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The Selection of Lactic Acid Bacteria to be used as Starter Cultures for *Ting* Production

By

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I, declare that the thesis herewith submitted for the degree M. Sc. (Microbiology) at the University of Pretoria, has not previously been submitted by me for a degree at another university or institution of higher education.

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LIST OF ABBREVIATIONS

CO_2 :	carbon dioxide
cm :	centimetres
Ed :	edition
Eds :	editions
Eg. :	Example
Fig. :	Figure
g :	gram
h :	hour
HNO_3 :	nitric acid
$HCLO_4$:	perchloric acid
HCL :	hydrochloric acid
H_2SO_4 :	hydrogen sulphate
HPLC :	high pressure liquid chromatography
KOH :	potassium hydroxide
La_20_3 :	lanthanum oxide
M :	molarity
min :	minutes
ml :	millilitres
mm :	millimetres
N :	normality
NDF :	neutral detergent fibre
nm :	nanometres
NaOH :	sodium hydroxide
S :	sample
UV :	ultraviolet
¼ :	quarter
% :	percentage
µl :	microlitres

DEDICATION

This dissertation is dedicated to my father Mr. Uriel Morwakgomo Ramaite; a man who loved the Lord and operated in faith. He was my pillar of strength, a mentor and a friend; I could have never had a better father. Even though my life has made a turn for the worst, I will forever treasure your memories dad; most of all remember your love, till we meet again someday. And in death, as in life, he has the final word:

"Whatever your hand finds to do, do it with all your might, for in the grave, where you are going, there is neither working nor planning nor knowledge nor wisdom"

Ecclesiastes 9: 10

THE SELECTION OF LACTIC ACID BACTERIA TO BE USED AS STARTER CULTURES FOR *TING* PRODUCTION

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SUMMARY

Most of the traditional foods in Africa are fermented before consumption. Fermentation is an old technology; however, during this process, especially in traditional fermented cereal based products with special emphasis on *Ting*, there is very little control involved during the processes. Fermentation is thus left to chance inoculation from the environment. *Ting* is a sorghum based product that is a result of LAB fermentation and has 0.6-0.7 % lactic acid with a final pH of 3.5-4.0. However, there is presently no adequate information on the employment of starter cultures for most South African traditional fermented foods. The aims of this research were therefore to evaluate the use of different isolates of LAB that had been previously isolated from *Ting* as potential starter cultures for *Ting* production, to evaluate whether these would result in a product with sensory characteristics similar to those of the naturally fermented *Ting* and also to determine the nutritional composition of *Ting*. Six isolates LAB isolates (*Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30) previously isolated from *Ting* were used in this study. When inoculated into sorghum mash to initiate *Ting* fermentation, the LAB starter cultures reduced the pH from 6.5-6.8 to levels

below 4.5 within a reduced fermentation time of 12 h instead of 48-72 h as is the case with the naturally fermented *Ting*. The same starters increased the amount of lactic acid present in the samples from 0.02 to 0.3% within 12 h, reaching up to 0.5 % after 72 h of fermentation. The nutritional composition of all the products was similar. The minerals calcium, phosphorous, magnesium and iron were analysed and phosphorous was the highest followed by magnesium; with calcium and iron being the lowest. Among all the amino acids analysed, glutamic acid was the highest in all the samples, followed by proline and leucine with cystine and lysine being the least. Generally, *Ting* was found to be high in protein and energy although with a low fat content. Based on the results of the consumer acceptability study, of all of the six LAB isolates; the LAB isolate *L. cellobiosus* 4.35 could be the best option when considering a starter culture for *Ting* production since the sample had the highest consumer acceptability results similar to the naturally fermented *Ting* sample.

Chapter 1

1.1 INTRODUCTION

The microorganisms implicated in lactic fermentation of foods in Africa belong to four major genera; namely *Lactobacillus, Lactococcus, Leuconostoc* and *Pediococcus* (Oyewole, 1997). These form part of the major group of microorganisms generally referred to as lactic acid bacteria (LAB). The organisms occur naturally in a wide range of food materials and in some circumstances they can be a cause of spoilage. However, they are often deliberately introduced or their growth is encouraged in order to produce a wide range of fermented foods that include all the major commodity groupings such as fish, meat, cereals, fruits, vegetables and legumes (Campbell-Platt, 1987; Oyewole, 1997). Lactic acid bacteria are widely used in the production of fermented foods where they are highly employed as starter cultures (Hansen, 2002). These organisms are able to fermentatively utilize carbohydrates and thus forming lactic acid as their major end product (Adams and Nicolaides, 1997).

Generally, fermented foods may be described as palatable and wholesome foods that are prepared from raw or heated raw materials by processes of microbial fermentation (Hammes, 1990). Such food products constitute a major portion of the people's diets in Africa since they generally form part of the daily main dishes of an average individual and also constitute the bulk of foods given to children (Sanni, 1993; Oyewole, 1997). Fermented foods are mostly cereal-based products including porridges, bread as well as both alcoholic and non-alcoholic beverages. In Africa, the common fermented cereals include maize, sorghum, millet, tef and occasionally rice and wheat (Oyewole, 1997). Fermented foods are of great significance because they provide vast quantities of nutritious foods in a wide diversity of flavours, aromas and textures that enrich the human diet (Steinkraus, 1997). They are also treasured as major dietary constituents in numerous developing countries primarily because of their keeping quality under ambient conditions, and also for their safety and traditional acceptability (Holzapfel, 2002).

The process of fermentation in most instances and especially in the rural communities where it is highly practiced is however spontaneous and uncontrolled thus resulting in products of varying quality (Mugula *et al.,* 2002). This process has served to improve the shelf life and safety of foods and enable people in the moderate and cooler regions to survive winter seasons and drought periods (Campbell-Platt, 1987). There is relatively little information available on starter culture traditions in Sub-Saharan Africa. There are however various studies in the development of starter cultures for other cereal based fermented foods consumed in other countries such as *Uji* (Mbugua *et al.,* 1984), *Garri* (Okafor *et al.,* 1998), *Kivunde* (Kimaryo *et al.,* 2000), *Ogi* (Teniola and Odunfa, 2001). A starter culture is a preparation or material that contains large numbers of viable microorganisms, which may be added to accelerate a fermentation process (Holzapfel, 1997).

No starter cultures are commercially available for the production of most African fermented food products (Holzapfel, 1997); with special emphasis on *Ting.* This is a sour cereal porridge usually made from maize, millet or sorghum that is consumed in the West, Central, East and Southern Africa (Campbell-Platt, 1987). Being adapted to the substrate, a typical starter culture facilitates improved control of a fermentation process and predictability of its products. Starter cultures also facilitate control over the initial phase of a fermentation process; they also enable stricter control of a fermentation process, the outcome of which may thus be predictable (Holzapfel, 1997).

The objectives of this study were:

- To determine the application of LAB isolated from a sorghum based fermented product called *Ting* as potential starter cultures for the production of *Ting*
- To evaluate the consumer acceptability
- To determine the nutritional composition of the different fermented *Ting* samples

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Chapter 2

LITERATURE REVIEW

2.1 FERMENTATION

Gibson (1995) defines fermentation as the process by which microorganisms propagate themselves utilizing their external medium as a source of nutrients. Fermentation is an old technology and a process dependent on the biological activity of microorganisms for the production of a range of metabolites that can suppress the growth and survival of undesirable microflora in foodstuffs (Klaenhammer and Fitzgerald, 1994; Fox, 1993). During the process of fermentation, the presence of fermentative microbes such as the lactic acid bacteria (LAB) causes acidification of the fermented food and production of bacteriocins (Gibbs, 1987). As the tolerance of microorganisms to widely differing pH levels varies naturally, the pH selects the species or group of fermentative microorganisms that will predominate in unaltered food products.

Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes; particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavors, aromas and textures pleasant and attractive to the human consumer. However, if the products of enzyme activities have unpleasant odours or undesirable, unattractive flavours or the products are toxic or disease producing, the foods are described as spoiled (Steinkraus, 1997). The raw materials traditionally used for fermentation are diverse and these include fruits, cereals, honey, vegetables, milk, meat and fish. It is possible to obtain a large variety of different food products by selecting different raw materials, starter cultures and fermentation conditions (Hansen, 2002).

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Fermentations by LAB generally provide a form of bio-control by inhibiting the growth of undesirable spoilage and pathogenic organisms and thus preserving the products (Smith and Palumbo, 1981). Due to this form of bio-control which fermentation provides, it is thus considered to be a relatively safe form of food preservation and the products are not commonly associated with food poisoning (Dillon and Cook, 1994). Lactic fermentation of food of plant or animal origin is a widely accepted method of preservation that also results in desirable nutritional properties to the fermented product (Cooke *et al.,* 1987).

Fermentation is also a means of improving the sensory quality and acceptability of many raw materials to such an extent that several foods are preferred in a fermented state than in an unfermented one (e.g. *Tempeh* rather than the unfermented soybeans and *Gari* rather than the unprocessed cassava tubers). There are also further attributes obtained through fermentation such as the reduction of toxic or undesired food components such as linamarin in cassava or antinutrition factors in legumes (Hammes, 1990).

2.1.1 HISTORICAL PERSPECTIVE OF FERMENTATION

The process of fermentation has been practiced for the millennia with the result that there is tremendous variety of fermented foods ranging from those derived from meat and plants to those derived from milk and dairy products (Ray and Daeschel, 1992). Traditionally, fermented foods have contributed greatly to both variety and safety in the human diet. Fermentation can be traced back thousands of years and has been used as a means of improving the keeping quality of food for more than 6 000 years (Klaenhammer and Fitzgerald, 1994; Holzapfel, 1997). The essential role of microorganisms in the fermentation process was realized in 1861 AD during the development of pasteurisation (Klaenhammer and Fitzgerald, 1994).

Together with drying and salting, fermentation is one of the oldest methods of food preservation. Its importance in modern day life is underlined by the wide spectrum of fermented foods marketed both in developing and industrialised countries; not only for the benefit of preservation and safety, but also for their highly appreciated sensory

attributes (Holzapfel, 2002). Fermentation has enabled our ancestors in temperate and cooler regions to survive winter season and those in the tropics to survive drought periods, by improving the shelf life and safety of foods (Campbell-Platt, 1994). Through the ages, fermentation has had a major impact on the nutritional habits and traditions, on culture and also on the commercial distribution and storage of food. Traditional fermentation still serves as a substitute where refrigeration or other means are not available for the safe keeping of food (Holzapfel, 2002).

Fermentation is one of the traditional methods of preserving crops and producing food, particularly in the tropical countries where high temperature and high humidity; coupled with unsanitary conditions favour food spoilage (Van de Sande, 1997). Indigenous fermented foods were developed through traditional or village art methodologies, which were preserved over the years in order to maintain the uniqueness and identity of these foods (Valyasevi and Rolle, 2002). Over the ages, processes as well as raw materials used have been adjusted to local opportunities and restrictions, the result being a highly diversified traditional food processing system providing income for many people including fermented food producers, retailers and related activity contractors such as millers and transporters. In some areas, at least food processing is the major source of employment and income for women (Van de Sande, 1997).

However, food technologists are convinced that they are able to improve traditional fermentation processes. This is based on their scientific understanding of the fermentation processes and their expertise in breeding and of microorganisms for strain improvement and the use of pure starter cultures (Hesseltine and Wang, 1983). They have however proven the ability to influence the duration of fermentation processes and the taste and food value of end products. To date, lactic fermentation of food is carried out through traditional village-art methods (Odunfa, 1985). Processing usually involves either soaking of the raw materials, submerged in water contained in a fermenting vat (usually clay pots) for a length of time or an initial size reduction of the raw material by grating or milling in the wet form before being allowed to ferment without being soaked in water.

2.1.2 EFFECTS OF FERMENTATION ON FOOD

Apart from preserving different food types, fermentation is also responsible for the sensory and nutritional characteristics of different foods and can thus result in new or improved flavours, aroma and texture. Fermented foods often have distinct organoleptic properties as compared to the unfermented products. The aroma and flavour compounds of fermented foods may include acids, carbonyl compounds, esters, ethanol, fusel oils, ketones, lactones, and pyrazines. During fermentation, both texture and organoleptic properties may be altered; for example cereals into bread and soya beans into soy sauce or Indonesian *Tempe* (moulded soya bean cake). The fermentative action of microbes may bring about a significant nutritional enrichment including increases in protein levels (particularly amino acids) or vitamins (Cook, 1993).

Cook (1993) also states that fermentation can increase the protein content of high-starch substrates and also cause the loss of starch solids leading to the doubling of the protein content on a dry mass basis. Fermentations of cereal/legume composites are particularly important nutritionally because the balance of amino acids is often improved. The process of fermentation can also lead to improvements in some vitamins like in the sorghum and maize beer fermentations of Southern Africa, the increase in levels of thiamine in Indonesian *Tape* fermentation of rice and vitamin B_{12} in Korean Kimchi vegetable fermentation and Indonesian Soya bean *Tempe* fermentation. These increases in vitamins are of greater importance to people who consume a limited diet. Most fermented foods provide a valuable source of readily available energy, glucose, maltose, ethanol and organic acids. The process of fermentation may also lead to a nutritionally more favourable balance of fatty acids, with increases in levels of polyunsaturated acids (Nout and Rombouts, 1992).

2.1.3 BENEFITS OF FERMENTATION

Fermented foods may be generally described as palatable and wholesome foods, prepared from raw or heated raw materials by microbial fermentation. Microorganisms, by virtue of their metabolic activities, contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture, shelf life and safety (Hammes, 1990).

According to Nout (1994) fermentation plays the following five roles:

1) Enrichment of the human diet through the development of a wide diversity of flavours, aromas and textures in food.
2) Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid and alkaline fermentations.
3) Enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamins.
4) Detoxification during food fermentation processing.
5) A decrease in the cooking times and fuel requirements.

2.2 FOOD FERMENTATION IN AFRICA

Fermentation is indigenous to the African countries and has been practiced for many centuries (Oyewole, 1997). Therefore, fermented foods and beverages are said to constitute a major portion of the people's diets in Africa (Sanni, 1993; Oyewole, 1997). In Africa, various types of cereal grains including maize, millet and sorghum are common substrates during fermentation and these are used to produce numerous staple foods and beverages known by different names (Odunfa and Adeyele, 1985). Fermented sorghum or millet-based foods, alcoholic and non-alcoholic drinks or beverages are prepared in many African countries for human consumption (Odunfa *et al.,* 1996; Steinkraus, 1996; Usha *et al.,* 1996).

Fermented foods are treasured as major dietary constituents in numerous developing countries primarily because of their keeping quality under ambient conditions, and also for their safety and traditional acceptability (Holzapfel, 2002). African countries require food-processing technologies that meet the challenges of their peculiar food security problems. They should be of low-cost in order to be affordable by the poor sectors of the community and should also be able to address the problems of food spoilage and food-borne diseases, which are prevalent in the continent. Therefore, the process of food fermentation on its own is one such important food processing technology that meets these challenges (Oyewole, 1997).

The production of lactic fermented foods in Africa is still largely home based and is usually left to chance inoculation from the environment. There is very little or no control involved at all in the processing. Instead, the fermentation vessel of previous ferments usually acts as the source of inoculum for the initiation of another fermentation cycle. There are no starter cultures available; and instead old stocks of previous ferments are used in some cases to initiate fermentation in new batches (Oyewole, 1997). In general, fermentation is a spontaneous and uncontrolled process that then results in a product of variable quality (Mugula *et al.,* 2002). Unlike in other parts of the world, lactic fermentations of vegetables, fish and meat are not common in Africa (Oyewole, 1997).

Fermented foods are essential components of the diet in a number of developing countries, and are consumed either as main dishes or as condiments (Steinkraus, 1996). They are prepared from both plant and animal materials, using processes in which microorganisms play an active role in the physical, nutritional and organoleptic modification of the starting material (Aidoo, 1994). However, due to the lack of scientific and technological know-how, fermented foods are generally evaluated on the basis of qualitative attributes such as odour and flavour (Valyasevi and Rolle, 2002).

As compared with foreign imported foods, the traditional indigenous foods sometimes lack appeal because of their presentation, unhygienic production practices and irregular quality. The prestige of traditional foods among these groups is low, and thus in many cases lead to their substitution by the more prestigious foreign imported foods (Nout, 1981). Upgrading traditional processes may provide an alternative far superior to food imports because it would avoid the dependency of the urban populations on expensive foreign foods, maintain employment opportunities in the sector itself reduce logistical problems in food distribution and also provide farmers with an opportunity to sell their product locally (Van de Sande, 1997).

2.2.1 IMPORTANCE OF LACTIC ACID FERMENTATION IN AFRICA

Amongst the various fermentation processes in Africa, lactic acid fermentation is one of the oldest and most widespread. This technology has developed indigenously for an extensive range of products (Dirar 1992; Oyewole, 1997).

According to Oyewole (1997), lactic acid fermentation processes in Africa have survived throughout the centuries because of the following benefits that this technology possesses:

- It serves as a household technology for improving food safety.
- It serves as a low-cost method of food preservation.
- It contributes to the improvement of nutritional value and digestibility of raw materials.

2.3 CLASSIFICATION OF FERMENTED FOODS

Fermented foods can be classified in a number of ways including classifications according to categories, classes or even commodity (Dirar, 1993). A few examples of such classifications are stated below and similarly apply for different fermented foods found around the world (Steinkraus, 1997).

2.3.1 CLASSIFICATION BASED ON DIFFERENT CLASSES

Campbell-Platt (1987) classified fermented foods into the following six classes:

1) Beverages
2) Cereal products
3) Dairy products
4) Fish products, fruit and vegetable products
5) Legumes and
6) Meat products

2.3.2 CLASSIFICATION BASED ON COMMODITY

Odunfa (1988) classified the fermented products according to commodity as follows:

1) Fermented starchy roots
2) Fermented cereals
3) Alcoholic beverages
4) Fermented vegetable proteins and
5) Fermented animal protein

2.4 FERMENTED CEREALS

A large number of lactic fermented products in Africa are cereal-based and the products include porridges, bread and both alcoholic and non-alcoholic beverages (Oyewole, 1997). Many of the indigenous cereal based fermented products are valued for the taste and aroma active components produced, and are used as seasoning and condiments. The majority of cereal crops produced worldwide includes wheat, rice, maize, barley, sorghum, oats, millet, and rye. Asia, America, and Europe produce more than 80% of the world's cereal grains. However, wheat, rice, sorghum, and millet are produced in large quantities in Asia; corn and sorghum are principal crops in America, and barley, oats and rye are major crops in the former USSR and Europe (Chaven and Kadam, 1989).

The natural fermentations of African cereal doughs are dominated by heterolytic bacteria; as is the case in wheat and rye sourdoughs (Steinkraus, 1995; Vogel, 1997). Sorghum [(*Sorghum bicolour*) L. Moench] and finger millet (*Eleusine coracana*) are important food crops in the arid and semi-arid regions of the world (Sulma *et al.,* 1991; Mukuru, 1992; Usha *et al.,* 1996; Owuama, 1997). Sorghum is a cereal crop belonging to the cultivated members of the monocotyledonous grass family *Poaceae*. The members of this family produce a dry indehiscent fruit known as caryopsis, which is also commonly called a grain or kernel. The average size of the sorghum grain is about 4 mm long, 2 mm wide and 2.5 mm thick; with a weight of 25–35 mg (Rooney and Serna-Saldivar, 1991). Sorghum appears to have originated from Ethiopia, in Africa (Hulse *et al*., 1980).

Fermented cereals are particularly important as weaning foods for infants and as dietary staple foods for adults. The major cereals grown in Africa include maize, rice millet and sorghum (Table 2.1). Cereals are more widely utilized as food in African countries than in the developed world and account for as much as 77% of the total caloric consumption. They also contribute substantially to the dietary protein intake in a number of these countries. A majority of traditional cereal-based foods are processed by natural fermentation and are particularly important as weaning foods for infants and as dietary staples for adults.

Fermented foods including fermented cereals contribute to about one-third of the diet worldwide (Campbell-Platt, 1987). Cereals are particularly important substrates for fermented foods in all parts of the world and are staples in the Indian subcontinent, in Asia, and in Africa (Steinkraus, 1994). Acid porridges prepared from cereals are still eaten in varying amounts in different parts of the world, particularly in the developing countries, where they may represent the basic diet (Steinkraus, 1996).

Table 2.1 summarizes the amounts of cereals produced in Sub-Saharan Africa (thousand metric tons).

Table 2.1 Production of cereals in Sub-Saharan Africa (FAO, 1997)

Cereal	**1997**	**% of world production**
Maize	24,798	4.2
Millet	10,950	38.9
Rice	11,321	2.0
Sorghum	17,400	28.2
Wheat	3,140	0.5

The fact that starch is the main carbohydrate in cereals and other crops such as cassava led to the idea that amylolytic lactic acid bacteria (ALAB) could play a major role in their fermentation (Calderon *et.al.,* 2002). In natural fermentations, ALAB could help increase the availability of easily fermented carbohydrates through partial starch hydrolysis, since their alpha-amylases are able to hydrolyze raw starch granules (Rodriguez Sanoja *et al.,* 2000). Traditional African cereal sourdoughs are also used as complimentary foods with examples of such being Poto-Poto in Congo or Ogi in Burkina Faso (Calderon *et al.,* 2002). The partial hydrolysis of starch in natural fermentation is not enough to markedly reduce the dietary bulk, which is one of the main issues to be addressed in child nutrition (Lorri and Svanberg, 1993; Tréche and Mbome, 1999).

Natural fermentations of cereal doughs are mixed substrate fermentations of starch, sucrose, fructose and glucose (Calderon *et al.,* 2002). Fructose can act as an alternative electron acceptor for heterofermentative LAB (Hammes *et al.,* 1996; Dols *et al.,* 1997; Erten, 1998); and limited amylase production has been observed on glucose, fructose, and sucrose (Calderon *et al.,* 2001). The fermentation of binary mixtures of starch with; glucose, fructose or sucrose was first studied by Calderon *et al.* (2002).

Cereal crops are energy dense, containing 10 000-15 000 kJ/Kg; about 10-20 times more energy than most succulent fruits and vegetables. Nutritionally, they are important sources of dietary protein, carbohydrates, the B complex of vitamins,

vitamin E, iron, trace minerals, and fibre. It has been estimated that global cereal consumption directly provides about 50 percent of protein and energy necessary for the human diet, with cereals providing an additional 25 percent of protein and energy through livestock intermediaries. Cereals have a variety of uses as food. The most general use of cereals is in cooking, either directly in the form of grain, flour starch, or semolina. Another common usage of cereals is in the preparation of alcoholic drinks such as whiskey and beer (barley; sorghum), vodka (wheat), American bourbon (rye) and Japanese sake (rice). A variety of unique fermented foods, other than leavened breads and alcoholic beverages, are also produced in regions of the world that rely mainly on plant sources of protein and calories. In developed countries, that obtain most of their protein from animal products, cereals are increasingly used as animal feed. More than 70% of the cereal crop produced in developed countries is fed to livestock; whereas, in developing countries, 68-98 % of the cereal crop is used for human consumption (Betschart, 1982; Chaven and Kadam, 1989).

2.4.1 FERMENTED SOUR CEREAL PORRIDGES

Common cereals that are fermented in Africa include maize, sorghum, millet, tef, and occasionally rice and wheat (Oyewole, 1997). A wide variety of sour cereal porridges can be prepared by the fermentation of maize (corn), sorghum, millet or cassava. Such products can be known by different names according to their locality in addition to the raw materials used. Some examples of these are summarized in Table 2.2 (Baningo and Muller, 1972; Campbell-Platt, 1987; Dirar, 1993; Lorri, 1993; Steinkraus, 1996).

Table 2.2 Different types of fermented sour cereal porridges

Product Name	**Type of Cereal**	**Country**
Akamu	Sorghum or Millet	Nigeria
Akasa	Maize	Ghana
Akatsakoe	Maize	Ghana
Amodeogboe	Maize	Ghana
Edi	Sorghum or Millet	Uganda
Ekuegbemi	Maize	Ghana
Kamu	Sorghum or Millet	Nigeria
Koklui	Maize	Ghana
Kouron	Sorghum or Millet	Cameroon
Kannu tzamia	Millet	Nigeria
Lesheleshele	Maize or Sorghum	South Africa
Mpapa	Maize	Ghana
Nasha	Sorghum or Millet	Sudan
Nyuka	Millet, Sorghum or Maize	Kenya
Oblayo	Maize	Ghana
Obungi bwakawa	Sorghum or Millet	Uganda
Obushes	Sorghum or Millet	Uganda
Ogi	Maize	Nigeria
Poporsu	Maize	Ghana
Ting	Sorghum or Millet	Lesotho and South Africa
Ucuru	Millet, Sorghum or Maize	Kenya
Ucuu	Millet, Sorghum or Maize	Kenya
Uji	Maize or Sorghum	Kenya and Tanzania

2.4.1.1 Production of the fermented sour cereal porridges

Campbell-Platt (1987) states that the sour cereal porridges are usually made from maize (*Zea mays*), millet (*Pennisetum species*) or sorghum (*Sorghum vulgare*). Maize, millet or sorghum is soaked in water for a period of 18-24h; and then milled before being sieved to remove the bran. The cereal grain is again soaked in about 3 times its own volume of water and then left to ferment at ambient temperatures ranging from 25-34˚C for a period of 18-24h and even up to 72 h depending on the required sourness of the end product.

After fermentation is complete, the fermented meal is boiled in water until the starch gelatinises and thickens into porridge. Alternatively, the fermented dough may be thinned down with boiling water into porridge. Herbs or spices such as pepper, garlic or cloves may be added, as may groundnut paste, sugar or milk (Campbell-Platt, 1987).

2.4.1.2 Consumption of the cereal porridges

The sour cereal porridges are mostly eaten with stew for lunch or dinner, or sometimes with milk and sugar as sweet porridge that is mostly taken for breakfast; or rather as weaning or invalid food in West, Central, East and Southern Africa.

2.4.1.3 Composition and nutritive value of the fermented sour cereal porridges

The fermented sour cereal porridges have a moisture content of 88-94 %. They have a protein content of 7-12g, fat content of 1-7g, with a carbohydrate content of 75-83g, the fibre is between 1-8g, ash content of 1-6g (all per 100g dry matter); and 1.5-1.6MJ of energy. The products also contain the minerals calcium, phosphorous and iron, with the amounts; 30-800mg, 30-1000mg and 3-30mg respectively (Campbell-Platt, 1987).

2.4.1.4 Microbiological and biochemical composition of the fermented sour cereal porridges

The microorganisms that have been implicated in fermentation of the sour cereal porridges are LAB. These include Lactobacillus acidophilus as the principal organism. However, *L. cellobiosus*, *L. buchneri*, *L. fermentum*, *Pediococcus acidilactici* and *P. pentosaecus* are usually also active. The cereal porridges have a total acidity of 0.6-0.7% lactic acid, 0.1-0.2% acetic acid and a final pH of 3.5-4.0 (Campbell-Platt, 1987).

Table 2.2 lists the different types of the fermented sour cereal porridges, the type of cereal used and the area where such products are consumed. Figure 2.1 further illustrates a flow diagram for the traditional preparation of one of the products in Table 2.2; a sorghum based product known as *Ting* (Campbell-Platt, 1987; Steinkraus, 1996).

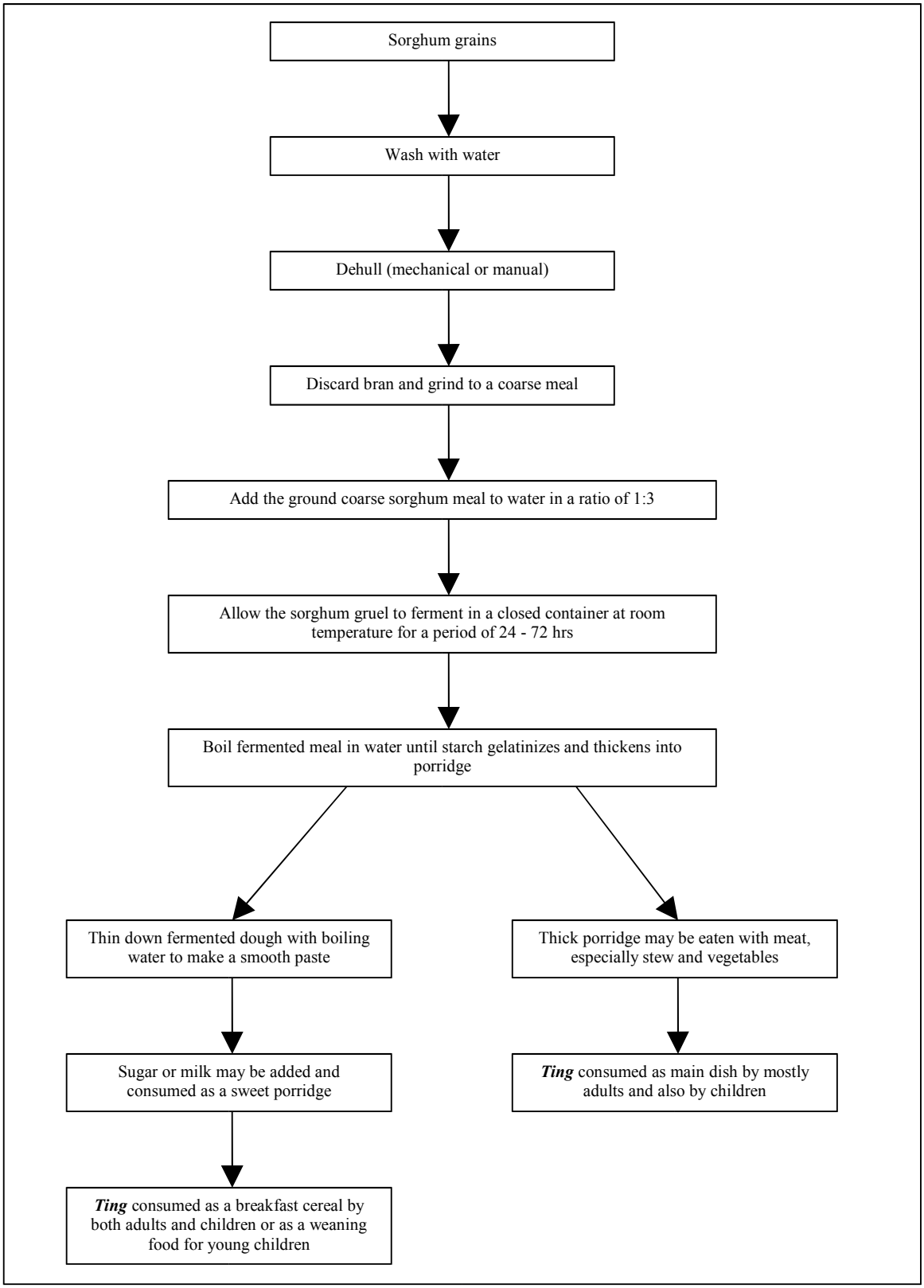

Figure 2.1 Flow diagram of the traditional preparation of *Ting*.

2.5 THE CONCEPT OF STARTER CULTURES

The inoculation of raw materials with a residue from a previous batch, also known as back-slopping; can accelerate the initial fermentation with the cultures used in the original fermentation (Holzapfel, 1997). Through trial and error, traditional skills have been developed for controlling technical parameters during fermentation processes. In order to accelerate fermentation and its predictability, process control is required and this can be achieved through ecological or environmental control. According to Nout and Rombouts (1992), ecological control involves the enrichment of starter organisms in food by natural selection. Recycling part of the previous fermented product (also called back-slopping) results in a highly competitive and well adapted multiple strain starter culture.

The process of back-slopping has traditionally been used and still is in different households, to ferment different cereal foods which are staple foods in most of the African countries. Usually 5 to 10% of the previous fermented product is used for back-slopping. According to Nout *et al*., (1987), this results in improved and predictable acidification and in general a fermentation process that can be better controlled. The acidification process stabilizes within three to five consecutive fermentation cycles (Nout, 1994). This in turn improves the quality and safety as well as increasing the shelf life of the final product. However, the use of starter cultures in fermentation would result in a more predictable fermentation and product of invariable quality.

Holzapfel (1997) defines a starter culture as a preparation or material that contains large numbers of viable microorganisms, which may be added to accelerate a fermentation process. The application of a strong starter culture would probably minimize dry matter losses, avoid contamination with pathogenic and toxigenic bacteria and moulds and also have the ability to minimize the risk of incidental microflora that cause off-flavour. Starter cultures also facilitate control over the initial phase of a fermentation process; and enable stricter control of a fermentation process, the outcome of which may thus be predictable. These are however specifically

selected for a specific substrate or raw material e.g. milk, meat, cereal, legumes, roots and tubers (Holzapfel, 1997).

Modern large-scale production of fermented foods and beverages is dependent almost entirely on the use of defined strain starters, which have replaced the undefined strain mixtures that were traditionally used for the manufacture of these products (Klaenhammer and Fitzgerald, 1994). Modern starter cultures are selected either as single or multiple strains, specifically for their adaptation to a substrate or raw material. The primary activity of the culture in food fermentation is to convert carbohydrates to desired metabolites such as lactic acid, alcohol, acetic acid or CO_2. Alcohol and organic acids are good natural preservatives (Hansen, 2002).

The first pure starter cultures (*Lactococcus lactis*) were introduced simultaneously in Denmark and Germany in 1890. These were used for the industrial fermentation of milk for cheese and sour milk processing. By acidification, metabolic abilities of the selected strains were utilized to support the technical process and to obtain a desired quality of the end product. Adaptation to the substrate and other beneficial attributes such as flavour and texture improvements also served as criteria for strain selection, and as an approach of back-slopping. Because of the long tradition and high standards attained, starter cultures in the dairy industry served as an example for the application of starters to other food commodities (Holzapfel, 1997).

There are no LAB starter cultures available yet for the small scale processing of most African traditional foods (Holzapfel, 1997). There are however studies in the development of starter cultures for the production of other cereal based fermented foods consumed in other countries such as *Ugi* (Mbugua *et al.,* 1984), *Garri* (Okafor *et al.,* 1998), *Kivunde* (Kimaryo *et al.,* 2000) and *Ogi* (Teniola and Odunfa). It is necessary to use starter cultures to avoid variations in the quality of traditional fermented products (Zorba *et al.,* 2003). Different microorganisms are used as starter cultures in the fermentation of different products and these include; bacteria, moulds and also yeast.

2.5.1 MOULDS

As compared with most Asian countries, moulds play a minor role in fermented foods in Africa. Fermented foods such as *Miso*, *Tempe* and *Shoyu* are typical examples of traditional Asian foods produced with moulds as starter cultures. They are produced on an industrial scale and are well known worldwide. In Europe, traditional mould ripened foods are mainly restricted to blue-mould (*Penicillum roqueforti*) and white-mould (*Penicillum camemberti*). Most of the cheeses and mould-ripened fermented sausages contain either *Penicillum nalgiovense* or *Penicillium chrysogenum* (Holzapfel, 1997).

2.5.2 YEASTS

Plant materials containing fermentable sugars provide suitable substrates for yeast species of *Saccharomyces, Candida, Torula* and *Hansenula* (Holzapfel, 1997). For the industrial production of Western style beers and even traditional African beers, selected strains of *Saccharomyces cerevisiae* have been used. Dehydrated yeast is also readily available on the market throughout Africa, mainly for bread making; although it is also used for small scale beer brewing.

2.5.3 BACTERIA

Among the bacteria associated with food fermentation, 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. Their association with the human environment and their beneficial interactions, both in food and in the human intestinal tract, combined with the long tradition of lactic fermented foods in many cultures, have led to the general conclusion that this group may be generally recognized as safe (GRAS). However, there are no LAB starter cultures that are commercially available yet for the small scale processing of most of the traditional African foods (Holzapfel, 1997).

2.6 LACTIC ACID BACTERIA IN CEREAL FOODS

The microorganisms implicated in the lactic fermentation of foods in Africa belong to four major genera, namely *Lactobacillus, Lactococcus, Leuconostoc* and *Pediococcus*; and together they form the major part of LAB (Oyewole, 1997). Lactic acid bacteria are mostly the predominant microorganisms and the main producers of the acid and flavour compounds in many cereal fermentations (Halm *et al.,* 1993). Although a wide variety of LAB are found, including mainly *Lactobacillus, Leuconostoc* and *Lactococcus* species (Hounhouigan *et al.,* 1993; Johansson *et al.,* 1995); *Lactobacillus fermentum* has repeatedly been reported as one of the dominant microorganisms in cereal sourdoughs (Hounhouigan *et al.,* 1993; Halm *et al.,* 1996; Hamad *et al.,* 1997; Hayford *et al.,* 1999). Lactic acid bacteria found in fermented foods are a rich source of antimicrobial compounds including organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins (Holt *et al.,* 1994).

Lactic fermented food products constitute the bulk of foods given to children and generally form part of the daily main dishes of the average individual in Africa. One example of such is a lactic fermented maize-based gruel called Ogi, which is a major indigenous traditional weaning food common in the whole West Africa. Many people also consume Ogi as a breakfast meal and it serves as the food of choice for the sick. Apart from Ogi another important product is Gari, which is consumed in a large part of the continent; where it serves as one of the three main dishes of most families. Lactic acid fermentation can therefore be said to be widespread in the African continent (Oyewole, 1997). Most bacterial fermentations produce lactic acid; while yeast fermentation results in alcohol production (Chaven and Kadam, 1989).

Lactic acid bacteria occur naturally in a wide range of food materials and in some circumstances they may result in the spoilage of the particular food material. These organisms are however often deliberately introduced, or their growth is encouraged; thus producing a wide variety of fermented foods that include the major commodity groupings such as fish, meat, cereals, fruits, vegetables and legumes (Campbell-Platt, 1987). These organisms are widely used in the production of fermented foods, and

they constitute the majority of the volume and the value of the commercial starter cultures (Hansen, 2002).

Lactic acid bacteria are best known for their use as starter cultures in the manufacture of dairy products such as acidophilus milk, yoghurt, buttermilk, cottage cheese, soft and hard cheeses as well as in a wide variety of other fermented food products (Jay, 1986). These organisms are also important commercially in the processing of meats, alcoholic beverages and vegetables; which include sausages, cured hams, wines, beer, fortified spirits, prickles, and sauerkraut (Jay, 1982). Although most of the LAB have beneficial effects in the food industry, they can be a nuisance as contaminants by producing off-flavours (Kandler and Weiss, 1986).

Lactic acid bacteria play an essential role in food technology in that they can improve the aroma and texture of food and also inhibit the growth of spoilage bacteria (Clewell, 1990). However, not all LAB are useful, some of them are involved in food spoilage or may even be pathogens (Clewell, 1990; Schleifer *et al.,* 1995). The amount of lactic acid formed during fermentation is critical in food manufacture and plays a prominent role in the taxonomic classification of LAB (Rose, 1981).

2.6.1 LACTIC ACID BACTERIA AND THEIR METABOLISM

According to Aguirre and Collins (1993), the term lactic acid bacteria (LAB) refers to a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, which are usually non-motile. Lactic acid bacteria are collectively assigned to the family *Lactobacteriaceae* (Holt *et al*., 1994). They are morphologically heterogenous, including long and short rods as well as cocci. Members of the *Lactobacteriaceae* do not form spores except *Sporolactobacillus inulinus*; and are non motile (Schlegel, 1986). These organisms may generally be characterized as Gram-positive, aerobic to facultatively anaerobic, asporogenous rods and cocci which are oxidase, catalase, and benzidine negative; lack cytochromes, do not reduce nitrates to nitrite, are gelatinase negative and are unable to utilize lactate. Frequently they are termed the *Lactics* and constitute a diverse group of microorganisms associated with meat, plant as well as dairy products (Sharpe, 1981; Collins and Lyne, 1984).

Lactic acid bacteria are obligate fermenters and depend on carbohydrates for their energy supply. They produce lactic acid as their major end product. Although they are anaerobic, they are aero-tolerant and are at times referred to as micro-aerophillic (Schlegel, 1986). Although this group of bacteria is not strictly defined taxonomically, they are however phylogenetically related and have a number of physiological and ecological features in common. Lactic acid bacteria are composed of a number of genera including *Lactococcus, Lactobacillus, Enterococcus, Streptococcus, Leuconostoc,* and *Pediococcus* (Adams and Nicolaides, 1997).

2.6.2 ANTIMICROBIAL COMPOUNDS PRODUCED BY LAB

The antimicrobial compounds produced by LAB include many organic acids such as lactic, acetic and propionic acids produced as end products which provide an acidic environment; unfavourable for the growth of many pathogenic and spoilage microorganisms (Doores, 1993). The preservative action of starter strains in food and beverage systems is attributed to the combined action of a range of antimicrobial metabolites produced during a particular fermentation process. These have a very broad mode of action and inhibit both gram-positive and gram-negative bacteria as well as yeast and moulds (Caplice and Fitzgerald, 1999).

According to Adams and Nicolaides (1997) the various antimicrobial factors associated with LAB are as follows:

- Low pH
- Organic acids
- Bacteriocins
- CO_2
- Hydrogen peroxide
- Ethanol
- Diacetyl
- Low redox potential
- Nutrient depletion
- Crowding

2.6.3 ISOLATION AND IDENTIFICATION OF LAB

Lactic acid bacteria are chemoorganotropic and thus grow only in complex media. These organisms can easily dominate in an appropriate environment and they can be isolated by enrichment on selective media (Schlegel, 1986; Schleifer *et al.*, 1995). The cultivation of LAB may be accomplished using enrichment broths and selective or non-selective media, depending on a need to isolate either a particular genus from a mixture of microorganisms or to maintain isolates in culture. Although most of the LAB are acid tolerant, this property is not unlimited, therefore; because of the large amounts of lactic acid produced, the media must be well buffered; especially when pure cultures are to be maintained (Kandler and Weiss, 1986).

An agar medium that detects the type of lactic acid formed exists (Jehano *et al.,* 1992). The use of deMan, Rogosa and Sharpe (MRS) broth or agar has gained acceptance as the all-purpose media because of its ability to support a variety of LAB. The MRS name originates from the formula of deMan, Rogosa and Sharpe (1960). MRS broth may be used as a general culture medium and also as a basal medium and for performing tolerance tests such as temperature, pH, alcohol, salt and teepol concentration. Other biochemical tests based on MRS include arginine hydrolysis and acetoin tests (Riebel and Washington, 1990; Collins *et al.,* 1993). A pH of less than 4.5 may be used to differentiate *Lactobacillus* and *Pediococcus* from *Leuconostoc*; the latter requires a pH of 4.5 or greater. Both *Lactobacillus* and *Pediococcus* will grow at a pH of less than 4.5 (Holzapfel, 1992).

2.6.4 METHODS USED FOR THE TAXONOMY OF LAB

2.6.4.1 Phenotypic identification

Traditionally, LAB have been identified and grouped by means of key biochemical, physiological and morphological tests (Sharpe, 1979). Briggs (1953) based an early classification system for LAB on only six physiological tests which included the production of gas from glucose and citrate, hydrolysis of arginine, growth and survival at 15, 45, 48, 60 and 65˚C and the tolerance of 4, 6 and 8% NaCl.

The use of rapid identification systems, such as the API examines isolates for a wider range of carbohydrate fermentation characteristics; but because of the broad application of these systems for examining many different genera, many tests may be redundant. These methods by themselves therefore cannot be used for taxonomic studies of novel LAB isolates (Lee and Simard, 1984).

2.6.4.2 Serological classification

Serological classification of LAB has generally been restricted to the *lactobacilli* and *streptococci* of clinical origin only, since the differentiation of non-pathogenic LAB by means of serology is regarded as an unsatisfactory method of classification (Sharpe, 1979; Garvie, 1986). Serogroups are determined by the reactivity of particular species to antisera produced against whole cells of other species (Sharpe, 1979). Sharpe (1955) classified the *lactobacilli* into six groups and one sub-group on the basis of serology, but this method was not widely accepted (Kandler and Weiss, 1986).

2.6.4.3 Nucleic acid based techniques

Determination of the DNA base composition of LAB by electrophoresis is a well-known technique (Garvie, 1984). This technique however has limited use in bacterial taxonomy since the base composition of two biologically unrelated organisms as determined by these methods may be the same (Owen and Pitcher, 1985). Examination of bacteria at the molecular level by DNA homology techniques is generally regarded as one of the most reliable indications of natural relationships between different strains and represents the main criterion for species definition (O' Donell *et al*., 1988; Wayne *et al.,* 1987).

2.6.5 NOMENCLATURE OF LACTIC ACID BACTERIA

According to Marshall (1987), the LAB involved in the various food fermentations can be divided into two general categories based on their temperature requirements as follows:

2.6.5.1 Mesophilic organisms

This category of LAB is composed of those organisms that have an optimum growth temperature of 30˚C to 34˚C. These include the organisms under the two genera *Lactococcus* (formerly the Group N *Streptococci*) and *Leuconostoc*.

2.6.5.2 Thermophilic organisms

The organisms under this category are those with an optimum growth temperature of 35˚C to 40˚C. They include the two genera *Lactobacillus* and *Streptococcus*.

However, Kandler and Weiss (1986) state that LAB may also be called hetero-fermentative or homo-fermentative depending on the pathways they use to metabolise glucose (Embeden-Meyerhof or phosphoketolase pathways) and also on the resulting end product of their fermentation (Schleifer *et al.,* 1995).

2.6.6 CARBOHYDRATE METABOLISM AND FERMENTATION PRODUCTS OF LAB

Schlegel, (1986) grouped a number of LAB as either homo-fermentative or hetero-fermentative according to their end products of fermentation as well as the way in which they metabolise carbohydrates as their major source of energy metabolism.

2.6.6.1 Homofermenters

The homofermenters are also known as the homofermentatives and include the genera *Lactococcus* and *Pediococcus*. *Lactococcus* produces L (+) lactate whilst *Pediococcus* produces DL lactate. They possess the enzyme aldolase and are able to ferment glucose more directly to lactic acid as compared to the heterofermenters (Jay, 1986). These organisms use fermentable carbohydrates as energy sources and they then degrade these carbohydrates into lactate as the major end product of their fermentation (Schleifer *et al.,* 1995). Homofermenters are those LAB that produces more than 90% lactic acid as their major end product (Kandler and Weiss, 1986).

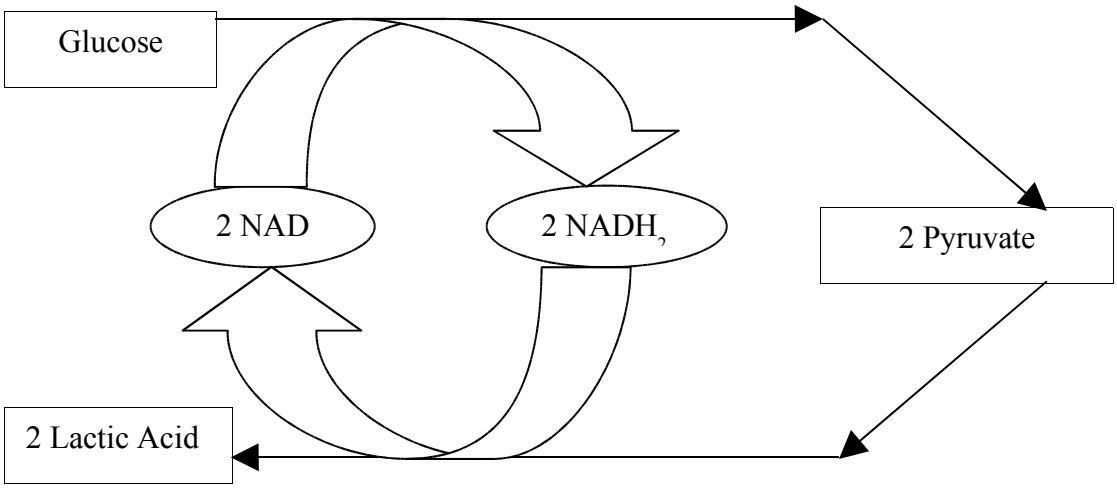

Figure 2.2 Homo-fermentative lactic acid fermentation (Marshall, 1987).

Figure 2.2 is a schematic presentation of the homo-fermentative pathway. In this pathway, only a small portion of pyruvate is decarboxylated and converted to acetic acid, ethanol, carbon dioxide or acetoin (Marshall, 1987). The extent, to which these subsidiary products are produced, depends on the oxygen supply. Thus, in a product made using homo-fermentative fermentation, low or rather undetectable levels of gas and volatile compounds are produced. Such a product is mainly characterised by a lactic acid flavour.

2.6.6.2 Heterofermenters

The heterofermenters are also known as the heterofermentatives and include the genus *Leuconostoc* and a subgroup of the genus *Lactobacillus* that is the *betabacteria*. *Leuconostocs* produce D (-) lactate whereas the betabacteria produce DL lactate. Heterofermenters use fermentable carbohydrates as energy sources and then degrade them into not only lactic acid but also produce gases and other organic acids such as acetate, ethanol, CO_2 and also formate or succinate (Schleifer *et al.,* 1995). They use the alternate pentose monophosphate pathway, to convert the six carbon sugars (hexoses) to five carbon sugars (pentoses) by the enzyme phosphoketolase producing in the process both aldehyde and diacetyl; which are highly desirable aromatic and flavour-enhancing substances. Heterofermenters are often used in the dairy industry because of these flavour-enhancing substances (Jay, 1986).

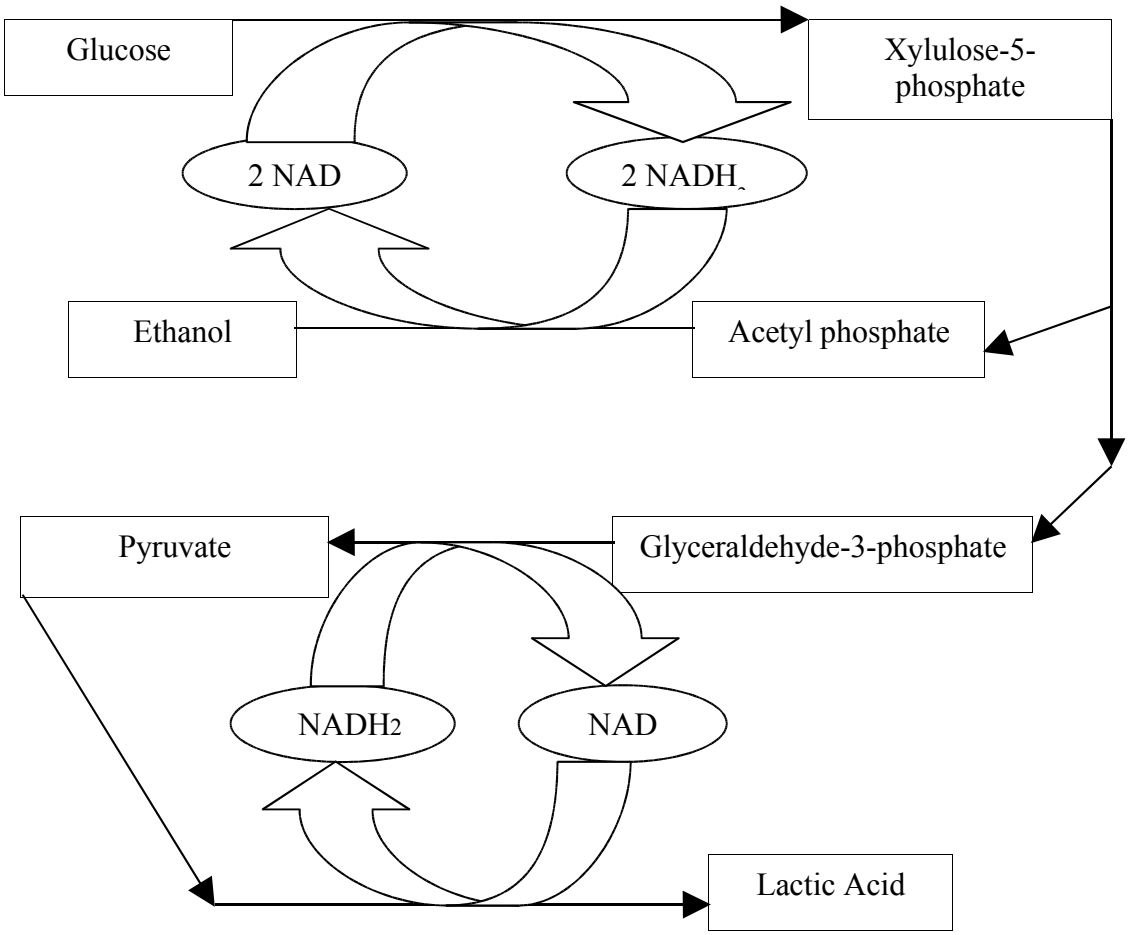

Figure 2.3 Hetero-fermentative lactic acid fermentation (Marshall, 1987).

According to Marshall (1987), the group of lactic acid bacteria that lack the necessary enzymes for the Embden-Meyerhof pathway are called hetero-fermentative. Figure 2.3 is a schematic presentation of the hetero-fermentative pathway. The initial glucose degradation is entirely through the pentose phosphate pathway via glucose-5-phosphate to xylulose-5-phosphate producing CO_2. The xylulose-5-phoshate is then cleaved to glyceraldehyde-3-phosphate and acetyl phosphate. The former is then reduced to lactic acid via pyruvate while the latter is degraded via acetyl CoA and acetaldehyde to ethanol. In some LAB, acetyl phosphate may also be converted to acetic acid. A hetero-fermentation is, therefore; characterized by the production of several products that include gas, acids and volatile compounds.

Table 2.3 Lactic acid bacteria grouped as either homo-fermentative or hetero-fermentative (Schlegel, 1986).

Genera and Species	**Homo-fermentative**	**Hetero-fermentative**
Lactobacillus		
L. leichmanii	+	-
L. lactis	+	-
L. helveticus	+	-
L. acidophilus	+	-
L. bulgaricus	+	-
L. delbrueckii	+	-
L. casei	+	-
L. plantarum	+	-
L. brevis	-	+
L. fermentum	-	+
Sporolactobacillus		
S. inulinus	+	-
Streptococcus		
S. salivarius	+	-
S. pyogenes	+	-
S. thermophillus	+	-
Leuconostoc		
L. mesenteroides	-	+
L. cremoris	-	+
Pediococcus		
P. cerevisiae	+	-
Lactococci		
S. lactis	+	-
S. cremoris	+	-
Enterococcus		
S. faecalis	+	-

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Chapter 3

Use of starter cultures of lactic acid bacteria for the production of *Ting*, a sour cereal fermented product from sorghum

3.1 ABSTRACT

Six isolates of lactic acid bacteria (*Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30) that were previously isolated from naturally fermented *Ting* were tested singly for their ability to ferment sorghum gruel to produce *Ting*. All the isolates showed an ability to ferment the gruel as judged by lowering the pH from 6.7 to between 4 and 4.5 within 12 h and increasing the titratable acidity from 0.07% to 0.7% within 72 h. The number of LAB increased from 7 to 9 log units during the first 12 h of the fermentation process in the starter culture inoculated *Ting* samples and thereafter remained constant around 10^9 and 10^{10} cfu for the entire 72 h fermentation period. There was also an increase in the fungal counts from 3 to 7 log units after 72 h. However, the number of coliform bacteria decreased to nil with an increase in fermentation period. The naturally fermented *Ting* sample however showed slower reduction of pH (6.5-3.7) and slower increase in titratable acidity (0.02-0.7) after 72 h. This sample also had low numbers of LAB and higher numbers of fungal colonies as compared to the starter culture inoculated samples. The number of coliform bacteria also decreased to nil in this sample, although the decrease was only after 24 h.

Key words: Fermentation, Lactic acid bacteria, Sorghum, Starter cultures, *Ting*

3.2 INTRODUCTION

According to Hammes (1990), fermented foods can be generally described as palatable and wholesome foods prepared from raw or heated raw materials by a process of microbial fermentation. Traditional fermented food products constitute a major portion of the people's diet in Africa and form part of the daily main dishes of the average individuals. However, they are also largely employed as weaning foods for young children. Most of these food products are cereal based and include porridges, bread and both alcoholic and non-alcoholic beverages (Oyewole, 1997). Many of the traditional foods in Africa are fermented before consumption and thus Africa is probably the continent with largest spectrum and richest variety of lactic fermented foods (Holzapfel, 1997). The most common fermented cereals in Africa include maize, sorghum, millet, tef, rice and wheat (Oyewole, 1997).

In Africa, food fermentation is still largely home based and is usually left to chance inoculation from the environment also known as spontaneous fermentation. In some cases, since there are no starter cultures employed; old stocks of previous ferments are used to initiate the fermentation in new batches. Fermentation vessels of previous ferments can also be used and these serve as the source of inoculum for the initiation of the fermentation in a process known as back-slopping. However, in both processes there is little involvement in the processing and the result thereof is usually a finished product of inconsistent quality, poor hygiene and doubtful safety (Onyekwere *et a*l., 1989; Oyewole, 1997). Therefore, in an attempt to address some of the problems associated with traditional foods in Africa, there is need to study the technological properties of relevance in food processing organisms associated with the fermentation of foods and thus the development of starter culture is one of the prerequisites for the establishment of small-scale industrial production of fermented foods in Africa (Sanni, 1993).

Among the various fermentation processes in Africa, lactic acid fermentation is one of the oldest and most widespread (Dirar, 1992). Lactic fermentation technology can be defined as the fermentation process involving the activities of a group of Gram positive, non-motile, catalase-negative, non-aerobic, organisms that are collectively

known as lactic acid bacteria (LAB). These organisms are able to ferment carbohydrates and thus producing lactic acid as their major organic acid (Oyewole, 1997). Lactic acid bacteria constitute a major part of microorganisms involved in the fermentation of most traditional foods in the continent and also constitute the majority of the volume and the value of commercial starter cultures. They are commonly involved either as a single group or in combination with other bacteria, yeasts and moulds in processes associated with indigenous fermented foods (Holzapfel, 1997; Hansen, 2002).

Metabolic by-products of LAB have been shown to inhibit the growth of several important pathogens and to increase product shelf life (Daeschel, 1989; Abee *et al.,* 1995). LAB fermented food products may also be healthy because of their probiotic effects; since several LAB genera such as *Lactobacillus*, *Leuconostoc* and *Lactococcus* have been proved to possess the probiotic activity. Probiotic species such as *Lactobacillus acidophilus* have been used safely for more than 70 years (Hammes and Tichaczek, 1994; Salminen *et al*., 1998).

According to Sanni (1993) and Kimaryo *et al*., (2000), the use of starter cultures would be an appropriate approach for the control and optimization of the fermentation processes in order to alleviate the problems of variations in organoleptic quality and microbiological stability observed in most African indigenous fermented foods. Holzapfel (2002) defines a starter culture as a preparation or material containing large numbers of viable microorganisms that may be added to accelerate a fermentation process. A typical starter culture facilitates the improved control of a fermentation process and also the predictability of the end products (Holzapfel, 1997).

Industrialized countries have benefited from a century of experience with starter cultures, particularly those selected for the large-scale industrial food fermentation (Holzapfel, 2002). Thus one of the prerequisites for the establishment of small-scale industrial production of fermented foods in Africa should be the development of starter cultures (Sanni, 1993). The knowledge gained with controlled starter cultures may also benefit those operating at a very small scale and practicing back slopping. A strong starter culture would probably minimize dry matter losses, avoid contamination

with pathogenic and toxigenic bacteria and moulds and also minimize the risk of incidental microflora that cause off-flavour. Some authors independently concluded that LAB originally isolated from certain food products would be the best choice for starter cultures for those same products, because they would be more competitive than LAB from other sources (Jeppesen and Huss, 1993; Vignolo *et al.,* 1993).

Cereal grains including sorghum, maize and millet are common substrates for lactic acid fermented gruels and beverages; with *Ting* being one example of such. *Ting* is a traditional fermented sour cereal porridge usually made from maize, millet or sorghum that is consumed in the West, Central, East and Southern Africa (Campbell-Platt, 1987). However, according to locality, cereal used and the area where it is consumed the same product can also be known by different names; *Ting* (South Africa), *Ogi* (Nigeria), *Uji* (Kenya and Tanzania), *Togwa* (Tanzania and Uganda) (Mugula *et al*., 2002; Steinkraus, 1996).

There are no LAB starter cultures commercially available yet for the small-scale processing of most traditional African foods (Holzapfel, 1997). There are however various studies in the development of starter cultures for other related cereal based fermented foods in other countries (Mbugua *et al.,* 1984; Okafor *et al.,* 1998; Kimaryo *et al.,* 2000; Teniola and Odunfa, 2001). Presently, there is no adequate information on the spectrum of microorganisms associated with the fermentation of sorghum to produce *Ting*. Such knowledge is essential for the development of a product with improved quality for increased consumption and commercial production and marketing. Therefore, the objectives of this study were to determine the application of LAB previously isolated from *Ting* as potential starter cultures for its production, determine the change in pH, amount of lactic acid produced and also the number of coliforms, fungi as well as LAB during the different fermentation cycles.

3.3 MATERIALS AND METHODS

3.3.1 Bacterial strains

Six strains of LAB that had been stored at 25ºC in MRS broth (Merck) were used as starter cultures during fermentation of sorghum for the production of *Ting*. These cultures were previously isolated and identified from naturally fermented *Ting*. The LAB isolates were *Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30 (Table 3.1).

3.3.1.1 Preparation of inoculum suspensions

Preparation of the inoculum suspensions to be employed as starter cultures was done according to a method by Mugula *et al.* (2003) although with slight modifications. The LAB isolates were first sub-cultured by streaking on MRS agar (Merck) and then incubated anaerobically (Anaerocult A, Merck) at 37°C for 24 h. A colony was picked from each pure culture plate and then grown in MRS broth (Merck) at 37°C for 24 h. The 24 h broth culture was then centrifuged at 655 X g/15 min (Eppendorf, 5804 R) and the supernatant liquid carefully tipped off. The pellet was then washed in sterile physiological salt solution (1/4 strength ringer); centrifuged again before tipping off the supernatant liquid. The resultant pellet was then redistributed into 10ml of the sterile ¼ strength Ringers solution.

3.3.2 Preparation of sorghum gruel

Sorghum grits used for purposes of this experiment were obtained from a local supermarket (Pick `n Pay Hatfield, Pretoria). These were weighed in weighing boats sterilized by wiping with 70% ethanol. The weighed sorghum grits were then combined with sterile tap water in six sterile flasks with plastic screw caps in ratios of 1:3 parts of sorghum to water respectively. The seventh bottle only contained normal tap water and sorghum in the same ratio as the previous samples although no control in was involved in this case. The ratio 1:3 parts of sorghum to water is an approximate of that which is used for the traditionally fermented *Ting* in the rural communities as was discovered during some informal interviews with some rural people who consume *Ting* as a staple food.

3.3.3 Fermentation

Sterile sorghum gruel in each of the six flasks was inoculated with washed cells of LAB (10 ml of each of the bacterial suspensions). No starter culture was introduced into the seventh flask and this was the control sample. Sorghum gruel in all seven flasks was allowed to ferment at 30°C for 72 hours. From all the seven flasks, 10 ml of fermentation sample was withdrawn at 6 h intervals starting at zero until 72 h for purposes of chemical and microbiological analyses. The samples are listed in table 3.2 below.

Table 3.2 Fermented *Ting* samples inoculated with different starter cultures of LAB

Sample	Starter culture
S1	*Lactobacillus collinoides* 1.42
S2	*Lactobacillus cellobiosus* 3.42
S3	*Leuconostoc mesenteroides* 2.35
S4	*Lactobacillus cellobiosus* 4.35
S5	*Lactobacillus cellobiosus* 3.30
S6	*Lactobacillus curvatus* 5.30

3.3.4 Chemical analysis

After every 6 h interval of the fermentation processes, equal amounts of samples from the six fermenting *Ting* gruels inoculated with different LAB starters as well as from the un-inoculated control were analyzed for change in pH and also the amount of acid present in each of the samples, which was expressed as percentage lactic acid.

3.3.4.1 pH measurements

The pH was determined with a pH meter equipped with a glass electrode. The pH meter was calibrated against standard buffer solutions at pH 4.0 and 7.0 (Merck). The pH meter was dipped into each of the 10 ml aliquots that had been withdrawn as described at top of page to measure the pH after each fermentation cycle.

3.3.4.2 Determination of the total lactic acid

The total amount of acid present in each of the fermented samples during the 6 h intervals was determined by titration against a 0.1 N NaOH (Byaruhanga, 1998); where 2 g of sample was measured in triplicate into 250 ml flasks. To this 8 ml of distilled water was added. Three drops of phenolphthalein indicator were then added and the mixture was thoroughly mixed. The mixture was then titrated against 0.1 N NaOH to a pink colour that then marked the end point. The percent lactic acid present in the samples was calculated using the formula:

$$\% \text{ lactic acid} = \frac{N \times V \times ME \text{ of lactic acid} \times 100}{\text{Weight of sample (g)}}$$

Where: N = the normality of the sodium hydroxide

V = sodium hydroxide in ml used to reach the titration end point

ME is the milli-equivalent of lactic acid

$$= \frac{\text{Molecular weight of lactic acid}}{1000}$$

$$= 0.09008$$

3.3.5 Microbiological analysis

The numbers of LAB, yeasts and moulds and also coliform bacteria were monitored in all the fermented *Ting* samples at the end of each of the 6 h fermentation cycles starting from 0 h until a total fermentation period of 72 h was achieved.

3.3.5.1 Preparation of dilutions

Tenfold serial dilutions of the samples were made by aseptically transferring 1 ml of sample into sterile 9 ml ¼ strength Ringer solution and thoroughly mixing (vortex) mixer; and thus resulting in a dilution of 10^{-1} (IDF, 1992). Further tenfold dilutions were made by transferring 1 ml of successive serial dilutions into test tubes containing 9 ml of sterile saline until a required dilution was reached.

3.3.5.2 Determination of LAB counts

MRS agar (Merck) was used for the enumeration of LAB. This medium was prepared according to the manufacturer's instructions and left to cool down to a temperature of approximately 55 °C +/- 5 °C.

Appropriately marked sterile petri dishes were inoculated with 100µl of the appropriate tenfold serial dilution of the samples and this was done in duplicate. MRS agar at room temperature (+/- 15 ml) was then added to the inoculated petri dishes. The plates were mixed by moving them in a circular movement on a flat horizontal surface before allowing them to solidify for about 30 minutes. The plates were then anaerobically incubated (Anaerocult A, Merck) in an inverted position at 37 °C for a period of 24 h. After the 24 h incubation period, the colonies were counted and expressed as number of colony forming units per millilitre (cfu/ml) of sample

3.3.5.3 Determination of fungal counts

Potato dextrose agar (Merck) was used for the enumeration of yeasts and moulds in the fermenting *Ting* samples. The medium was prepared according to the manufacturer's instructions and 12 to15 ml was equally distributed into marked petri plates. The plates were allowed to dry overnight.

The dried plates were inoculated with 100 µl of the relevant serial dilutions of each sample in ¼ strength Ringer solution as above. Each inoculum was then spread over the surface of the dried PDA plates using a sterile (dipped in ethanol and flamed) bent glass rod ("hockey stick"). The plates were then incubated in an inverted position at 28 °C for a period of 48 hours. After incubation, the colonies were counted and expressed as number of colony forming units per millilitre (cfu/ml) of sample

3.3.5.4 Determination of coliform counts

Violet red bile agar (Merck) was used for the enumeration of coliforms in the samples. The medium was prepared according to the manufacturer's instructions and distributed into marked Petri dishes. These were allowed to dry overnight at room temperature.

From appropriate serial dilutions in ¼ strength Ringers solution as previously; 100 µl amount of sample was inoculated onto VRBA plates, the procedure used was as used in the case of the yeast and moulds count. The plates were then incubated in an inverted position at 37°C for 24 hours. After the incubation period, the colonies were counted and expressed as number of colony forming units per millilitre (cfu/ml) of sample

For purposes of all experiments above, the samples were analysed in duplicate and the mean count thereof was recorded.

3.4 RESULTS AND DISCUSSION

3.4.1 Chemical analysis

3.4.1.1 pH

The changes in the pH of *Ting* during its fermentation are shown in Fig. 3.1. The initial pH of unfermented *Ting* gruel at 0 h was around 6.7 (Fig. 3.1). In the naturally fermented product S7, the pH remained above 4.5 for the first 24 h of the fermentation process (Fig.3.1). The pH in this same sample dropped off gradually below 4.5 from 30 h reaching a pH of around 3.7 after a period of 72 h (Fig. 3.1). However, the pH declined from around 6.5 to around 4.5 within the first 12 h of the fermentation process for all the samples inoculated with starter cultures of LAB (Fig. 3.1.). For all these samples, the pH then remained low (between 4 and 4.5) for the remainder of the fermentation process; the pH however, did not continue to drop as was the case with the naturally fermented sample.

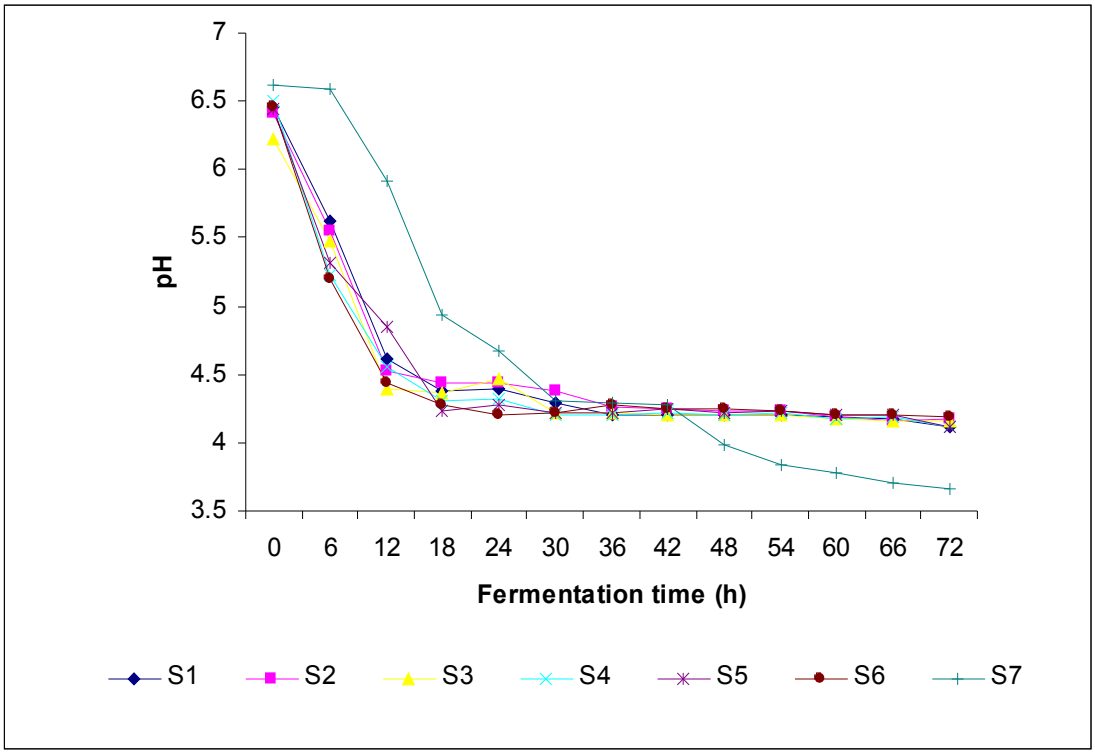

Figure 3.1 pH changes in fermenting *Ting* gruel inoculated with different starter cultures of LAB.

3.4.1.2 Lactic acid analysis

Figure 3.2. shows the changes in titratable acidity (expressed as % lactic acid) during the 72 h fermentation process at 30°C in samples of fermented *Ting* gruel inoculated with starter cultures of LAB (S1-S6) and also in the un-inoculated naturally fermented sample (S7). A general increase in titratable acidity was observed from 0, 07 to around 0.3 % during the first 12 h of fermentation in the starter culture inoculated samples. Thereafter, the amount of lactic acid present in these samples increased gradually up to about 0.7 % after 72 h of fermentation. However, in the un-inoculated control, the initial concentration of lactic acid was 0.02 % and increased to 0.3 % after 24 h. Thereafter, the amount of lactic acid present in this sample reached a value of 0.6 % after 72 h of fermentation. This was similar to the starter culture inoculated fermentation, which reached 0.7 % of lactic acid after the same time.

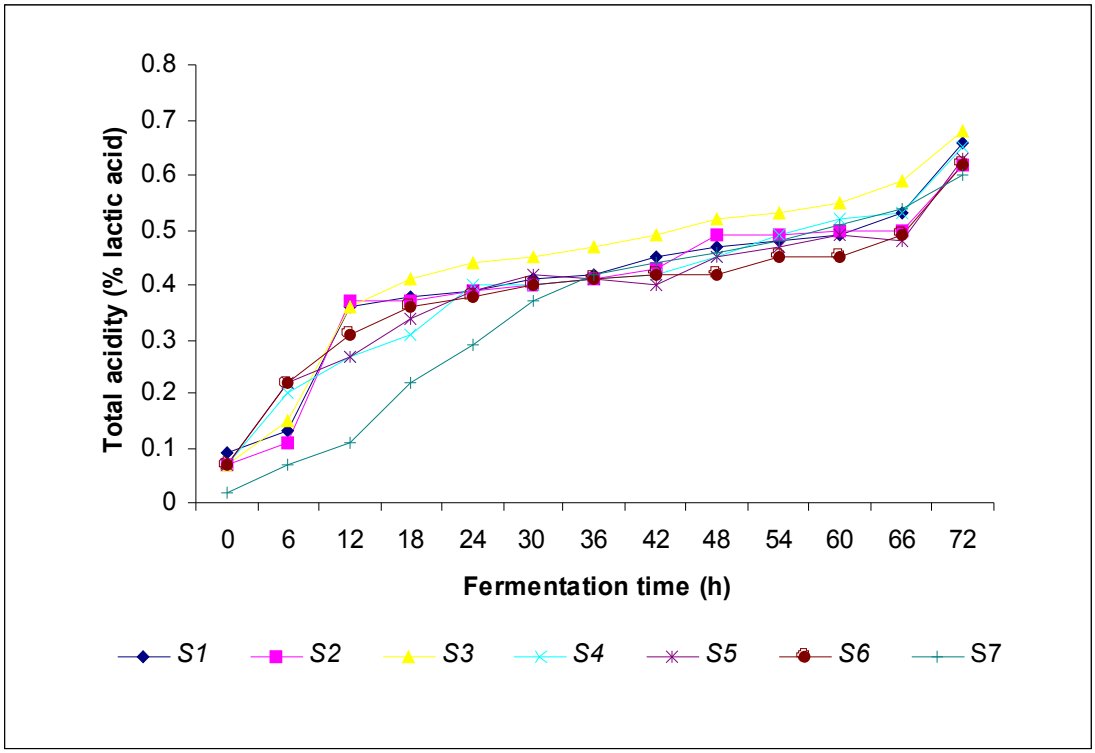

Figure 3.2 Acidity changes in fermenting *Ting* gruel inoculated with different starter cultures of LAB.

3.4.2. Microbial growth

3.4.2.1 Lactic acid bacteria

Bacterial counts of, *Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30; increased from 7 to 9 log units during the first 12 h of the fermentation process. At zero hour of the fermentation process, the number of LAB in the starter-inoculated samples was 10^7 cfu/ml; however in the naturally fermented sample, which acted as a control, there were very low numbers of LAB (e.g. 10^1 cfu/ml). After 12 h the number of LAB in the starter culture inoculated samples increased to 10^9 cfu/ml. The LAB counts remained around 10^9 cfu/ ml up to 54 h and thereafter increased by only 1 log unit until 72 h when the fermentation process was terminated. However, in the case of the naturally fermented sample, the LAB counts only reached 10^7 cfu/ml after 24 h and thereafter increased in no specific sequence up to 10^9 after 72 h (Fig. 3.3). This explains the delay in the pH decrease and acidity production in the naturally fermented product.

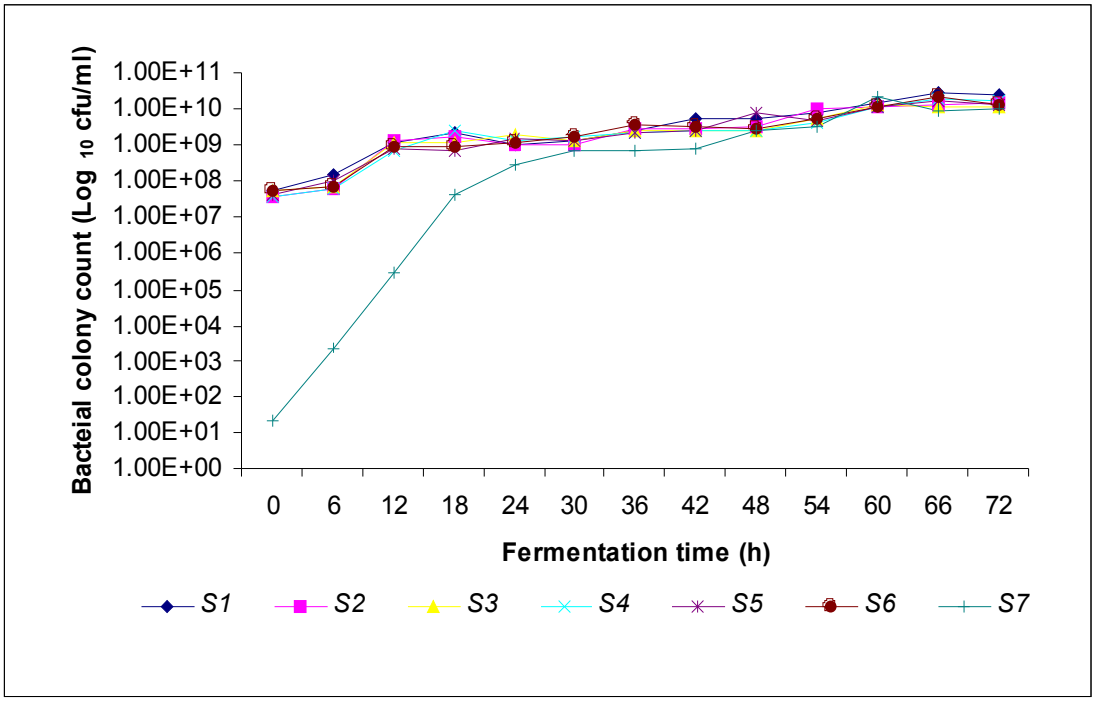

Figure 3.3 Time-course of LAB development in fermenting *Ting* gruel inoculated with different starter cultures of LAB.

3.4.2.2 Fungal counts

At 0 h of fermentation, fungal counts were 10^3 cfu/ml and increased by 1 log unit after 6h. Overall, fungal counts increased from 3 to 7 log units after 72 h in fermented samples inoculated with starter cultures of LAB. However, the un-inoculated naturally fermented sample had the highest fungal counts after 72 h of fermentation (Fig. 3.4).

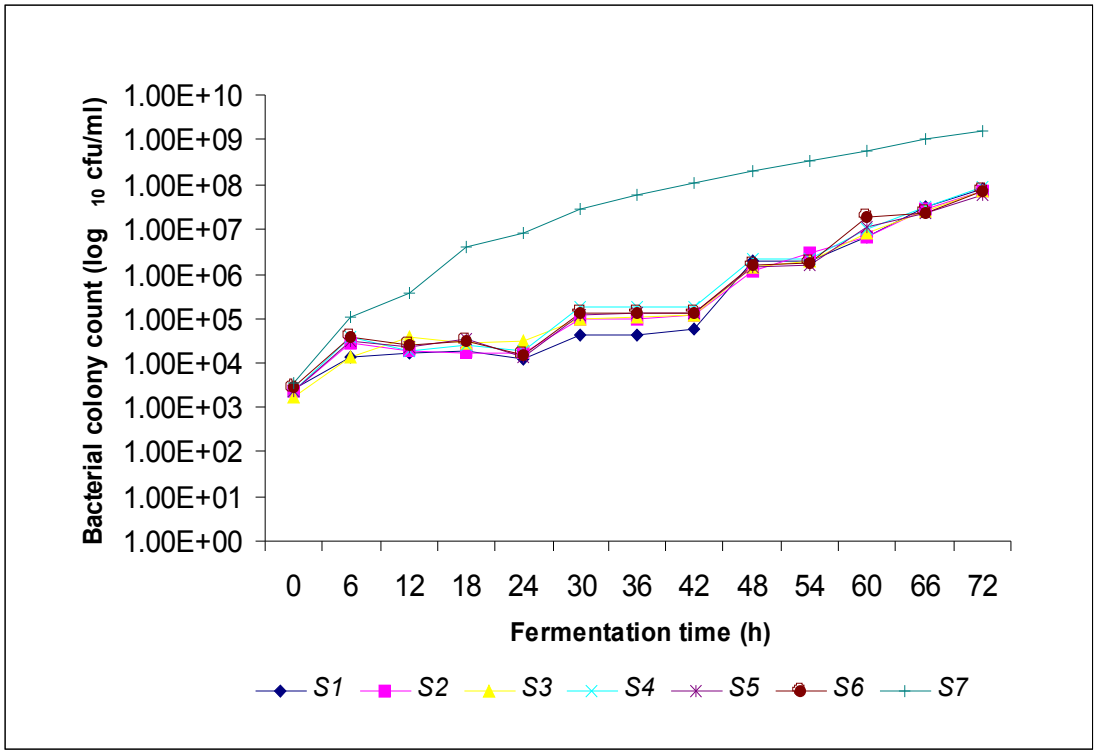

Figure 3.4 Time-course of fungi development in fermenting *Ting* gruel inoculated with different starter cultures of LAB.

3.4.2.3 Coliform counts

Figure 3.4.1 shows the number of coliforms determined in fermented samples inoculated with different LAB starter cultures. *Ting* gruel was contaminated with coliforms (10^3 cfu/ml). The coliform bacterial counts continued to increase by 1 log unit after 6 h and thereafter started to decline (Table3.1) in the fermentations inoculated with the starter cultures. In the un-inoculated naturally fermented sample, the number of coliforms only started declining after 30 h of fermentation (Fig. 3.4.2), and as soon as the pH decreased, the numbers of coliform bacteria disappeared.

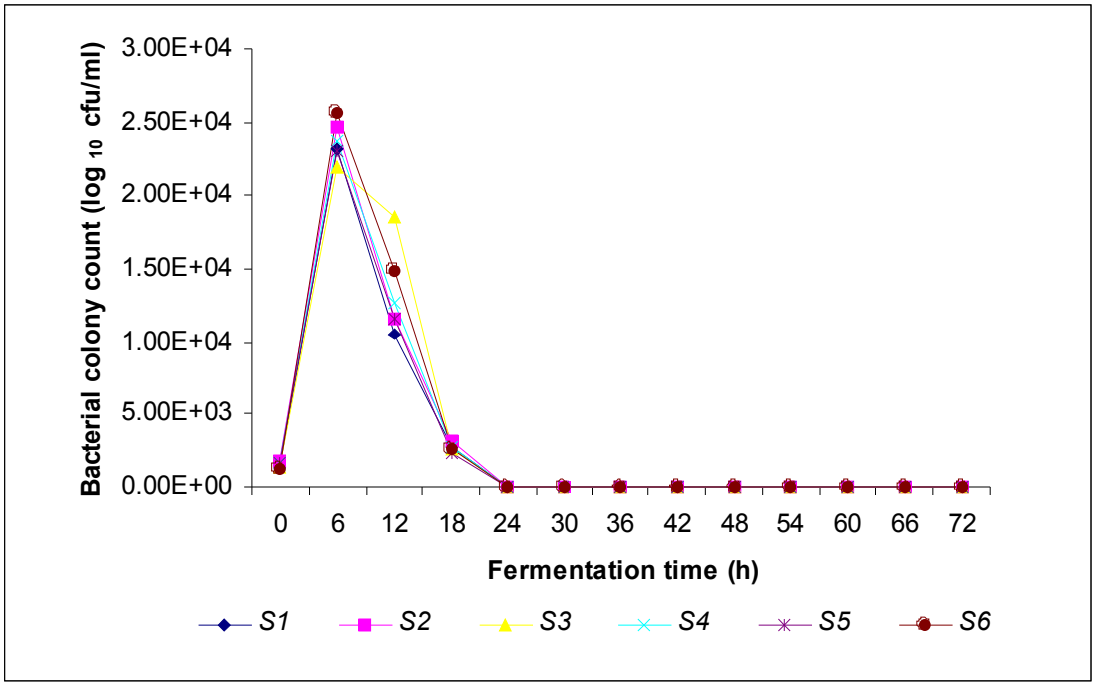

Figure 3.5.1 Time-course of coliform development in fermenting *Ting* gruel inoculated with different starter cultures of LAB

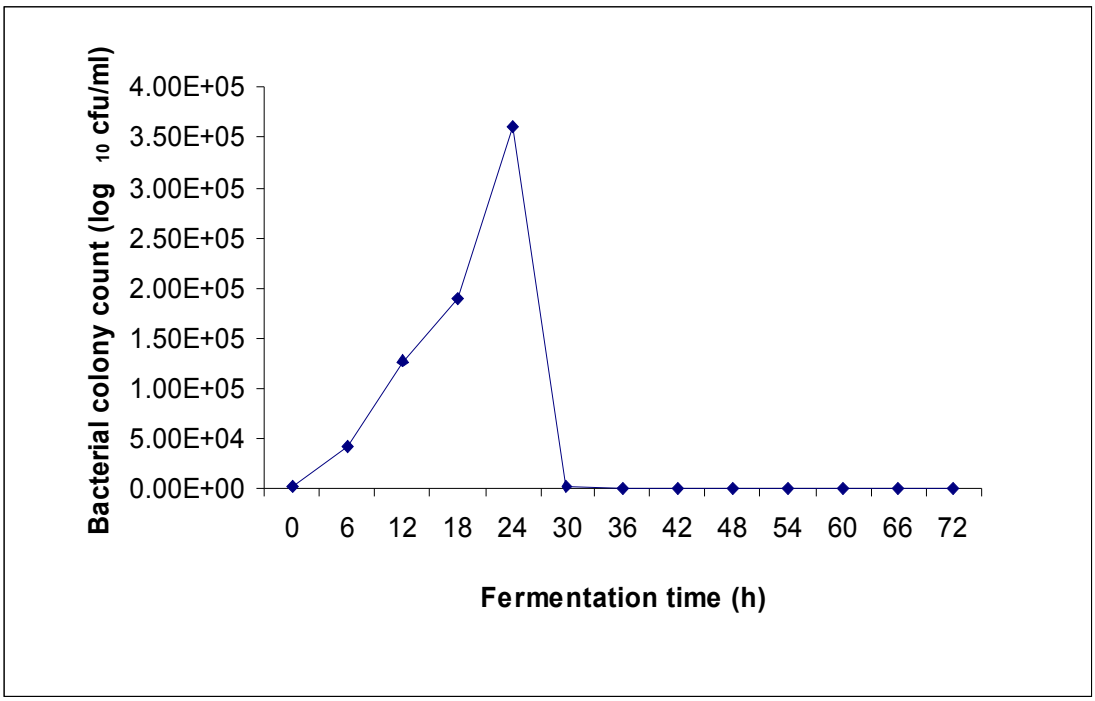

Figure 3.5.2 Time-course of coliform development in naturally fermented *Ting* gruel.

Table 3.3 The number of coliforms present in fermented *Ting* samples

Sample	Number of colonies (cfu/ml)						
	S1	S2	S3	S4	S5	S6	S7
Time (h)							
0	$1.3x10^3$	$1.8x10^3$	$1.3x10^3$	$1.7x10^3$	$1.6x10^3$	$1.2x10^3$	$2.4x10^3$
6	$2.3x10^4$	$2.8x10^4$	$2.1x10^4$	$2.4x10^4$	$2.3x10^4$	$2.6x10^4$	$4.2x10^4$
12	$1.1x10^4$	$1.2x10^4$	$1.9x10^4$	$1.3x10^4$	$1.7x10^4$	$1.5x10^4$	$4.9x10^4$
18	$2.7x10^3$	$3.1x10^3$	$2.6x10^3$	$2.7x10^3$	$2.3x10^3$	$2.6x10^3$	$1.9x10^5$
24	ND	ND	ND	ND	ND	ND	$3.6x10^5$
30	ND	ND	ND	ND	ND	ND	$1.6x10^4$
36	ND	ND	ND	ND	ND	ND	ND
42	ND	ND	ND	ND	ND	ND	ND
48	ND	ND	ND	ND	ND	ND	ND
54	ND	ND	ND	ND	ND	ND	ND
60	ND	ND	ND	ND	ND	ND	ND
66	ND	ND	ND	ND	ND	ND	ND
72	ND	ND	ND	ND	ND	ND	ND

cfu/ml = colony forming units per millilitre of sample; ND= Not Detected.

3.5 CONCLUSION

In the starter culture inoculated samples, the pH decreased after 12 h and thereafter was more or less stable between 4 and 4.5; probably this was because there was no competition amongst microorganisms in these samples and also as a result of the high number inoculated. However, in the case of the naturally fermented samples, the pH continued to decrease. Here there might have been competition amongst the various organisms present in the naturally fermented product such that they inhibited the fermentation process until one organism became dominant and out-competed the others by a process of natural selection and succession. It was also attributed to the natural inoculum which had a limited number of bacteria (10^1 cfu/ml initially).

There was an increase in the number of LAB and yeasts in fermented *Ting* over a period of 72 h. This may be due to the proliferation of yeasts in foods, which is favoured by the acidic environment created by LAB. According to Mugula *et al.* (2003), the presence of yeasts stimulates the growth of bacteria and may also provide growth factors such as vitamins and nitrogen compounds. The association of LAB

and yeasts during fermentation may also contribute metabolites, which could give desirable taste and flavour to foods. Production of acids and other antimicrobial components in the cereal gruel during fermentation may also improve microbiological safety of the resulting fermented product as was indicated by the elimination of coliforms in the fermentations.

The results obtained indicated that, judging from the lowering of pH value and production of lactic acid; all the six LAB isolates could be used singly as starter cultures to produce *Ting* within a shorter fermentation period of 6-12 h instead of 24 h and longer. However, the potential of mixed cultures of the same isolates in fermentation of *Ting* could be exploited. Molecular techniques will also be used in future research to further identify the isolates.

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Chapter 4

Sensory analysis of fermented *Ting* inoculated with starter cultures of lactic acid bacteria

4.1 ABSTRACT

Sensory evaluation was conducted on seven samples of *Ting*. The first six *Ting* samples were produced using starter cultures of lactic acid bacteria (LAB) that were previously isolated from *Ting*. The cultures were *Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30. The seventh sample was naturally fermented for a period of 72 h. The aim of this work was to determine the consumer acceptability of the starter culture produced *Ting* samples as compared to the un-inoculated naturally fermented *Ting* sample. A total of 50 volunteers were recruited within the University of Pretoria community, to participate in the sensory analysis of *Ting* samples. A nine point hedonic scale was used to rate each of the seven samples in terms of appearance, colour, taste, sourness and overall acceptance. There were no significant differences in consumer acceptance between the fermented *Ting* sample inoculated with a starter culture of *Lactobacillus cellobiosus* 4.35 and the naturally fermented *Ting* sample; these were the most preferred samples of all seven. The least preferred sample of all seven samples was the one inoculated with a starter culture of *Lactobacillus cellobiosus* 3.42.

Key words: *Ting*, Sensory evaluation, Starter cultures, Lactic acid bacteria.

4.2 INTRODUCTION

Cereals are particularly important substrates for fermented foods in all parts of the world and are staple foods in Africa, the Indian Subcontinent and Asia. Fermented foods are palatable products prepared from raw or heated raw materials acquiring their characteristic properties by a process in which microorganisms are involved (Hammes, 1990). This occurs through a process of fermentation. Fermentation is one of the oldest techniques used to preserve foods. The process can also cause major changes in food quality indices including colour, aroma, flavour, texture, appearance, nutrition and safety. Such changes are brought about by the metabolic activities of different kinds of microorganisms. One example of such is a group of bacteria collectively known as lactic acid bacteria (LAB) (Beuchat, 1995). These organisms play a major role during most food fermentations and are as such among the most important groups of microorganisms in food processing. This is mainly due to their established roles in flavour development and preservation of foods (Sandine, 1987).

In addition to altering the flavour, texture and appearance of foods, LAB are also known to inhibit the growth of other organisms thus reducing contamination (Hammes and Tichaczec, 1994). In most instances, the development of desired sensory qualities in indigenous fermented foods depends on a composite contribution of two or more types of microorganisms. Although LAB are very common in the sequence of fermentative microorganisms; other microorganisms such as yeast and moulds are also often present during food fermentations. Yeasts and moulds are also necessary for proper flavour and aroma development (Beuchat, 1995).

Lactic fermentation of foods of plant or animal origin, which results from these organisms, is a widely accepted method of preservation. This process may also result in the production of desirable sensory and nutritional properties in the fermented product (Cooke *et al.,* 1987). According to Beuchat (1995), LAB can either be commonly involved as a singular group or in combination with other bacteria and yeasts and moulds in processes associated with indigenous fermented food products. Yeasts and moulds are sometimes also necessary for proper flavour and aroma development.

According to Carpenter *et al*. (2000), the term sensory analysis can be described as the identification, scientific measurement, analysis and interpretation of the properties of a product; as perceived through the five senses of sight, smell, taste, touch, and hearing. In addition to chemical and microbiological parameters, sensory properties of food are also extremely important. They can also contribute to studies in the development of proper flavour compounds during the process. These properties are the ones that determine consumer acceptance and thus are essential in terms of quality (Kuti *et al*., 2004).

The previous experiments in chapter 3 showed that the six isolates of LAB when used as starter cultures can reduce the fermentation period of *Ting* in a similar pattern. However, the samples might have different organoleptic characteristics and there is a need to evaluate this aspect. Therefore, this work aimed to evaluate the sensory acceptance of fermented *Ting* inoculated with different starter cultures of LAB and the un-inoculated naturally fermented *Ting* and also to use statistics to determine if there are significant differences in the consumer acceptability of the products.

4.3 MATERIALS AND METHODS

4.3.1 Experimental design

Six strains of LAB previously isolated and identified from naturally fermented *Ting* were used in this study. These were employed as starter cultures for the initiation of *Ting* fermentation. The organisms were *Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30 (Table 3.1). *Ting* is a sour cereal fermented product produced from different cereals; maize, sorghum or millet. This product is consumed in the West, Central, East and Southern Africa. This same product is also known by different names according to locality, cereal used and also the area where it is consumed (Campbell-Platt, 1987). However, for purposes of this experiment, *Ting* was prepared using sorghum grits purchased from a local supermarket (Pick `n Pay, Hatfield, Pretoria).

Preparation of inoculum suspensions for the initiation of *Ting* fermentation was carried out according to a method by Mugula *et al*. (2003) with slight modifications as described in the previous chapter under sub-heading 3.3.1.1. Ten millilitres of each of the six LAB inoculum suspensions in Ringers solution was then inoculated into the sorghum gruels contained in six flasks prepared as in the previous chapter under sub-heading 3.3.2.

4.3.2 Fermentation and preparation of *Ting*

Inoculated sorghum gruel in all six flasks was allowed to ferment at 30º C for a period of 12 h after which a pH that was below 4.5 was reached in each of the samples. However, the naturally fermented sample was left to ferment for 72 h at room temperature after which it also had a pH that was below 4.5 as in the case of the other starter culture inoculated samples. This then also acted as a control since no LAB starter culture was inoculated. The pH was measured with a pH meter equipped with a glass electrode. The pH meter was calibrated against standard buffer solutions at pH 7.0 and 4.0 (Merck).

After fermentation was terminated, all seven fermented sorghum gruels were cooked in seven steel pots on an electric stove at the same temperature conditions (75°C). This was achieved by boiling the fermented sorghum gruel in water until the starch gelatinized and thickened into porridge. The thickened porridge was left for a few minutes at low heat in order to allow the sorghum to be well cooked and absorb enough heat before consumption. *Ting* samples contained in the steel pots were then removed from the heat and left to cool slightly at room temperature (+/- 55°C).

After cooling, equal amounts from each of the seven cooked *Ting* samples were packed into appropriately marked plastic containers. These were then used for sensory analysis.

4.3.3 Sensory analysis

Sensory evaluation of fermented *Ting* samples was carried out to determine their organoleptic characteristics. This was conducted on all of the six *Ting* samples produced with starter cultures of LAB as well as on the seventh naturally fermented

Ting sample in order to evaluate the consumer acceptability of the different samples in terms of their appearance, colour, taste, sourness and overall acceptance. A nine point hedonic scale, varying from "dislike extremely" (score 1) to "like extremely" (score 9) was used according to Stone and Sidel (1992).

A total of 50 panellists participated in the study and these were from the University of Pretoria community; including university students and staff. The panellists were all volunteers in this regard and 60% were females. Although there was no formal training of panellists prior to tasting, a majority of them were familiar with *Ting* and actually consumed it in their homes as a staple food.

Ting samples were served side by side in random order in plastic containers that were coded with three digit numbers. In addition to differentiating amongst the different samples, the three digit codes were used to blindfold the panellists and to avoid bias. The panellists were then instructed to determine their acceptance of *Ting* samples presented to them. Each panellist evaluated all seven samples of *Ting* and scored them for appearance, colour, taste, sourness and overall acceptance using the score sheets presented to them (Figure 4.1). Sample tasting was carried out in two groups each consisting of 25 people.

4.3.4 Statistical analysis

Friedman's analysis of variance test using a significance level of $\alpha<0.05$ was applied to the results of the hedonic tests to determine whether or not the preferences were significantly different. The Friedman test is a nonparametric test that compares three or more paired groups. It is also called the Friedman two-way analysis of variance by ranks (O`Mahony, 1986).

All statistical analysis was performed using BMDP Statistical Software.

4.4 RESULTS AND DISCUSSION

Mean scores obtained for sensory evaluation of the seven fermented *Ting* samples are presented in Table 4.1.

Tables 4.2.1 to 4.2.5 show the frequency of the hedonic scale scores for appearance, colour, taste, sourness and overall acceptance obtained for the seven different fermented *Ting* samples. Figures 4.2.1 to 4.2.5 further presents frequency percentages of hedonic scale scores for only three of the fermented *Ting* samples (S2, S4 and S7). This is because these were the samples that showed the most statistically significant differences as compared to all the other four samples (S1, S3, S5 and S6).

4.4.1 Appearance

The fermented *Ting* sample S2 that was inoculated with a starter culture of *L. cellobiosus* 3.42 had the lowest mean of 5.20 in terms of appearance (Table 4.1). The sample that had the highest mean of 7.00 in this regard was S4; this was the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 4.35. The fermented *Ting* sample S3 that was inoculated with a starter culture of *Leuc. mesenteroides* 2.35 had the second highest mean of 6.74 (Table 4.1).

There were statistically significant differences between sample S2 and samples S4 and S7; which represented fermented *Ting* samples inoculated with starter cultures of *L. cellobiosus* 3.42, *Leuc. mesenteroides* 2.35, *L. cellobiosus* 4.35 and the un-inoculated naturally fermented *Ting* sample respectively. According to Friedman`s analysis of variance test, there were statistically significant differences between samples S2 and S3 (Table 4.1) and also between samples S2 and S4 that were at the 95% level ($p<0.05$). However, in the same regard; significant differences between samples S2 and S7 were at 90% level ($p<0.10$).

The frequency of hedonic responses for appearance were more concentrated between scores 7 and 9 for the fermented *Ting* sample that was inoculated with a starter culture of *L. cellobiosus* 4.35 (S2) and also for the un-inoculated naturally fermented *Ting* sample (S7) (Figure 4.2.1). Friedman's analysis of variance test indicated that the

appearance of the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus 4.35* was acceptable just as the appearance of the un-inoculated naturally fermented *Ting* sample which acted as a control in this regard.

For the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42; the frequency of responses were more concentrated at score 4 (Figure 4.2.1) and this also confirmed the results of its appearance where this sample had the lowest mean. These results show that the panellists preferred *Ting* sample S2 the least in terms of appearance as compared to all the other fermented *Ting* samples, especially sample S4 whose appearance was the most preferred.

4.4.2 Colour

The sample that had the lowest mean of 5.34 in terms of colour (Table 4.1) was the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2). The fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 4.35 (S4) had the highest mean score of 7.00 followed by the naturally fermented *Ting* sample (S7) with a mean score of 6.80 (Table 4.1).

Statistically significant differences between the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2) and another fermented *Ting* sample that was inoculated with a starter culture of *L. cellobiosus* 4.35 (S4) and also between the sample S2 and the un-inoculated naturally fermented *Ting* sample (S7) were both at 95% level ($p<0.05$).

For the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 4.35 (S4) and the un-inoculated naturally fermented *Ting* sample (S7) the frequency of responses in terms of how the respondents rated the colour of these two samples were concentrated between scores 7 and 8 (Figure 4.2.2). However, for the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2), the frequency of responses were more concentrated between scores 5 and 7.

Frequency results obtained for the three samples in terms of how the respondents rated their colour also confirm the results obtained for colour where fermented *Ting* sample

S2 was the least preferred whereas *Ting* samples S4 and S7 were the most preferred ones.

4.4.3 Taste

Fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2) had the lowest mean of 5.32 (Table 4.1). In this regard, in terms of the taste of the different fermented *Ting* samples, the highest mean of 6.92 was found to be in the un-inoculated naturally fermented *Ting* sample (S7) (Table 4.1). The second highest mean score of 6.62 was for the fermented *Ting* sample S4 (Table 4.1). This sample was inoculated with a starter culture of *L. cellobiosus* 4.35, prior to its fermentation.

Statistically significant differences between fermented *Ting* samples inoculated with starter cultures of *L. cellobiosus* 3.42 (S2) and the fermented *Ting* sample inoculated with starter cultures of *L. cellobiosus* 4.35 (S4) and also between sample S2 and the un-inoculated naturally fermented *Ting* sample were at the 95% level ($p<0.05$).

The frequency of responses in terms of taste for the sample inoculated with a starter culture of *L. cellobiosus* 4.35 (S4) were more concentrated between the scores 7 and 8 in terms of the taste (Figure 4.2.1). However, in the case of the un-inoculated naturally fermented *Ting* sample (S7), the frequency of the responses were more concentrated between the scores 7 and 9 (Figure 4.2.1). In the case of the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42; the frequency of the respondents were more concentrated in the score 6.

The results obtained for fermented *Ting* sample S4 and S7 were similar. These results show that a large percentage of the respondents preferred the taste of samples S4 and S7 rather than that of the sample S2 and thus also confirming the results obtained above for the mean values of the samples.

4.4.4 Sourness

In terms of the sourness of the different fermented *Ting* samples, the sample with the lowest mean of 5.46 was the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2). As was the case with taste, the un-inoculated naturally

fermented *Ting* sample (S7) again had the highest mean of 6.56 for sourness as well. The second highest mean score of 6.20 was for the fermented *Ting* sample S4 that was inoculated with a starter culture of *L. cellobiosus* 4.35 (Table 4.1). The same sample S4 also had the second highest mean score secondary to sample S7 as in terms of taste. Statistically significant differences between all the tested samples for sourness were only between samples S2 and S7. Statistically significant differences between these samples were at the 90% level ($p<0.05$).

Figure 4.2.4 clearly illustrates that the frequency results of the respondents for sourness was more concentrated between the scores 6 and 8 for the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 4.35 (S4). Similar results in frequency were observed for the un-inoculated *Ting* sample (S7). This *Ting* sample also had a concentration of frequency of respondents at score 7. Together these results show that the respondents preferred the sourness of the two samples S4 and S7.

The fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2), in terms of sourness was almost the same as S4; although the frequency of respondents was only concentrated at score 6. This showed that a high percentage of the respondents in this regard slightly preferred the sourness of this sample. However, considering the mean comparison test results obtained for this sample it is clear that although the sample's taste was slightly preferred, it was overall still the least preferred one in comparison with all the other fermented *Ting* samples.

4.4.5 Overall acceptance

In terms of the overall acceptance of all the seven fermented *Ting* samples, the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2) had the lowest mean score of 5.24 (Table 4.1). The highest mean score of 6.64 was found to be in the sample S4, which was the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 4.35 (Table 4.1). The second highest mean score of 6.62 was for the overall acceptance of fermented *Ting* sample S7 that was the one with no LAB starter culture introduced. This sample was second highest in terms of mean scores for overall acceptance and colour secondary to sample S4.

Statistically significant differences between the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2) and that inoculated with a starter culture of *L. fermentum* 42.4 (S4) was at the 90% level ($p<0.10$). However, the differences between sample S2 and the un-inoculated naturally fermented *Ting* sample was at the 95% level ($p<0.05$).

The frequency of responses were more concentrated between scores 7 and 8 for the fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 4.35 (S4) in terms of overall acceptance of the product (Figure 4.2.5). The un-inoculated naturally fermented *Ting* sample (S7) also had a high concentration in frequency of respondents at score 9 (Figure 4.2.5). The fermented *Ting* sample inoculated with a starter culture of *L. cellobiosus* 3.42 (S2) was least preferred since the frequency of responses was more concentrated at 4 (Figure 4.2.5).

The overall acceptance of the samples S4 and S7 was similar. This similarity between the overall acceptance of fermented *Ting* samples S4 and S7 is also confirmed by the results obtained for the mean of the samples in terms of their overall acceptance.

Table 4.1 Mean scores for sensory acceptance of different fermented *Ting* samples

	S1	S2	S3	S4	S5	S6	S7
Appearance	6.12_a	5.20_b**	6.74_b**	7.00_b**	6.12_a	6.26_a	6.70_b*
Color	6.18_a	5.34_b**	6.50_a	7.00_b**	6.30_a	5.86_a	6.80_b**
Taste	6.06_a	5.32_b**	6.12_a	6.62_b**	5.86_a	5.84_a	6.92_b**
Sourness	5.82_a	5.46_b*	6.16_a	6.20_a	5.96_a	5.78_a	6.56_b*
Overall acceptance	6.00_a	5.24_b*	6.28_a	6.64_b*	5.68_a	5.78_a	6.62_b**

1) For purposes of all experiments in this chapter, the samples S1 to S7 represents different fermented *Ting* samples as illustrated in Table 3.2.
2) Means followed by different subscript letters are significantly different by Friedman analysis of variance test at either **95% level ($p<0.05$) or at *90% level ($p<0.10$).

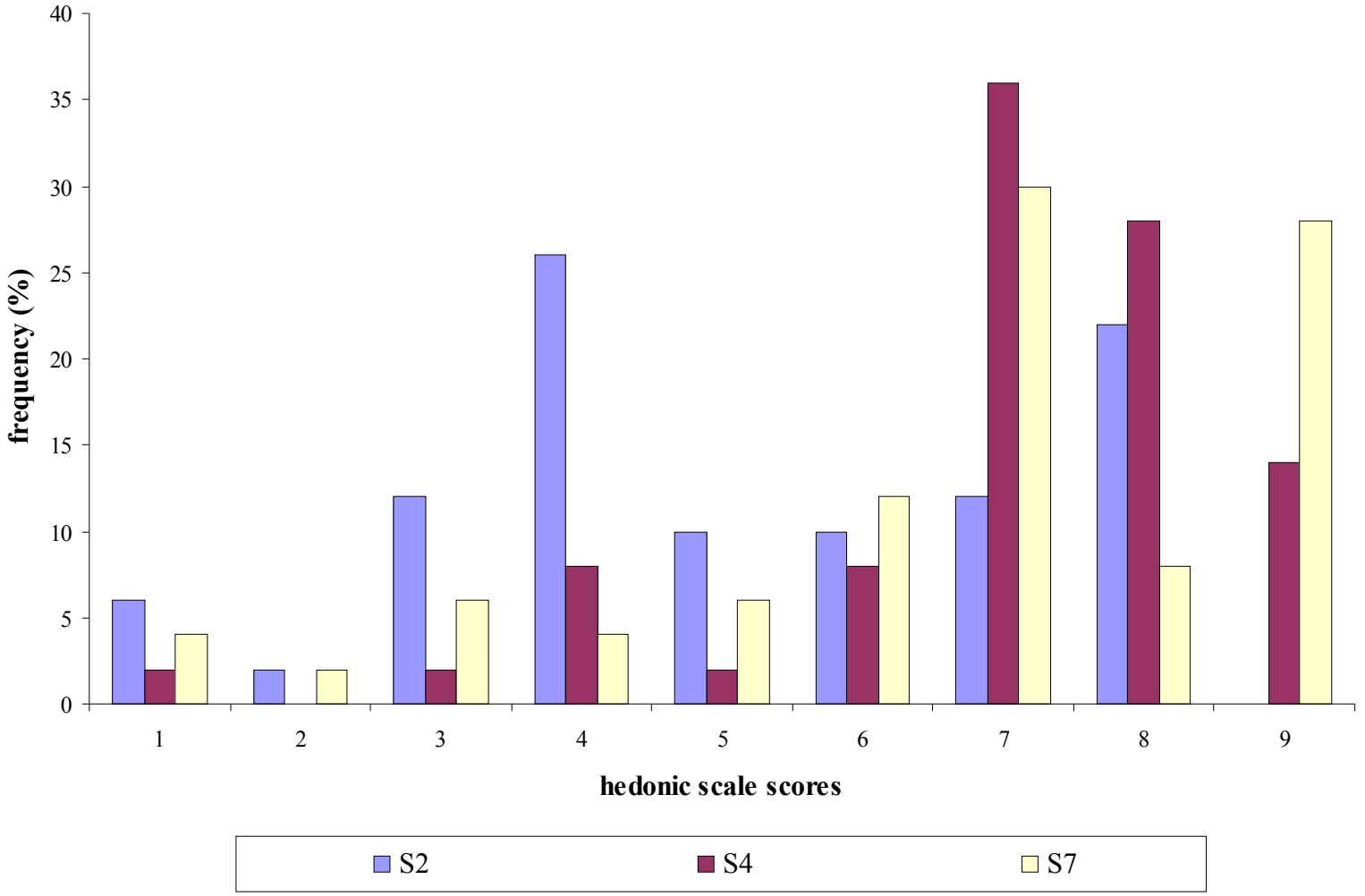

Figure 4.2.1 Frequency of hedonic scale scores of fermented *Ting* samples for appearance (1=dislike extremely; 9=like extremely)

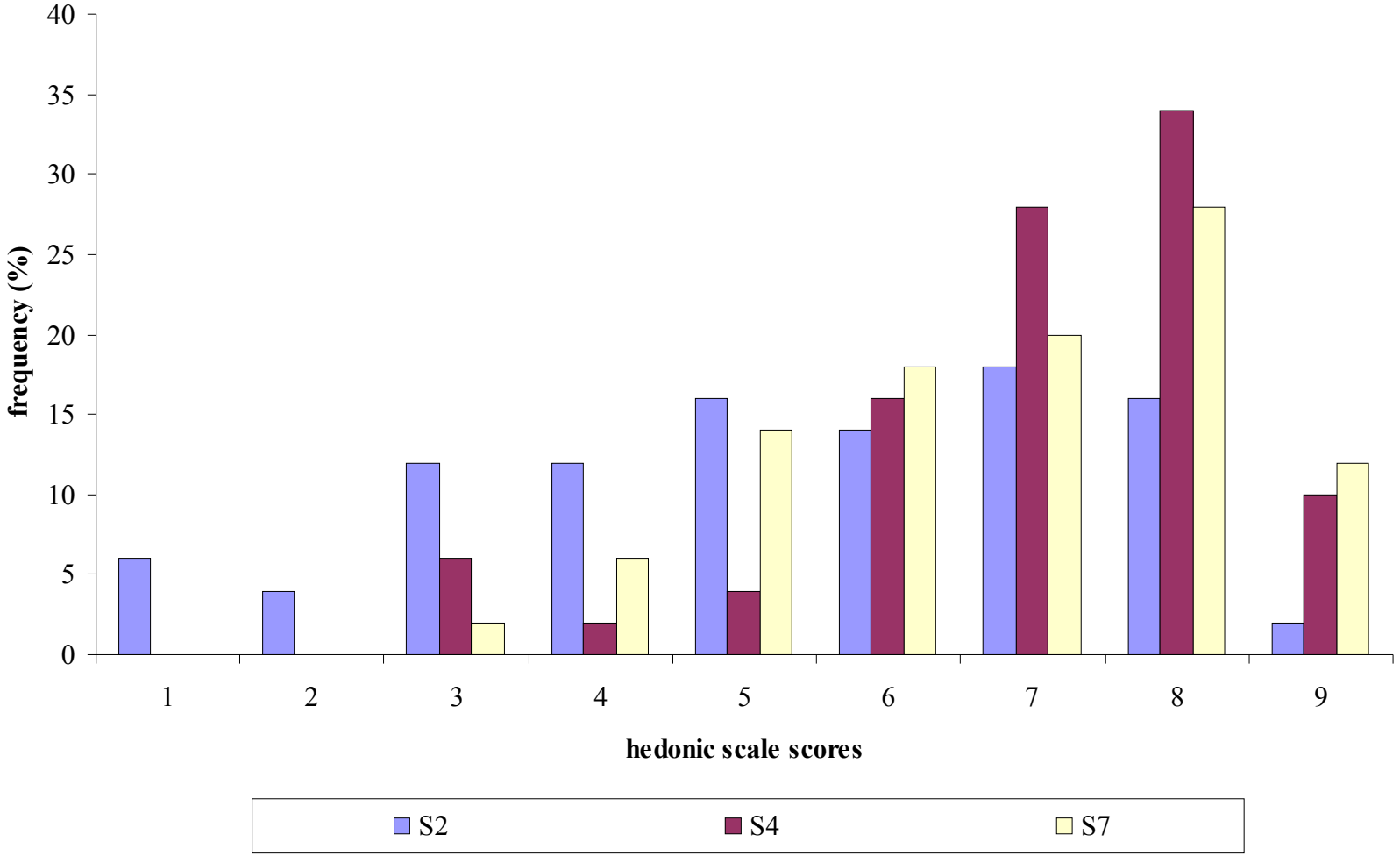

Figure 4.2.2 Frequency of hedonic scale scores of fermented *Ting* samples for colour (1=dislike extremely; 9=like extremely)

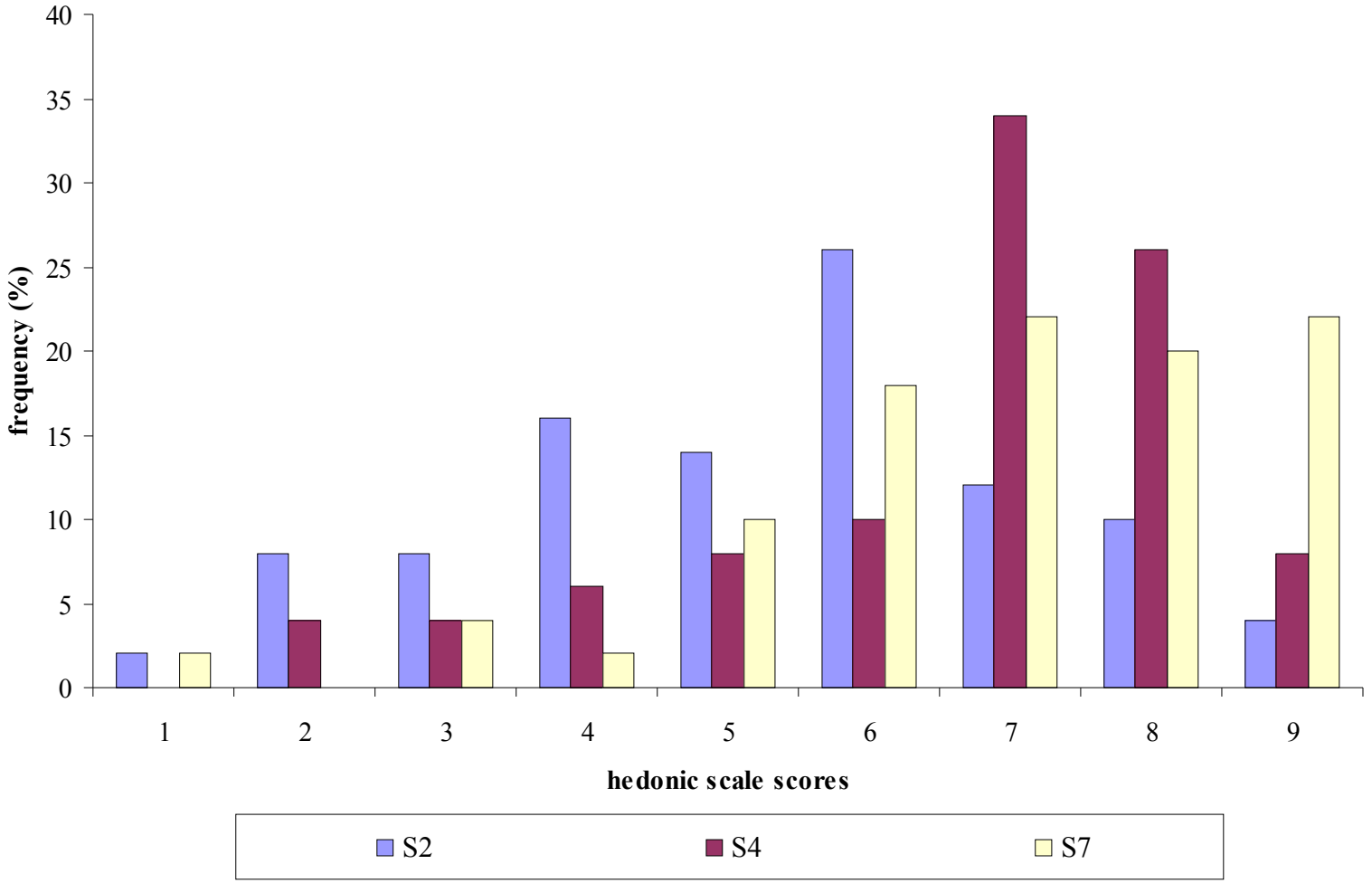

Figure 4.2.3 Frequency of hedonic scale scores of fermented *Ting* samples for taste (1=dislike extremely; 9=like extremely)

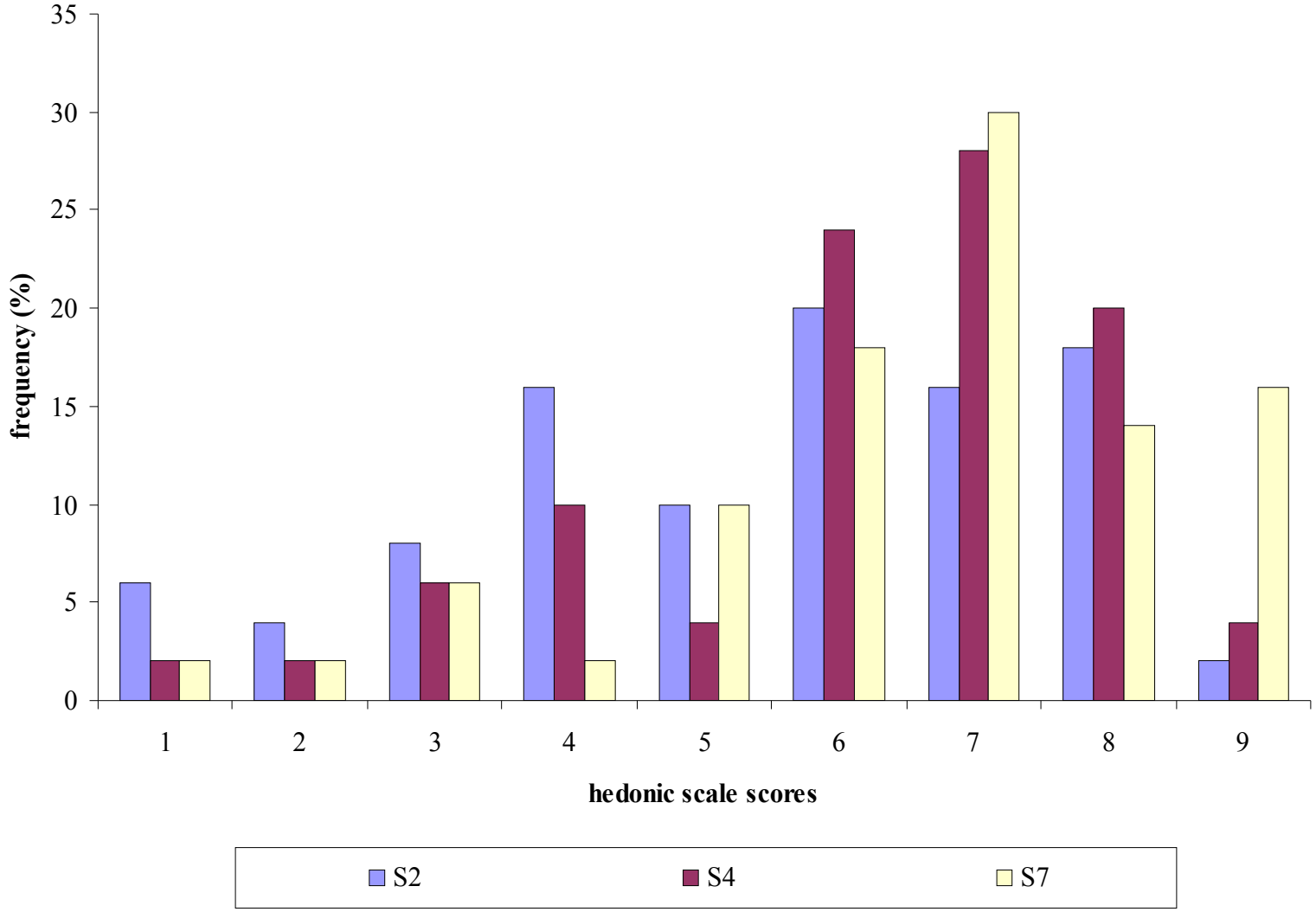

Figure 4.2.4 Frequency of hedonic scale scores of fermented *Ting* samples for sourness (1=dislike extremely; 9=like extremely)

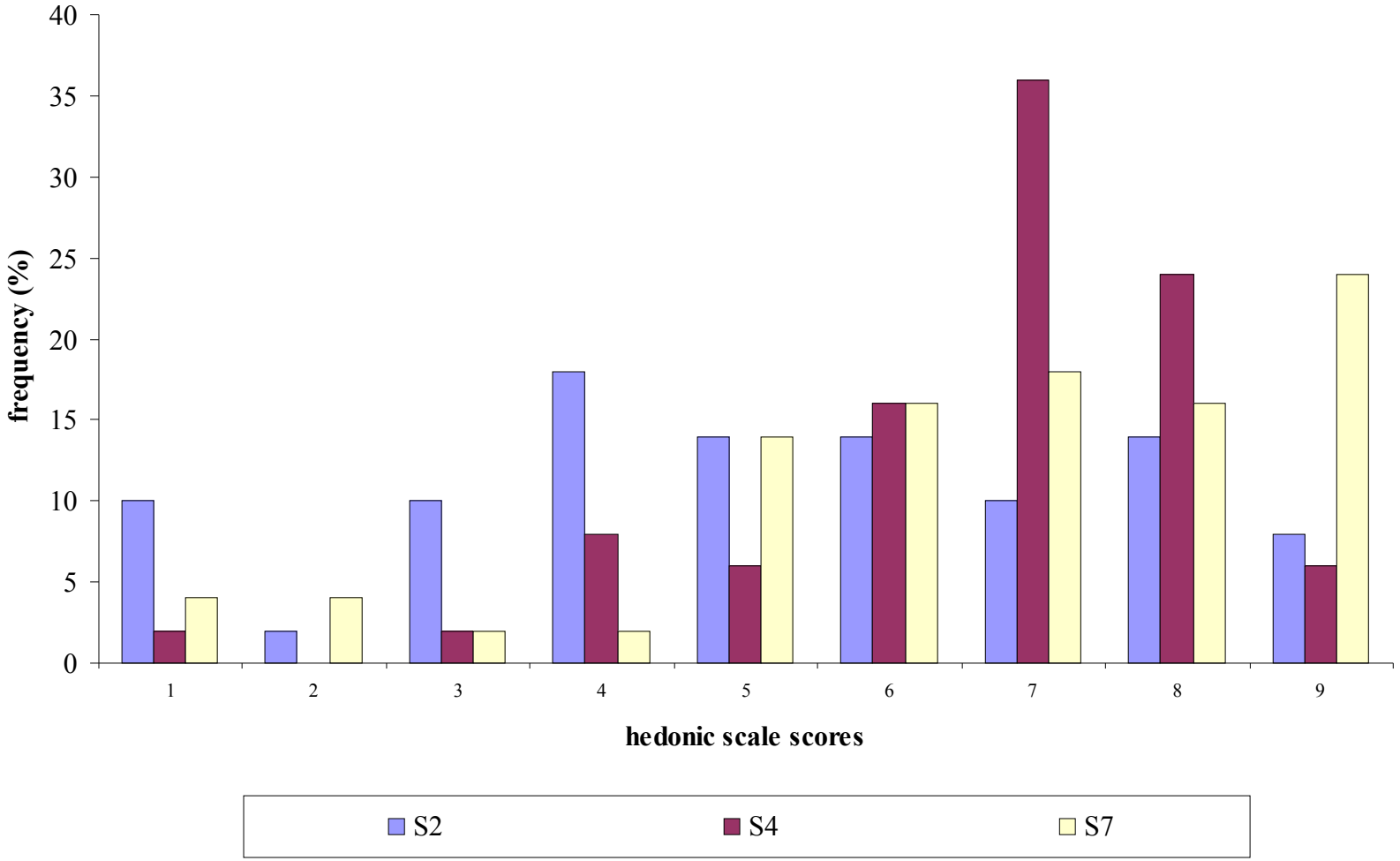

Figure 4.2.5 Frequency of hedonic scale scores of Fermented Ting samples for overall acceptance (1=dislike extremely; 9=like extremely)

4.5 CONCLUSION

Sensory acceptance results indicated that consumer's acceptance to the naturally fermented *Ting* sample and fermented *Ting* samples inoculated with different starter cultures of LAB varied depending on the starter culture inoculated. Sample S4 was the most preferred one having the highest acceptance scores for its appearance, colour and overall acceptance. Sample S2 was the least preferred of all the seven tested samples with the lowest acceptance scores. The taste and sourness of the un-inoculated naturally fermented *Ting* sample were the most preferred. This sample received the highest acceptance scores of all the samples tested.

Although sample S4 received the highest acceptance scores for appearance, colour and overall acceptance whilst sample S7 had the highest acceptance scores for taste and sourness; the two samples showed many similarities in terms of consumer acceptance. In all instances, where either one of the samples S4 or S7 had the highest acceptance scores; the other sample would have the second highest, score except for appearance, where S3 had the second highest score following S4. However, even in this instance the sample S7 still had a higher acceptance score in relation to S4 than the other three remaining samples.

According to the results obtained, for the production of a starter culture to initiate the fermentation of *Ting*, the organism *L. cellobiosus* 3.42 would not be the best option since it does not result in desirable sensory attributes. However, *L. cellobiosus* 4.35 would be the best option for the production of such a starter culture because it had sensory characteristics that were closely related to those of the un-inoculated naturally fermented *Ting* sample as compared to the other six samples. Since sample S4 had desirable sensory attributes related to the naturally fermented *Ting*, the organism *L. cellobiosus* 4.35 can thus be employed in the production of *Ting* starter culture resulting in a reduced fermentation period of 12 hours instead of 72 hours and still yield similar sensory characteristics to the naturally fermented *Ting*.

The results obtained in this regard also support a conclusion by Jeppesen & Huss, 1993 and Vignolo *et al.,* 1993; that LAB originally isolated from certain food products would be the best choices as starter cultures for those same products. The sensory

attributes obtained for the different fermented *Ting* samples may be useful in future studies. Further studies should be done on these starter organisms to evaluate the effectiveness of combined starter cultures of LAB in *Ting* production since these might result in more acceptable sensory acceptance to consumers.

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Chapter 5

NUTRITIONAL ANALYSIS OF *Ting*

5.1 ABSTRACT

Ting was prepared in the laboratory with starter cultures of LAB (*Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30). The aim of this work was to determine the nutritional composition of different samples of *Ting* in terms of the amount of crude protein, moisture, crude fat, dry matter, ash, fiber, gross energy, amino acids and also the minerals calcium, phosphorous, magnesium and iron. All samples analysed had a pH below 4.5 and total lactic acid of 0.7% after 72 h of fermentation. The nutritional content of different samples of *Ting* was determined and the products were found to be high in protein and a good source of energy. However, the minerals analysed were almost in a similar composition with phosphorous being the highest and calcium the lowest. In general, fermentation did not result in any significant change in the total nutrient composition of the products.

Key words: Lactic acid bacteria, Starter cultures, Fermentation, Nutritional analysis.

5.2 INTRODUCTION

In many developing countries, especially in tropical Africa, infant weaning foods as well as foods for adults are based on local staple diet made from cereals, roots and tubers of cassava and potatoes. These foods are usually prepared as thick porridges for adults or as liquid gruels for infants (Sanni *et. al.,* 1999). One such product is *Ting,* a fermented sour cereal porridge made from sorghum through a process of natural fermentation (Campbell-Platt, 1987). Natural fermentations are spontaneous processes caused by microorganisms derived from the raw materials, or from the environment itself (Buckenhűskes, 1993). There is very little or no control at all during such processes.

The major cereals grown in Africa include maize, rice, sorghum and millet. Cereals are more widely utilized as food in African countries than in the developed world. They account for as much as 77% of the total caloric consumption in African countries (Mitchell and Ingro, 1993) and also contribute substantially to dietary protein intake in a number of these countries. A majority of traditional cereal based foods consumed in Africa are processed through natural fermentation.

Fermentation was one of the first methods used by man to produce and preserve foods. Microbial fermentations have played an important role in food processing for thousands of years. The process of fermentation provides a way to preserve food products, to enhance nutritive value, to destroy undesirable factors, to make a safer product, to improve the appearance and taste of some foods, to salvage material otherwise not usable for human consumption and also to reduce the energy required for cooking (Lopez, 1992). The microbial interactions therefore play an important role in the nutritional, safety and sensory characteristics of the end product (Hall, 1989).

Most cereals are limited in essential amino acids such as threonine, lysine and tryptophan and thus making their protein quality poorer compared with animal products (Horn and Schwartz, 1961). During fermentation, the microorganisms secrete hydrolytic enzymes into the substrates and assimilate some of the fatty acids,

amino acids, and simple sugars thus liberated. These are converted into microbial structural components and secondary metabolites. Lactic acid fermentation is an ancient process whereby a varied group of bacteria known as lactic acid bacteria (LAB) ferment carbohydrates, producing lactic acid as their major end product. During fermentation, lactic acid accumulates, with a concomitant increase in acidity and decrease of dry matter yields. Due to the crude methods of analysis, the proximate composition of foods does not change much during fermentation. The proteolytic activity of bacteria in traditional fermentations degrades complex proteins into simpler proteins, peptides and amino acids (Lopez, 1992). Fermented foods are also expected to be more digestible than their unfermented counterparts.

Although there has been extensive research on most fermented weaning foods from several African countries, not many reports exist on traditional fermented foods consumed in the rural and informal settlements of South Africa. However, these products continue to be the major basic diets of adults and infants in most of the developing countries. One example of such products is *Ting,* a sorghum based product that is consumed in most parts of South Africa. Such knowledge is essential for the development of *Ting* with improved nutritional quality. There is great need of efforts to be directed towards improving the nutritional status of the food products. The objective of this research was to determine the nutritional composition of *Ting.*

5.3 MATERIALS AND METHODS

5.3.1 Sample preparation

A total of eight samples were prepared for the determination of the nutritional quality. The first six fermented *Ting* samples were prepared by mixing sorghum and water in a ratio of 1:3 and inoculating with six different starter cultures each of LAB to initiate the fermentation process. The samples were then allowed to ferment for 12 h at a temperature of 30º C. Another sample of naturally fermented *Ting* was prepared by mixing sorghum and water in the same ratio as above but no starter culture was introduced and the sample was allowed to ferment for 72 h. The last sample was unfermented.

All eight samples were placed in aluminium containers and left in a freezer overnight. The samples were then removed from the freezer and placed in a freeze drier for 5 days. Freeze dried samples were milled with an automatic grinder to pass through a sieve with circular openings of 1mm diameter. These were then stored in airtight containers and kept at room temperature until analysed.

For purposes of all the samples analysed, unless otherwise specified; the experiments were done in duplicate. Furthermore, unless otherwise specified, the products were analysed by standard methods (AOAC, 1984).

5.3.2 Dry matter determination

Crucibles were dried in the oven for one hour and then cooled for at least 30 minutes in a desiccator. These were then weighed to determine the dry mass of the empty crucibles. One gram of sample was weighed into each of the crucibles. The sample containing crucibles were then placed in an oven to dry for 24 hours at 105º C. After 24 hrs, the crucibles were again allowed to cool in a desiccator for 30 minutes and then the mass of the crucible plus sample was determined and recorded.

The percentage dry matter was calculated using the following formula:

$$\% \text{ dry matter} = \frac{\text{mass of sample after drying}}{\text{initial mass of sample}} \times \frac{100}{1}$$

5.3.3 Moisture

The moisture content was determined by the AACC-44-19 method (American Association of Cereal Chemists, 1983). This was calculated from the results obtained for the dry matter determination above. Loss in weight was reported as moisture.

The following formula was used to calculate the percentage moisture:

$$\% \text{ moisture} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times \frac{100}{1}$$

5.3.4 Ash determination

Determination of ash in the samples was done according to the AACC method 08-01 (American Association of Cereal Chemists, 1983).

Crucibles with dry sample from the dry material determination were placed in a furnace. They were incinerated at 600º C for 4 hours. After the 4 hrs, the furnace was switched off and allowed to cool off overnight to a temperature below 250° C before removing the crucibles and placing them in a dessicator to cool off for half an hour. After cooling, the mass of the crucible plus ash was weighed and then recorded. The ash content was then calculated by mass difference.

The percentage ash present in the samples was calculated as follows:

$$\%\text{Ash} = \frac{(\text{Weight of crucible + ash}) - (\text{Weight of empty crucible})}{\text{Sample weight}} \times \frac{100}{1}$$

5.3.5 Crude protein

The amount of crude protein present in each of the *Ting* samples analysed was determined using the Dumas method (AOAC, 2000). The principle of the method is that N_2 is freed by pyrolysis and subsequent combustions; it is then swept by a CO_2 carrier into a nitrometer. CO_2 is then absorbed in KOH and the volume of residual N_2 is then measured and converted to equivalent protein by a numerical factor.

Approximately 0.2 g of each sample was weighed onto tin foil that was then carefully folded and inserted into a Leco F428 that then measured the amount of protein in the samples.

5.3.6 Crude fat determination

Crude fat was determined by the Soxhlet method 30-10 (American Associatiation of Cereal Chemists, 1983). The principle of the method is that petroleum ether with a boiling point of 40-60°C dissolves all the lipid material in the sample and then evaporates over a boiling water bath leaving the lipid material as sediment in the container. The amount of fat extracted is then calculated by mass difference.

Approximately 3 g of each sample analysed was weighed into a Whatman filter paper no.1. The sample containing filters were folded and placed in extraction thimbles that were then placed in the Soxhlet extraction unit. Clean flat-bottomed flasks that were dried in an oven overnight were allowed to cool down in a dessicator for half an hour before their mass was determined. The flasks were then filled with approximately 175 ml petroleum-ether. The flat-bottom flask and extraction tubes were connected to the apparatus and the extraction lasted for 4 hrs. The flat-bottomed flasks with fat were dried in an oven at 70ºC for 1 h. These were then cooled in a dessicator for 30 min before their mass was determined. A difference between the mass of the flask before and after extraction is the mass crude fat. All experiments for the crude fat determination were done in triplicate.

The percentage of crude fat in the samples was calculated as follows:

$$\% \text{ Fat} = \frac{\text{Mass crude fat}}{\text{Sample mass}} \times \frac{100}{1}$$

5.3.7 Crude fibre determination

The determination of crude fibre was done as described by the AOAC (2000), using a Fibertec 2010, hot extractor. Crude fibre is defined as the residue after treatment with hot H_2SO_4 (concentration 1.25%) and hot NaOH (concentration 1.25%).

Approximately 1g of sample was weighed into clean and dry "sintered glass" crucibles. The sample containing crucibles were then placed in the hot extraction unit ensuring that all the crucibles fitted snugly and thus could not be turned by hand. All valves of the system were placed in a closed position before the condenser cooling water system was turned on. To each crucible, 150 ml warm 0.128M H_2SO_4 was then added into the condensation tube above the crucible, with a funnel and the heating element was turned to maximum. The sample was then brought to boiling point and the heat was adjusted to 450º C and boiled for 30 min. The heating element was then turned off after 30 min and the sample was drained of excess liquid using vacuum pump filters.

Thereafter, the sample was flushed three times with 30 ml warm distilled water per flush. The second solution, which was NaOH, was added to the samples and then left to boil for 30 min at 450º C as before, prior to rinsing three times with 30ml warm distilled water per flush, and then filtered through a dry vacuum pump. The crucibles were then placed in a drying oven where they were dried overnight at 105ºC before cooling in a dessicator for 30 min. The mass of each crucible plus sample was determined and recorded before ashing the samples in a furnace at a temperature of 550ºC for three hours. The furnace was left to cool down overnight to a temperature below 250ºC, before removing the samples and cooling them in a dessicator for 30 min before determining the mass of the crucible plus ash.

The percentage crude fibre was calculated using the following formula:

$$\% \text{ Crude fibre} = \frac{Wrd - Wra}{Ws} \times \frac{100}{1}$$

Where: Wrd = weight of residue after drying in crucible
Wra = weight of residue after ashing in crucible
Ws = weight of initial sample

5.3.8 Neutral detergent fibre determination

Neutral detergent fibre (NDF) is defined as the residue after treatment with a neutral detergent solution (Cetyl trimethylammonium bromide in sulphuric acid solution). The NDF determinations were done as described by the AOAC (1980). All experiments for NDF determination were done in triplicate.

Approximately 1 g of each sample was weighed into sintered glass crucibles before placing them on a warm extraction unit in such a way that each fitted tightly. The water-cooling system was opened to ensure that the reagent did not boil over. The NDF solution was then added into the condensation tube above the crucible, with a plastic bottle. The lid of the heating unit was then placed into position and the heating element turned to maximum only to bring the samples to a boiling point; and

thereafter reducing the temperature to 550º C and the samples continued to boil at 550º C for 1 h.

After the samples had boiled for an hour, the heating element was then turned off, and the excess solution filtered from the samples using a vacuum pump. The samples were then flushed three times with warm distilled water per flush. The crucibles were then placed into the drying oven and dried overnight at 105ºC. Thereafter, the samples in crucibles were cooled down in desiccators for 30 minutes before determining their mass. The samples were then ashed at a temperature of 550ºC for three hours in a furnace. After allowing the furnace to cool down to a temperature below 250ºC, the crucibles were removed and then left to cool down in desiccators for 30 minutes before determining their mass.

The percentage NDF in the samples was calculated using the following formula:

$$\% \text{ Neutral detergent fibre} = \frac{Wrd - Wra}{Ws}$$

Where: Wrd = weight of residue after drying in crucible
Wra = weight of residue after ashing in crucible
Ws = weight of initial sample

5.3.9 Gross Energy determination

The gross energy was determined using a water bomb calorimeter (MC 1000). Approximately 0.5 g of the samples to be analysed was weighed and each placed in a clean metal crucible. A wool string was attached to the wire between the electrodes and the crucibles were placed on the o-ring of the cap. The other points of the string were inserted into the crucible and covered with the sample. The wool string helps in the ignition of the sample. The bomb cap together with the sample in the metal crucibles were placed in the bomb and the sample mass entered into the computer. Before the bomb can ignite the sample, it goes through different stages i.e. testing temperature, pre-period 1 and 2, bomb fired, main period, cooling and washing the bomb. The bomb runs for approximately 5 min to ignite the sample and then the gross energy is shown on the screen of the computer.

5.3.10 Minerals

The minerals that were analysed were calcium (Ca), phosphorus (P), magnesium (Mg) and iron (Fe).

5.3.10.1 Sample preparation

Approximately 0.5 g of each sample was weighed in triplicate into clean dry 250 ml calibrated volumetric flasks. The samples were wet ashed for the analysis of the above-mentioned minerals using nitric acid (HNO_3) and perchhloric acid ($HCLO_4$) solutions. Twenty-five ml of HNO_4 was added to every tube before placing these on the extraction block digestor (BD-20; capable of maintaining a temperature of 410ºC and digesting 20 samples at a time in 250 ml calibrated volumetric tubes constricted at top) for 10 min after which it was removed and cooled down for five minutes before adding 10 ml $HCLO_4$ and placing it on the block for further 20 min. The samples were cooled for 30 min before diluting each test tube to 50 ml with distilled water. The samples were stored in acid washed glass bottles with screw tops until analysed.

5.3.10.1.1 Calcium

For the determination of the amount of Ca present in the samples, 5X dilutions of the digested samples were made in 0.25% lanthanum chloride ($LaCl_3$). The amount of Ca was then determined using an atomic absorption spectrophotometer (Perkin-Elmer 5100 PC) with a photon hollow cathode lamp (P809) at a wavelength of 422.7 nm.

5.3.10.1.2 Phosphorus

The amount of P in the digested samples was determined colometrically using an Auto Analyser (Technicon Autoanalyser II); with molibdovanadate used as a colour reagent.

5.3.10.1.3 Magnesium

For the determination of the amount of Mg present in the samples, 5X dilutions from the digested samples were made in 0.25% lanthanum chloride ($LaCl_3$). The amount of Mg was then determined using an atomic absorption spectrophotometer (GBC 905AA) with a photon hollow cathode lamp (P831) at a wavelength of 285.2 nm.

5.3.10.1.4 Iron

The amount of iron in the samples was determined using an atomic absorption spectrophotometer (GBC 905AA) with a photon hollow cathode lamp (P826) with a wavelength of 248.3 nm.

5.3.11 Amino acid analysis

The Pico Tag-Method (Bidlingmeyer *et al*., 1984) was used to determine the amino acid composition of the fermented *Ting* samples. The method quantitatively determines the amino acid composition of a sample protein.

Approximately 10-20mg of sample was weighed in duplicate into hydrolysis flasks. The protein was first hydrolysed using 6 M HCL under vacuum in an oven at 110ºC for 24 h to yield free amino acids that were then derivatised using phenylisothiocyanate. This was then followed by the separation of the constituent amino acids on the basis of their hydrophobicity using reverse-phase liquid chromatography (HPLC) and thus finally quantified in the ultraviolet (UV) spectrum.

Two pumps were used to form a gradient for optimum separation and the entire process was monitored at 254 nm. All experiments were done in duplicate and system gold was used for calculations. The column used was a PICO.TAG column for hydrolysate amino acid analysis Part number 88131 (3.9 mm x 15 cm).

5.4 RESULTS AND DISCUSSION

5.4.1 pH and titratable acidity

Table 5.1 Effect of starter cultures on the pH and titratable acidity of *Ting*

	pH			Titratable acidity		
Sample	0h	12h	72h	0h	12h	72h
S1	6.3	4.2	4.0	0.08	0.35	0.66
S2	6.3	4.3	4.1	0.07	0.36	0.62
S3	6.4	4.4	4.2	0.07	0.35	0.68
S4	6.4	4.3	4.2	0.07	0.27	0.65
S5	6.4	4.4	4.2	0.07	0.27	0.63
S6	6.3	4.3	4.2	0.07	0.31	0.62
S7	6.8	6.0	4.0	0.02	0.11	0.63
S8	6.8	6.8	6.8	0.02	0.002	0.002

For purposes of all experiments performed in this chapter, S1 to S6 represents fermented *Ting* samples inoculated with different starter cultures of lactic acid bacteria (Table 3.2). S7, un-inoculated naturally fermented *Ting* sample, S8, un-fermented *Ting* sample.

Table 5.1 shows the changes in pH and titratable acidity of the different fermented *Ting* samples over time. The initial pH of *Ting* was 6.8 with a titratable acidity of 0.02 % as lactic acid. After 12 h of fermentation, fermented *Ting* samples inoculated with different starters of LAB resulted in a reduction of pH from 6 to below 4.5. This is the pH required to inhibit the growth of other undesirable microorganisms. The pH was almost constant for these samples during the fermentation cycles between 6 and 72 h. The naturally fermented *Ting s*ample also attained a pH below 4.5 after 72h.

The amount of lactic acid in the samples increased from below 0.1 up to 0.7 % in all fermented *Ting* samples. However, in the naturally fermented *Ting* sample this increase in acidity was slower. In general, the use of starter cultures of LAB in *Ting* fermentation resulted in reduction of the pH within a period of 12 h instead of 72 h as with the naturally fermented *Ting*.

5.4.2 Proximate composition

Table 5.2 The chemical composition of *Ting* samples (g/100 g)

Component	*S1*	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*
Dry matter	99.850	99.750	99.550	99.101	99.300	99.399	99.400	98.751
Moisture	0.150	0.250	0.450	0.899	0.700	0.601	0.600	1.249
Ash	0.999	0.701	0.800	0.799	0.900	0.800	0.800	0.800
Crude protein	9.451	9.351	9.082	9.390	9.725	9.808	9.951	9.551
Crude fat	1.796	1.815	1.796	1.847	1.700	1.738	1.522	1.670
Crude fibre	0.99	1.03	1.00	1.00	0.90	1.10	1.95	1.00
NDF	6.782	6.383	9.008	15.814	7.549	12.489	9.924	6.340
Gross energy	17.968	17.951	17.914	17.901	17.865	17.853	17.960	17.751

S1 to S6 represents the same *Ting* samples as mentioned previously in Table 5.1. S7, un-inoculated naturally fermented *Ting* sample; S8, un-fermented *Ting* sample.

Table 5.2 shows that the different *Ting* samples did not differ greatly in their chemical composition. Although most of the fermented samples were inoculated with different starter cultures of LAB, this did not appear to affect the composition of the samples as compared to the naturally fermented *Ting* sample. Furthermore, the samples still had almost a similar composition of dry matter, moisture, ash, crude protein, crude fat, crude fibre and gross energy in relation to the naturally un-fermented sorghum sample. Therefore, in this case fermentation did not result in any major change in the composition of the samples. This was also found by Lopez (1992). The NDF results were variable amongst the samples.

Ting was found to be high in protein and energy with a low fat content. The low levels of fat in the samples are desirable to enhance the storage and keeping quality of the fermented product. Lipids yield a considerable amount of energy for microorganisms when oxidised. Microorganisms can oxidise lipids to obtain energy for their metabolic activities.

5.4.3 Minerals

Table 5.3 Mineral composition in *Ting* (%)

Mineral	*S1*	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*
Calcium	0.016	0.016	0.017	0.017	0.017	0.015	0.017	0.017
Phosphorous	0.182	0.174	0.179	0.182	0.144	0.178	0.167	0.186
Magnessium	0.06	0.05	0.05	0.06	0.05	0.05	0.05	0.06
Iron (mg/kg)	57.9	35.9	41.6	36.4	37.9	39.9	27.7	37.9

S1 to S6 represents the same *Ting* samples as mentioned previously in Table 5.1. S7, un-inoculated naturally fermented *Ting* sample; S8, un-fermented *Ting* sample.

Table 5.3 shows the mineral composition in the fermented *Ting* samples. Of all the four minerals analysed, phosphorous was found to be the highest in all the samples followed by magnesium. Iron and calcium were the lowest in all sorghum samples analysed. The sample S1 had the highest Fe content of 57.9 mg/kg with all the other samples having less than 42 mg/kg. Generally, fermentation in this case did not show a great effect in the amounts of Ca, P, Mg and Fe present in the samples since all the samples had almost similar contents.

5.4.4 Amino acid composition

Table 5.4 Amino acid composition of protein in fermented *Ting* (g per 100 g protein)

Amino acid	*S1*	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*
Aspartic acid	0.26	0.27	0.21	0.27	0.28	0.27	0.30	0.29
Glutamic acid	1.00	1.18	0.99	1.15	1.19	1.16	1.23	1.24
Serine	0.25	0.29	0.24	0.28	0.29	0.28	0.29	0.31
Glysine	0.14	0.16	0.14	0.16	0.17	0.16	0.17	0.18
Histidine*	0.10	0.11	0.09	0.10	0.11	0.11	0.12	1.12
Arginine	0.19	0.19	0.19	0.20	0.21	0.21	0.21	0.21
Threonine*	0.15	0.18	0.14	0.17	0.19	0.18	0.20	0.10
Alanine	0.32	0.42	0.32	0.37	0.40	0.36	0.39	0.39
Proline	0.41	0.59	0.46	0.58	0.67	0.59	0.68	0.66
Tyrosine	0.20	0.27	0.21	0.28	0.31	0.29	0.31	0.25
Valine*	0.17	0.25	0.19	0.25	0.28	0.25	0.27	0.22
Methionine*	0.07	0.10	0.10	0.13	0.09	0.13	0.07	0.08
Cystine	nd	0.02	0.02	0.03	0.03	0.03	0.05	0.02
Isoleucine*	0.11	0.17	0.13	0.17	0.18	0.15	0.18	0.16
Leucine*	0.41	0.58	0.43	0.57	0.62	0.53	0.60	0.63
Phenylalanine*	0.16	0.23	0.18	0.23	0.26	0.21	0.25	0.26
Lysine*	0.04	0.05	0.04	0.05	0.06	0.05	0.05	0.06

* Essential amino acids

S1 to S6 represents the same *Ting* samples as mentioned previously in Table 5.1. S7, un-inoculated naturally fermented *Ting* sample; S8, un-fermented *Ting* sample, nd, not detected.

Table 5.4 shows the amino acid composition of the fermented *Ting* samples and this consists of essential and non-essential amino acids. Among all the amino acids analysed, glutamic acid was the highest in all the samples, followed by proline and leucine with cystine and lysine being the least.

Although the amino acid amounts in all samples were almost constant, samples S1 and S3 showed to have slightly lower contents for most of the amino acids as compared to the other samples. Methionine and cystine amounts were not quantitative even though sample S1 did not show to have any cystine. However, fermentation and especially inoculation with different starter cultures of LAB did not have a high impact on the amino acid composition of the proteins in the samples analysed.

The amounts of all the amino acids analysed varied in the following order: glutamic acid > proline > leucine > alanine > serine > aspartic acid > tyrosine > valine > phenylalanine > arginine > threonine > glysine > isoleucine > histidine > methionine > lysine > cystine.

However, the amounts of essential amino acids varied in the following order: leucine > valine > phenylalanine > threonine > isoleucine > histidine > methionine > lysine.

5.5 CONCLUSION

Ting was produced from fermented sorghum gruel using starter cultures of LAB. An inhibitory pH for bacterial growth is considered to be 3.6 – 4.1 (Lopez, 1992) and this was reached within 12 h in *Ting* samples inoculated with starter cultures of LAB. The relatively shorter period of fermentation (12 h) compared to the traditional spontaneous fermentation process (48-72 h) was in agreement with a report by Sanni *et al.,* (1994). There was also an increase in the titratable acidity expressed as percentage lactic acid from less than 0.1 % to 0.7 % within 72 h.

Fermentation did not result in any significant change in the total nutrient composition (protein/amino acid content in *Ting*). These results are similar to those by Mugula *et al.* (2003) for *Togwa,* which is a fermented product related to *Ting.* Also in this study, there was no significant change caused by fermentation in the crude protein content.

From the results, *Ting* was found to be rich in protein and phosphorous. The product is also a good source of energy. However, the crude fibre in the samples was found to be very low and as a result, the NDF determination gave variable results.

The results obtained for the composition and nutritional composition of *Ting* were also related to those reported by Campbell-Platt (1987) for related products although with different names and from different cereals. Due to the crude methods of analysis, the proximate composition of fermented food was reported not to change much during fermentation (Lopez, 1992). The results obtained can be used for further research in enhancing the nutritional status of *Ting* should the need arise.

5.6 REFERENCES

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Chapter 6

GENERAL DISCUSSION

Ting, a sorghum based cereal product, consumed in most parts of South Africa is fermented for 48-72 h before consumption. It has an initial pH of 6.8 and 0.02 % of lactic acid. However, within 12 h of fermentation with starter cultures of LAB (*Lactobacillus collinoides* 1.42, *Lactobacillus cellobiosus* 3.42, *Leuconostoc mesenteroides* 2.35, *Lactobacillus cellobiosus* 4.35, *Lactobacillus cellobiosus* 3.30 and *Lactobacillus curvatus* 5.30), the pH of these samples decreased to levels below 4.5 where they remained constant for the remainder of the fermentation period of 72 h. This pH level falls within the range of the inhibitory pH for bacterial growth (3.6-4.1), which renders such products safe. The use of starter cultures resulted in a shorter fermentation period. The reduction in pH after 12 h fermentation could be due to the high inoculum numbers of LAB in the starter cultures. This resulted in the reduction of the numbers of coliform bacteria in the samples as determined on VRBA. The LAB starters out-competed the coliforms and as a result the numbers of coliform bacteria in the samples were eventually reduced to nil. Starter cultures occupy vital niches, thereby discouraging the colonisation of undesired microorganisms. The introduction of starter cultures in *Ting* fermentation resulted in the samples having constant numbers of LAB (10^9-10^{10}) after 12 h for the remainder of the fermentation period. The naturally fermented *Ting* sample had a slow reduction in pH and this resulted also in a slow reduction in the numbers of coliform bacteria. This was due to competition of the microorganisms in the sample. However, also in this instance, these coliform bacterial numbers declined to nil after 72 h of fermentation. This is because of the development of LAB, which have an inhibitory effect. However, in both cases of the naturally fermented samples, there were yeasts and moulds present as cultured on PDA. Yeasts and LAB can be found in co-culture since the presence of yeasts stimulate the growth of these bacteria and thus providing vitamins, desirable taste and flavour to foods. Since the starter cultures of LAB employed reduced the pH and the numbers of coliform bacteria, increased the total concentration of lactic acid and the number of yeasts and moulds in almost similar patterns, there was need to evaluate the nutritional composition of *Ting* products and also determine their

organoleptic characteristics. The samples were all almost similar in their nutritional composition although they were low in the minerals; Ca and Mg and high in P. and Fe. *Ting* is a good source of energy and thus is consumed as a main meal in most countries. This product is also high in protein and is thus fed to children as a weaning food in most parts of the country. The sensory acceptance results indicated that the sample inoculated with a starter culture of *L. cellobiosus* 4.35 was preferred and the results were not significantly different from those for the naturally fermented sample. This indicated that when choosing a starter culture for *Ting* production, *L. cellobiosus* 4.35 would be the best option of all six samples since it yielded organoleptic characters similar to those of the naturally fermented sample.

APPENDIX

Table 3.1 LAB isolates from *Ting* as identified by the API 50 CH

Isolate	Species Name	Identification (%)
1.42	*Lactobacillus collinoides*	60.3
	Lactobacillus cellobosus	28.7
	Leuconostoc mesenteroides	8.8
3.42	*Lactobacillus cellobiosus*	98
	Lactobacillus brevis	0.8
2.35	*Leuconostoc mesenteroides*	99.9
	Lactobacillus fermentum	0.1
4.35	*Lactobacillus cellobiosus*	80.4
	Lactobacillus fermentum	11.3
3.30	*Lactobacillus cellobiosus*	99.3
	Lactobacillus brevis	0.3
5.30	*Lactobacillus curvatus*	72.5
	Lactobacilllus collinoides	20.5
	Leuconostoc lactis	3.1

Table 4.2.1 Frequency of hedonic scale scores of fermented *Ting* samples for appearance (1=dislike extremely; 9=like extremely)

SAMPLE	FREQUENCY (%)								
	1	2	3	4	5	6	7	8	9
S1	2	0	8	12	12	24	10	24	8
S2	6	2	12	26	10	10	12	22	0
S3	0	4	2	12	6	10	22	28	16
S4	2	0	2	8	2	8	36	28	14
S5	2	2	4	10	12	26	20	18	6
S6	2	4	4	10	12	14	30	6	18
S7	4	2	6	4	6	12	30	8	28

Table 4.2.2 Frequency of hedonic scale scores of fermented *Ting* samples for colour (1=dislike extremely; 9=like extremely)

SAMPLE	FREQUENCY (%)								
	1	2	3	4	5	6	7	8	9
S1	2	4	6	6	12	20	22	20	8
S2	6	4	12	12	16	14	18	26	2
S3	0	6	0	14	10	14	18	20	18
S4	0	0	6	2	4	16	28	34	10
S5	2	4	2	6	12	22	28	14	10
S6	4	6	6	12	12	12	22	16	10
S7	0	0	0	6	14	18	20	28	12

Table 4.2.3 Frequency of hedonic scale scores of fermented *Ting* samples for taste (1=dislike extremely; 9=like extremely)

SAMPLE	FREQUENCY (%)								
	1	2	3	4	5	6	7	8	9
S1	2	0	12	4	8	38	12	16	8
S2	2	8	8	16	14	26	12	10	4
S3	4	8	0	14	10	8	24	18	14
S4	0	4	4	6	8	10	34	26	8
S5	2	4	4	6	18	32	18	12	4
S6	4	4	2	24	6	16	22	8	14
S7	2	0	4	2	10	18	22	20	22

Table 4.2.4 Frequency of hedonic scale scores of fermented *Ting* samples for sourness (1=dislike extremely; 9=like extremely)

SAMPLE	FREQUENCY (%)								
	1	2	3	4	5	6	7	8	9
S1	4	2	8	6	10	34	22	8	6
S2	6	4	8	16	10	20	16	18	2
S3	4	6	4	12	6	12	24	18	14
S4	2	2	6	10	4	24	28	20	4
S5	2	4	0	10	20	24	20	18	2
S6	4	8	4	14	12	14	24	2	18
S7	2	2	6	2	10	18	30	14	16

Table 4.2.5 Frequency of hedonic scale scores of fermented *Ting* samples for overall acceptance (1=dislike extremely; 9=like extremely)

SAMPLE	FREQUENCY (%)								
	1	2	3	4	5	6	7	8	9
S1	2	0	10	8	14	34	6	14	12
S2	10	2	10	18	14	14	10	14	8
S3	4	4	2	12	12	10	22	18	16
S4	2	0	2	8	6	16	36	24	6
S5	4	4	0	14	12	38	14	12	2
S6	4	8	6	12	14	16	12	10	18
S7	4	4	2	2	14	16	18	16	24

Place an X in the box that best describes how much you like or dislike each attribute

SAMPLE

How much do you like or dislike the look/appearance of this *Ting*?

			Any comments?
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		

How much do you like or dislike the colour of this *Ting*?

			Any comments?
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		

How much do you like or dislike the taste of this *Ting*?

			Any comments?
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		

How much do you like or dislike the sourness of this *Ting*?

			Any comments?
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		

How much do you like or dislike this *Ting*?

			Any comments?
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		

Is there anything specific that you really disliked about this *Ting*?

Is there anything specific that you really liked about this *Ting*?

Figure 4.1 Score sheet for acceptance test