fermented foods and beverages. *FEMS Yeast Research*, 3: 191-200.

Johansson, M.L. 1995. Systematic and starter culture selection of *Lactobacillus* for human intestine and Nigerian *ogi* with special reference to *Lactobacillus plantarum.* PhD thesis. Lund University.

Johansson, M.L., Sanni, A., Looner, C., Molin, G. 1995. Phenotypically-based taxonomy using API 50 CH of lactobacilli from Nigerian ogi, and occurrence of starch fermenting strains. *International Journal of Food Microbiology*. 25: 159-168.

Johnson, P.N.T. and Halm, M. 1998. Maize quality requirement of producers of six traditional Ghanaian maize products. *Ghana Journal of Agricultural Science,* 31: 203-209.

Kalchayanand, N., Sikes, T., Dunne, C.P. and Ray, B. 1994. Hydrostatic pressure and electroporation have increased efficiency in combination with bacteriocin. *Applied Environental Microbiology*. 60: 4174-4177

Kalui, C.M., Mathara, J.M., Kutima, P.M., Kiiyukia, C., and Wongo, L.E. 2009. Functional characteristics of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* from ikii, a Kenyan traditional fermented maize porridge. *African Journal Biotechnol*, 8(17): 4363-4373.

Kandler, O. 1983. Carbohydrate metabolism in lactic acid bacteria. *Antonie van Leeuwenhoek Journal of Microbiology*, 49: 209-224.

Kandler, O., and Weiss, N. 1986. Regular, nonsporing Gram positive rod.*In* P. H. A. Sneath., N.S. Mair, M.E. Sharpe, and J.G. Holt (ed.), Bergey's manual of systematic bacteriology. Vol. 2.Williams & Wilkins, Baltimore, Md. pp. 1208-1234.

Kazitawa, T. and Ittoh, T.H. 1992. B-cell mitogenic activity of slime forming encapsulated lproducts produced from *Lactococcus lactis* subsp. cremoris. *Journal of Dairy Science*, 75: 2946-2951.

Kingamko R, Sjogren E, Svanberg U, Kaijser B. 1994. pH and acidity in lactic fermenting cereal gruels: effects on viability of enteropathogenic microorganisms. *World Journal Microbiol. Biotechnol* 10: 664-669.

Kingamkono R, Sjoegren E, Svanberg U & Kaijser B. 1996. Inhibition of different strains of enteropathogens in a lactic-fermenting cereal gruel. *World Journal of Microbiology & Biotechnology*, 11: 299-303.

Klaenhammer, T.R. 1993. Genetics of bacteriocins produced by lactic acid bacteria.*FEMS- Microbiological Reviews*, 12(1-3), 38-85.

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

Knoshaug, E.P., Ahlgrent, J.A. and Trempy, J.E., 2000. Growth associated exopolysaccharide expression in *Lactococcus lactis* subsp *cremoris* ropy 352. *Journal of Diary Science*, 83:633.

Konlani, S., Delgenes, J.P., Moletta, R., Traore, A. and Doh, A. 1996a Isolation and physiological characterization of yeasts involved in sorghum beer production. *Food Biotechnology* ,10: 29-40.

Kostinek M., Specht I., Edward V.A., Schillinger U., Hertel C. and Holzapfel W.H. 2005. Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of *gari*, a traditional African food. *Systematic and Applied Microbiology,* 28: 527–540.

Krebs, H.A., Wiggins, D., Sole, S. and Bedoya, F. 1983. Studies on the antifungal action of benzoates. *Biochem Journal*. 214: 657-663.

Kreger- van Rij, N.J.W. 1984. “The Yeasts, A Taxonomic Study”. Elsevier, Amsterdam, The Netherlands.

Kurtzman, C.P. 1994. Molecular Taxonomy of Yeasts.*Yeasts,* 10: 1727-1740.

Kurtzman, C.P. and Fell, J.W. 1998. *The Yeasts; A Taxonomic Study*. 4th Ed. *Elsevier, Amsterdam*. pp. 1035.

La Anyane, S. 1956. An historic account of agricultural development in the Gold Coast before 1900. .*New Gold Coast Farmer*, 1: 62-65.

Lartey, B.L. 1967. Evaluation of the kenkey production project. Food Research Institute Bulletin, CSIR, Accra, Ghana.

Lartey, B.L. 1975. Some technological transformation and research needs in Ghana’s indigenous food industries. UNIDO Joint Consultation on Promotion of Industrial Research and Services in Africa. Lagos 15, 22 September 1975. UNIDO.

Lei V, Jakobsen M 2004. Microbiological characterization and probiotic potential of koko and koko sour water, an African spontaneously fermented millet porridge and drink. *Journal of Applied Microbiol*, 96: 384-397.

Leistner L. 1990: Mould-fermented foods: recent developments. (In `Proceedings of the International Conference on Biotechnology & Food' [see FSTA (1991) 23 4B18].) .*FoodBiotechnology,* 4: 433-441.

Leroi, F., Pidoux, M., 1993b. Characterisation of interaction between Lactobacillus hilgardii and Saccharomyces florenticus isolated from sugary kefir grains. *Journal of Applied Bacteriology*, 74: 54–60.

Lindgren, S.E. and Dobrogosz, W.J. 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. *FEMS Microbiology Reviews,* 87: 149-164.

Litopoulou-Tzanetaki, E. 2000. Changes in numbers and kinds of lactic acid bacteria during ripening of Kefalotyri cheese. *Journal of Food Science,* 55: 111-113

Lorri W & Svanberg U. 1994. Lower prevalence of diarrhoea in young children fed lactic acid fermented cereal gruels. Food and Nutrition Bulletin. 15: 57-63

Maisnier-Patin, S., Deschamps, N., Emin, P., Gratadoux, J.J. and Richard, J. 1992. Utilisation en technologie fromage're, de bacte'ries productrices de bacte'riocines pour inhiber les bacte'ries inde'sirables dan les fromages. In: *Proceeding of the SFM Meeting Gestion des populations microbiennes dans les Industries Agroamentaries Dijon,* France. pp. 337-382.

Mante, E.S., Sakyi-Dawson, E. and Amoa-Awua, W.K. 2003. Antimicrobial interactions of microbial species involved in the fermentation of cassava dough into *agbelima* with particular reference to the inhibitory effect of lactic acid bacteria on enteric pathogens. *International Journal of food Microbiology*, 89: 41-50.

Marshall, V.M. 1987. Lactic acid bacteria: starters and flavours. *FEMS Microbiology Review*, 46: 327-360.

Martinez-Anaya MA, José Torner M, Benedito de Barber C, 1990.Microflora of the sour dough wheat flour bread. XIV. Changes in volatile compounds during fermentation of doughs prepared with pure microorganisms and their mixtures. Z. L. Unters. *Forsch*, 190: 126-131.

Masha, G.K., Ipsen, R., Petersen, M.A. and Jakobsen, M. 1998. Microbial, rheological and aromatic characteristics of fermented Uji (an East African Sour Porridge). *World Journal of Microbiology and Biotechnology*, 14: 451-456.

Mbugua S.K and Njenga J. 1991**.** The antimicrobial activity of fermented uji. *Ecology of Food Nutrition*, 28: 191-198.

Mbugua, S.K and Njenga, J. 1992. The antimicrobial activity of fermented uji. *Ecology of Food Nutrition*, 28: 191-198.

Mbugua, S.K. and Ledford, R.A. 1984. Application of pure lactic cultures on the fermentation of Uji (an East African sour cereal porridge). *Lebensmittel Wissenschaft und Technologie,* 17: 252-256.

McGee, H. 1984.On Food and Cooking. Charles Scribne's Sons, New York. pp. 684.

McKay, L.L. and Baldwin, K.A. 1990. Applications for biotechnology: present and future improvements in lactic acid bacteria. FEMS *Microbiology Reviews.* 87: 3-14.

Meignen B, Onno B, Gélinas P, Infantes M, Guilois S, Cahagnier B, 2001.Optimization of sourdough fermantation with *Lactobacillus* brevis and baker's yeast. *Food Microbiol*, 18 : 239-245.

Mensah P. 1990. Microbiological studies of fermented Ghanaian maize dough. PhD thesis. London School of Hygiene and Tropical Medicine, London.

Mensah P, Tomkins A.M., Drasa B.S., Harrison T.J. **(**1991)**.** Antimicrobial effect of fermented Ghanaian maize dough. *Journal of Applied Bacteriology,* 70: 203-210.

Mensah, P.P.A., Tomkins, A.M., Drasar, B.S. and Harrison, J.T. 1990. Fermentation of cereals for reduction of contamination of weaning foods in Ghana. Lancet 336, 140-143.

Metchnikoff, E. 1908. The prolongation of life .1st (Ed.) G.P Putnam's Sons, New York.

Ministry of Food and Agriculture, Statistics, Research and Information Directorate (SRID), April 2010. pp.14.

Montville, T.J and Winkowski, K. 1997.Biologically based preservation systems and probiotic bacteria. In: Doyle, M.P., Beuchat, L.R., Montville, T.J. (Ed.), Food Microbiology Fundamentals and Frontiers. ASM Press USA, pp. 557-576.

Nanson, N.J and Field, M.L. 1984. Influence of temperature on the nutritive value of lactic acid fermented cornmeal. *Journal of Foods Science,* 49: 958-959.

Nche, P.F., Odamtten, G.T., Nout, M.J.R. and Rombouts, F.M. 1994. Dry milling and accelerated fermentation of maize for industrial production of "Kenkey", a Ghanaian cereal food. *Journal of Cereal Science,* 20: 291-298.

Nche, P.F., Odamtten, G.T., Nout, M.J.R., and Rombouts , R. 1996. Soaking of maize determines the quality of aflata for kenkey production. *Journal of Cereal Science*. pp. 291-297.

Nout, M.J.R. 1980. Microbiological aspects of the traditional manufacture of Busaa, a Kenyan opaque maize beer. *Chemie Mikrobiologie Technologie der Lebensmittel,* 6: 137-142.

Nyarko- Mensah, B. and Muller, H.G. 1972. Studies on kenkey, a Ghanaian cereal food. *Journal of Science Food Agriculture,* 24: 2-4.

Nyarko, K.O. and Danso, O.K. 1991. Role of added yeasts in the acceptability of naturally fermented corn dough. In: *Proceedings of a Regional Workshop on Traditional*

African Foods- Quality and Nutrition. International Foundation for Science. (Eds.) Wesby, A. and Reilly Stockholm: IFS. pp. 19-21.

Nyarko, K.O. and Obiri- Danso, K. 1992. Role of added yeast in naturally fermented corn dough. Proc. Regional Workshop Traditional African Foods: Quality and Nutrition, 25-29 November 1991. Ed. Wesby, A. and Reilly P.J.A. *International Foundation for Science*, Stockholm.

Obiri-Danso K. 1994. Microbiological studies on corn dough fermentation. *American Association of Cereal Chemists*, Inc. pp. 21-25.

Obiri-Danso, K., Ellis, W.O., Simpson, B.K. And Smith, J.P. 1997. Suitability of high lysine maize, Obantanpa for kenkey production. *Food Control* ,8: 125-129.

Odunfa S A., Adeyele S. 1985. Microbiological changes during the traditional fermentation of ogi-baba,a West African fermented sorghum gruel. *Journal of Cereal Science*, 3: 173-180.

Odunfa S.A., Oyewole O.B. 1998. African fermented foods. In J. B. Woods (Ed.), *Microbiol of fermented foods*, pp. 713–752. London: Blackie Academic and Professional.

Odunfa, S.A. 1985. African fermented foods. In: *Microbiology of fermented foods.*Wood, B.J.B. (ed). Vol. I, Elsevier Applied Science Publishers, New York. pp. 712-752.

Odunfa, S.A., Nordstrom, J. and Adeniran, S.A. 1994. Development of starter cultures for nutrient enrichment of Ogi, a West African fermented cereal gruel. Report submitted to HBVC research grants program. USAID, Washington, U.S.A.

Ofosu, A.1971. Changes in the levels of niacin and lysine during the traditional fermentation of kenkey from maize grain. *Ghana Journal of Agricultural Science.* pp. 153-158.

Okamura, N., Ijioma, B. and Oyolu, C. 1986. Interaction of *Shigella* with bifidobacteria. *Bifidobacteria* Microflora, 5 (1): 51-55.

Olasupo, N,A., Akinsanya, S,M., Oladele, O,F. and Azeez, M,K. 1996. Evaluation of nisin for preservation of "nono", a Nigerian fermented milk product. *Journal of Food Processing and Preservation*, 20: 71-78.

Olasupo, N.A., Olukoya, D.K and Odunfa, S.A. 1997. Identification of *Lactobacillus* species associated with selected fermented African foods. *Verlag der Zeitschrift fur Naturforchung*, pp. 105-108.

Olsen, A., Halm, M. and Jakobsen, M. 1995. The antimicrobial activity of lactic acid bacteria from fermented maize (*kenkey*) and their interactions during fermentation *Journal of Applied Bacteriology*, 79(5): 06-51.

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

Olukoya, D.K., Ebigwei, S.I., Olasupo, N.A., Ogunjimi, A.A. 1994. Production of Dogik: an improved ogi (Nigerian fermented weaning food) with potentials for use in diarrhoea control. *Journal of Tropical Pediatrics*, 40: 108-113.

Owusu Ansah, J., Van de voort, F.R. and Bediako-Amoa, B. 1980. Banku-its degree of gelatinization and development of a quick cooking product. *Canadian Institute of Food Sccience and Technology Journal*, 13: 131

Oyewole, O.B. 1990. Optimization of cassava fermentation for fufu production: effects of single starter cultures. *Journal of Applied Bacteriology*, 68: 49- 54.

Oyewole, O.B and Odunfa, S.A. 1990. Characterisation and distribution of lactic acid bacteria in cassava fermentation during fufu production. *Journal of Applied Bacteriology,* 68:145-152.

Patricia, R.M., Jeroen, H., Pieternela, Z. 2002. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *International Diary Journal*, 12: 163-171.

Pederson, C. S. 1971. Microbiology of food fermentation. AVI Publishing Company Inc. Westport, Connecticut.

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

Pidoux, M., Marshal, V.M., Zanoni, P. and Brooker, B., 1990. *Lactobacilli* isolated from sugar *keffir* grains capable of polysaccharide production and minicell formation. *Journal of Applied. Bacteriology,* 69: 311-320.

Plahar, W.A. and Leung, H.K. 1982. Effect of moisture content on the development of carboxylic acids in traditional maize dough fermentation. *Journal of Science Food and Agriculture,* 33: 555-558.

Plahar, W.A. and Leung, H.K. 1983. Composition of Ghanaian fermented maize meal and the effect of soy fortification on sensory properties. *Journal of Science Food and Agriculture,*34: 407-411.

Plahar, W.A., Nti, C.A. and Annan, N. 1997. Effect of soy- fortification method on the fermentation characteristics and nutritional quality of fermented maize meal. *Plant Foods for Human Nutrition*, 51: 365-380.

Priest, F.G. and Pleasant, J.G. 1988. Numerical taxonomy of some *Leuconostoc* and related bacteria isolated from Scotch whisky distilleries. *Journal of Applied Bacteriology,* 64: 379-387.

Roberfroid, M.B., Van Loo, J.A.E. and Gibson, G.R. 1998. The bifidogenic nature of chicory inulin and its hydrolysis product.*Journal of Nutrition*. 128: 11-19.

Rusell, J.B. 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. *Journal of Applied Bacteriol*. 71 191-201.

Saavedra, J.M., Bauman, N.A., Oung, I., Perman, J.A. and Yolken, R.H. 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* for infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344: 1046-1049.

Salmond, C. V., Kroll, R.G., Booth, I.R. 1984. The effect of food preservatives on pH homeostasis in *Escherichia coli*. *Journal of General Microbiology,* 130: 2845-2850.

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

Sanni, A.I. 1993. The need for process optimization of African fermented foods and beverages. *International Journal of Food Microbiology*, 18: 85-95.

Sanni AI, Lonner C, Mark Hinder I, Johansson M.L. and Molin G. 1994. Starter cultures for the production of 'Ogi', a fermented infant food from maize and sorghum. *Chemic Microbiol Technol de Lebensmittel*, 16 (1/2): 29-33.

Sanni, A,A,I., Morlon-Guyot, J. and Guyot, J,P. 2002. New efficient amylase producing strain of *Lactobacillus plantarium* and *L. fermentum* isolated from different Nigerian traditional fermented foods. *International Food of Microbiology,* 7: 53-62.

Sahl, H.G., Jack, R.W., Bierbaum, G. 1995. Biosynthesis and biological activities of lantibiotics with unique post- translational modifications. *European Journal of Biochem*. 230: 827-853.

Salminen, S., Deighton, M.A., Benno, Y., Gorbach, S.L. 1998.Lactic acid bacteria in health and disease. In: Salminen, S., Wright, A. (Eds.), Lactic Acid Bacteria: Microbiology and functional Aspects. Marcel Dekker, New York, pp. 211-253.

Saunders, R.M., Ng, H. And Kline, L. 1972. The sugars of flour and their involvement in the San Francisco sourdough French bread process. *Journal of Cereal Science*, 18: 237-244.

Savadogo, A., Ouattara, C.A.T., Savadogo, P.W., Barro, N., Ouattara, A.S. and Traore, A.S. 2004. Identification of exopolysaccharides-producing lactic acid bacteria from Burkina Faso fermented milk samples. *African. Journal of Biotechnol*, 3(3): 189-194.

Sawadogo-Lingani H., Diawara B., Glover R.K., Tano-Debrah, K., Traoré, A.S. and Jakobsen M. 2010. Predominant lactic acid bacteria associated with the traditional malting of sorghum grains. *African journal of Microbiology Research*, 4 (3) 169-179.

Sawadogo-Lingani H., Diawara B., Traoré, A.S. and Jakobsen M. 2008. Technological properties of *Lactobacillus fermentum* involved in the processing of dolo and pito, West African beers, for the selection of starter culture. *African journal of Applied Microbiology* 104: 873-882.

Sawadogo-Lingani, H., Lei, V., Diawara, B., Nielsen, D.S., M□ller, P.L., Traoré, A.S. and Jakobsen, M. 2007.The biodiversity of predominant lactic acid bacteria in dolo and pito wort, for production of sorghum beer. . *Applied Microbiology*, 103:765-777.

Schillinger, U and Lucke, F.K. 1987. Identification of *Lactobacilli* from meat product. *Journal of Food Microbiology*, pp. 4, 199-208.

Schillinger, U. and Lucke, F.K. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Applied Environ Microbiology*, 55: 1901-1906.

Schleifer, K.H., Kraus, J., Dvorak, C., Kilpper, R., Collins, M.D. and Fisher, W. 1985. Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen. nov. *System of Applied Microbiology*, 6: 183-195.

Schweigart, F. 1970. Activity of lactic acid bacteria cultures used in the preparation of Mahewu. *Food Industries of S. Africa*, 12: 25-27.

Schweigart, F. 1971. The drying of lactic acid bacteria cultures for Mahewu production. *Lebensmitte*l *Wissenschaft und Technologie,* 4: 20-23.

Schweigart, F. and Fellingham, J.A. 1963.A study of fermentation in the production of Mahewu, an indigenous sour maize beverage of Southern Africa.*Milchwissenschaft*,18: 241-246.

Sefa-Dedeh, S. 1993. Traditional food technology. In Macrae R., Robinson R. and Sadler M. (Eds.), *Encyclopedia of Food Science, Food Technology and Nutrition,* New York: Academic Press. pp. 4600–4606.

Sefa-Dedeh, S. and Mensah, M. 1989. Cereal Processing Methods in Greater Accra Region: A technology Economis Survey. *Kellogg International Fellowship Programme in Food Systems*.Research Report. No. 03-88.

Sefa-Dedeh, S. and Plange, H. 1989.Processing of Ga *kenkey (komi*) in Greater Accra Region.A techno-economic study.*Kellog International Report* 03/1988 1–36.

Sefa-Dedeh, S., Frimpong, K., Afoakwa, E.O., and Sakyi-Dawson, E. 2000. Cowpea fortification of traditional foods. Book of Abstracts, World Cowpea Research conference III, Ibadan, Nigeria, 4-7 September.

Sharpe, E.M. 1979. Identification of lactic acid bacteria. In Identification Method for Microbiologists, 2nd. Ed. Soc. Appl. Microbacteriol .Technical Series, No. 14. Ed. Skinner, F.A and Lovelock, D.W. London: academic press. pp. 246-255.

Sikkema, J. and Oba, T.1998.Extracellular polysaccharides of lactic acid bacteria. *Snow Brand R&D Rep*, 107: 1-31.

Spicher, G and Schroder, R. 1980. The microflora of sourdough. Lebensmittel-Untersuchung und –Forschund. 170: 119-123.

Stam, H., Hoogland, M., Laane, C. 1998. Food flavours from Yeast. In: *Microbiology of fermented foods*. Brown, B.J.B. (Ed.), Black Academic and Professional, London. pp. 506-542.

Stevens, K.A., Sheldon, B.W., Klapes, N.A. and Klaenhammer, T.R. 1992. Nisin treatment for inactivation of *Salmonella* species and other Gram negative bacteria. *Applied Environmental Microbiology*. 57: 3613-3615.

Stille, M.E and Holzapfel, W.H. 1997. The lactic acid bacteria of food and their current taxonomy. *International Journal of Food Microbiology*, 36: 1-29.

Straford, M., and Anslow, P.A. 1998. Evidence that sorbic acid does not inhibit yeast as a classic “weak acid”preservative. *Lett. Applied Microbiology*. 27: 203-206.

Sulma, I., Larry, R.S. and Kirleris, A. 1991. Isolation and characterization of microorganisms associated with production of Sudanese *kirsa*. *Applied Environmental Microbiology Lett*, 238: 159-165.

Tanasupawat, S. and Komagata K. 1995. Lactic acid in fermented foods in Thailand. *World Journal of Microbiology Biotechnology*, 11: 253-256.

Teniola, O.D. and Odunfa, S.A. 2001. The effects of processing methods on the levels of lysine, methionine and the general acceptability of Ogi processed using starter cultures. *International Journal of Food Microbiology,* 63: 1-9.

Theron, J. and Cloete, T.E. 2000. Molecular Techniques for Determining Microbial Diversity and Community Structure in Natural Environments. *Critical Reviews in Microbiology*, 26(1): 37-57.

Thiele, C., Ga’’nzle, M.G. and Vogel, R.F. 2003. Fluorescence labelling of wheat proteins for determination of gluten hydrolysis and depolymerisation during dough processing and sourdough fermentation. *Journal of Agricultural and food Chemistry*, 51: 2745-2752.

van den Berg, D.J.C., Robijn, G.W., Jansser, A.C., Guiseppin, M.L.F., Vreeker, R., Kamerling, J.P., Vliegenthart, J.F.G., Ledeboer, A.M. *et. al.* 1995. Production of novel extracellular polysaccharides by *Lactobacillus sakei* 0-1 and characterization of the polysaccharides. *Applied Environmental Micrbiology*, 61: 2840-2844.

van der Merwe, A. le R., Schweigart, F. and Cachia, V.A. 1964. Mahewu- it’s industrial production and its value as a nutrient. *Vitalsoffe zivilsationskrankenheiten*.pp. 7.

van Noort, G. and Spence, C. 1976. The Mahewu Industry. *South Africa Food Review*, 10: 129-133.

Vandamme, P., Pot, B., Gill, M., de Vos P., Kerster, K., Swings, J. 1996. Polyphastic taxonomy, a consensus approach to bacteria systematic. *Microbiology*. Rev. 60: 407-438.

Wakil S.M. and Daodu, A.A. 2011. Physiological properties of a microbial community in spontaneous fermentation of maize (*Zea mays*) for *ogi*. *International Journal of Microbiology*, 2(3): 109-115.

Webster, G., Newberry, C.J., Fry, J.C. and Weightman, A. 2002. Microbial diversity in deep sub-saefloor sediment assessed by denaturing gradient gel electrophoresis. Narural Environment Research Council.

Whitby, P. 1968. Foods of Ghana.Food Research Institute Bulletin, CSIR, Accra, Ghana.

Wieser, H., Vermeulen, N., Gaertner, F., Vogel, R. 2008. Effects of different lactobacillus and enterococcus strains of chemical acidification regarding degrading of gluten proteins during sourdough fermentation. *European Food Research and Technology*, 226: 1495-1502.

Wood B.J.B and Hodges M.M, 1985. Yeast-lactic acid bacteria interactions and their contribution to fermented foodstuffs. In: Wood BJB (Editor) *Microbiology of fermented Foods*, Vol 1, Elsevier Science Publishers, London, New York, pp 263-293.

Appendix 1

Acceptability of white kenkey (*Nsiho*)

Name: **Date:**

Please taste all samples in the order indicated on your questionnaire and rate the taste, odour, texture and overall acceptability of each of them according to the scale below. Please rinse your mouth in-between sample tasting.

1. _____________ 2. _____________ 3._____________ 4. _____________5. _____________
6. _____________ 7. _____________ 8._____________ 9. _____________10. _____________

Aroma/Odour:

Sample code	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
9. Like extremely										
8. Like very much										
7. Like moderately										
6. Like slightly										
5. Neither like nor dislike										
4. Dislike slightly										
3. Dislike moderately										
2. Dislike very much										
1. Dislike extremely										

Taste:

Sample code	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
9. Like extremely										
8. Like very much										
7. Like moderately										
6. Like slightly										
5. Neither like nor dislike										
4. Dislike slightly										
3. Dislike moderately										
2. Dislike very much										
1. Dislike extremely										

Texture

Sample code	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
9. Like extremely										
8. Like very much										
7. Like moderately										
6. Like slightly										
5. Neither like nor dislike										
4. Dislike slightly										
3. Dislike moderately										
2. Dislike very much										
1. Dislike extremely										

Overall Acceptability

Sample code	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
9. Like extremely										
8. Like very much										
7. Like moderately										
6. Like slightly										
5. Neither like nor dislike										
4. Dislike slightly										
3. Dislike moderately										
2. Dislike very much										
1. Dislike extremely										

Overall liking:

Which of the samples do you like most? ______________

Why? ____

Thank you for participating

Appendix 2

Pattern of carbohydrate utilization of the dominant lactic acid bacteria from n*siho* using API 50 CHL

	Isolates						
Carbohydrte	*L. fermentum1*	L. *fermentum2*	*L. brevis 1*	L. brevis 2	L. plantarum	P. acidilactici	P. pentosaceus
Glycerol	-	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-	-
D-arabinose	-	-	-	-	-	-	-
L-arabinose	+	?	+	+	+	-	+
Ribose	+	+	+	+	+	+	+
D-xylose	?	+	+	+	-	+	+
L-xylose	-	-	-	-	-	+	?
Adonitol	-	-	-	-	-	-	-
β methyl-xyloside	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	-	-
D-Glucose	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+
D-mannose	+	+	-	-	+	+	+
L-sorbose	-	-	-	+	-	-	+
Rhamnose	-	-	-	-	-	+	-
Dulcitol	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	?
Mannitol	?	-	-	-	+	-	?
Sorbitol	-	-	-	-	+	-	?
α methyl-D-mannose	-	-	-	-	?	-	?
α methyl-D-glucoside	-	-	-	-	-	+	-
N acethyl glucosamide	-	-	-	-	+	-	+
Amygdaline	-	-	+	?	+	-	+
Arbutin	-	-	+	-	?	?	+
Esculin	?	+	?	+	+	+	+
Salicin	+	+	-	-	+	+	+
Cellobiose	-	-	+	-	+	+	+
Maltose	+	?	+	+	+	+	?
Lactose	+	+	+	?	+	-	+
Melibiose	+	+	+	+	+	-	+
Saccharose	+	+	+	+	+	-	+
Trehalose	-	+	+	-	+	+	+
Inulin	-	-	-	-	-	-	-
Melezitose	-	-	-	+	+	-	-
D-raffinose	+	+	?	+	-	-	-
Amidon	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-
Xylitol	-	-	-	-	+	-	-
β gentiobiose	+	-	+	-	-	+	+
D-turanose	-	-	-	+	+	-	-
D-lyxose	+	+	-	-	-	-	-
D-tagatose	-	-	-	-	-	+	+
D-fucose	-	-	-	-	-	-	-
L-fucose	-	-	-	-	-	-	-
D-arabitol	-	-	-	-	-	-	-
L-arabitol	-	-		-	-	-	-
Gluconate	+	+	+	+	+	-	-
2 ceto-gluconate	-	-	+	+	-	-	-
5 cetoglunate	+	+	?	+	-	-	-

(+) positive, (-) negative, ?

Appendix 3

Whole grains

Dehulled grains

Steeped dehulled maize

Fermenting dough

Dehulling machine

Milling machine

Slurry for ohu preparation

Preparation of ohu

Cooking of ohu

Mixing of ohu and fresh dough

Corn husk

Washed husk for wrapping ohu

Mixture of ohu and fresh dough

Moulding of ohu

Moulding of nsiho

Balls of nsiho ready for steaming/cooking

Perforator for steaming

Perforator and cooking pot

Nsiho packed in cooking bowl

Nsiho on fire

Ball of n*siho*

Cooking of nsiho

Cooked nsiho packed in basket

Prepared n*siho* for hawking

Nsiho in plate

Preferred fish for consuming nsiho