A STUDY ON THE USE OF MODIFIED FERMENTED CEREAL GRUEL FOR CONTROL OF DIARRHOEA

BY

OPERE, BOLANLE OLAITAN
(B.Sc., M.Sc, LAGOS)

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CERTIFICATION

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By

OPERE, BOLANLE OLAITAN
in the Department of Botany and Microbiology

OPERE, BOLANLE OLAITAN		08.01.09
AUTHOR'S NAME	SIGNATURE	DATE
DR. E.O. IGBOSUAH		08/01/09
1ST SUPERVISOR'S NAME	SIGNATURE	DATE
Dr O.O. Aboaba		08/01/09
2ND SUPERVISOR'S NAME	SIGNATURE	DATE
DR. R.E. UZEH		08/01/09
1ST INTERNAL EXAMINER	SIGNATURE	DATE
Dr A.A. Adekunle		08/01/09
2ND INTERNAL EXAMINER	SIGNATURE	DATE
Dr. A.A. Onifade		08 January 2009
EXTERNAL EXAMINER	SIGNATURE	DATE
DR. A.O. LAWOLARE		08/01/09
SPGS REPRESENTATIVE	SIGNATURE	DATE

DEDICATION

To

My parents, Deacon John Oke Agbede (Of blessed memory)

and

Mrs. Elizabeth Oluwabamise Oke Agbede

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ABSTRACT

Two species of *Lactobacillus, L. pentosus* and *L. acidophilus* were used as starter cultures for the fermentation of cereals – corn and sorghum, to produce a cereal-based gruel, a popular weaning food in many parts of West Africa. All the fermented gruels produced with either *L. acidophilus* or *L. pentosus* or both (mixed culture) yielded lactobacilli count > 10^7 cfu/g on day 4 post fermentation. The lowest mean counts (2.6×10^7 cfu/g) and highest (3.1×10^7cfu/g) were observed in *L. pentosus*-fermented gruel and mixed culture of *L. pentosus* and *L. acidophilus* respectively while spontaneous fermentation accounted for lactobacilli count <10^7 cfu/g in the gruels tested. Those produced by spontaneous fermentation elicited microbial contamination by fungal species of *Aspergillus* and *Penicillium,* yeasts *Saccharomyces cerevisiae* and *Candida* spp. and bacterial species of *Staphylococcus aureus, Bacillus* sp., and *E. coli.* All samples showed increased levels of proteins, amino acids, and reducing sugars. The total protein value was highest in those cereals fermented with mixed culture having an average percentage (%) value of 13.13 ± 4.33 on day 3 , followed by *L. acidophilus*-fermented with a value of 10.97 ± 4.37, then *L. pentosus*-fermented ones with a value of 9.62 ± 4.50 and the locally fermented having the lowest value of 7.78 ± 2.15. Essential amino acids, lysine, tryptophan and methionine and a semi-essential isoleucine were elicited in all fermented samples, showing desirable nutritional status. The average amino acid content (mg/100g) of cereals spontaneously-fermented was found to be lowest with a value of 78.89 ± 5.13 on day 3, followed by *L. pentosus* -fermented 283.03 ± 4.65, then the *L. acidophilus*-fermented 338.51 ± 8.13 and the cereals fermented using the mixed culture having the highest value of 498.50 ± 9.69. There was no significant difference in results of reducing sugar content of *Lactobacillus*-fermented cereal gruels by day 3, while the spontaneously fermented cereal gruels were significantly different from others. The flavour-enhancing acetoin and diacetyl in the products were significantly increased using the starter culture organisms individually, but was exceptionally higher ($p>0.05$) when combined culture was used. Gruels made from *Lactobacillus* fermentation elicited significantly ($P<0.05$) higher organoleptic scores for each of the sensory attributes investigated compared to the spontaneously fermented. The fermented samples showed the highest total acidity of 15.36µmol/ml in mixed culture-fermented sample and lowest value of 0.36 µmol/ml in the naturally - fermented sample. There was decrease in the pH values of the various gruel samples as fermentation progressed from 5.75 – 6.1 to 3.2. The *Lactobacillus* species were found *in vitro* to inhibit the growth of pathogenic agents

Escherichia coli, Shigella, Salmonella, Vibrio cholerae, and *Staphylococcus aureus* within 6 hours. The spontaneously-fermented gruels supported the growth of *Vibrio cholerae* for 14 hours, *E. coli* for over 40 h and all others up to 30 h. The albino mice (neonates) were fed with the fermented gruels for 8 days, and immediately challenged with cells of *Shigella dysenteriae.* Survival rates of 100%, 90% and 80% were found in those fed with cereals fermented with the mixed culture, *L. acidophilus* and *L. pentosus* respectively after 20 days. This result suggests the possible use of *Lactobacillus* starter cultures, especially of mixed cultures in the development of locally – based cereal weaning food against shigellosis, a common form of diarrhoea in children in developing countries.

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CHAPTER ONE

INTRODUCTION AND BACKGROUND OF STUDY

1.0 INTRODUCTION AND BACKGROUND OF STUDY

1.1 BACKGROUND OF STUDY

Diarrhoeal diseases are among the major causes of death in most developing countries as well as being a major factor in malnutrition in young children (UNICEF 1988, Lorri and Svanberg, 1994, Olukoya *et al.*, 1994, Anon, 1998: Elliot and Dalby-Payne, 2004, Quadri *et al.,* 2005). Diarrhoea is defined as the passage of loose or watery stool usually with increased frequency and is an important clinical manifestation of a large number of gastrointestinal disorders (Molbak *et al.,* 1994). Diarrhoea could be either acute or chronic. The acute one has been reported to account for more than 4 million deaths in children less than five years of age (Biswas *et al.,* 1996). Diarrhoea is a major cause of protein calories malnutrition in the world. Diarrhoea of infectious origin induces an average daily negative nitrogen balance of 0.9g/kg/day, as muscle protein is converted to glucose through gluconeogenesis by the liver; this glucose is used as a fuel by tissues to sustain the hypermetabolism associated with fever (Martinez and Tomkins, 1995). The number of acute diarrhoea episodes in Africa, Asia (except China) and Latin America was estimated at 774 million in 1980 with 4.6 million deaths in which 80 per cent of these deaths occurred in children below the age of two years (Snyder and Merson, 1982; Tumwine *et al.,* 2003). Surveillance in large number of communities in most developing countries has detected a diarrhoea incidence peaking between the ages of 6 months to 3 years with an annual individual rate ranging from two to twelve episodes averaging four per year (Nader de Macias *et al.*, 1992, Svanberg *et al.,* 1992, Dalby-Payne and Elliot, 2003). Matthews *et al.* (1996) reported that diarrhoeal diseases are most frequent in children aged 6 to 11 months, an age range when weaning foods are introduced. It has also been reported that diarrhoea ranks second as major cause of morbidity and mortality amongst children in Nigeria and according to the Federal Statistics Bulletin, 300 children die every day from dehydration and malnutrition caused by diarrhoea (Ogunsanya *et al.*, 1994; Akinyemi *et al.*, 1998 and Sandhu, 2001).

A large part of the diarrhoeal diseases is foodborne due to unhygienic food practices and the insanitary environment. Foodborne diseases are a major global public health problem with developing countries bearing the brunt of the problem. Although statistics on the incidence of foodborne diseases in developing countries are not available, the high prevalence of diarrhoeal diseases particularly in infants and young children in these parts

of the world is an indication of an underlying safety problem (Ekanem *et al.*, 1991, Motarjemi, 2002).

The aetiological agents responsible for foodborne diseases are broad and include bacteria, viruses, and protozoa. Some of the principal pathogens responsible for diarrhoeal diseases are pathogenic strains of *Escherichia coli, Shigella, Salmonella, Vibrio cholerae, Campylobacter jejuni,* and enterotoxigenic *Staphylococcus aureus,* protozoa such as *Giardia lamblia, Cryptosporidium* spp., *Entamoeba histolytica* and viruses such as Rotavirus and Hepatitis A and E (Svanberg *et al.*, 1992, Molbak *et al.*, 1994, Ogunsanya *et al.*, 1994, Alabi *et al.*, 1998, Akinyemi *et al.*, 1998, Motarjemi, 2002, and Quadri *et al.*, 2005). Although antibiotic therapy and non-specific measures such as oral rehydration therapy, and breastfeeding are employed in management of diarrhoeal diseases diarrhoea remains a public health problem especially in children, and infants in sub-Saharan Africa. This is as a result of the use of subtherapeutic levels of antibiotics for prophylaxis especially broad-spectrum antibiotics a measure which aids rapid spread of resistance in diarrhoeal agents. In Nigeria, misuse of antibiotics through self-medication, sale of fake drugs, and ease of availability of these drugs are all contributory factors to antibiotic resistance by pathogens. Many children survive the acute diarrhoeal illness only to succumb later to persistent diarrhoea and undernutrition (Nchito *et al.*, 1998).

Fermented foods have been reported to help gut mucosal recolonisation in malnourished children whose microfloral balance has been altered due to acute diarrhoea (Isolauri *et al.*, 1991, Oyetayo *et al.*, 2003). Fermentation is one of the oldest and most economical methods of producing and preserving foods and inhibiting the growth of pathogenic bacteria even under conditions where refrigeration and other means of storage are not available. Fermentation also destroys antinutritional factors, enhance nutritive value, to improve appearance and taste of some foods, and to salvage material otherwise not fit for human consumption. It also reduces energy required for cooking and makes available safer products (Chavan and Kadam, 1989). Lactic Acid Bacteria particularly *Lactobacillus* have been reported to be involved in the production of many African fermented foods (Odunfa, 1985), probably due to its biopreservative effects, enhancement of nutritive value of foods and beneficial effects on human health. Despite the fact that most African fermented foods have *Lactobacillus* spp. as the predominant organism, the above-mentioned properties of this organism do not reflect. For most people in developing

countries, the major source of food is cereals as dairy products are limited to a very small segment of affluent groups. Presumably, the reports of food as the origin of diarrhoea refer to cereal-based diets, as all cases cited came from developing countries (Rowland *et al.*, 1978, Kingamkono *et al.*, 1994; Lorri and Svanberg, 1994).

1.2 STATEMENT OF THE PROBLEM

In spite of the declining rate of childhood morbidity and mortality due to diarrhoeal diseases in many developing countries, including Nigeria, diarrhoea ranks second among the notifiable diseases as a major cause of morbidity (Ogunsanya *et al.*, 1994). This however could be an underestimate as very few patients have access to very limited number of hospitals and health centres available coupled with the fact that diarrhoeal control programmes in Nigeria have been compromised by inadequate surveillance systems for diarrhoeal illnesses such as shigellosis (Iwalokun *et al.*, 2001) and campylobacteriosis (Smith *et al.*, 1999). This scenario has tremendous therapeutic implications concerning the management of diarrhoea in children and risk of treatment failures. It has also been reported that a Nigerian child under 5 years of age experienced on the average 4.3 episodes of diarrhoea annually (Babaniyi, 1991). This high incidence of diarrhoea in this part of the world stems from diverse socio-economic and environmental problems. The environmental problems include inadequate waste disposal methods, non-availability of potable water in many communities, poor personal hygiene, and lack of programmes on health and strategies of infection control (Olasupo *et al.*, 1997). Diarrhoea persists as a cause of morbidity and mortality in Nigerian infants and children because they are weaned to foods with poor probiotic efficacy coupled with their unhygienic preparation and distribution as well as poverty (Olasupo *et al.*, 1997). The dietary habits of Nigerians may also play important role in their susceptibility to infection by these diarrhoeagenic bacteria. There is also inadequacy of energy and protein intakes among children, which is an indication of economic regression in the country (Mbata *et al.*, 2006). It was reported that the caloric intakes of rural children in Nigeria was between 80 and 95% of the Recommended Daily Allowance (RDA) while the daily protein intake falls between 57 and 75%.

Traditional weaning foods in most African countries are based on the local staple foods, usually a cereal such as maize, millet, sorghum or rice. This staple food is commonly prepared as a thick porridge for adults and older children or as a liquid gruel for

younger children. While the thick porridge contains about 30% flour with an energy density of about 1.2kcal/g, the liquid gruel with flour concentration of about 5% provides only 0.2kcal/g even lower than the energy density of 0.75kcal/g for breast milk. The thick porridge could provide enough energy but its thickness may prevent young children from consuming adequate quantities, thus the thin gruel that is more easily consumed has a too low energy density to meet the energy requirements of young children (Svanberg, 1992, Onilude *et al.,* 1999). It is therefore necessary for children to be fed on balanced meal, and food rich in protein. There is a need to look inwards to see how children can be well fed.

It is therefore necessary for children to be fed on balanced meal, and food rich in protein. It has also been established in developing countries that acute diarrhoea drastically alters the microfloral balance in malnourished children and lasts longer in poorly nourished children (Isolauri *et al.,* 1991). Malnutrition is common in Nigeria as evidenced by "marasmus" and "kwashiorkor". Marasmus means wasting or loss of weight, resulting from an overall deficiency in both protein and energy intake and is characterized by emaciation. Kwashiorkor refers to an inadequate protein intake with a fair intake of energy. Both marasmus and kwashiorkor are also characterized by stunted growth. In these cases, fermented products could help as functional food and contribute to the attainment of a positive nutritional status. Fermented foods have been reported to aid recolonisation in malnourished children whose microfloral balance has been altered due to acute diarrhoea (Oyetayo *et al.,* 2003). Poverty has been found to breed poor personal hygiene and limit the choice of weaning foods by mothers in developing countries. However, a large percentage of African diets are fermented foods, which are mostly prepared by spontaneous fermentation and which lack bacteriologic precision regarding aetiology of fermentation and probiotic substances produced.

"Ogi" a fermented gruel is popular as the major weaning food and staple food in West Africa. It can be made using cereals such as maize, sorghum, and millet. Cereals generally have protein of low quality, yet supply more than 70% of protein needs of man. Most cereals are deficient in one or more of the essential amino acids e.g. maize is deficient in lysine, tryptophan, and methionine all of which are major determinants of protein quality. Thus, children who consume it as a major part of their diet may be

deficient in these nutrients and may suffer from malnutrition. Malnourished children have less resistance to infection than well-nourished ones.

Microbial fermentations have played an important role in food processing for thousands of years and may have offered the most and simple economical means to improve the quality and utilization of cereals. However, simple fermentation conditions need to be standardized to obtain a product with acceptable sensory properties and improved nutritional quality.

Furthermore, the emergence of bacterial resistance to chemotherapeutic agents constitutes one of the major limitations to their successful therapeutic use in diarrhoeal control.

1.3 PURPOSE/OBJECTIVE OF THE STUDY

1.3.1 Purpose of Study

The broad goal of this research was to develop local cereal-based weaning food, with improved nutritional qualities and with potentials for use in control and probably treatment of infantile diarrhoea. The study was therefore set out to achieve the following objectives:

1.3.2 Objective of Study

3.1 To produce fermented gruels from different types of cereals using *Lactobacillus acidophilus* and *L. pentosus* as starters in different combinations.

3.2 To detect and quantify the possible secondary metabolites required for organoleptic qualities.

3.3 To investigate the nutritional status of the gruels, i.e. protein content, amino acid content, and reducing sugar concentration.

3.4 To carry out sensory evaluation and assess consumer acceptability and the shelf-life of the fermented cereal gruels.

3.5 To assess the rate of survival of test organisms (diarrhoeagenic agents) in the products over a period of time.

3.6 To use products as feed to experimental animals previously challenged with a diarrhoeagenic agent and study the survival pattern of the diarrhoeagenic agent.

1.3 SIGNIFICANCE OF STUDY

This study will enhance the use of our local cereals to produce fermented cereal gruels of improved nutritional status and acceptable sensory properties with antibacterial potentials and probiotic qualities that can be of use in diarrhoeal control.

1.4 RESEARCH QUESTIONS

Several research questions were asked on the effects of fermentation in the development of local-based cereal foods.

- Which cereal type will be best for development of weaning foods?
- Which fermentation methods will give product with desirable qualities?
- What are the antibacterial potentials of the fermented products?
- Can the fermenting organisms have probiotic qualities?
- What are the prospects of the fermented products in diarrhoeal control?

CHAPTER TWO

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Diarrhoea and Hygiene

Diarrhoea is an intestinal disorder characterized by abnormal fluidity and frequency of faecal evacuations, generally the result of increased motility in the colon. It may be an important symptom of such underlying disorders as dysenteric diseases, lactose intolerance, gastrointestinal (GI) tumors, and inflammatory bowel disease. It is the passage of watery stools, usually at least three times in a 24 hour period. However, it is the consistency of the stools rather than the number that is most important. Frequent passing of formed stools is not diarrhoea. Babies fed only on breast milk often pass loose, "pasty" stools; this also is not diarrhoea. Many problems are associated with diarrhoea especially amongst the poor in developing countries where it is a major killer. It was reported that in 1998, diarrhoea was estimated to have killed 2.2 million people, most of whom were under 5 years of age (WHO, 2000). Each year there are approximately 4 billion cases of diarrhoea worldwide.

The treatment of diarrhoea is most practically based on the *clinical type* of the illness, which can easily be determined when a child is first examined. Four clinical types of diarrhoea can be recognized, each reflecting the basic underlying pathology and altered physiology:

- **acute watery diarrhoea** (including cholera) which lasts several hours or days: the main danger is dehydration; weight loss also occurs if feeding is not continued;

- **acute bloody diarrhoea** (also called dysentery): the main dangers are intestinal damage, sepsis and malnutrition; other complications, including dehydration, may also occur;

- **persistent diarrhoea** (which lasts 14 days or longer): the main danger is malnutrition and serious non-intestinal infection; dehydration may also occur;

- **diarrhoea with severe malnutrition** (marasmus or kwashiorkor); the main dangers are: severe systemic infection, dehydration, heart failure and vitamin and mineral deficiency.

It is imperative for the management of each type of diarrhoea to prevent or treat the main danger(s) that each presents (WHO, 2000).

Despite the intensive activities of the National Diarrhoeal Control Programme, there is a high incidence of childhood diarrhoea in Nigeria (Ogunsanya *et al.,* 1994). The low level of personal hygiene and public sanitation as well as the low socio-economic status of most families compound the incidence and prevalence of diarrhoeal diseases in this country. Another report has it that over 315,000 lives of Nigerian children under the age of five years are lost annually due to diarrhoeal diseases (Babaniyi, 1991). Thus improved food hygiene during the weaning period seems to be one important way to prevent transmission of foodborne diarrhoea-causing pathogens. Indeed, several studies have confirmed that cereal-based weaning foods and water are important sources of pathogens: the contamination of weaning foods therefore constitute potential sources of diarrhoea in children (Motarjemi, 2002). In this respect, lactic acid bacteria - fermented foods may offer a high potential especially in developing countries since fermentation is a traditional household-level technology that can easily be adapted (Holzapfel, 2002). There is a growing interest in the use of traditional fermented foods for weaning as it is thought that bacterial pathogens likely to cause diarrhoea could not survive for long periods in them because of the substances produced during fermentation as well as using these foods for treating infected children. However, a large percentage of fermented foods in Nigeria are mostly prepared by spontaneous fermentation (Olasupo *et al.,* 1997) and there is need for standardisation by using starter cultures to improve the food quality (Olukoya *et al.,* 1994).

2.1.1. Diarrhoea in Children

Diarrhoea is a symptom of infection caused by a host of bacterial, viral and parasitic organisms most of which can be spread by contaminated water. It is more common when there is a shortage of clean water for drinking, cooking and cleaning and basic hygiene is important in prevention. Thus food contamination is one major route for transmission of enteropathogens especially under hygienic conditions prevailing in a rural setting. Sources of food contamination are diverse and include polluted water, dust, flies, domestic animals, dirty utensils and food handlers. Raw foods may also be a source of contaminants as many foods harbour pathogens or originate from infected animals (Parveen and Hafiz, 2000). Moreover, during food preparation there is an added risk of cross contamination.

One major factor leading to food contamination during food preparation and storage is time-temperature abuse, which results in the survival, growth, and production of toxins by pathogens. In many developing countries, diarrhoea is one of the major precipitating factors of child morbidity and mortality. It also significantly contributes to the high prevalence of malnutrition in young children. Available information suggests that a major part of all diarrhoea episodes is associated with bacterial contamination of weaning foods (Black *et al.*, 1982).

The following bacteria have been reported among the causative agents: enterotoxigenic *Escherichia coli* (ETEC), *Shigella, Salmonella,* and *Campylobacter,* while among the viruses, rotavirus seems to be the most common (Mensah *et al.,* 1990, Lorri and Svanberg, 1994). Many workers reported Rotavirus as the most frequently encountered pathogen in children diarrhoea. Alabi *et al.,* (1998) carried out a pilot study to identify the role of viral, bacterial, and parasitic agents responsible for diarrhoea episodes in children in Lagos State within the age range of 13 days to 48 months. Results showed forty-six (63.9%) of the diarrhoea stools contained recognised enteropathogens against seven (18.9%) of the control. Rotavirus was the most commonly isolated and found to be responsible for 37.5% of diarrhoea against 10.8% in the control group. Of the bacterial agents, *Salmonella* spp was the most frequent followed by *E. coli,* then *Shigella* spp, *Enterobacter*, and *Klebsiella,* with *Shigella* being isolated only from diarrhoeaic patients. This agrees with the reports of Lorri and Svanberg, (1994), Ogunsanya *et al.,* (1994), Biswas *et al.* (1996), Akinyemi *et al.* (1998) and Kingamkono *et al.* (1999). It has been made clear that shigellosis is an important cause of severe diarrhoea in children from developing countries; hence, whenever it is found in stool samples it is of great significance (Ebrahim, 1990, Iwalokun *et al.*, 2001).

2.2 Cereals and cereal-based products

Most African traditional foods are formulated based on local staple usually cereal grains such as maize, sorghum, millet and rice and stem and root tubers such as yam and cassava. Cereals and cereal-based products are a major source of inexpensive dietary energy and nutrients worldwide. In Nigeria as in other parts of West Africa, cereal grains lack two essential amino acids, lysine and tryptophan, thus making their protein quality poorer compared to that of animals (Mbata *et al.,* 2006). Many cereal products such as boiled or steamed rice, porridge, paste, cookies etc. are made without

undergoing any fermentation process, Apart from non-fermented cereal foods, fermented products constitute the staple food in almost every civilisation reflecting in a huge variety of different products especially in developing countries. Fermentation is a low cost method for enhancing food quality, safety, and shelf life (Olasupo *et al.*, 1997). However, there are a number of cereal-based products made by lactic acid bacteria (LAB) fermentation. Such include European sour rye bread, various Asian flat breads, and the numerous types of fermented sour porridge, dumplings and non - alcoholic beers common in Africa and elsewhere (Steinkraus, 1996; FAO, 1999). In these applications the LAB, contribute technological and nutritional benefits, as well as affecting flavour and keeping properties of the products. Cereals generally have protein of low quality, hence consumers of cereal – based diets may suffer from malnutrition, having less resistance to infection than well-nourished ones (Olukoya *et al.*, 1994). Several methods have been employed to improve the nutritional quality of cereals of which fermentation has been shown to be effectively used for improving the sensory and nutritional qualities of cereals and their products (Adebawo, et *al.*, 2000, Mbata *et al.*, 2006). The sensory or organoleptic characteristics are important in acceptability and utilization of any natural or processed food. Fermentation with natural microflora or pure cultures for a limited period has been found to be highly beneficial in improving the quality of cereal-based foods (Chavan and Kadam, 1989). Adebawo *et al.* (2000) reported higher yield of available amino acids in maize fermented with starter culture of *Lactobacillus plantarum* compared to the spontaneously traditionally fermented one. Most fermented cereal-based products are heat-treated after fermentation and the bacteria are killed. However, there are also such indigenous lactic acid fermented cereal-based products that contain live LAB.

2.3 Fermentation

The term fermentation was first applied to the production of wine more than a thousand years ago, being based on the conversion of sugar to carbon dioxide gas. Early research on fermentation dealt mostly with carbohydrates and reactions that liberated carbon dioxide. It was soon recognized, however, that microorganisms or enzymes acting on sugars did not always evolve gas. Further, many of the microorganisms and enzymes studied also had the ability to breakdown non-carbohydrate materials such as proteins and fats, which yielded carbon dioxide, other gases, and a wide range of additional materials (Hassan *et al.*, 2006).

Currently, the term fermentation is used in various ways which require clarification. When chemical change is discussed at the molecular level, in the context of comparative physiology and biochemistry, the term fermentation is correctly employed to describe the breakdown of carbohydrate materials under *anaerobic* conditions. In a somewhat broader and less precise usage, where primary interest is in describing the end products rather than the mechanisms of biochemical reactions, the term fermentation refers to breakdown of carbohydrate and carbohydrate-like materials under either *anaerobic* or *aerobic* conditions (Holzapfel, 2002). Conversion of lactose to lactic acid by *Streptococcus lactis* bacteria is favoured by anaerobic conditions and is true fermentation; conversion of ethyl alcohol to acetic acid by *Acetobacter aceti* bacteria is favoured by aerobic conditions and is more correctly termed an oxidation rather than a fermentation.

Fermentation has remained an important technology throughout the history of mankind and many benefits are attributed to it. It preserves and enriches food, improves digestibility, and enhances the taste and flavour of foods (Muller, 2000). It is also an affordable technology and is thus accessible to all populations. Furthermore, fermentation has the potential of enhancing food safety by controlling the growth and multiplication of a number of pathogens in foods even under conditions where refrigeration and other means of storage are not available. Thus, it makes an important contribution to human nutrition, particularly in developing countries, where economic problems pose a major barrier to ensuring food safety (Hammes, 1990; Sanni, 1993a; Motarjemi, 2002). Fermentation is often part of a sequence of food processing operations, which may include unit operations such as cleaning, grinding, soaking, salting, cooking, packaging, and distribution.

The potential of fermentation for improving nutritional quality and safety of foods should therefore be viewed within the context of the complete food processing operation. Fermentation also provides a way to reduce volume of material to be transported, to destroy undesirable factors to enhance nutritive value, to improve appearance and taste of some foods, to salvage material otherwise not usable for human consumption, to reduce energy required for cooking and to make a safer product (Chavan and Kadam, 1989). Fermentation may offer the most simple and economical means to improve the quality and utilization of cereals. However, simple fermentation conditions need to be standardized to obtain a product with acceptable sensory

properties and improved nutritional quality. In addition, the type of raw materials used for fermentation, methods or conditions of fermentation, and sensory qualities of finished products vary greatly from culture to culture in various regions of the world (Muller, 2000).

Generally, fermented foods can be described as palatable and wholesome foods prepared from raw or heated raw materials usually appreciated for attributes such as pleasant flavour, aroma, texture, and improved cooking and processing properties (Holzapfel, 1997, 2002). 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. Enzymes indigenous to the raw materials may play a role in enhancing these characteristics. Fermentation may serve to improve the nutritional value of cereal staples through the reduction of antinutritive factors as reported for a number of foods of plant origin (Chavan and Kadam 1989, Westby and Choo, 1994, Olasupo *et al.*, 1997). The fermentation of cereals and cereal-based products in the traditional way depends largely on chance inoculation, involving mixed cultures of bacteria, yeast, or both. The common fermenting bacteria are the species of *Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus* and *Bacillus.* Most bacteria fermentations are usually lactic acid fermentations while the yeasts bring about alcoholic fermentation. In addition, some common mould species such as *Aspergillus, Penicillium, Fusarium,* and *Cladosporium* may be involved in certain products.

2.3.1 Lactic acid fermentations

Lactic acid bacteria (LAB) comprising the genera *Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Lactococcus, Enterococcus, Carnobacterium, Sporolactobacillus,* and *Bifidobacterium* are all Gram positive rods or cocci, anaerobic, microaerophilic or aerotolerant, catalase negative organisms which have the capability of producing lactic acid from the energy-yielding fermentation of sugars. These bacteria use carbohydrates as an energy source producing more than 85% lactic acid as the sole major product of metabolism or as the major end product, other products being carbon dioxide, acetate, and ethanol, in equimolar amounts; the former is known as Homolactic Fermentation and the latter Heterolactic Fermentation (Kazana and Fields, 1981, Madigan *et al.,* 1997). Lactic acid bacteria are commonly found on the mucous membranes of humans and animals, in dairy products and naturally on some plant

(Ray and Daeschel, 1992). In comparison to acetic acid, lactic acid the major end-product in Heterolactic fermentation is mild in flavour but strongly decreases pH and in doing so affects the antimicrobial properties of the system (Jay, 1986). Heterofermentative lactobacilli lack aldolase, a key enzyme of glycolysis, but instead they have phosphoketolase and produce carbon dioxide and ethanol/acetate from glucose in addition to lactate (Madigan *et al.*, 1997).

2.3.1.3 Lactic acid-fermented traditional cereal-based foods other than bread

Lactobacilli are important organisms recognized for their fermentative ability as well as their health and nutritional benefits. They produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin or bactericidal proteins during lactic fermentations. The antimicrobial properties of lactobacilli are of special interest in developing strongly competitive starter cultures for food fermentation. Lactobacilli exert strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens (Ogunbanwo *et al.*, 2003). Fermentation with lactic acid bacteria thus provides a cost effective method of food preservation; inhibiting spoilage and pathogenic microorganisms by a combined activity of pH reduction, lowering of oxidation-reduction potential, competition for essential nutrients and production of inhibitory compounds like antibiotics, diacetyl, bacteriocins, and hydrogen peroxide.

Fermentation of foods using LAB is common in Africa most probably due to the following benefits intrinsic to the usage. Preservation of fermented food by lactic acid bacteria is due primarily to sugars being converted to organic acids (lactic, acetic) causing a reduction in pH and removal of carbohydrates as nutrient sources. For example, 'ogi' – (a fermented cereal product) can keep for upwards of 14 days by decanting and replacing the supernatant water (Olasupo *et al.*, 1997). The lactic and acetic acids formed, lower the pH thereby inhibiting the growth of foodborne pathogens and other pathogens of medical importance such as *Escherichia coli, Salmonella typhimurium, Shigella* spp, *Staphylococcus* spp, *Proteus, Klebsiella,* and *Vibrio* spp . Selected lactobacilli can be added to the food, in the viable state where they grow very slowly antagonizing undesirable spoilage microorganisms (Cooke *et al.*, 1987, Ray, 1992, Davidson and Hoover, 1998). The exact mode of action of diacetyl in preservation has not yet been determined in live cells, but *in vitro* studies indicated that diacetyl deactivated enzymes from several microorganisms (Ray and Daeschel, 1992). Alcohol

dehydrogenase and adenylate cyclase from yeasts, and glutamate dehydrogenase and transketolase in pentose cycle have been reported to be deactivated by diacetyl (Ray and Daeschel, 1992). It was reported that 344ppm of diacetyl treatment reduced the viability loss of strains of *Listeria monocytogenes*, pathogenic *Escherichia coli, Yersinia enterocolitica, Salmonella sp.*, and *Aeromonas hydrophila* from 0>95% during a 24 hour incubation at 4^0C, and that seven of the ten strains studied had over 50% viability loss at this low diacetyl concentration (Motlagh *et al.*, 1991).

Bacteriocin production is a common phenomenon among lactic acid bacteria. Klaenhammer (1988) defined bacteriocins as antimicrobial proteins or peptides produced by some Gram positive and Gram-negative bacteria, with bacteriocidal activity directed against species that are closely related to the 'producer microorganisms'. In addition, bacteriocins are heterogenous compounds, which vary in molecular weight, biochemical properties, activity spectra and mechanism of action. Bacteriocin-producing strains are sometimes used as protective cultures to improve the microbial safety of foods. The crude or purified form of bacteriocins may also be applied directly as food protective agents. Bacteriocins are of food origin and are thus of great importance in food processing, food safety, and food preservation. Despite the enormous application potential of bacteriocins in food processing, only nisin, a bacteriocin produced by certain strains of *Lactococcus lactis subsp. lactis* has been of practical application in food processing to date as a food preservative (Klaenhammer, 1993, Cleveland *et al.*, 2001).

2.4 Benefits of LAB Fermentation to Man

2.4.1 Enhanced nutritional value – In some cases, the nutritional value or the digestibility of the raw material is increased, while the vitamin B levels rise in many cases in the fermented foods (Salmien *et al.*, 2000). Since whole cereals are used in some fermentation, the fibre content is high. Among the amino acids essential to humans, lysine is limiting in all cereal grains. Lactic fermentation has been shown to lower the levels of proteinase inhibitors in cereal porridges thereby increasing the availability of essential amino acids such as lysine, leucine, isoleucine, methionine and even tryptophan, and consequently improving the protein quality of cereal grains (Holzapfel, 2002).

2.4.2 Detoxification of food – LAB fermentation is known to decrease or eliminate toxic components in some foods. The degradation or inactivation of toxins by pure cultures during fermentation has received considerable attention. For example, the consumption of raw or unprocessed cassava may cause severe intoxications, due to the naturally occurring toxins such as cyanogenic glucosides (linamarin and lotaustralin) (Oyewole *et al.*, 1988, 1991). Westby and Choo (1994) showed that detoxification during cassava fermentation is brought about primarily by microbial activity, although endogenous linamarinase enzymes present in cassava play a significant role in the process. *Lactobacillus* spp., yeasts, and moulds play an important role in cassava processing (Amoa-Awua 1995; Essers *et al.*, 1995 and Olasupo *et al.*, 1997). Kimaryo *et al,* (2000) while comparing the effects of spontaneous fermentation, back slopping and use of starter cultures for the reduction of cyanogenic glucosides in cassava revealed a starter culture consisting of *L. plantarum* giving the best results though others contributed significantly too.

2.4.3 Rendering inedible foods edible – Fermentation removes antinutritional factors in foods that are inedible in their unfermented state. It has been established that there are nutrient-rich crops in Nigeria, which are fermented and used as food condiments, which are generally not used as foods in their unfermented state because some of them contain toxic or antinutritional factors (Iwuoha and Onyekwere, 1996).

2.4.4 Production of variety in flavour – Fermentation in some cases provide a variety in flavours. Diacetyl is a compound associated with the desirable characteristic flavour of many fermented dairy products. Members of the genus *Lactobacillus* constitute a most important member within the LAB group and one of the most useful microorganisms in the food industry. Lactobacilli are characterized as Gram positive, non-spore forming rods, catalase and oxidase negative, usually non-motile, do not reduce nitrate and utilize glucose fermentatively (Kandler and Weiss, 1986). They are strictly fermentative and have complex nutritional requirements; needing to be supplied with carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives and vitamins (Hammes *et al.*, 1992).

Food fermentation is a traditional art in Africa and Asia with numerous fermented foods reported. Lactic acid bacteria particularly *Lactobacillus* have been involved in the production of many African fermented foods such as fufu, lafun, chickawangue, kocho,

gari, kenkey, uji, mahewu, kishk, merissa, iru, leban, kisra, nono, obusera – all African foods spanning over 10 countries. Odunfa (1985) classified about 40 different African fermented foods into five groups: Fermented starchy root crops, Fermented non alcoholic cereal substrate, Fermented alcoholic beverages, Fermented vegetable proteins and Fermented animal proteins.

2.4.5 Beneficial effects on human health – *Lactobacillus* is used as prophylactic and therapeutic means in disease treatment.

a) The antimicrobial activity of lactobacilli would permit the use of fermented products as a simple method for the treatment and prevention of gastrointestinal infections e.g. viable *Lactobacillus acidophilus* has been reported to be effective in the treatment of different types of diarrhoea especially *E. coli*-mediated diarrhoea in infants. It was reported that administration of *Lactobacillus*-fermented milk to children with *Salmonella* and *Shigella dysenteriae* led to the elimination of the cases of dysentery (Olasupo, 1997). In one study, by Isolauri *et al.* (1991), the effect of *Lactobacillus* GG of human origin was investigated on recovery of infants from acute diarrhoea mainly due to rotavirus. The results demonstrated a significant reduction in the duration of rotaviral diarrhoea by administration of the human *Lactobacillus* GG. Kaila *et al.* (1992) concluded that intestinal implantation of lactic acid bacteria may reverse mucosal dysfunction caused by enteral infection, as they found that *Lactobacillus* GG survived the passage through the gut during rotavirus diarrhoea in infants.

b) Ability to detoxify carcinogens – *Lactobacillus acidophilus* has been found to have the capacity to suppress the metabolic activity of the colonic microflora and may reduce the formation of carcinogens in the intestine (Salmien *et al.*, 2000).

c) Secretion of lactase, which might facilitate lactose digestion assist in lactose intolerant humans (Savaino 1984, Martini *et al.*, 1991).

d) Enhancement of Immune Response – In immunocompromised hosts including immune stimulation and colonization resistance to pathogens which may give incentives for their use to protect immunodeficient patients from pathogenic microbes (Salmien *et al.*, 2000).

2.5 *Lactobacillus* in Food Fermentation

In fact, many Nigerian foods are fermented before consumption. These fermentation products include alcoholic and non-alcoholic beverages, non-alcoholic main course meals, food condiments etc (Odunfa, 1985, 1988, 2002). Their use in food production is perhaps one of the oldest examples of biotechnology. Lactobacilli are responsible for the fermentation of dairy products such as yoghurt, soured milk, and hard cheeses. The ordinary consumer may not realise that they are ingesting millions of lactobacilli while eating these foods but they are also commensal colonizers of the human digestive system (Hammes *et al.*, 1992). Their historical use was probably to preserve foods and retard spoilage as well as improve flavour and texture. The reasons for the widespread use of *Lactobacillus* in the preparation of foods and other fermentation processes can be summarized as follows:

1. Enhancement of the organoleptic properties of the fermented products such as production of desired flavour which is associated with diacetyl.
2. Retardation of spoilage and reduction of contamination by (a) suppressing the growth of foodborne pathogens. (b) Suppression of growth of undesirable spoilage organisms.

Lactobacillus has been found to produce substances that inhibit the growth of foodborne pathogens and other pathogens of medical importance such as *Escherichia coli, Salmonella typhimurium, Shigella* spp., *Staphylococcus* spp., *Proteus, Klebsiella, Pseudomonas* spp. and *Vibrio.* There is now a renewed interest in using lactobacilli as a natural food preservative. Selected lactobacilli can be added to food, in the living state where they grow very slowly antagonizing undesirable spoilage microorganisms (Ray, 1992). The mechanisms include

e) Reduction of pH as a result of the production of lactic acid.

f) Production of complex antimicrobial polypeptides or proteins known as bacteriocins e.g. acidophin produced by certain strains of *Lactobacillus acidophilus*.

g) Competitive removal of substrates used by other microbes.

h) Production of hydrogen peroxide.

i) Removal of large amounts of carbohydrate by fermentation.

j) Production of specific antimicrobial acids such as lactic acid and acetic acid.

k) Alteration of the oxidation-reduction (redox) potential.

2. Enhancement of the nutritional value of foods

Despite the fact that most African fermented foods have *Lactobacillus* spp. as the predominant organism, the above-mentioned properties of this organism do not reflect because of the high incidence of childhood diarrhoea in Nigeria as reported by Alabi *et al.* (1998). The low level of personal hygiene and public sanitation as well as the low socio-economic status of most families compound the incidence and prevalence of diarrhoeal diseases in Nigeria. Thus improved food hygiene during the weaning period seems to be one important way of preventing the transmission of foodborne diarrhoea-causing pathogens. In this respect, lactic acid fermented foods may offer a high potential especially in developing countries (like Nigeria), since fermentation is a traditional household-level technology that can easily be adopted. Unfortunately, spontaneous fermentations (employed at household level) are neither predictable nor controllable. Initiations of a spontaneous fermentation process takes a relatively long time (24 - 48h) with high risk of failure during which contaminating microorganisms on raw materials, utensils, and from the environment, slowly increase in number and compete for nutrients in order to produce metabolites. This phase can be shortened by inoculation either through backslopping or with use of selected starter cultures.

The microorganisms involved in spontaneous or natural fermentation of cereals and cereal-legume blends are essentially the surface flora of grains/seeds. The types and the nature of microorganisms harboured by these grains depend on many factors such as climatic conditions under which the grains are produced, the soil in which the plants are grown, biological environment and weather conditions during and after harvesting, conditions and duration of storage (Muller, 2000). A wet mash of grains is said to undergo an acid fermentation by lactic acid bacteria (LAB) and by coliforms, with the highest number of coliforms on the second day but eliminated by the fourth day of fermentation (Kingamkono *et al.,* 1994). The nature of microflora involved in natural fermentation is complex varying from crop to crop. Lactic Acid Bacteria (LAB) are the part of the flora that dominate in water/cereal meal mixtures (Lei and Jakobsen, 2004). Since food-poisoning flora and coliforms also grow with the LAB during natural fermentation, these microorganisms need to be eliminated to make fermented foods safe for consumption. Earlier researches in this field indicated that lactic acid fermented cereal-based and dairy foods, significantly suppressed the growth of foodborne pathogens (Nout *et al.,* 1989, Svanberg *et al.,* 1992, Olukoya *et al.,* 1994). Lactobacilli are used as starters in several fermented foods to inhibit the growth and activity of several microorganisms (Lewus *et al.,* 1991, Vignolo *et al.,* 1993).

2.6 Starter Cultures

A starter culture may be defined as a preparation or material containing large numbers of viable microorganisms, which may be added to accelerate a fermentation process and enable control of the fermentation and give a predictable process (Salmien *et al.,* 2000). Starter cultures in addition can also impart desirable sensory attributes, antagonism against pathogenic organisms, degradation of antinutritional factors, detoxification, and probiotic qualities. Starter cultures are subcultures, and depending on their concentration, they are inoculated directly or require further subculture before use. Historically, a starter culture was simply a sample of fermented food, retained for inoculating the next batch of product as in production of ***kenkey***. This process was known as "backslopping" and gave a variable outcome. The disadvantage of uncontrolled fermentation is the recurring variation in product quality and stability leading to product inconsistency and inability to predict the fermentation

(Westby and Choo, 1994). Nowadays, fermentation has to be predictable and consistent to ensure the maintenance of product quality and this underpins the importance of starter cultures in food production.

Traditionally, starter cultures were associated with lactic acid fermentation. Starter cultures are most extensively used in the dairy industry, but are also used in the production of other fermented foods, including meat, wine, vegetables, and cereals (Motarjemi, 2002). Bacteria are selected for use in the food industry by virtue of their properties. Starter cultures perform reactions that chemically alter raw food material into a desirable edible product. By their growth and metabolism these microbes, either alone or in combination, also alter the physical and biological composition of food. The important features of a microbe that ensure its use as a starter microbe include non-pathogenicity, lack of production of toxic chemicals, absence of production of chemicals that reduce desirability of product, ease of storage and transport period required for raw material to be converted to final edible product. Others include genetic stability of the species, some degree of natural resistance to inhibitory factors present in the raw material and the accumulating product as fermentation proceeds, ability to grow symbiotically, with other starter microbes if need be, production reproducibility between different batches of cultures grown and lack of spoilage of the product as it matures and during storage (Holzapfel, 2002). Being adapted to the substrate, a typical starter facilitates improved control of a fermentation process and predictability of its products (Holzapfel, 1997; 2002). In addition, starter cultures facilitate control over the initial phase of a fermentation process.

There is also a growing interest in the use of traditional fermented foods for weaning as it is thought that bacterial pathogens likely to cause diarrhoea could not survive for long periods in them because of the inhibitory activities of the substances produced during the fermentation. The possible mechanisms for the antagonistic effects of Lactobacilli that have been proposed are:

1. Organic Acid Production – e.g. lactic and acetic acids with pH < 4.0. During fermentation of cereal-based products, pH decreases with a concomitant increase in acidity as the lactic acid and other organic acids accumulate due to microbial activity. The antimicrobial effect of organic

acids produced by lactobacilli is due to the undissociated form of the acid, which can penetrate the membrane and liberate hydrogen ions in the neutral cytoplasm, which then leads to inhibition of vital cell functions and the specific effect of the molecule itself (Barber *et al.*, 1989). It has been reported that at pH ranges around 4.0 – 4.7, acetic acid (pKa – 4.75) is a much more powerful inhibitory substance than lactic acid (pKa – 3.86). The antimicrobial effects are thus pronounced at pH values below the pKa value for the acid. In cereal-based food fermentations, both acids may act synergistically (Gobbetti *et al.*, 1995).

2. Diacetyl Production - is not only important because it is responsible for the desirable flavour in many foods, but also because it has antimicrobial property. Diacetyl is produced by some species and strains of Lactic Acid Bacteria (LAB) as a metabolic end product synthesized from the intermediary metabolite – pyruvate. LAB produce diacetyl and acetoin primarily as a means of converting excess pyruvate into non-toxic neutral compounds that are of little or no use to the microbes that produce them (Ray and Daeschel, 1992). Jay (1982) reported the antimicrobial effects of diacetyl on some bacteria associated with food spoilage and foodborne diseases. These bacteria include *Enterobacter aerogenes, Escherichia coli, Pseudomonas* spp., *Salmonella* spp. *and Staphylococcus aureus.* He also reported diacetyl to be unique as an antimicrobial compound being more effective against Gram-negative bacteria and fungi than against Gram-positive bacteria with lactobacilli being the least sensitive. Motlagh *et al.*, (1991) and other researchers reported that the antimicrobial property results only at relatively high concentrations but is ineffective and can even be metabolized or destroyed by some microorganisms at lower concentrations. Diacetyl at present is available primarily as a starter culture distillate and can only be used alone or in combination with other antimicrobial treatments to increase the destruction of microorganisms during sub pasteurization heat treatment of foods. *In-vitro* studies have indicated that diacetyl deactivates enzymes from several microbes by blocking or modifying the catalytic sites (Guyot *et al.*, 1996). It can exist in monomer, dimer, and trimer forms, with trimer being the most effective as an

arginine- modifying agent with the maximum antimicrobial activity at pH 5.0.

3. Hydrogen Peroxide Formation – Hydrogen peroxide also has antibacterial action and is usually produced by lactobacilli as a protective mechanism, through pyruvate and others. It accumulates because *Lactobacillus* does not produce catalase. It was reported that inhibited growth of *Staphylococcus aureus* by *L. bulgaris* and *L. lactis* was due partially to hydrogen peroxide. It was also reported that storage at low temperature favoured the formation of hydrogen peroxide and that there is an inverse relationship between it and acid production. It functions in the lactoperoxidase system of dairy products.

4. Bacteriocin production.

5. Production of antibiotic-like substances.

2.7 Probiotics

The role of probiotics in human nutrition has been increasingly recognized together with the growing public awareness of certain health benefits of fermented foods. Today, a broad variety of products containing microorganisms with probiotic function is available in the market.

Fuller (1989), defined "probiotics" as a "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance". This definition has also been extended to human nutrition and probiotics have now been defined as "live microbes which when ingested, enhance the well-being of the host through their effect on the intestinal microflora". However, Klaenhammer (2001) defined probiotics as "live microbial cell preparations that when applied or ingested in certain numbers exert a beneficial effect on health and well-being", while Rolfe, (2000) defined probiotics as viable microorganisms that have a beneficial effect in the prevention and treatment of specific pathologic conditions when they are ingested. Metchnikoff first popularized the concept of probiotics at the turn of the 20th century: who proposed that a normal, healthy gastrointestinal microflora in humans and animals provided resistance against "putrefactive" intestinal pathogens. He theorized

that the intestinal flora influences the incidence and severity of enteric infections and either enhances or slows atrophy and aging processes (Klaenhammer, 2001).

The probiotics that can be applied in human nutrition belong to the group of Lactic Acid Bacteria (LAB) only. At present, it has been shown that probiotic effects could be attributed primarily to certain species and strains of lactobacilli, bifidobacteria, enterococci, and lactococci. It is important that this function must not be considered as a general property of a defined genus, but of defined strains (Breidt and Fleming, 1992, Kneifel *et al.,* 1999). In addition to LAB, *Saccharomyces bourlardii* is used as a probiotic. This species of yeast isolated from litchi fruits in Indonesia is used as a probiotic (Tomasik and Tomasik, 2003).

Probiotics have been examined for their effectiveness in the prevention and treatment of a diverse spectrum of gastrointestinal disorders. These include antibiotic-associated diarrhoea (including *Clostridium difficle*-associated intestinal disease), infectious bacterial and viral diarrhoea (including diarrhoea caused by rotavirus, *Shigella, Salmonella,* enterotoxigenic *E. coli, Vibrio cholerae*), and human immunodeficiency disorder, enteral feeding diarrhoea, *Helicobacter pylori* gastroenteritis, sucrase - maltase deficiency, inflammatory bowel disease, irritable bowel syndrome, small bowel bacterial overgrowth and lactose intolerance (Rolfe, 2000). The human gastrointestinal tract (GIT) forms a very variable ecosystem with a wide range of different conditions. This naturally affects both the microflora and the physiological responses of the host in each particular intestinal location. The digestive tract is composed of four major microbial population categories defined as;

- Autochthonous microflora- populations of microbes that are present in high levels and permanently colonize the host.
- Normal microflora- microorganisms that are frequently present, but can vary in number and sporadically absent.
- True pathogens- microorganisms that are periodically acquired, but can persist, causing infection and disease.
- Allochthonous microflora- microbes of another origin and present temporarily (most probiotic cultures are allochthonous) (Holzapfel *et al.,* 2001).

The natural microflora of the host thus forms the background for the action of each probiotic strain, which may survive, colonize, and act in the typical environment of the human host. LAB are normal residents, of the GIT of human being among the facultative flora (Kneifel *et al.,* 1999, Salmien *et al.,* 2000). Gram-positive bacteria such as *Bifidobacterium, Eubacterium,* and Gram-negative species such as *Bacteroides* dominate the microflora of the oral cavity, and *Fusobacterium* common in the lower parts of the intestinal tract can be found among the mouth microflora.

However, it has been reported that during the course of evolution, a certain balance has been established between the host and the intestinal microflora. Although the intestinal tract forms an ideal ecological habitat for numerous bacterial species, their presence and metabolic activities have several important consequences- some beneficial, others potentially harmful for the host, for instance, a positive function is the prevention of bacterial and viral infections. The normal intestinal flora of man performs an important protective function, as the presence of resident bacteria prevents pathogens establishing themselves or colonizing the intestinal tract. Therefore, any bacterial strain administered as a probiotic must compete with the established flora, for nutrients, atmospheric requirements and attachment sites. The pH of an empty stomach (normally < 3) very effectively eliminates most microbes. However, the buffering effect of foods during meals allows for the survival of both salivary bacteria and of microbes present in the ingested foodstuffs.

2.7.1 Characteristics of Probiotics

To be effective a probiotic strain of LAB requires several basic properties, the most important of which are:

i. The ability to survive passage through or colonisation of the oral cavity, stomach, small and large intestines.

ii. The stability to bile acids, thus the strains must be stable in gastric conditions and resistant to acid.

iii. Adhesion to intestinal epithelial cells.

iv. Stabilization of the intestinal microflora.

v. Amenable to cultivation on an industrial scale

vi. Ability to multiply fast with either permanent or temporary colonisation of the GIT.

vii. Ability to survive in foodstuffs and possible of production of pharmacopoeia lyophilized preparation.

Most evidence for the beneficial probiotic action has been obtained from cases where there has been a severe disturbance in the normal intestinal microbial balance i.e. bacterial and viral diarrhoeas, lactose malabsorption, antibiotic or surgical interventions or radiotherapy-induced intestinal disturbance (Salmien *et al.,* 1998). Though the mechanisms of probiotic action are still unclear, various studies on probiotic strains of LAB revealed information on the probable mechanism of action.

2.7.2 Mechanism of Action

The proposed mechanisms of action include:

a) Suppression of harmful bacteria and viruses – Specific probiotics in foods have been shown to block the adhesion of pathogenic bacteria to human intestinal mucosa and to prevent enteroinvasive pathogens from invading mucosal cells by competitive exclusion, production of antimicrobials such as lactic acid, bacteriocins etc or cell aggregation, and competition for nutrients (Isolauri *et al.,* 2001). For example, *L. acidophilus* (NFCO 1748) and *L. casei* strain GG are included among the probiotics used on man. *Lactobacillus* GG has been reported to colonize the human intestinal tract and adheres more strongly to human intestinal and mucosal buccal cells than other strains of *Lactobacillus* or *Streptococcus* used as starter cultures in the dairy industry. Colonization with *Lactobacillus* GG is more efficient in infants and children than in adults. In a study of infants with rotavirus diarrhoea, feeding 10^9 cfu/day *Lactobacillus* GG as a freeze-dried powder resulted in colonization of all babies (Salmien *et al.,* 1993).

Kaila *et al.* (1991) found that *Lactobacillus* GG survived the passage through the gut during rotavirus diarrhoea in infants as all patients who

received *Lactobacillus* GG became colonized with the strain as measured by faecal *Lactobacillus* GG counts. They then suggested that *Lactobacillus* GG promotes the establishment of colonization resistance even during acute gastroenteritis. It was also suggested that *Lactobacillus* GG may prove to be beneficial supplement in the treatment and prevention of clinical disorders associated with increased intestinal permeability. In healthy newborns, similar colonization by *Lactobacillus* GG was observed and it was concluded that the composition of intestinal microflora of newborns can be influenced by early administration of *Lactobacillus* GG resulting in colonization of most newborns (Mikelsaar *et al.,* 1992).

b) Stimulation of local and systemic immunity – Lactobacilli are reported to be involved in the stimulation of phagocytic activity e.g., *L. casei* and *L. plantarum* given parenterally stimulate phagocytic activity. Increase in antibody levels was reported when germ-free mice were fed with *Lactobacillus*-fermented yoghurt. Lactobacilli are known to translocate and survive for many days in the spleen, liver, and lungs to have systemic effect. However, these findings of a systemic effect on immunity do indicate that probiotics have the potential, not only to affect the balance of the gut flora, but also to influence the pathogenesis of diseases, which occur in tissues remote from the intestinal tract. For example, administration of probiotics containing *Bifidobacterium bifidum* and *Streptococcus thermophilus* to children with rotaviral diarrhoea resulted in faster seroconversion within IgA and IgM accompanied by the growth of cells producing IgM antibodies that is increased production of IgA antibodies and increased macrophage activity (Isolauri *et al.,* 2001).

Lactobacillus is known to suppress the growth of urinary tract pathogens through the production of antimicrobial substances, and application of pure cultures of *Lactobacillus* has been suggested as a remedy for some vaginal infections in the face of increasing antibiotic resistance. Bruce and Reid (1988) indicate intravaginal and perineal implantation with *Lactobacillus* may be successful in preventing urinary tract infection in women by

excluding uropathogens from the vaginal flora (Fuller, 1989, Kaila *et al.*, 1991, 1992; Hammes and Tichaczek, 1994).

c) Alteration of gut microbial metabolic activity – is by increased and decreased enzyme activity as appropriate. Fuller (1989) reported that when *L. acidophilus* was fed to human subjects and selected enzymes were looked for this treatment suppressed the activity of β-glucuronidase, nitroreductase and azoreductase, which are responsible for the conversion of procarcinogens into carcinogens. Similarly, in rats associated with a human gut flora, there was a reduction in β-glucuronidase and β-glucosidase when they were dosed with the same strain of *L. acidophilus*. Lidbeck *et al.* (1991) gave a dose of fermented milk containing *L. acidophilus* NCFB 1748 to 14 patients with colonic cancer twice daily (10^9 cfu/day) for six weeks and observed significant increase in faecal *Lactobacillus* was observed in 10 of the patients.

Unhealthy fermentation commonly occurring in the large intestine produces poisons, which are absorbed and lead to deterioration of the tissues of the walls of the arteries, and so to senile changes and unduly early death. The use of "acidophilus" milk was used to arrest this unhealthy process in the intestine by introducing the lactic acid bacteria which on forming lactic acid renders the life and growth of the bacteria of those poisonous fermentation (which cannot flourish in an acidic environment) impossible (Isolauri *et al.*, 2004). Also, chronic constipation in elderly people has been treated using *Lactobacillus acidophilus* yoghurt supplemented with fibre and lactitol. Probiotics may also have their influence by increasing the activity of useful enzymes e.g. uses of β-galactosidase (lactase) in the alleviation of lactose (Rajala 1988, Hammes and Tichaczek; 1994, Salmien *et al.*, 1998).

The positive effect of probiotics is not limited solely to the gastrointestinal tract. Enzymatic hydrolysis with participation of bacteria increases bioaccessibility of lipids and proteins and reduces allergenicity of foodstuffs. Children receiving mixtures enriched in probiotics were seen to have exhibited significantly fewer allergy symptoms than the control group (Tomasik and Tomasik, 2003; Majama and Isolauri, 1997).

Other benefits of probiotic bacteria are:

- Reduction in allergic inflammation
- Protection against certain types of cancer
- Lowering serum cholesterol levels and reducing the incidence of coronary heart disease.
- Prevention and treatment of peptic ulcer disease.

Several studies have confirmed that cereal-based weaning foods and water are important sources of pathogens thus contamination of weaning foods constitute potential sources of diarrhoea in children (Svanberg and Lorri, 1994; Black *et al.*, 1982; Rowland *et al.*, 1978).

Odunfa and Adeyele (1985) reported that the fermentative microflora responsible for the pH decrease in Nigerian "ogi" production was dominated by *Lactobacillus*. Various dishes are made from "ogi" by boiling with water to make gels of variable stiffness (Odunfa, 1985, Adeyemi and Oluwamukomi, 1989, Hounhouigan, 1999). These include "akassa", a stiff gel-like product eaten with fish or meat stew, "agidi", a dumpling-like food wrapped in leaves and "koko", a porridge food used for breakfast. Due to time involved in food preparation and because food is too valuable to be thrown away large amounts of "ogi" are usually prepared for babies and kept for long hours before consumption. There is also the practice of diluting the "ogi" with unwholesome water before feeding it to the baby. This amongst other unhygienic practices further increases the potential of "ogi" to carry pathogens. The lack of refrigeration facilities at the household level enables rapid proliferation of microbial contaminants in foods. In infant foods, such as porridge, which is often prepared once or twice daily for use at intervals during the day, the growth of pathogenic bacteria may contribute to acute diarrhoea (Olukoya *et al.*, 1994). Thus improved hygiene during weaning period seems to be one important way to prevent the transmission of foodborne diarrhoea-causing pathogens. In this respect, the lactic acid-fermented foods may offer a high potential especially in developing countries, since fermentation is a household level technology that can easily be adopted. However, this "ogi" has a few problems (Olasupo, *et al.*, 1997):

1. Short shelf life due to the activity of spoilage organisms, contamination and poor hygiene.
2. Deficiency in some nutrients due to loss of important nutrients during processing.
3. Decrease in acidity on storage.

All these problems are due to spontaneous fermentation depending on chance inoculation from the environment, as starter cultures are not used and because of the activity of contaminating spoilage organisms. Thus to overcome these problems starter cultures are necessary to develop better weaning food.

Most of the studies evaluating the antagonistic effect of lactic acid – producing bacteria on intestinal pathogens have been based on dairy products. Although information on the effect of these bacteria from cereal-based products on the control of diarrhoea in the community is very limited, a few in vitro studies have reported inhibited growth of ETEC and diarrhoea-causing pathogens of genera such as *Campylobacter, Salmonella, Shigella, Staphylococcus,* and *Bacillus* (Mensah *et al.,* 1990, Svanberg *et al.,* 1992).

CHAPTER THREE

MATERIALS AND METHODS

3.0 MATERIALS AND METHODS

3.1 Sample Collection

3.1.1 Cereal Samples:

Eleven varieties of maize (*Zea mays* L.) used were collected from Maize Research Laboratory of the International Institute of Tropical Agriculture, (I.I.T.A.) Ibadan while the sorghum varieties (*Sorghum vulgare*) were purchased from Mile 12 Retail Market, in the Lagos metropolis and authenticated by Mr. O.K. Oluwa of the Department of Botany, Lagos State University, Ojo. The maize varieties are: TZPB-SR, TZB-SR-SE, EV8363-SRQPM, TZSR-W-1, TZB-SR, 8321-21, SUWAN-1-SRQPM, EV8766-SR-YQPM, TZSR-Y-1, 8321-18, EV8762-SR, and designated 1-11 respectively. The red, yellow, and white sorghum varieties were designated 12-14 respectively.

3.1.2 *Lactobacillus* Species (Starter Culture)

Two *Lactobacillus* species - *L. acidophilus* and *L. pentosus* obtained from the culture collection of the Biotechnology Unit of Nigeria Institute of Medical Research, Yaba, Lagos were used as starter cultures. These two organisms confirmed as bacteriocin-producing and amylase-producing were isolated from "wara" and "ogi" respectively and characterized by Olukoya (1994). The two bacteria maintained in 50% glycerol in de Mann Rogosa-Sharpe broth and stored at -20 ^{O}C were resuscitated by culturing in MRS broth incubated at 30 -32 ^{O}C for 36h, later subcultured microaerophilically on MRS agar at 37^{o}C before use.

3.1.3. Test Organisms:

The diarrhoeagenic agents used in this study were *Salmonella* (AP23118), *Vibrio cholerae*-01 (Eltor strain, AP23622), *Shigella* (AP22433), and *Staphylococcus aureus* (ATCC25923) *Escherichia coli* (ATCC25922), and *E. coli* 0157:H7, a food poisoning agent. These organisms were clinical isolates obtained from Nigerian Institute of Medical Research (NIMR) collections. They were reported as local isolates taken to International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB), where they were authenticated and coded as indicated above for repository purposes. *Shigella dysenteriae* isolated from diarrhoeal patients at Lagos University Teaching Hospital (LUTH), General Hospital Lagos, and Massey Children Hospital Lagos, was collected

from Genetics Division of NIMR, Yaba, Lagos. This was authenticated biochemically before use. Virulence disposition of the isolates was further assessed using the method of Sereny (1957). The bacteria were grown using appropriate media, prepared according to the manufacturers' instructions.

3.1.3.1 Maintenance of stock cultures

- **LAB** The two *Lactobacillus* organisms were maintained at at -80°C for 6 months as follows:

  2.5ml of 18h old culture of *Lactobacillus* in MRS broth was mixed with equal volume of 50% glycerol and stored at a temperature range of -80°C in Ultra Low freezer SO-LOW Model U85-18.
- **Test Organisms** The test organisms were subcultured on Nutrient agar slants incubated for 18h at and stored at 4-8°C in the refrigerator until used.

3.1.3.2 Assessment of degree of virulence of Test organisms

One full loop of *Shigella* cells was taken from overnight culture on Congo-red-TSA and instilled into the conjunctiva of a healthy guinea pig The animal was observed for 72h for the development of keratoconjunctivitis. Initially, for a positive virulence test, at 24 h the eye developed blood red colour and would be totally blind after 72h (Sereny, 1957).

3.1.3.3 Sensitivity Test

Antibiotic sensitivity testing of the isolates was carried out using Mueller Hinton agar. This was performed using the disc-diffusion method Bauer *et al.* (1966) according to the National Committee For Clinical Laboratory Standards (NCCLS) guidelines. The antibiotic sensitivity discs from Oxoid (England) consisting of tetracycline 25µg, ampicillin 30 µg, colistin sulphate 30 µg, chloramphenicol 30 µg and gentamicin 25 µg were placed on the solidified agar surface and incubated aerobically for 24h at 37°C. *E. coli* ATCC25922, a standard strain was used as the control. Results were interpreted as sensitivity (S) or resistance (R) based on (NCCLS) guidelines (NCCLS, 1995).

3.1.4 Experimental Animals:

One hundred and fifty, three weeks old male weaning Swiss albino mice (150), of weight ranging from 22 - 27g were obtained from the Animal Colony of the Nigerian Institute of Medical Research, Yaba-Lagos. The animals were kept in plastic cages in a well-ventilated room at temperature of 30-32^{0}C.

3.1.5 Bile:

The gall bladder of a Vet-certified disease-free, freshly killed cow was collected from Bariga abattoir in Lagos metropolis. This was punctured using a sterile needle and the bile drawn aseptically into a sterile container using sterile syringe and kept in the refrigerator at 4 - 8 ^{O}C until needed.

3.2 Sterilization and Aseptic Techniques

3.2.1 Glassware

All glassware including conical flasks, McCartney bottles, glass pipettes, glass funnels, glass petri dishes, screw-capped tubes, test tubes, Durham bottles and measuring cylinders were thoroughly washed in detergent solution, rinsed in distilled water, drained, and allowed to dry. After drying the glassware were wrapped in aluminium foil and placed in an oven (UNISCOPE SM 9053) set at 180^{0}C for 3h.

3.2.2 Growth Media

All growth media both solid and liquid and other solutions were prepared according to the manufacturer's instruction and sterilized in an autoclave (All American Model No. 75X) at a temperature of 121^{0}C at 1.0kg/cm^2 for 15 min, unless otherwise stated.

3.2.3 Bench Work

The work bench was thoroughly swabbed with absolute alcohol before each experiment in order to get rid of contaminants. The incubator (UNISCOPE SM 9082) was fumigated using mixture of phenol and absolute alcohol to reduce incidence of contamination during incubation.

3.2.4 Inoculating Loop

Inoculating loop was sterilized by flaming the platinum loop over a Bunsen flame until red-hot and then allowed to cool before use.

3.2.5 Glass Rod

Glass rod (hockey stick) was sterilized by dipping it in alcohol, igniting in a Bunsen flame and then allowing it to cool before use.

3.2.6 Filter Papers, Tips, Wire Mesh (sieve)

Filter papers, pipette tips, and sieve were wrapped in aluminium foil, and sterilized by autoclaving as described in section **3.2.1**.

3.3 Preparation of Cereal Samples

3.3.1 Processing of cereals

Five hundred grammes of sample were weighed into 1.5 litre of 5% (w/v) sodium metabisulphite solution placed in a 3 l conical flask. They were made to soak for 24 h to prevent microbial contaminants and to surface-sterilize the grains. The grains were then washed three times repeatedly with sterile distilled water, drained, blotted dry using sterile filter papers and then resoaked in 2 l of sterile distilled water for two days; Olukoya *et al.* (1994) and then wet-milled using a Philips HP-2815 Model Blender under aseptic conditions. Each of the wet-milled cereal was subsequently passed through a sterile wire mesh (0.2mm. pore size) to obtain fine slurry, with the pomace retained on the sieve. The pomace was discarded while the slurry was allowed to sediment in the flask.

3.3.2 Preparation of starter culture

The stock cultures of the two *Lactobacillus* spp were resuscitated by bringing the frozen MRS/Glycerol broth culture to room temperature of 30 ± 2 °C by leaving them to thaw. The organisms were subcultured on MRSA and incubated microaerophilically at 37°C for 24h. This 24h pure culture was further subcultured into MRS broth and incubated for 18h after which it was centrifuged using Refrigerated Centrifuge Model GL 18B at 3,000rpm for 10min to sediment the cells. The supernatant was discarded and the cells washed three times using PBS. The washed cells in PBS were used to prepare the inoculum.

3.3.3. Determination of inoculum size

The turbidity of the washed cells in PBS gave MacFarland Turbidity Standard of 2 (600×10^8). This was diluted serially using PBS to give a colony-forming unit (cfu) of (6×10^6) which served as starter culture in inoculating the wet-milled ogi.

3.3.4 Fermentation of processed cereals

The slurry of each cereal variety was divided into three parts and sterilized by filtration. To each part, the appropriate starter culture (*L. acidophilus* and *L. pentosus* singly and combined) of about 6×10^6 cfu/ml was added. Each preparation was allowed to ferment for 72h at room temperature of 30 ± 2 °C. All these operations were carried out under aseptic conditions.

3.3.5 Spontaneous fermentation of cereal types

Approximately 150g of each of the fourteen cereal varieties were weighed, soaked in tap water and prepared using the traditional method of Odunfa (1985). The preparation involved soaking of maize grains in water for 1 to 3 days followed by wet milling and sieving to remove bran, hulls and germ. The pomace was retained on the sieve and later discarded, while the filtrate was fermented (for 2-3 days) to yield ogi (Appendix 1). This spontaneous fermentation was at room temperature 30 ± 2 °C.

3.3.6 Processing and Analysis of Fermented Cereal Samples

3.3.6.1 Determination of microbial load during and after fermentation

The population of lactobacilli in each fermented gruel during fermentation (i.e. "ogi") was determined using the plate count technique and recorded as colony forming unit per gram of gruel (cfu/g). Each of the fermented gruels was thoroughly mixed with water and centrifuged using Refrigerated Centrifuge Model GL 18B. An aliquot of 0.1ml of each supernatant was taken and plated out on MRS agar. This was incubated under microaerophilic conditions at a temperature of 37°C for 24h. Counts were taken in triplicates and results expressed as mean of three.

3.3.6.2 Production of powdered samples

A quantity of each fermented cereal type was drained using muslin cloth and placed in sterile glass Petri dishes and left to dry using a UNISCOPE hot air oven Model SM9053 at 60^0C for 12h to produce flour.

3.4 Proximate Analysis of Fermented Cereal Gruel

3.4.1 Determination of Total Protein

The Micro-Kjeldahl method for protein determination was employed (Ma and Zuazaga 1942). Two-gramme flour of each "ogi" sample was weighed accurately on a filter paper and carefully wrapped to avoid losing any of the fermented gruel, dropped into a 500 ml Kjeldahl flask, and 8g of catalyst, mixture (K_2SO_4 and $CuSO_4$) added. Twenty ml of concentrated sulphuric acid was added and the flask placed on the heating mantle in an inclined position in a fume cupboard. Heat was first applied gently and later vigorously after initial frothing had stopped. The flask was swirled and shaken from time to time in order to wash down charred materials adhering to the flask and then heated continuously until the liquid became clear, after which it was again heated for 1 h. The flask was allowed to cool and the mixture diluted with about 150 ml of tap water. To the liquid, now in the distillation flask and ready to be distilled was added one large piece of granulated zinc (to minimize bumping during the distillation). Fifty ml of 2% boric acid was pipetted into 250 ml receiving flask and a few drops of screened methyl red indicator added. It was ensured that the delivery tube dropped below the acid solution and that all joints were tight. Through the top funnel, 75 ml of NaOH solution (45g NaOH in 75 ml water) was added. The funnel was closed after the liquid mixture had been confirmed to be alkaline. The alkaline liquid in the flask was boiled taking care to prevent undue frothing in the early stages and about 150 – 200 ml distilled. The top funnel was opened before turning off the gas. The receiving tube was washed down into the receiving flask and 10ml of the cold distillate was titrated with 0.1N H_2SO_4. The same procedure was carried out for the blank test without the sample but using 8g of mixed catalyst to filter paper and 20 ml of H_2SO_4 and allowed to digest.

Calculation of the protein was thus done.

mg/g Protein = %N × 5.7

$$\%\text{Nitrogen} = \frac{(Y-X) \times 1.4 \times N}{W}$$

Where N = actual standardised normality of H_2SO_4 used is 0.1107N

W = weight of sample

X = titre value of blank

Y = titre value of sample

5.7 – Gravimetric factor

1ml 0.1N acid is 1.401mg/N

3.4.2 Amino Acid Determination

Rosen (1957) method which is a modified Ninhydrin method was used in determining the amino acid profile of the cereal gruels. Approximately 2 g of slurry was suspended in 20 ml of phosphate buffer (pH 7.0) in a 250 ml beaker. The suspension was centrifuged with Refrigerated Centrifuge Model GL 18B at 3000 rpm for 10 min and the supernatant poured into a separating funnel, and shaken with 10 ml petroleum ether to remove the organic pigments. The top phase was discarded and the aqueous phase which contained protein and amino acids was retained. The protein was precipitated from the aqueous phase by adding 5 ml of 10% (v/v) trichloroacetic acid (TCA) to 5 ml of the extract. The mixture was shaken and kept in the freezer for 10 min. The precipitate formed was removed by centrifuging and the filtrate was used for total amino acid profile determination using thin layer chromatography (TLC).

3.4.3 Chromatographic separation of amino acids

The amino acid contents of the extracts were separated by thin layer chromatography using the method of Mikes and Chalmers (1989).

The thin layer plate was dried at 110^{0}C for 10min before use as TLC plates tend to pick up moisture from the air and this can considerably reduce their ability to adsorb the sample. Aliquots of 50 µl of each filtrate were spotted on thin layer glass plates along with 20 µl of reference standard amino acids. The standard amino acids (0.1% w/v) were isoleucine, lysine, tryptophan and methionine. One dimensional ascending chromatography was used. The solvent system employed for the separation was n-butanol: glacial acetic acid: water at a ratio of 4:1:2 (v/v/v) and separation was carried out for 3 h. The chromatograms were air-dried and the amino acids located by spraying with 0.2% (w/v) ninhydrin in ethanol. These were allowed to air-dry and then oven-dried at 100°C for 5 min for the spots to develop. Identification of the separated amino acids was done using the reference standard.

The quantitative estimation of the amino acids was done using the colorimetric method (Rosen, 1957). The supernatant of each fermented gruel was prepared and used to determine the concentration of each amino acid present. Each supernatant was made up to 2 ml with distilled water and 1 ml ninhydrin solution was added. The absorbance taken at 570 nm was read using a spectrophotometer (Spectronic Unicam Type Helios Gamma NC 9423 UNG Model). Absorbance readings were taken in triplicate. The control test tube contained 2 ml. distilled water and 1ml ninhydrin solution. The concentration of each amino acid was extrapolated from a standard curve previously prepared.

3.4.4 Determination of total reducing sugar:

This was carried out using the Nelson-Somogyi procedure (Somogyi, 1952).

Two gram of each cereal sample was weighed into different test tubes and thoroughly mixed using autovortex mixer (Stuart Vortex Model SA8. Volts-90-240). The supernatants were each treated to give a neutral protein-free solution. After 10 min, the tubes were centrifuged using Refrigerated Centrifuge (Model GL18B) at 3000 rpm for 5 min.

One millilitre of alkaline copper tartarate solution added. The tubes and the contents were incubated for 3 min in a boiling water bath and the colour change recorded. The tubes and contents were cooled and to each 1 ml of Nelson Reagent B added. All tests were done in triplicates and average taken. The absorbance was read at 556 nm using a Beckman spectrophotometer. The total reducing sugar was extrapolated from a standard curve previously prepared.

3.4.5 Detection and Quantitative estimation of diacetyl

The colorimetric method of Westerfield (1945) was used to determine the concentration of acetoin.

A weighed (0.5g) quantity of creatine was dissolved in 100 ml of distilled water. Three ml of filtrate from **3.5.1**(ii) was put into well-labelled test tubes, and 1ml. of the creatine solution was added to the filtrate. 0.5g. of α-Naphthol was dissolved in 10 ml 2.5 NaOH. 1ml of the α-naphthol – NaOH solution was added to the filtrate-creatine solution. Colour was allowed to develop at room temperature for 10 minutes (i.e. brown to pink colouration). Absorbance readings were taken in triplicate at 540nm for diacetyl using spectrophotometer (Spectronic Unicam Type

Helios Gamma NC 9423 UNG Model). A calibration curve was plotted and the quantity of diacetyl extrapolated from it.

Detection and Quantitative estimation of acetoin

The same procedure as for diacetyl was employed but the absorbance was taken at 540nm an hour after that of diacetyl in triplicates.

3.4.6 Sensory evaluation:

Sensory evaluation of the fermented cereal gruels was carried out by use of 10 untrained taste panellists in a special room prepared for the purpose (Ebuehi *et al.*, 2004). They were instructed to taste the fermented cereal samples and to rinse their mouth after each taste. They were requested to express their feelings about the samples by scoring the following attributes: appearance, texture, taste, aroma and overall acceptability. Questionnaires (Appendix II) were distributed to each of the panellists and sensory scores were based on a 9- point hedonic scale, where 1 is dislike extremely and 9 is like extremely (Watt *et al.*, 1989). The responses of the panellists with regard to their preference for the samples were statistically analyzed.

3.5 Physicochemical Determinations

3.5.1 pH Determination: The pH of each sample was determined using a pH meter (Metrohm 780pH model).

This was determined for the wet slurry as well as for the flour dispersed in water.

i. The wet samples, 10 ml of slurry was mixed thoroughly and allowed to sediment. Five ml of the supernatant water was taken and the pH determined.

ii. To 2g flour was added 10 ml of sterile distilled water. This mixture was thoroughly mixed using an auto vortex mixer (Stuart Vortex Model SA8. Volts-90-240) to enhance homogeneity and then filtered using Whatman No.1 filter paper. The pH of the filtrate was taken.

3.5.2 Determination of titratable acidity:

Ten millilitre of each slurry was mixed thoroughly with sterile distilled water and allowed to settle. To 5 ml of supernatant was added 3 drops of phenolphthalein indicator. Then 1 ml of 0.1 M NaOH was made up to 20ml. and 5ml of this was then made up to 20ml with distilled water. The supernatant-phenolphthalein solution was then titrated with the new

concentration of 0.1 M NaOH. The colour change, titre value and volume of supernatant used were noted. Readings were taken in triplicates.

Calculation:

Titre value= Base volume reading before titration – Base volume reading after titration.

$$\text{Titratable Acidity} = \frac{2.5 \times 10^{-3}\ \mu\text{mol/ml} \times \text{titre value}}{\text{Volume of Filtrate (ml)}}$$

3.6 Determination of rate of Survival of test organisms in the 56 fermented cereal samples (*In - Vitro Studies*)

Aliquots of each test organism were separately taken and centrifuged to allow the cells to sediment, the supernatant was discarded while each sediment was washed three times with phosphate-buffered saline (PBS). The cell suspension giving approximately 10^6 cfu/ml. of each organism was separately inoculated into 50ml. of fermented cereal gruel. The fermented gruel was prepared by thoroughly mixing 10ml of slurry with sterile distilled water and making it up to 50ml. Tenfold dilution of this was made in 0.1% peptone at various periods (hourly) for the detection of these test organisms by pour-plate method (Olukoya *et al.*, 1994). The plates were incubated aerobically at 30^0C for 40h. The procedure was in three replicates, and results expressed as mean of three readings.

A portion each of the flour of some cereals was also mixed with equal volume of water to form a paste, which was further diluted and cooked, and about 50ml of the cooked was then used to test if the flour retains the anti-diarrhoeagenic properties.

3.7 *In - Vivo* Studies

The method of Nader de Macias *et al.*, 1992). The fermented gruels of the following cereal varieties were used for the in vivo studies. The gruels have been fermented by the *Lactobacillus* species (both singly and combined) and spontaneously by chance inoculation.

They were TZB-SR, EV8766, QPM, and Red Sorghum.

3.7.1 Challenge Test:

All the fermented cereal samples were separately cooked at 100^0C for 5 min. and cooled down afterwards. The male mice of same age of mean group weight ranging from 22 – 25g were randomly divided into five groups (30 per group in

three replicates) I, II, III, IV, V. The control group designated Group V was fed directly from the mothers' breast milk. The other four groups were fed with the fermented cooked samples and thus designated.

Group I – *Lactobacillus pentosus*-fed

Group II – *Lactobacillus acidophilus*-fed

Group III – Mixed Culture of *L. pentosus & L. acidophilus*

Group IV – Spontaneously fermented-fed

The animals were housed in standard cages in the Animal Colony of the Nigerian Institute of Medical Research (NIMR) where the experiments were carried out. The temperature was 28° ± 2 °C. . The light was dim at night, but during the day broad daylight was maintained. The litter removal was done every 24h. Each animal was maintained on 50 ml. of cooked porridge containing 1.5×10^6 cfu/ml. of the fermenting organisms every four hours using oral catheter for 8 days.

After the feeding, the experimental and control mice were challenged orally with *Shigella dysenteriae* using a sterile catheter. The mice received a double challenge of 2×10^6 cells/ml at an interval of 6 h in peptone water and observed daily for symptoms of diarrhoea, change in stool texture, and the number of survivors counted everyday for a period of 20 days. The percentage survivors was calculated and plotted against time in days (Figure 4.43). (Nader de Macias *et al.*, 1992)

3.7.2 Recovery of Pathogen:

Wide-mouthed sterile glass funnels were fixed to the base of the cage to collect faecal samples from both experimental and control mice aseptically into plastic petri dishes at 24-h intervals. One gram of each sample was macerated and mixed with 100 ml. of normal saline in a sterile beaker. This was serially diluted up to 10^{-4} and 0.1 ml aliquot of this dilution was plated out on Salmonella-Shigella Agar (SSA) and incubated at 37 ± 2 °C for 24 h. The typical colonies of *Shigella dysenteriae* on this agar were counted using a colony counter. The log number was calculated and plotted against time in days.

3.8 Assessment of probiotic potentials of *Lactobacillus* species (Starter cultures)

3.8.1 Preparation of *Lactobacillus* species

The 18-hour cultures of the *Lactobacillus* strains were grown in sterile MRS broth for 24 h at 37 °C and then harvested by centrifugation using Refrigerated Centrifuge Model GL

18B at 3000rpm to sediment the cells. The cells were washed severally in sterile phosphate-buffered saline (PBS) and resuspended in same before use.

3.8.2 Test for Acid and Bile Tolerance

3.8.2.1 Acid Tolerance Evaluation

This was determined according to a modified method of Oh *et al.,* (2000) Thirty-two screw-capped test tubes were cleaned and into each 4ml of sterile MRS broth was dispensed. The tubes were divided into four groups, adjusted to pH values of 2, 4, 5, and 7 (control).To two of the tubes in each group was added 0.01ml aliquot of *Lactobacillus acidophilus* and to the other two *Lactobacillus pentosus* and incubated for 2, 4, 6, and 24 h in a water bath (Grant GLS 400 Model) at 37 ^{0}C. This procedure was in duplicates. After each incubation period, the growth of the strain was determined by measuring absorbance at 620nm. The acid tolerance was categorized as excellent, very good, good, fair, and poor based on their growth at different pH values.

3.8.2.2 Bile Tolerance evaluation

Ten mililitre each of MRS broth was dispensed into thirty-two screw-capped test tubes and grouped into four with 0 (control), 0.05, 0.15, and 0.3% of bile from disease-free, veterinary- certified cow. To two of the tubes in each group was added 0.1ml aliquot of *L. acidophilus* and *L. pentosus* to the other two. This procedure was in duplicates. The inoculated broth was incubated at 37^{0}C in a water bath model (Grant GLS 400). The growth of the isolates was monitored at 2, 4, 6, and 24 h by measuring absorbance at 660nm.

3.9 Storage Stability

The storage stability of both the wet and floury fermented gruels were determined after four weeks and three months respectively.

Two conditions were set up for the storage test of the wet fermented gruels. For one group the steep water (liquor) of the wet fermented gruels, was decanted daily for the four weeks of observation and for the other it was not.

The pH of the flour was measured every 10 days. Two gramme flour was thoroughly mixed with 10 ml sterile distilled water and the pH measured using a pH meter (Metrohm 780pH model).

Escherichia coli and *Staphylococcus* were chosen for the *in - vitro* survival test. *E. coli* because it is a common contaminant, and an indicator organism for food safety, and *Staphylococcus* because it is a producer of heat - resistant toxin and a food-poisoning agent.

3.10 Statistical Analysis

All results were subjected to descriptive statistical analysis for standard deviations, standard errors, based on Microsoft Excel statistical package.

For graphical presentations, errors were fixed at maximum standard deviation (SD) values for each range of readings.

CHAPTER FOUR

RESULTS

4.0 RESULTS

4.1 Antibiotic Susceptibility Pattern of Diarrhoeagenic Agents

Table 1 shows the antibiotics used and susceptibility patterns.

All the tested bacteria were resistant to tetracycline. *Shigella dysenteriae, Staphylococcus aureus,* and *Salmonella typhi, E. coli* 0157:H7 and *Vibrio cholerae* were resistant to all the antibiotics used except *E. coli* ATCC 25922 which was susceptible to colistin.

Table 4.1: Antibiotic susceptibility pattern of diarrhoeagenic bacteria tested with fermented cereal gruels.

Microorganism[a]	Local strain code	Susceptibility pattern[b]	Virulence
S. dysenteriae	AP22433	$Tet^{R}Amp^{R}Gen^{R}$.	+
E. coli	ATCC *25922*	$Tet^{R}Amp^{R}Col^{S}Chl^{R}$.	+
E. coli O157 :H7	STD	$Tet^{R}Amp^{R}Str^{R}$.	+
S. aureus	ATCC25923	$Tet^{R}Chl^{R}Col^{R}$.	ND
Salmonella typhi	AP23118	$Tet^{R}Str^{R}Col^{R}Chl^{R}$.	+
Vibrio cholerae	AP23622	$Tet^{R}Col^{R}$.	ND

R = Resistant, S = Sensitive, Tet = Tetracycline, Amp = Ampicillin, Chl = Chloramphenicol, Gen =Gentamicin, Str = Streptomycin. Col = Colistin sulphate. STD = Standard strain. +, keratoconjuctivitis induction, -, no keratoconjuctivitis induction; ND = Not determined.

4.2 Lactobacilli count in the fermented cereal gruels.

All the fermented gruels produced with either *L. acidophilus* or *L. pentosus* or both (mixed culture) yielded lactobacilli count > 10^7 cfu/g on day 4 post fermentation. Lowest (2.6 x 10^7) and highest (3.1 x 10^7) mean counts were observed in *L. pentosus*-fermented gruel and mixed culture of *L. pentosus* and *L. acidophilus* respectively. Spontaneous fermentation accounted for lactobacilli count of less than 10^7cfu/g in 75 % of the gruels tested. Unlike in laboratory adapted fermented gruels, those produced by spontaneous inoculation elicited microbial contamination by fungal species of *Aspergillus* and *Penicillium*, and bacterial species of *Staphylococcus aureus,* with yeasts *Saccharomyces cerevisiae* and *Candida spp.* being part of the fermenting flora. There was reduction in the growth of these contaminants as fermentation proceeded.

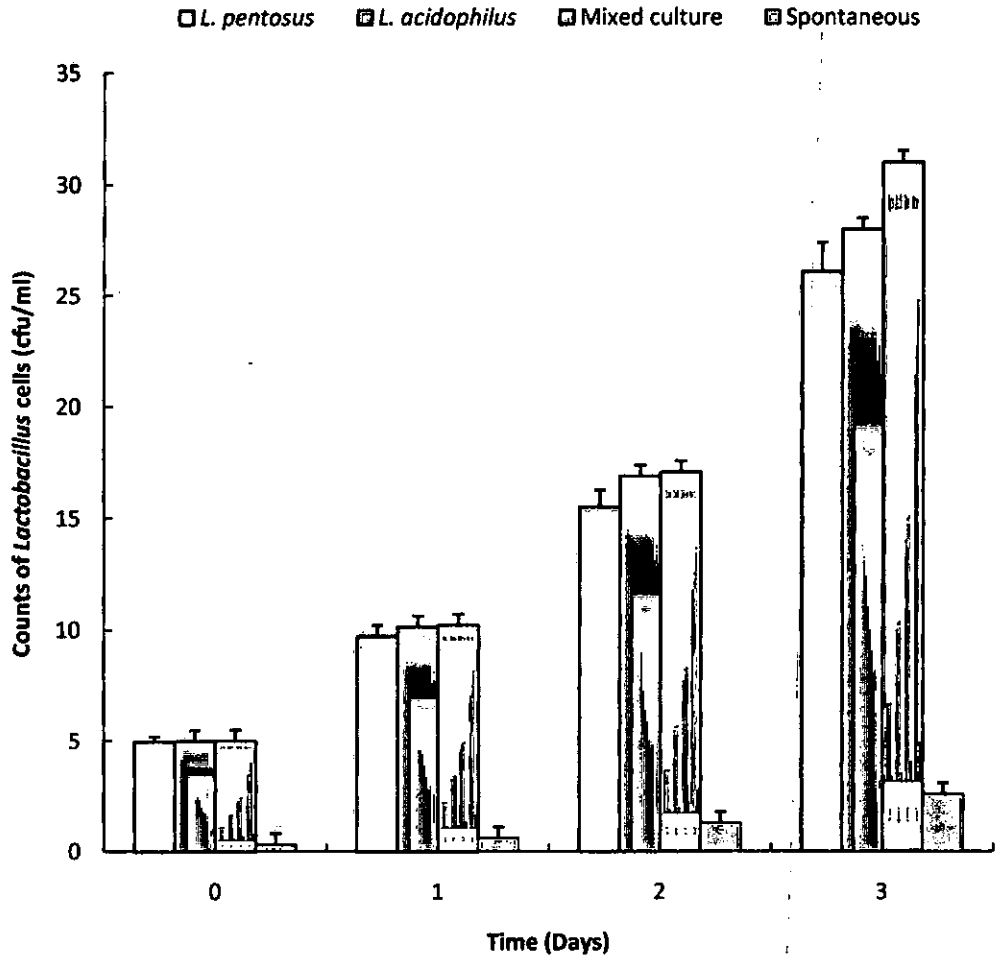

Figure 4.1: Time Course of Lactobacilli Count during Fermentation

4.3 Proximate Analysis of Fermented Gruel:

4.3.1 Total Protein Content

In this study, the protein contents of the cereal gruels after fermentation were found to range from 7.78 ± 2.15% to 13.13 ± 4.33%. Total protein value was highest in those fermented with mixed culture having an average percentage (%) value of 13.13 ± 4.33 on day 3, followed by *L. acidophilus*-fermented with a value of 10.97 ± 4.37, then *L. pentosus*-fermented ones with a value of 9.62 ± 4.50 and the locally-fermented having the lowest value of 7.78 ±2.15 (Figure 4.2).

The gruels from the cereal variety EV8363-SR, QPM which is quality protein maize had highest values of protein content in all the conditions of fermentation compared to the other two quality protein maize varieties (SUWAN-1-SR and EV8766-SR-Y, QPM). Though the highest value of (26.08 ± 0.03%) was obtained in the gruel fermented with mixed culture of *Lactobacillus* this value is not significantly different from those fermented singly by *L. pentosus* (24.65± 0.04%) and *L. acidophilus* (25.23± 0.02%) while the spontaneously – fermented had the lowest value of (13.79 ± 0.03%) (Figures 4.2 – 4.6).

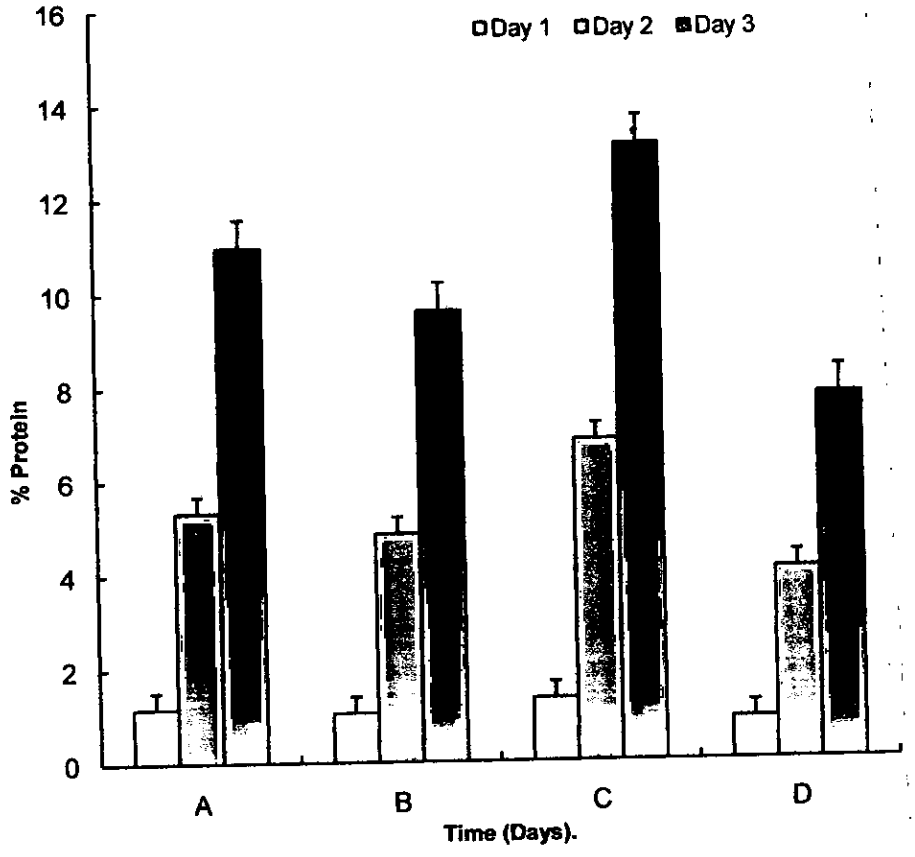

Figure 4.2: Variations in average total protein content with days of fermentation in four varieties of cereals.

A= cereals fermented with *L. acidophilus;* B= cereals fermented with *L. pentosus;* C= cereals fermented with mixed culture; D = Control. *P< 0.05 (compared to the control).

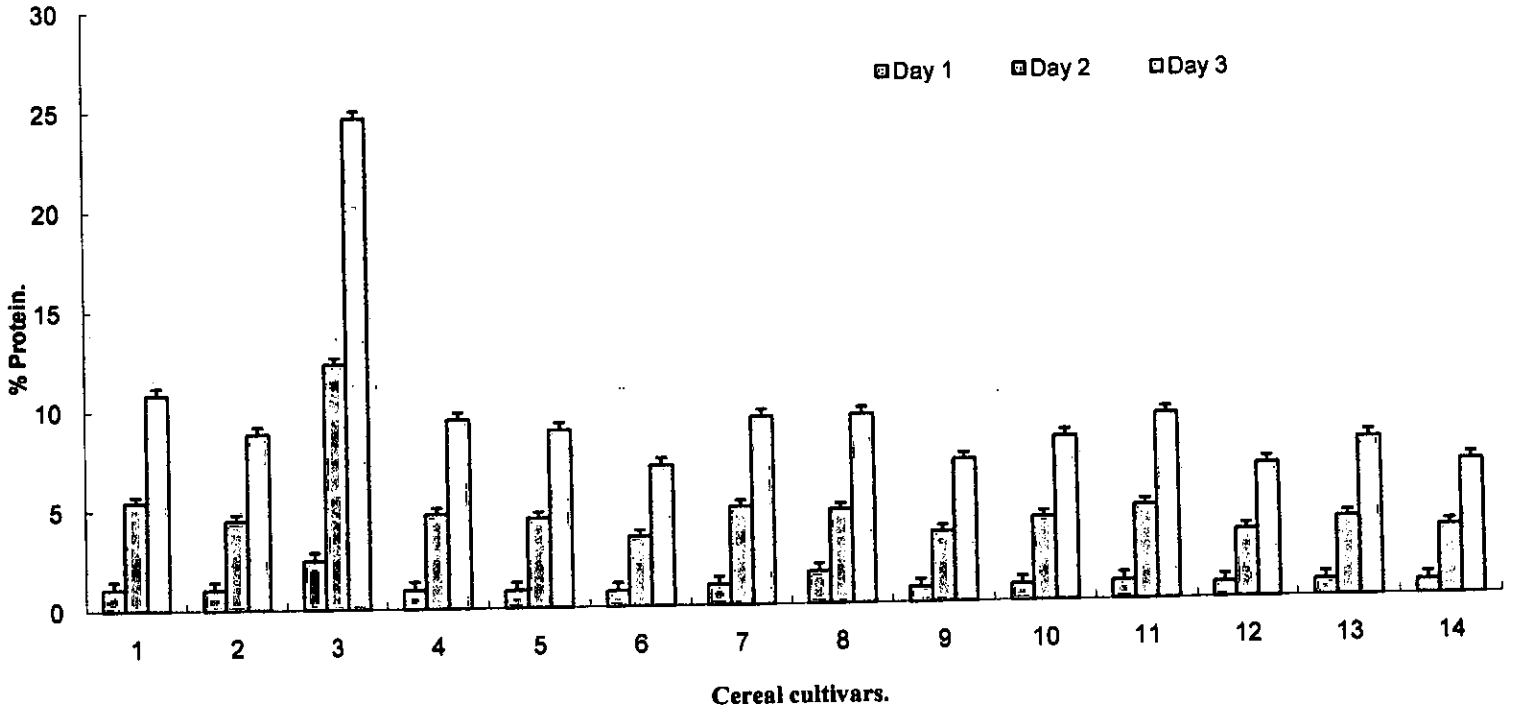

Figure 4.3: Post fermentation protein content of the various *L. pentosus* -fermented cereal gruels.

1=TZPB-SR, 2 =TZB-SR-SE, 3=EV8363-SR QPM, 4=TZSR-W-1, 5=TZB-SR,6=8321-21,7=SUWAN-1-SR,QPM, 8=EV8766-SR-Y,QPM, 9 = TZSR-Y-1, 10 = 8321-18
11 = QPM EV8762-SR, 12 = Sorghum (red), 13 = Sorghum (yellow), 14 = Sorghum (white).

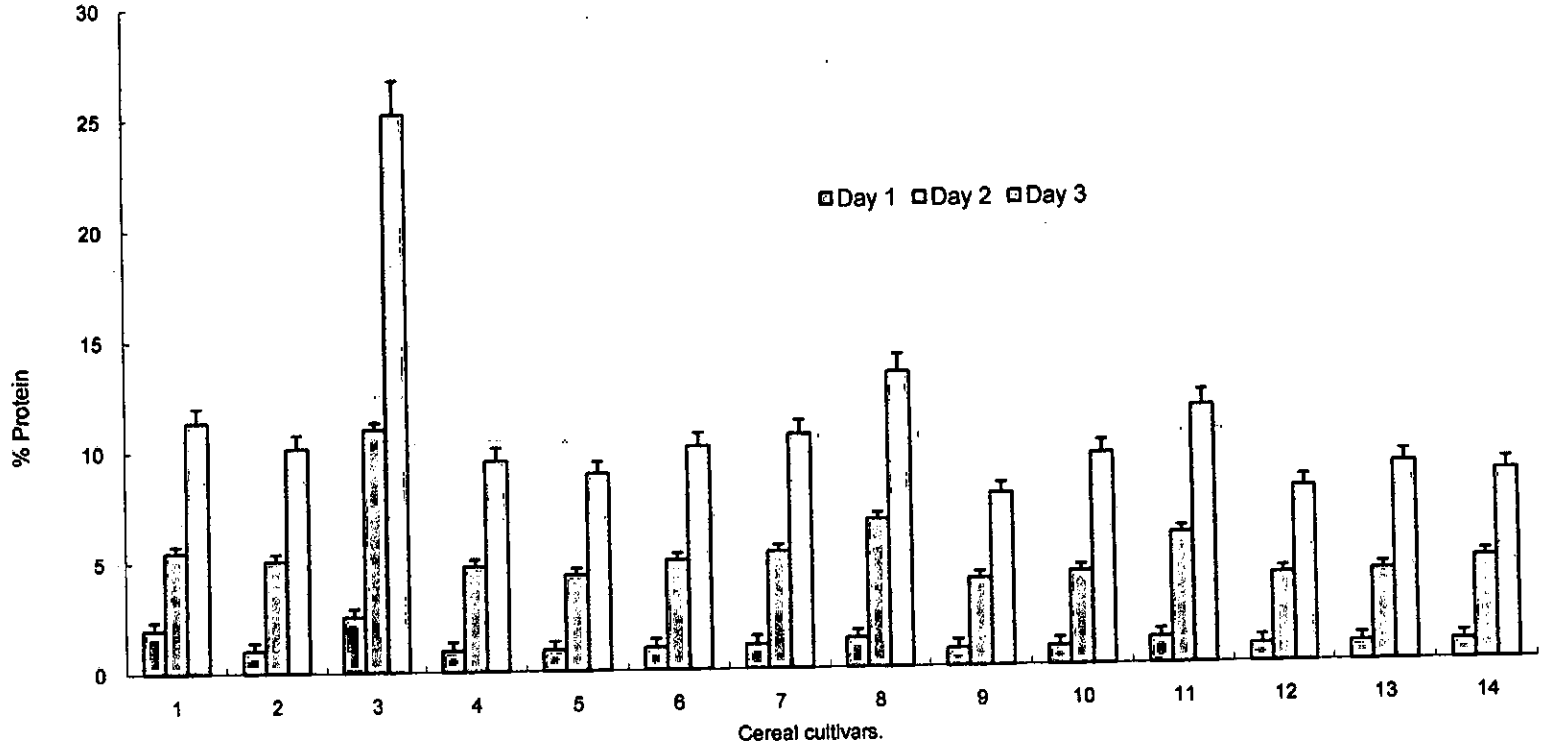

Figure 4.4: Post fermentation protein content of the various *L. acidophilus* -fermented cereal cultivars.
1 = TZPB-SR, 2 =TZB-SR-SE, 3 =EV8363-SR,QPM, 4 =TZSR-W-1, 5 =TZB-SR, 6 =8321-21, 7=SUWAN-1-SR,QPM, 8=EV8766-SR-Y- QPM, 9= TZSR-Y-1, 10=8321-18 11= QPM EV8762-SR, 12 = Sorghum (red), 13 = Sorghum (yellow), 14= Sorghum (white).

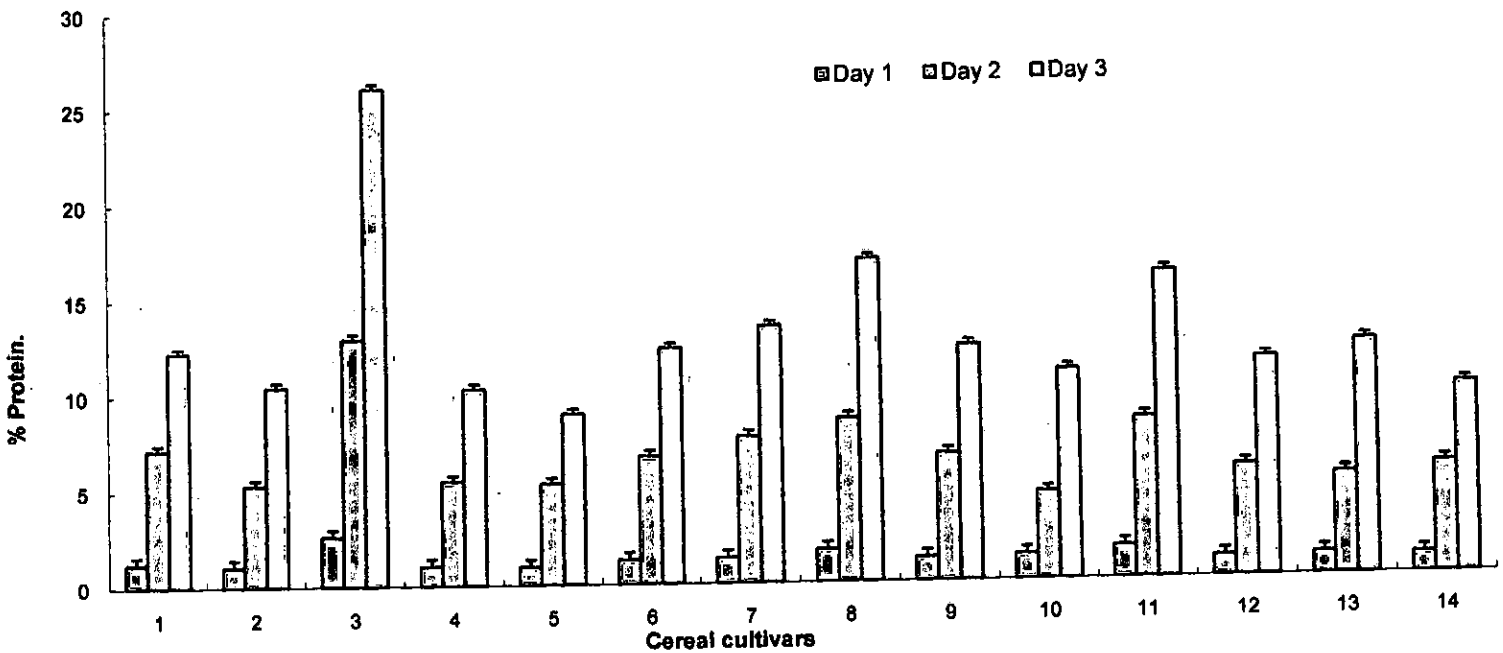

Figure 4.5: Post fermentation protein content of the various *L. acidophilus-L. pentosus* fermented cereal cultivars..

1 = TZPB-SR, 2= TZB-SR-SE, 3= EV8363-SR QPM, 4 = TZSR-W-1, 5A=TZB-SR, 6= 8321-21, 7 = SUWAN-1-SRQPM, 8= EV8766-SR-Y QPM, 9 = TZSR-Y-1, 10 = 8321-18, 11 = QPM EV8762-SR, 12 = Sorghum (red), 13 = Sorghum (yellow), 14 = Sorghum (white).

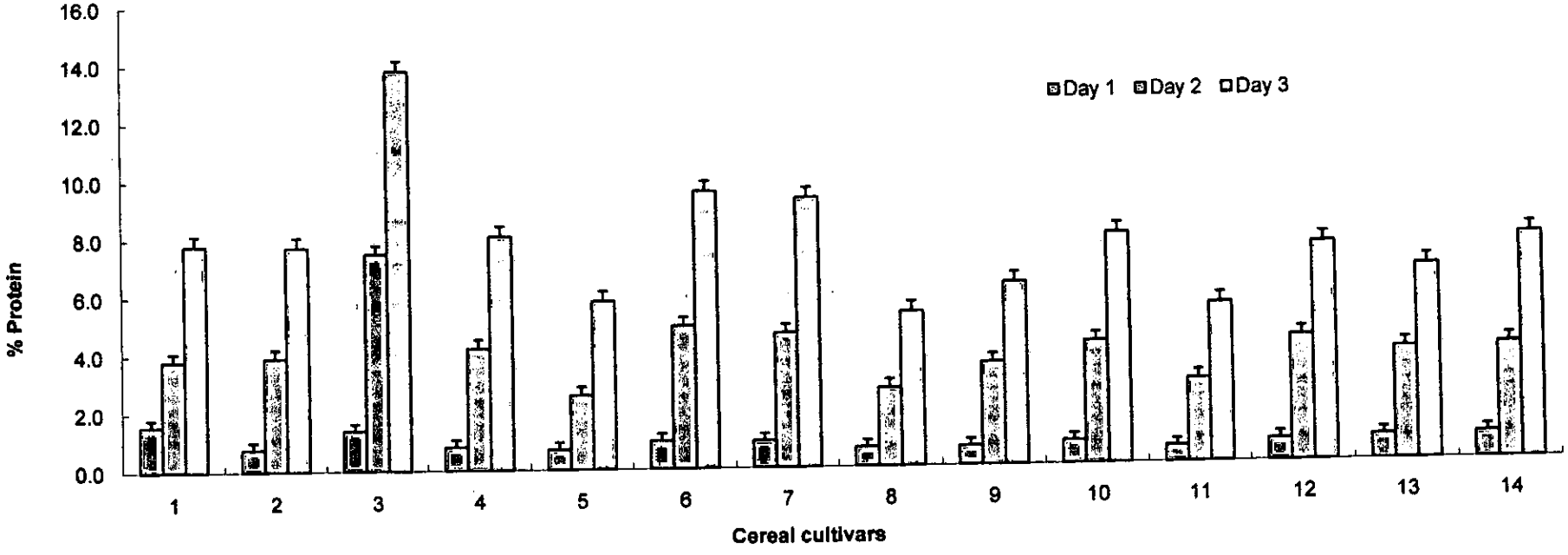

Figure 4.6: Post fermentation protein content of spontaneously-fermented cereal gruels

1 = TZPB-SR, 2 = TZB-SR-SE, 3 = EV8363-SR,QPM, 4 = TZSR-W-1, 5= TZB-SR, 6 = 8321-21, 7 = SUWAN-1-SR,QPM, 8= EV8766-SR-Y,QPM, 9 = TZSR-Y-1, 10 = 8321-18, 11 = EV8762-SR, 12 = Sorghum (red), 13= Sorghum (yellow), 14= Sorghum (white)

4.3.2: Amino acid determination

The average amino acid amount (mg/100g) of cereals spontaneously-fermented was found to be lowest with a value of 78.89 ± 5.13 on day 3, followed by *L. pentosus*-fermented 283.03 ± 4.65 then the *L. acidophilus*-fermented 338.51 ± 8.13, and the cereals fermented using the mixed culture having the highest value of 498.50 ± 9.69. Tables 4.2 and 4.3 show the result of quantitation of the amino acids present in all the fermented cereal gruels. Levels of lysine ranged from 195.7 - 720.7mg/100g in the *Lactobacillus*-fermented cereal gruels compared to 58.4 – 95.2mg/100g in the fermented cereal gruels produced by spontaneous inoculation ($p<0.05$) and methionine content ranged from 260.7 – 652.4mg/100g in *Lactobacillus*-fermented cereal gruels to 65.2 – 143.8mg/100g in the control. The values of isoleucine and tryptophan also followed the same pattern of 201.6 – 631.8mg/100g and 285.2 - 678.3mg/100g in *Lactobacillus*-fermented cereal gruels and 58.9 – 92.6mg/100g and 58.3 -143.8mg/100g in the cereal gruels fermented by chance inoculation respectively.

Further analysis revealed significant disparity ($p<0.05$) in the contents of these amino acids with respect to the inoculating species and the cereal cultivar tested. *Lactobacillus acidophilus* elicited the highest contents of lysine in 10 of 14 cultivars, methionine and tryptophan in 12 of 14, and isoleucine in 9 of 14, when compared with *L. pentosus*. When compared with the gruels fermented by mixed culture of *Lactobacillus*, *L. acidophilus* elicited highest lysine and isoleucine contents in only 1 of 14 cultivars, 8 of 14 in methionine, and 7 of 14 in tryptophan. *L. pentosus* had the highest values of tryptophan in 2 of 14 when compared with other fermented gruels. These two were the sorghum cultivars. *L. acidophilus* seemed to have elicited amino acid in the cereals more than *L. pentosus*.

4.3.3 Chromatographic separation of amino acids of the various gruels

The amino acid profile using thin layer chromatography revealed the presence of lysine, methionine, and tryptophan among the essential amino acids and isoleucine among the semi essential amino acids in all the fermented cereal gruels. However, arginine, a non-essential amino acid occurred in some *L. acidophilus*-fermented and mixed culture samples (Figures 4.7 - 4.10).

Table 4.2: Limiting amino acids (Lysine and Methionine)of fermented cereal cultivar gruels (Ogi).

	Amino acid (mg/100g)							
	Lysine				Methionine			
Cultivar[@]	A	B	C	D	A	B	C	D
TZPB-SR	275.1±33.4^{d}	210.7±28.2^{f}	340.1±35.3	85.7±22.4^{a}	382.8±30.5^{d}	305.4±35.8^{s}	397.4±35.5	92.7±22.5^{a}
TZB-SR-SE	208.3±19.5	195.7±28.3^{c}	250.6±20.8	72.8±25.1^{a}	425.2±27.4^{de}	302.7±18.2	385.4±29.7	58.3±22.7^{a}
EV8363-SR –QPM	410.5±20.8^{d}	320.5±32.2^{c}	505.6±19.6	80.5±14.2^{a}	520.5±28.4^{de}	425.2±19.7	380.5±28.1	125.2±25.7^{a}
TZSR-W-1	204.8±24.6^{b}	285.2±25.7^{c}	605.3±19.7	72.4±20.4^{a}	405.2±24.8^{d}	306.1±23.7^{c}	485.2±31.7	72.6±27.1^{a}
TZB-TZB-SR	250.8±22.5^{b}	328.6±38.2^{c}	385.1±18.7	60.4±20.6^{a}	350.6±28.3^{d}	205.8±21.8^{c}	325.8±25.6	80.4±9.6^{a}
8321-21	237.2±38.2^{b}	295.4±28.7^{c}	375.2±26.5	82.7±20.4^{a}	442.6±45.8^{d}	346.3±37.9^{c}	526.2±34.8	85.1±25.2^{a}
SUWAN-1-SR-QPM	302.5±36.8^{e}	245.3±24.6^{f}	625.6±21.3	86.4±19.1^{a}	480.4±34.6^{de}	338.4±21.6	285.2±34.8	85.1±25.2^{a}
EV8766-SR-Y-QPM	452.5±37.1^{d}	280.4±25.7^{c}	720.7±26.5	80.2±22.4^{a}	585.2±28.1^{de}	318.4±17.6^{c}	406.4±28.4	143.8±23.7^{a}
TZSR-Y-1	220.3±18.5^{d}	198.5±25.1^{c}	318.2±30.7	90.3±12.4^{a}	405.1±28.4^{de}	320.2±24.6^{c}	260.7±31.5	88.1±21.8^{a}
8321-18	315.7±28.7^{b}	208.8±19.7^{c}	570.4±26.2	80.2±26.4^{a}	480.4±34.6^{d}	375.2±26.4^{c}	502.7±51.7	78.5±28.2^{a}
EV8762-SR	245.3±24.6^{b}	302.5±36.8^{c}	625.6±21.3	86.4±19.1^{a}	405.6±28.3^{de}	340.2±19.6	285.2±34.8	85.1±25.2^{a}
RYW-TZ	516.5±25.2^{b}	406.6±25.2^{f}	610.6±26.4	72.8±27.2^{a}	375.2±28.2	530.5±21.5^{f}	340.3±21.7	64.8±23.8^{a}
YKT-TZ	385.3±24.6^{d}	302.5±36.8^{c}	625.6±21.3	58.4±20.7^{a}	362.8±30.2	530.5±21.5^{f}	340.3±21.7	64.8±23.8^{a}
WHG-TZ	714.3±18.3^{de}	381.7±42.1^{c}	420.4±24.5	95.2±22.6^{a}	652.4±42.5^{de}	422.6±27.8	415.3±26.3	65.2±18.3^{a}

Data is presented as mean ± standard deviation from the mean (SD) of three determinations and analyzed by student's t-test (2 – tail)[@]Cultivars of maize (1 – 11) as obtained from IITA. Red, yellow and white sorghum cultivars (12 – 14) were bought from the market. A = *L. acidophilus*; B = *L. pentosus*; C = Mixed culture; D = Control. $P < 0.05$ was considered significant. [a]$P<0.05$ (D vs. A or B or C), [b]$P<0.05$ (A vs. B or C), [c]$P<0.05$ (B vs. C), [d]$P < 0.05$ (A vs. B), [e]$P< 0.05$ (A vs. C), [f]$P<0.05$ (B vs. A or

Table 4.3: Limiting amino acids: (Isoleucine and Tryptophan) content of fermented cereal cultivar gruels (Ogi).

	Amino acid (mg/100g)							
	Isoleucine				Tryptophan			
Cultivar[@]	A	B	C	D	A	B	C	D
TZPB-SR	228.3±21.6	201.6±25.7^{c}	241.5±31.8	80.3±15.5^{a}	379.6±27.6^{d}	305.4±35.8^{s}	388.5±27.7	86.4±20.4^{a}
TZB-SR-SE	217.7±15.3^{b}	208.3±24.8^{c}	262.4±27.3	72.8±25.1^{a}	425.2±27.4^{de}	302.7±18.2	385.4±29.7	58.3±22.7^{a}
EV8363-SR QPM	445.7±26.2	320.5±32.2^{c}	480.4±29.4	81.7±16.8^{a}	520.5±28.4^{de}	425.2±19.7	380.5±28.1	125.2±25.7^{a}
TZSR-W-1	204.8±24.6^{b}	285.2±25.7^{c}	328.1±22.5	78.7±19.4^{a}	405.2±24.8^{d}	306.1±23.7^{c}	485.2±31.7	72.6±27.1^{a}
TZB-SR	276.4±22.6^{b}	260.3±31.7^{c}	309.3±19.4	70.5±21.5^{a}	350.6±28.3^{d}	205.8±21.8^{c}	325.8±25.6	80.4±9.6^{a}
8321-21	237.2±38.2^{b}	295.4±28.7^{c}	375.2±26.5	83.1±25.1^{a}	442.6±45.8^{d}	346.3±37.9^{c}	526.2±34.8	85.1±25.2^{a}
SUWAN-1-SR-QPM	245.3±24.6^{b}	302.5±36.8^{c}	602.7±40.8	83.7±20.5^{a}	480.4±34.6^{de}	338.4±21.6	285.2±34.8	85.1±25.2^{a}
EV8766-SR-Y-QPM	306.7±35.6^{d}	280.4±25.7^{c}	386.1±25.8	80.2±22.4^{a}	585.2±28.1^{de}	318.4±17.6^{c}	406.4±28.4	143.8±23.7^{a}
TZSR-Y-1	220.3±18.5^{d}	207.6±24.6^{c}	262.6±28.7	90.3±12.4^{a}	405.1±28.4^{de}	320.2±24.6^{c}	260.7±31.5	88.1±21.8^{a}
8321-18	252.7±25.6^{b}	218.4±22.6^{c}	286.4.8±27.1	80.2±26.4^{a}	480.4±34.6^{d}	375.2±26.4^{c}	502.7±51.7	78.5±28.2^{a}
EV8762-SR	245.3±24.6^{b}	302.5±36.8^{c}	625.6±21.3	86.4±19.1^{a}	405.6±28.3^{de}	340.2±19.6	285.2±34.8	85.1±25.2^{a}
RYW-TZ	406.6±25.2^{b}	516.5±28.4^{c}	610.6±26.4	72.8±27.2^{a}	375.2±28.2	530.5±21.5^{f}	340.3±21.7	64.8±23.8^{a}
YKT-TZ	392.7±20.6^{d}	298.5±34.3^{c}	615.3±20.3	58.9±19.5^{a}	328.5±37.1	527.4±24.5^{f}	340.3±21.7	64.8±23.8^{a}
WHG-TZ	631.8±13.9^{de}	278.3±36.1^{c}	405.7±22.8	92.6±21.8^{a}	678.3±42.5^{de}	422.6±25.2	384.5±24.9	65.6±15.4^{a}

Data is presented as mean ± standard deviation from the mean (SD) of three determinations and analyzed by student's t-test (2 – tail)
[@]Cultivars of maize (1 – 11) as obtained from IITA. Red, yellow and white sorghum cultivars (12 – 14) were bought from the market.
A = *L. acidophilus*; B = *L. pentosus*; C = Mixed culture; D = Control. $P < 0.05$ was considered significant. [a]$P<0.05$ (D vs. A or B or C), [b]$P<0.05$ (A vs. B or C), [c]$P<0.05$ (B vs. C), [d]$P < 0.05$ (A vs. B), [e]$P< 0.05$ (A vs. C), [f]$P<0.05$ (B vs. A or C)

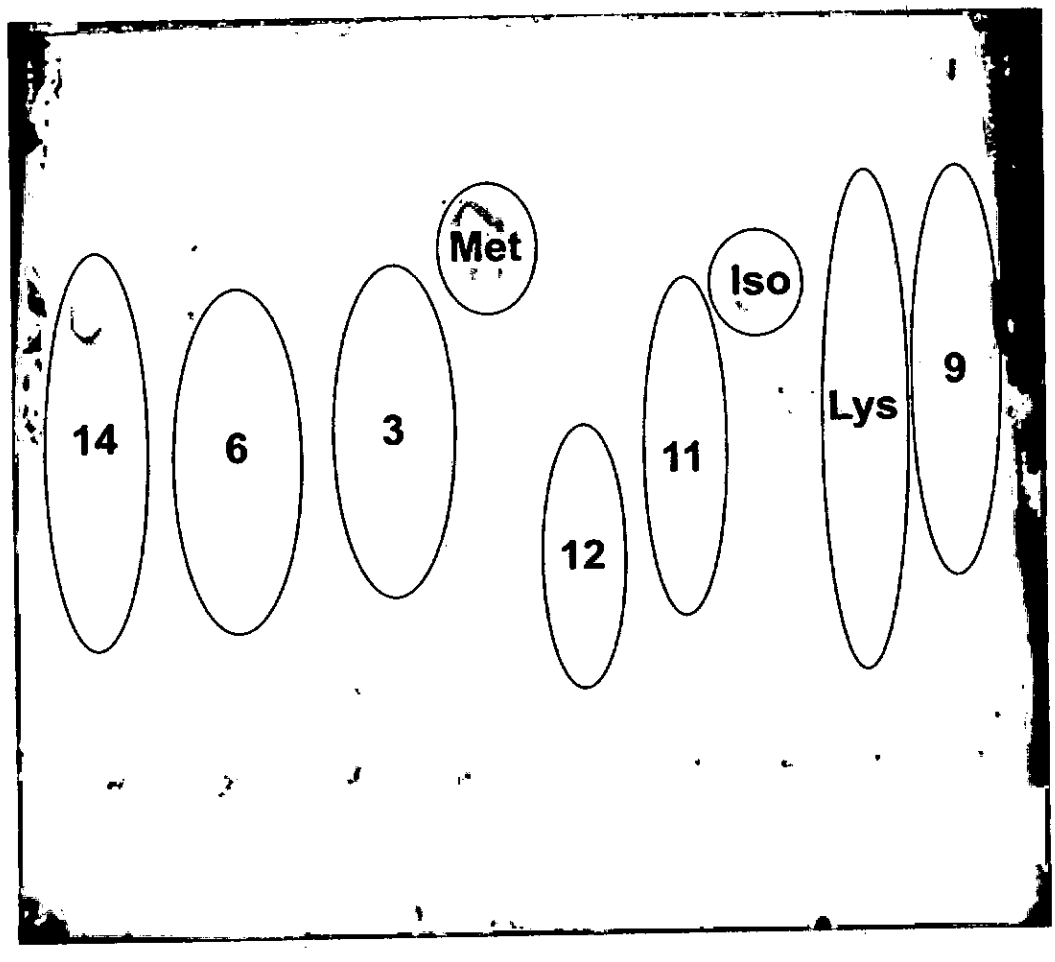

Figure 4.7: Amino acid paper chromatogram of some spontaneously-fermented cereal gruels. Try – Tryptophan, Iso – Isoleucine, Ly – Lysine, 3=EV8363-SRQPM, 7=SUWAN-1SRQPM, 12=Red Sorghum, 13=Yellow sorghum, 6=8321-21, 11=EV8762-SR, 14=White sorghum

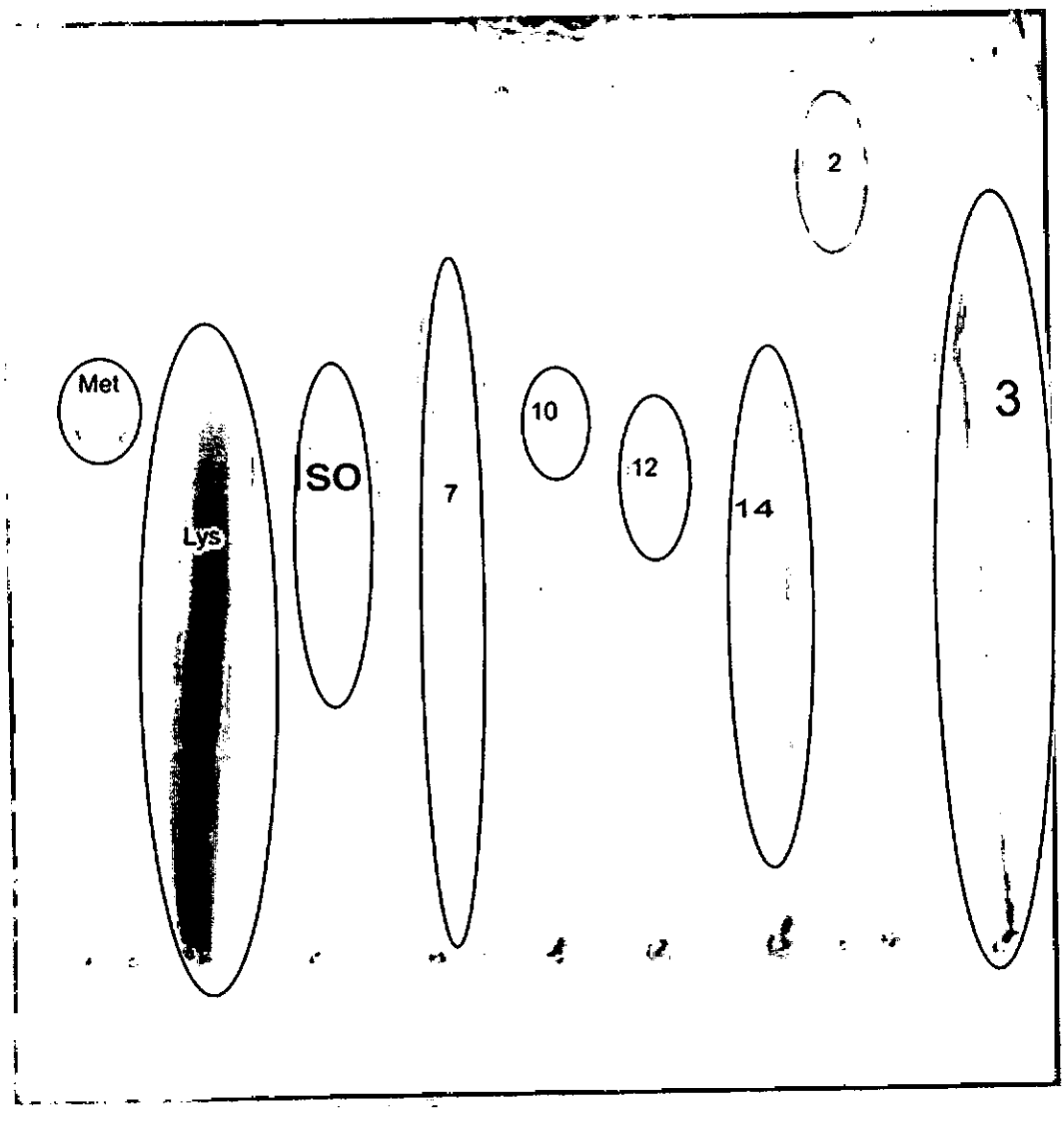

Figure 4.8: Amino acid paper chromatogram of some *L. acidophilus*-fermented cereal gruels. Try – Tryptophan, Iso – Isoleucine, Ly – Lysine, 3=EV8363-SRQPM, 7=SUWAN-1SRQPM, 12=Red Sorghum, 13=Yellow sorghum.

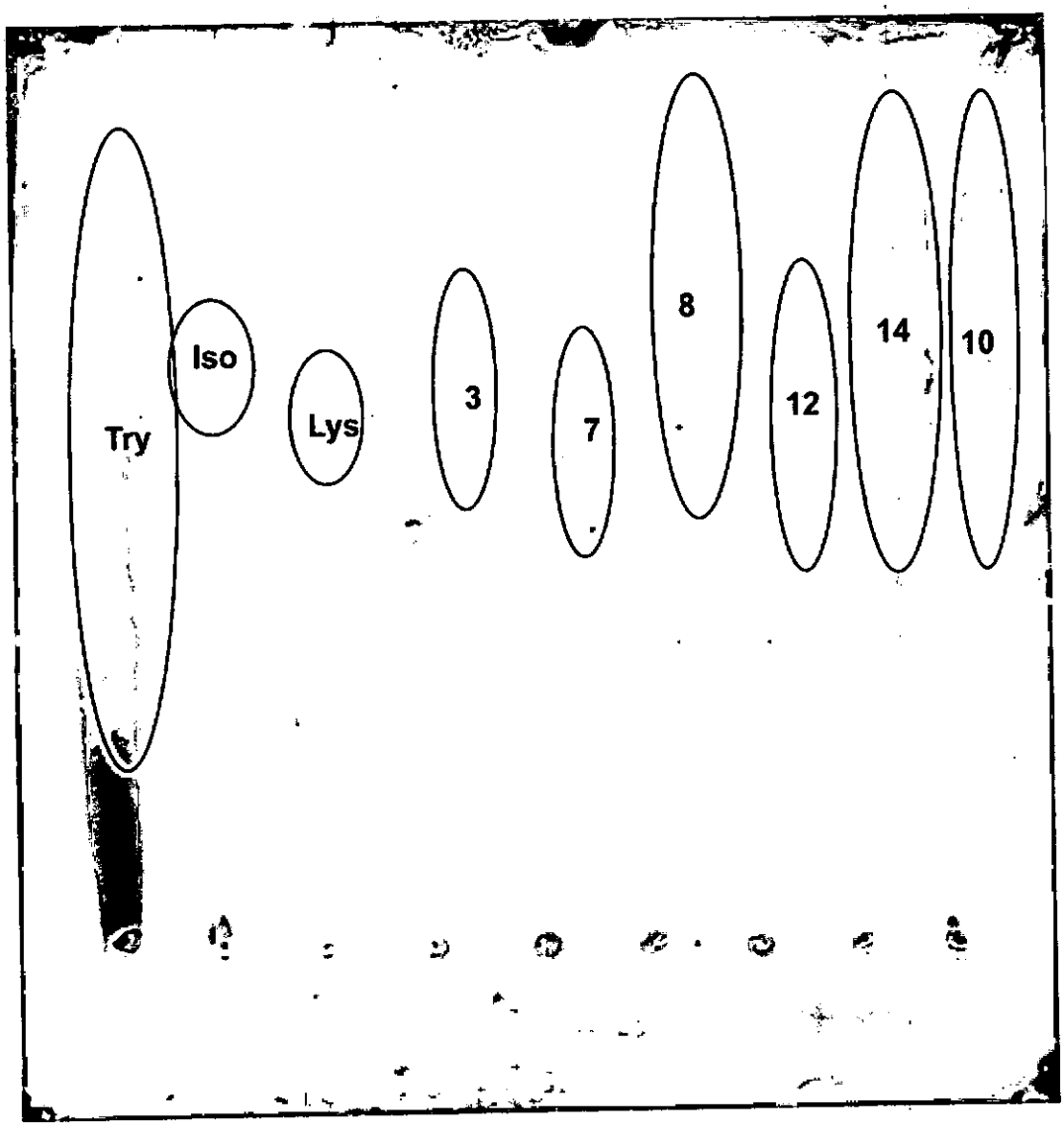

Figure 4.9: Amino acid paper chromatogram of some mixed culture-fermented cereal gruels. Try – Tryptophan, Iso – Isoleucine, Ly – Lysine, 3=EV8363-SRQPM, 7=SUWAN-1SRQPM, 12=Red Sorghum, 13=Yellow sorghum.

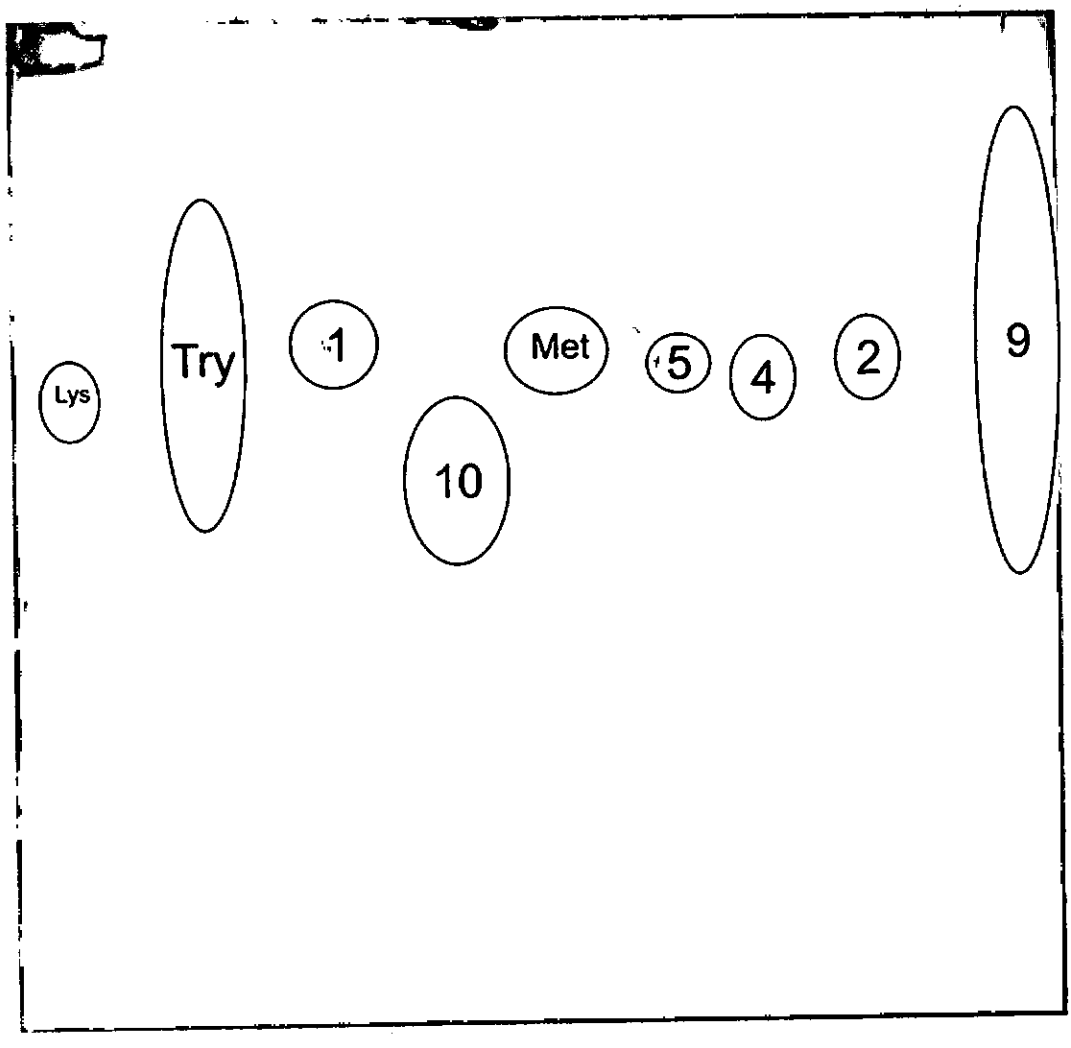

Figure 4:10: Figure 4.7: Amino acid paper chromatogram of some *L. pentosus*-fermented cereal gruels. Try – Tryptophan, Iso – Isoleucine, Ly – Lysine, 3=EV8363-SRQPM, 7=SUWAN-1SRQPM, 12=Red Sorghum, 13=Yellow sorghum.

4.4 Reducing sugar amount

Figures 4.11 - 4.14 revealed that EV8762-SR had the highest reducing sugar content of (84.64 ± 0.036 mg/ml) among the *L. pentosus*-fermented samples, (86.12 ± 0.026 mg/ml) in the *L. acidophilus*-fermented , and (91.28 ± 0.044 mg/ml) in the mixed culture – fermented samples respectively. TZB-SR-SE had the lowest value of (10.15 ± 0.22 mg/ml) in *L. pentosus* samples while the lowest value in the *L. acidophilus*-fermented samples was in TZB-SR with a value of (10.9 ± 0.044 mg/ml), while in the mixed culture – fermented samples TZB-SR had the lowest value of 11.45 ± 0.015 mg/ml. EV8766-SR-Y-QPM had the highest value of 63.05 ± 0.11 mg/ml and the lowest value of 9.14 ± 0.03 mg/ml was found in TZB-SR-SE in spontaneously-fermented samples (Figure 4.14). Figure 4.15 revealed *L. pentosus*-fermented samples to have an average of 44.9 ±31.26 mg/ml, *L. acidophilus*-fermented samples had 46.44 ± 31.77 mg/ml and those fermented by mixed culture 49.81±32.63 mg/ml, while the spontaneously-fermented had the lowest value of 29.58 ± 18.03 mg/ml.

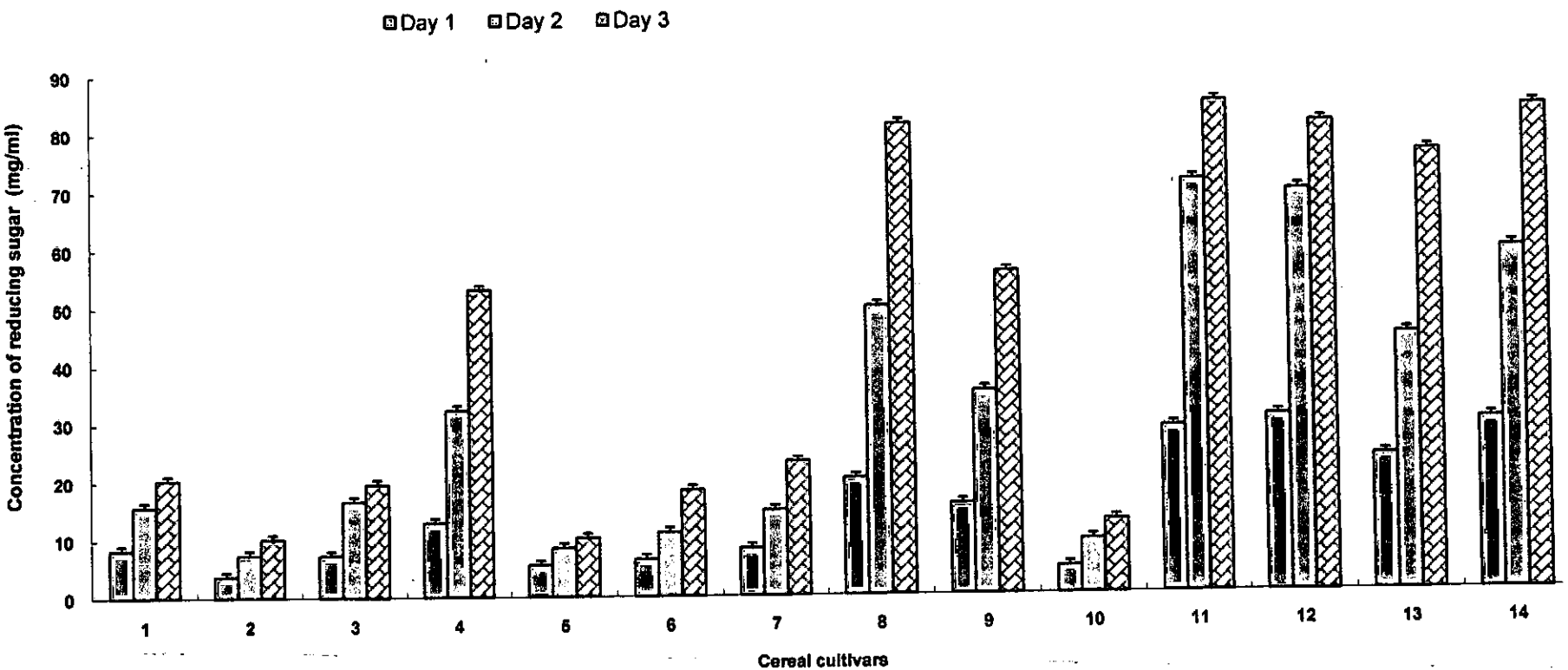

Figure 4.11. Reducing sugar content of *L. pentosus* – fermented cereal gruels.

1=TZPB-SR, 2=TZB-SR-SE, 3=EV8363-SR, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7= SUWAN-1-SR,QPM, 8= EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10= 8321-18, 11=EV8762-SR, 12= Red sorghum, 13= Yellow sorghum, 14= White sorghum

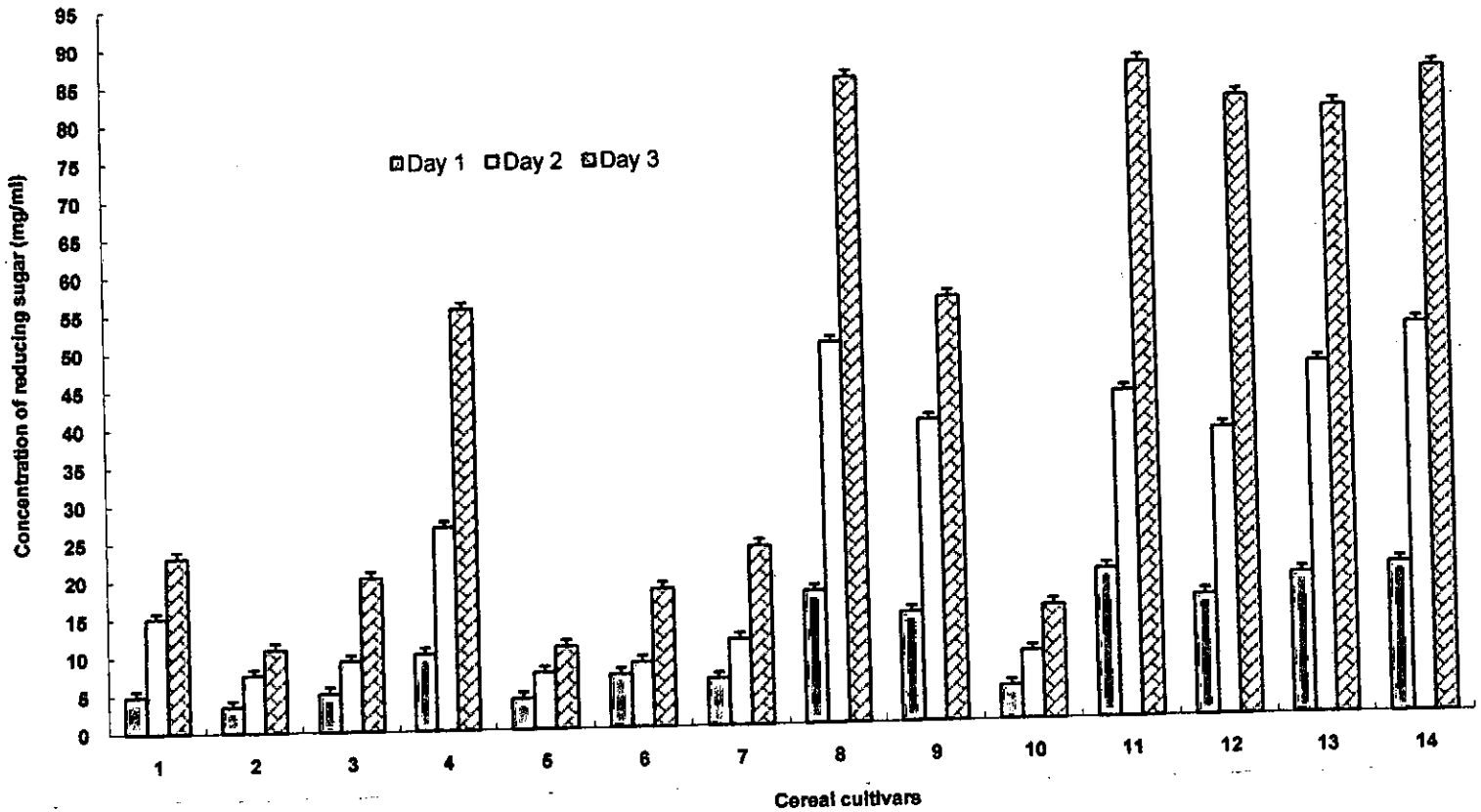

Figure 4.12: Reducing sugar content of *L. acidophilus* – fermented cereal gruels.

1=TZPB-SR, 2=TZB-SR-SE, 3=EV8363-SR, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7= SUWAN-1-SR,QPM, 8= EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10= 8321-18, 11=EV8762-SR, 12= Red sorghum, 13= Yellow sorghum, 14= White sorghum

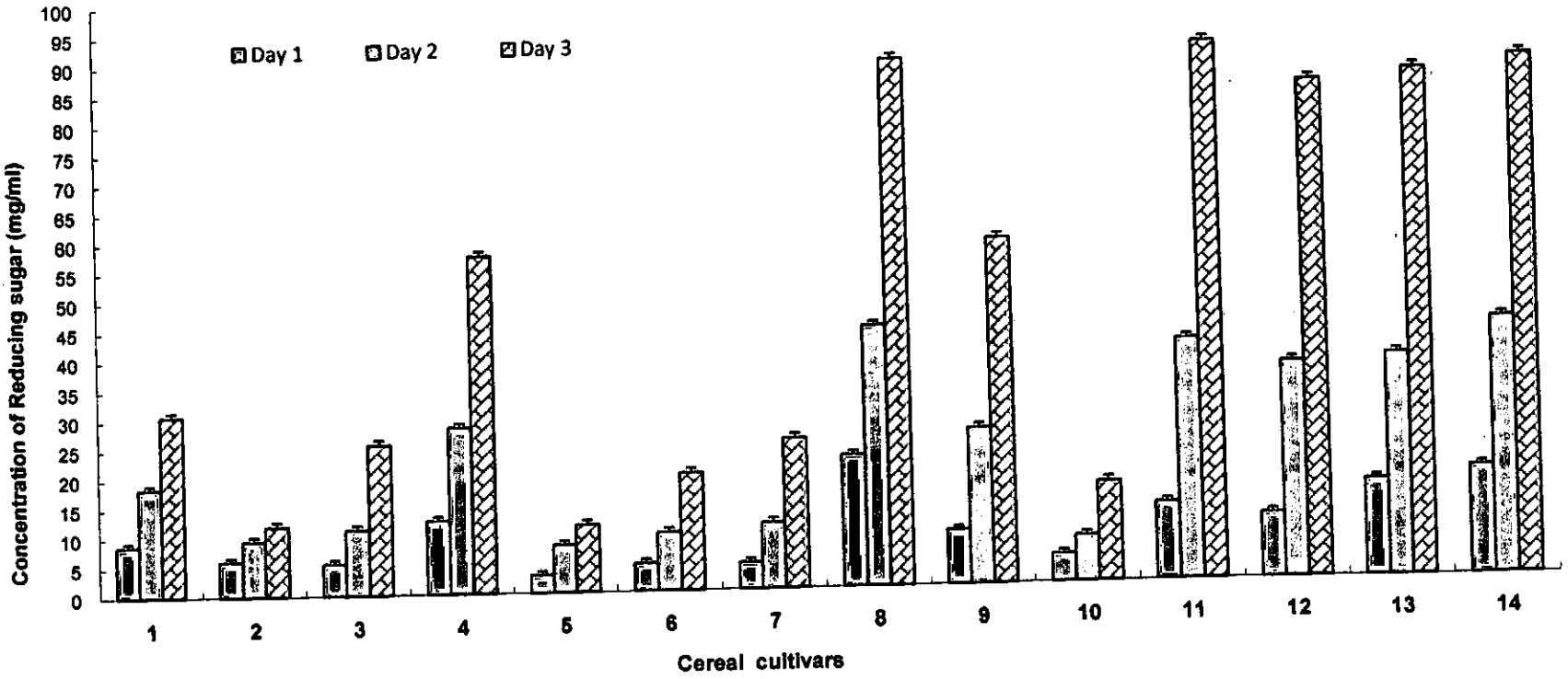

Figure 4.13: Reducing sugar content of cereal gruels fermented with mixed culture of *Lactobacillus*
Data was expressed as mean ± SD. 1=TZPB-SR, 2=TZB-SR-SE,3=EV8363-SR,4=TZSR-W-1,5=TZB-SR,6=8321-21,7=SUWAN-1-SR,QPM, 8=EV8766-SR-Y,QPM, 9=TZSR-Y-1,10=8321-18, 11=EV8762-SR, 12=Red sorghum,13=Yellow sorghum, 14=White sorghum

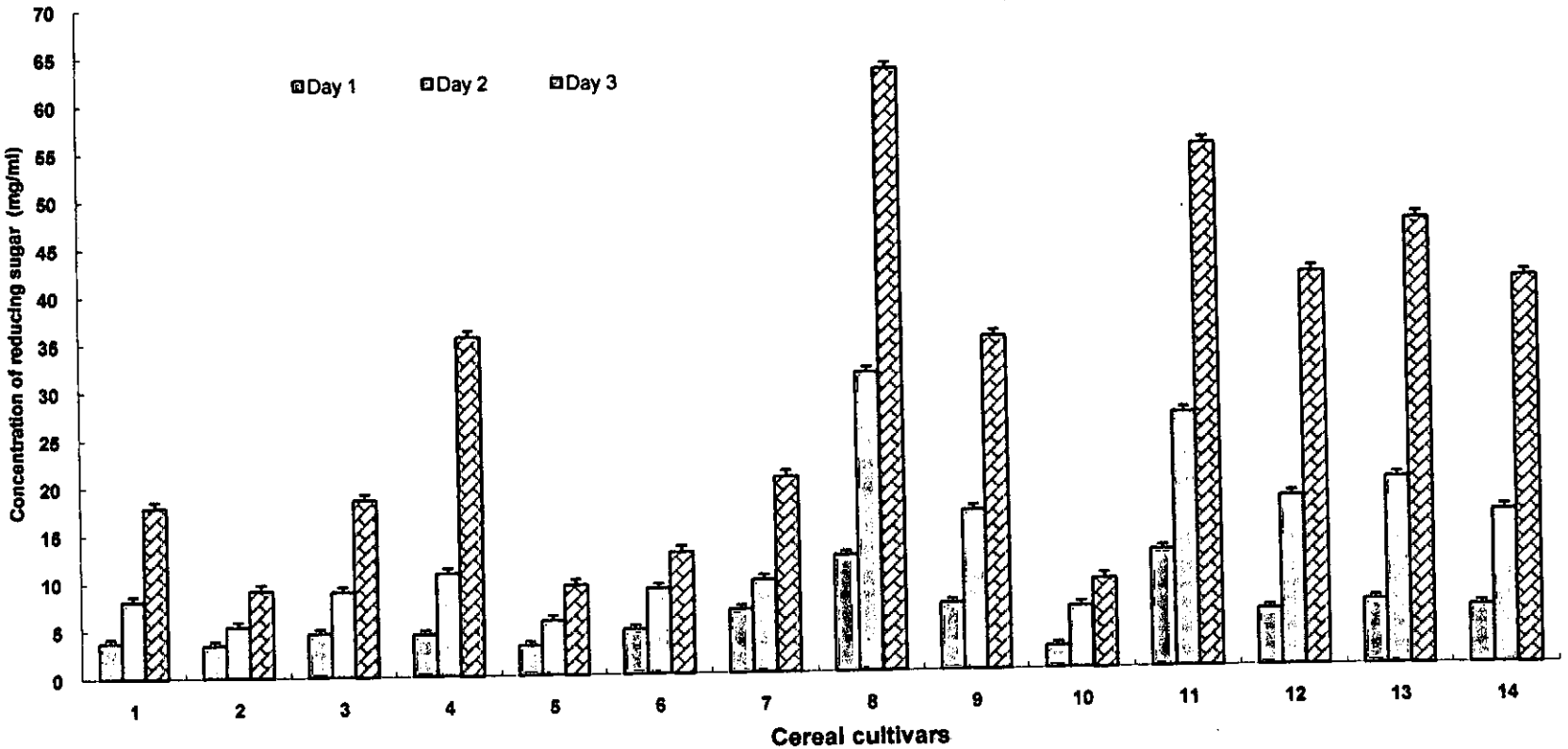

Figure 4.14: Reducing sugar contents of cereal gruels during spontaneous fermentation. Data was expressed as means ± SD.
*(Cereals designated 1-14) 1=TZPB-SR, 2=TZB-SR-SE, 3=EV8363-SR,QPM, 4=TZSR-W-1,5=TZB-SR,6=8321-21, 7=SUWAN-1-SR,QPM, 8=EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10=8321-18, 11=EV8762-SR, 12=Red sorghum, 13=Yellow sorghum, 14=White sorghum

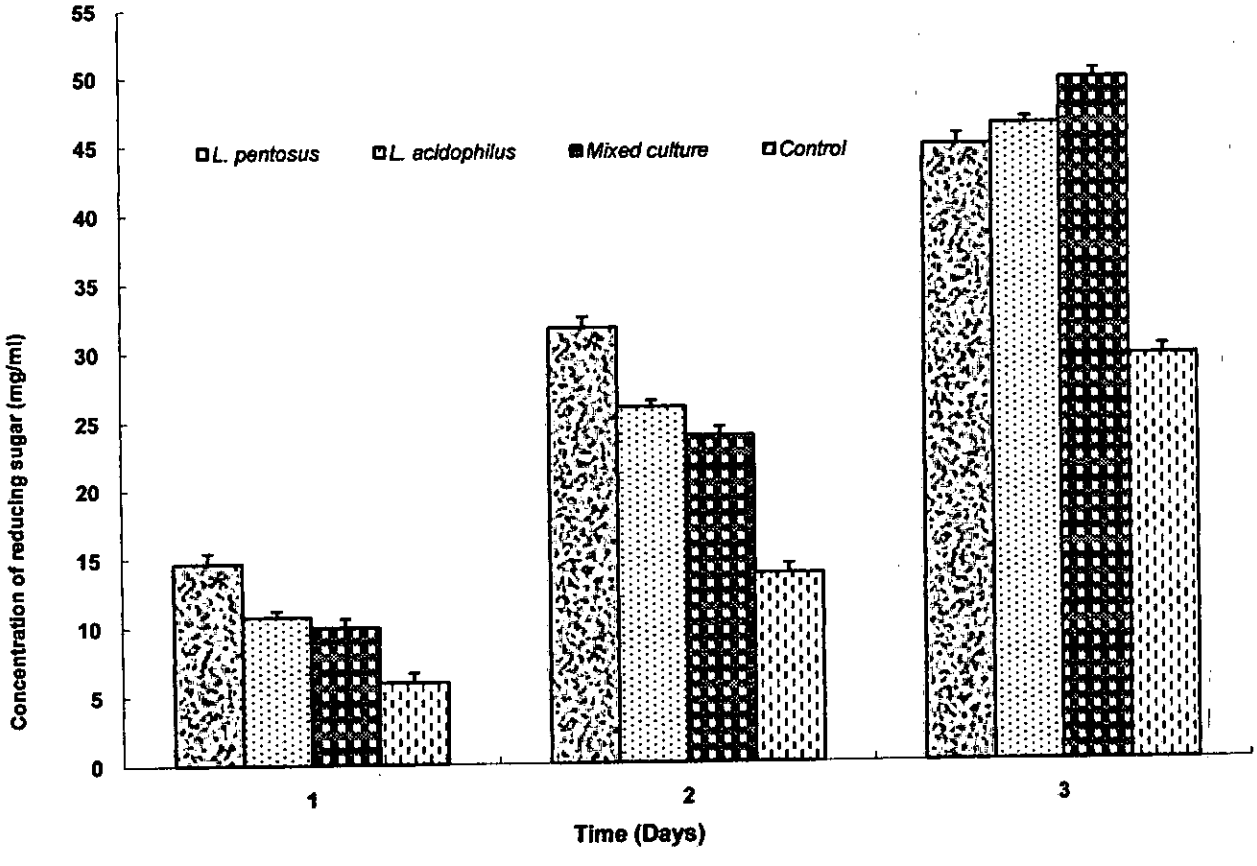

Figure 4.15: Concentration of reducing sugar in different fermented cereal gruels relative to fermentation time.
Data given as mean ± SD.

4.5 Quantitative Determination of Acetoin and Diacetyl

Flavour-enhancing diacetyl and acetoin levels in the products were significantly increased during fermentation using the starter culture strains individually but was exceptionally higher ($p>0.05$) when mixed culture was used (Figures 4.16 – 4.25).

The results of acetoin contents of the various fermented cereal gruels were expressed in Figures 4.16 to 4.20. Figure 4.17 showed TZSR-W-1 to have the highest value of 4.77 µg/ml in the *L. pentosus* –fermented gruels and SUWAN-1-SR-QPM to have the lowest value of 2.6 In the *L. acidophilus*-fermented gruels the highest value of 10.2µg/ml was obtained in cereal 8321-18 while EV8766-SR-Y-QPM had the lowest value of 3.4 (Figure 4.17). All gruels fermented with mixed culture exhibited higher values when compared with those fermented singly, with EV8386-SR,QPM exhibiting the highest value of 17.25 µg/ml and TZSR-W-1 having the lowest value of 6.6 µg/ml. . The amount of acetoin (µg) produced in cereals spontaneously fermented was found to be lowest with an average of (0.05 ± 0.027) on day 1 to (1.48± 0.48) on day 3, while the mixed culture–fermented had the highest values of (0.10 ± 0.027) to (9.42 ± 3.24) (Figure 4.20).

The diacetyl values followed the same pattern as those of acetoin (Figures 4.21 – 4.25).

The amount of diacetyl (µG) produced in spontaneously - fermented cereals was found to be lowest with an average of 1.16 ± 0.35 on day 3, followed by *L. pentosus*-fermented cereals 1.51± 0.69, followed by *L. acidophilus*-fermented cereals with average value of 1.92 ± 0.71 and highest in those fermented with the mixed culture with value of 3.50 ± 0.50 (Figure 4.25).

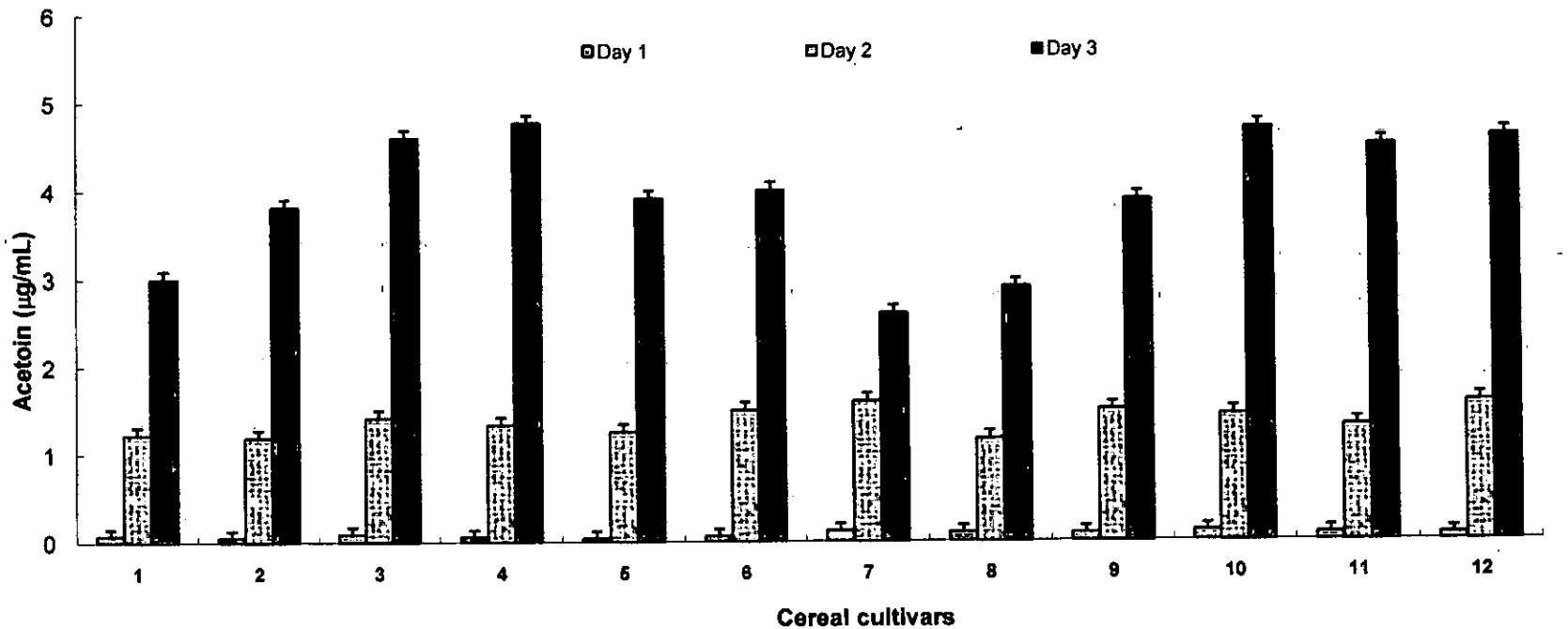

Figure 4.16: Acetoin concentration (µg/ml) in *L. pentosus* – fermented cereal gruels relative to fermentation time.

1=TZPB-SR, 2=TZB-SR-SE, 3=EV8363-SR, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7= SUWAN-1-SR,QPM, 8= EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10= 8321-18, 11=EV8762-SR, 12= Red sorghum, 13= Yellow sorghum, 14= White sorghum

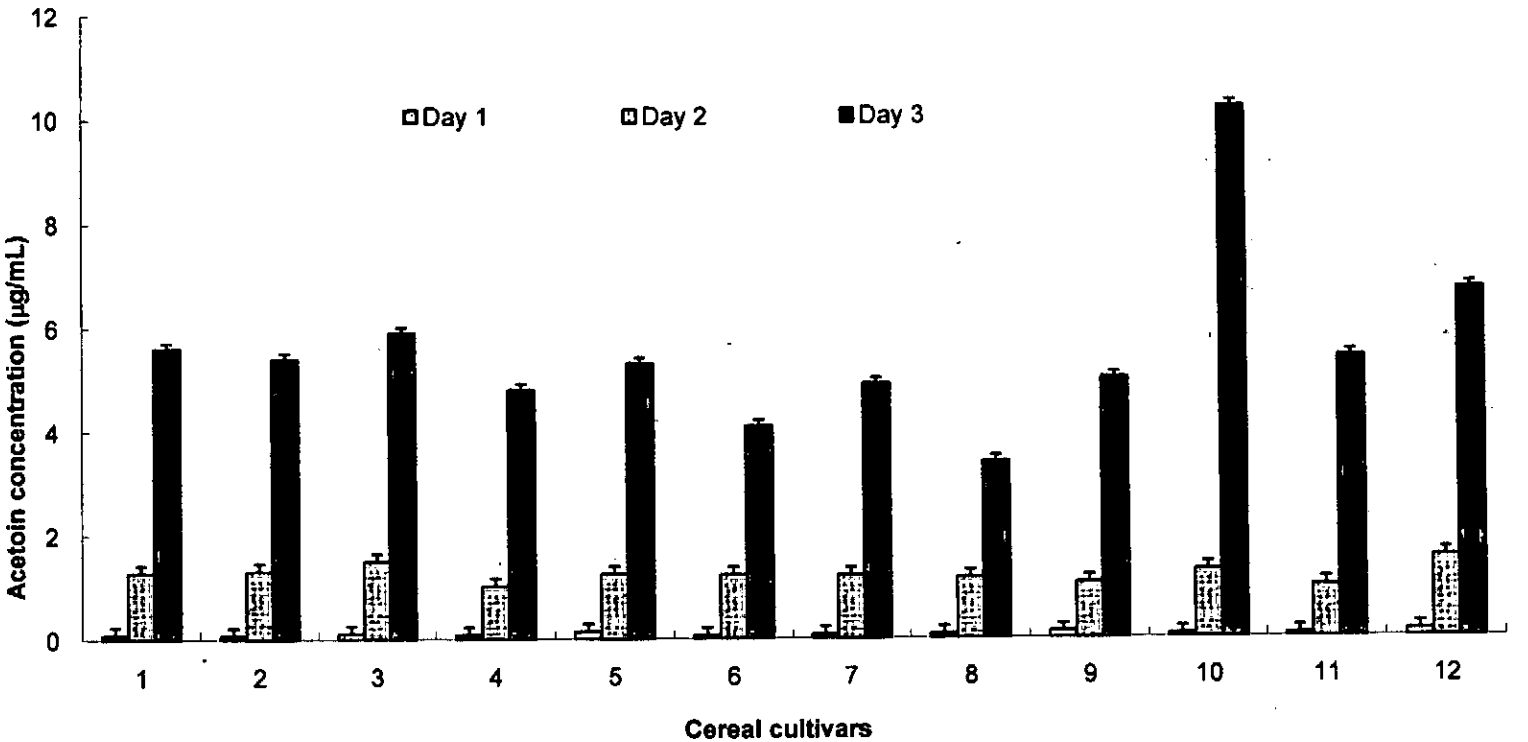

Figure 4.17: Acetoin concentration (µg/ml) in *L. acidophilus* - fermented cereal gruels relative to fermentation time.

1=TZPB-SR, 2=TZB-SR-SE, 3=EV8363-SR, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7= SUWAN-1-SR,QPM, 8= EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10= 8321-18, 11=EV8762-SR, 12= Red sorghum, 13= Yellow sorghum, 14= White sorghum

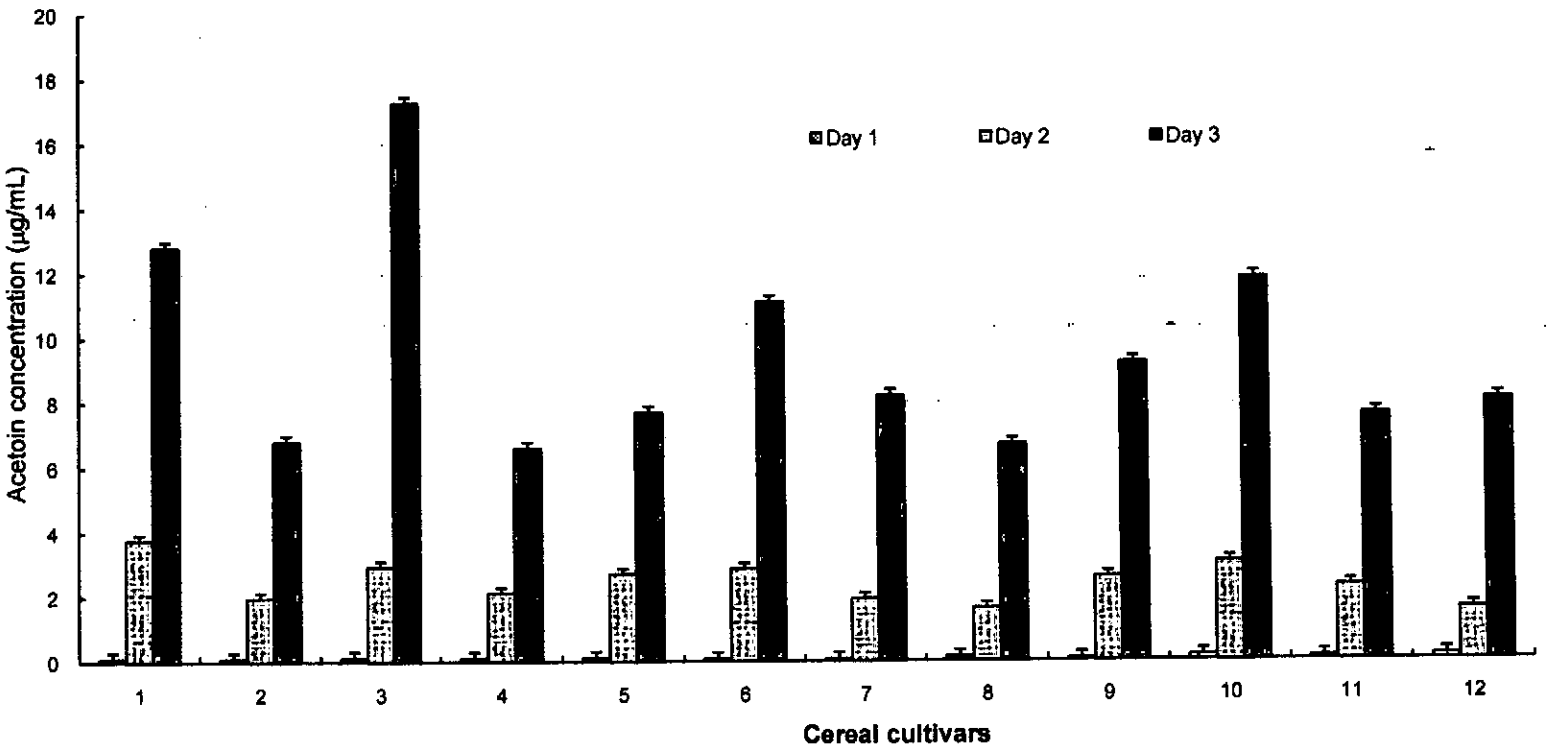

Figure 4.18: Acetoin concentration (µg/ml) in cereal gruels fermented with mixed culture of *Lactobacillus* relative to fermentation time.

1=TZPB-SR, 2=TZB-SR-SE, 3=EV8363-SR, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7= SUWAN-1-SR,QPM, 8= EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10= 8321-18, 11=EV8762-SR, 12= Red sorghum, 13= Yellow sorghum, 14= White sorghum

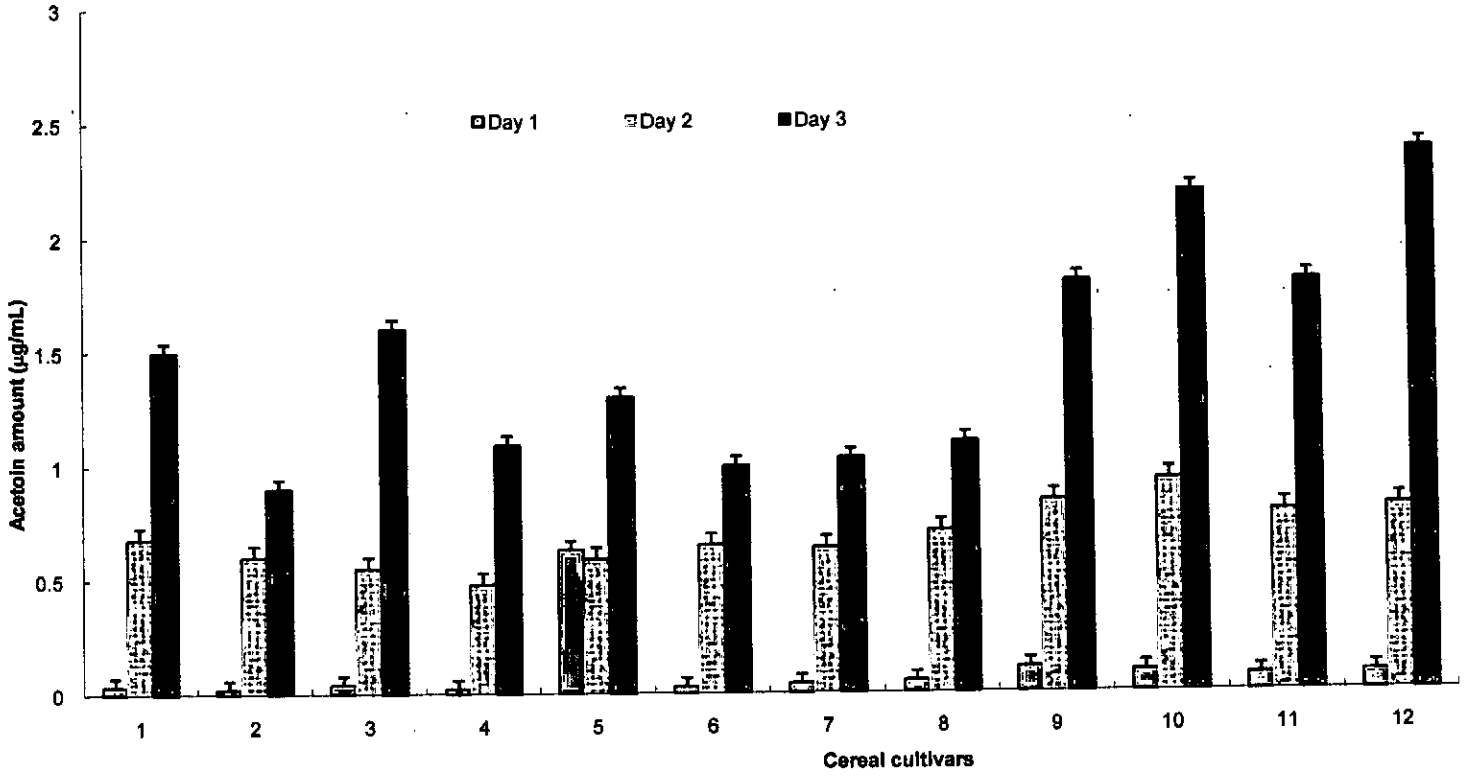

Figure 4.19: Acetoin concentration (µg/ml) in spontaneously- fermented cereal gruels relative to fermentation time.

1=TZPB-SR, 2=TZB-SR-SE, 3=EV8363-SR, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7= SUWAN-1-SR,QPM, 8= EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10= 8321-18, 11=EV8762-SR, 12= Red sorghum, 13= Yellow sorghum, 14= White sorghum

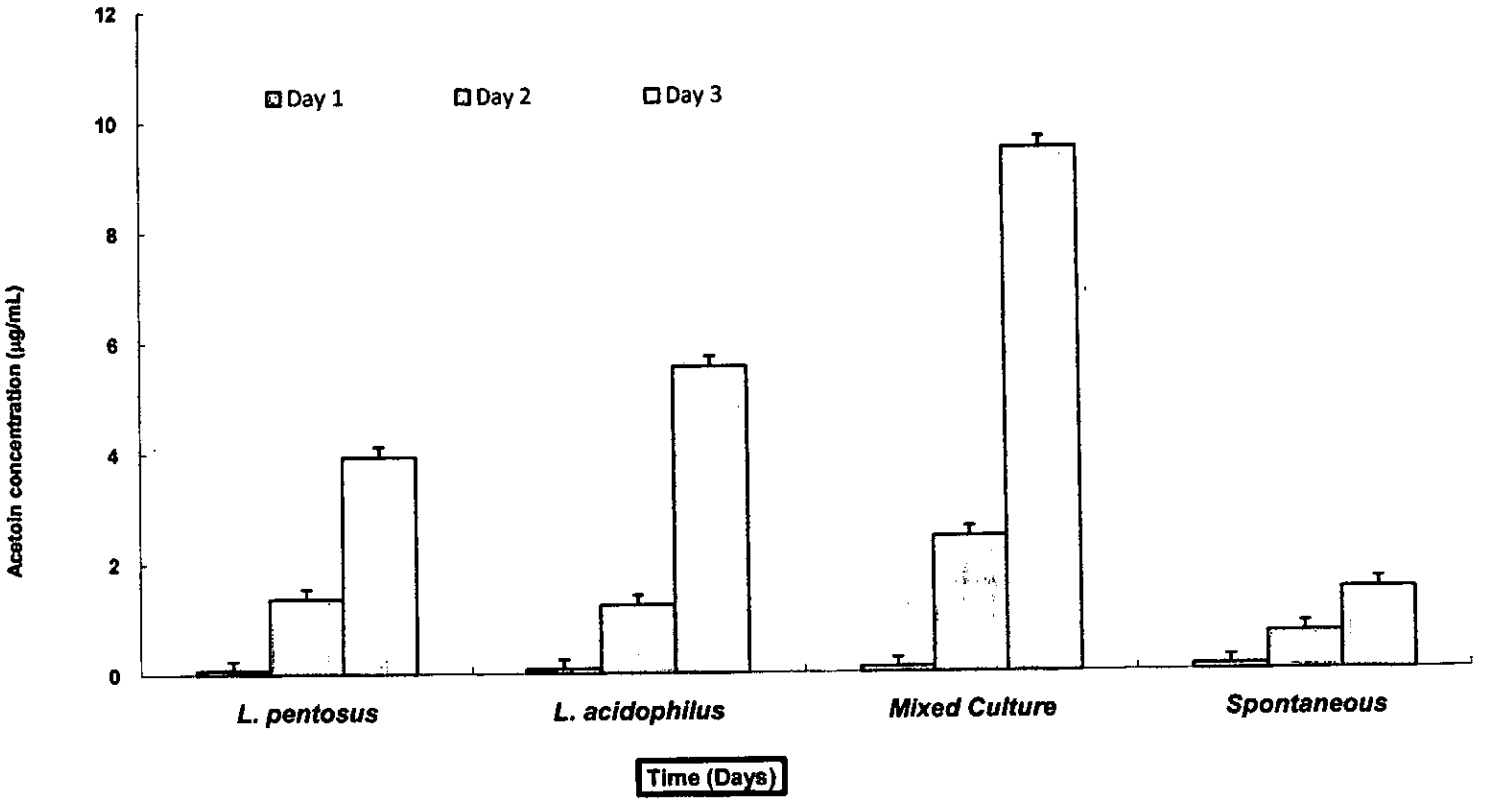

Figure 4.20: Total Acetoin concentration in fermented cereal gruels relative to fermentation time.

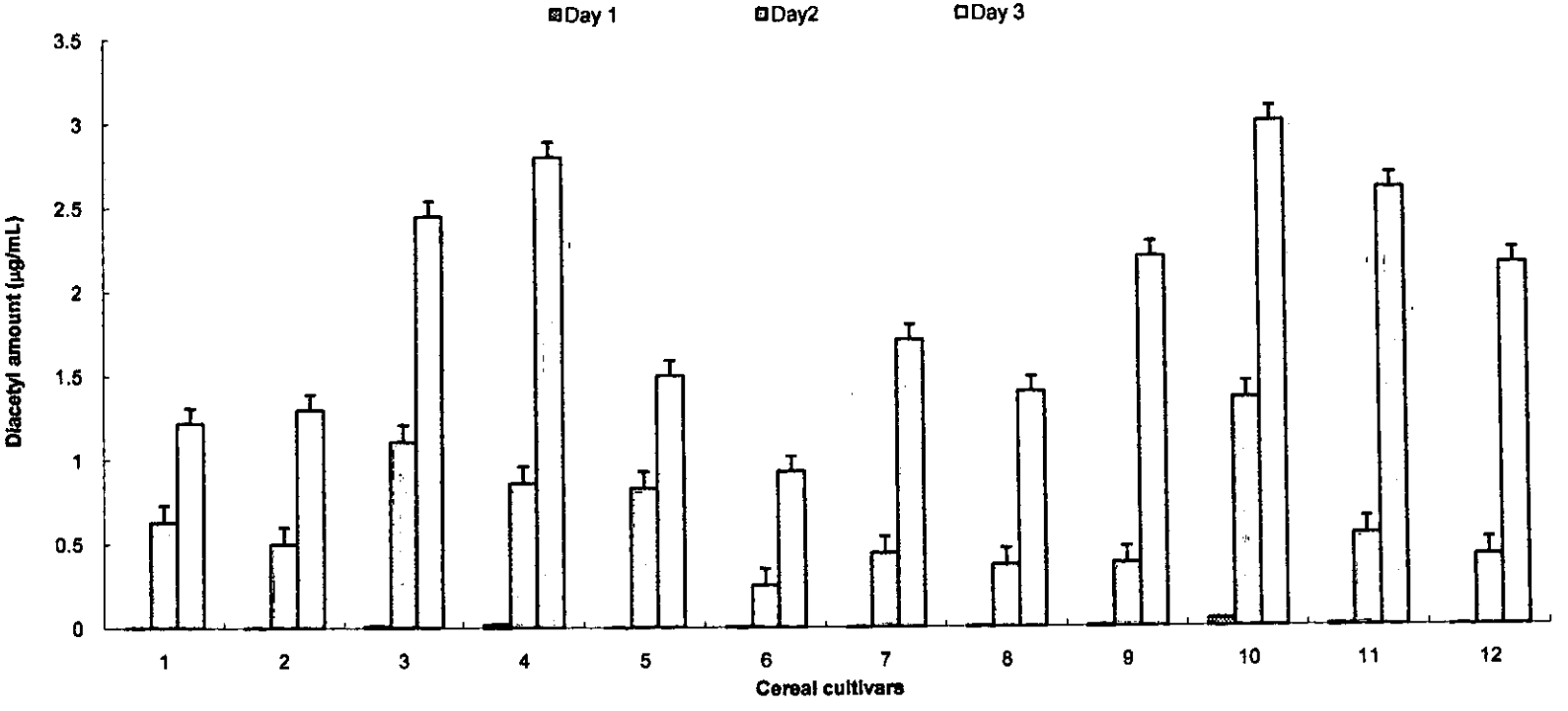

Figure 4.21: Diacetyl concentration in *L. pentosus*-fermented cereal gruels.

1 = TZPB-SR, 2 = TZB-SR-SE, 3 = EV8363-SR,QPM, 4 = TZSR-W-1, 5 = TZB-SR, 6 = 8321-21, 7 = SUWAN-1-SR,QPM, 8 = EV8766-SR-Y,QPM, 9 = TZSR-Y-1, 10 = 8321-18, 11= EV8762-SR, 12 = Sorghum (red)

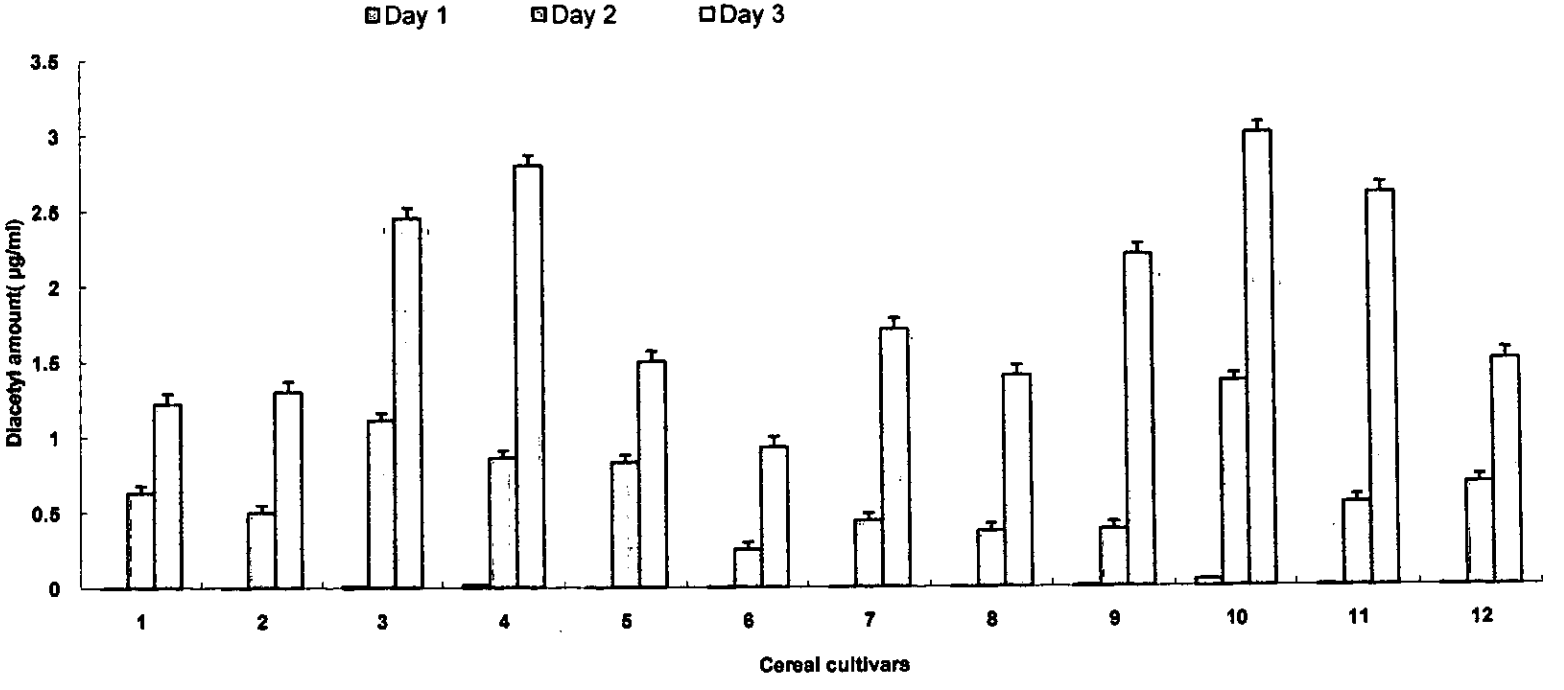

Figure 4.22: Diacetyl Concentration in *L. acidophilus* – fermented cereal gruels. Data was expressed as mean ± SD.
*(Cereals are designated 1 – 12) 1=TZPB-SR, 2=TZB-SR-SE,3=EV8363-SR,QPM,4=TZSR-W-1,5=TZB-SR,6=8321-21,7=SUWAN-1-SR,QPM,8=EV8766-SR-Y,QPM,9=TZSR-Y-1,10=8321-18,11=EV8762-SR, 12=Red sorghum,

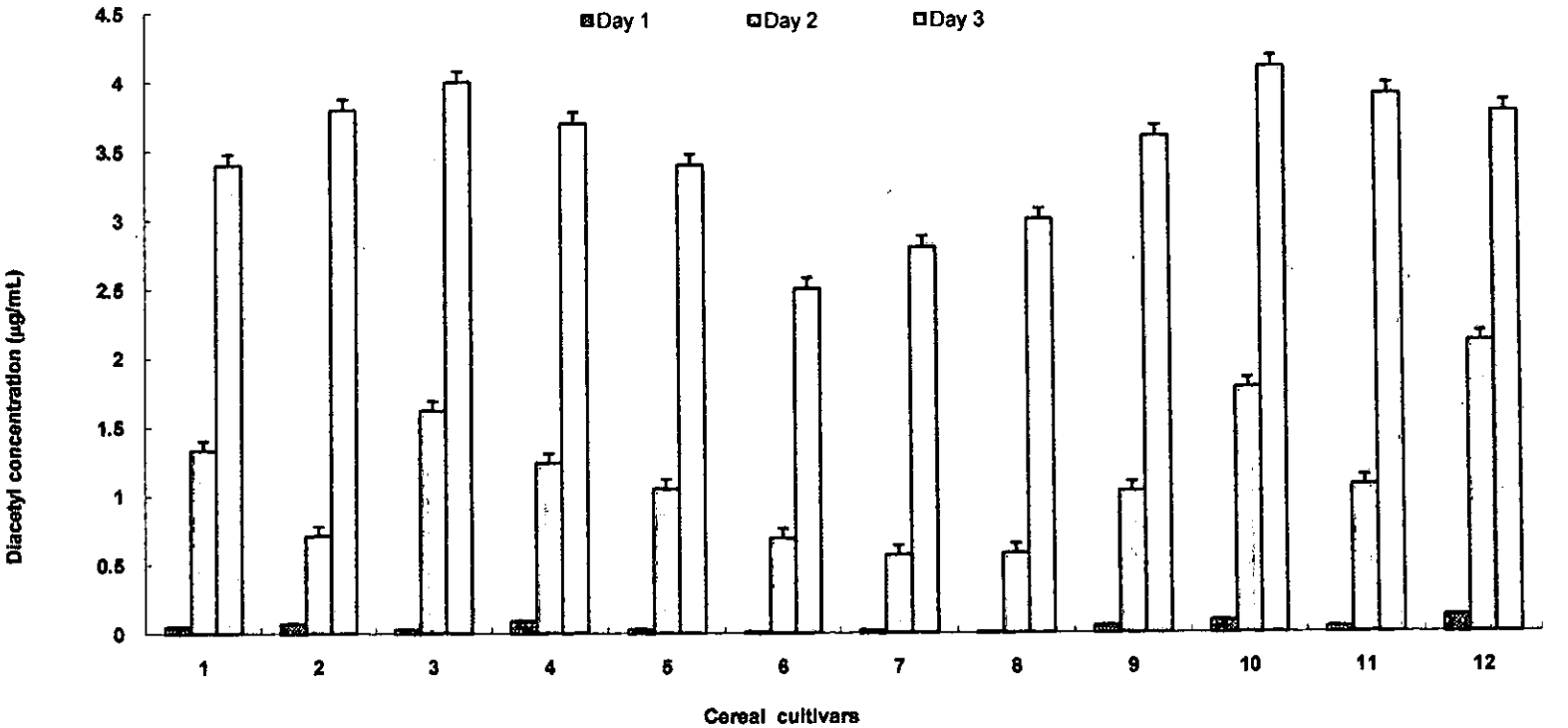

Figure 4.23: Diacetyl concentration in cereal gruels fermented with mixed culture of *Lactobacillus*

1D = TZPB-SR, 2D = TZB-SR-SE, 3D = EV8363-SR,QPM, 4D = TZSR-W-1, 5D = TZB-SR, 6D = 8321-21, 7D = SUWAN-1-SR,QPM, 8D = EV8766-SR-Y,QPM, 9D = TZSR-Y-1, 10D = 8321-18, 1 1D = EV8762-SR, 12D = Sorghum (red)

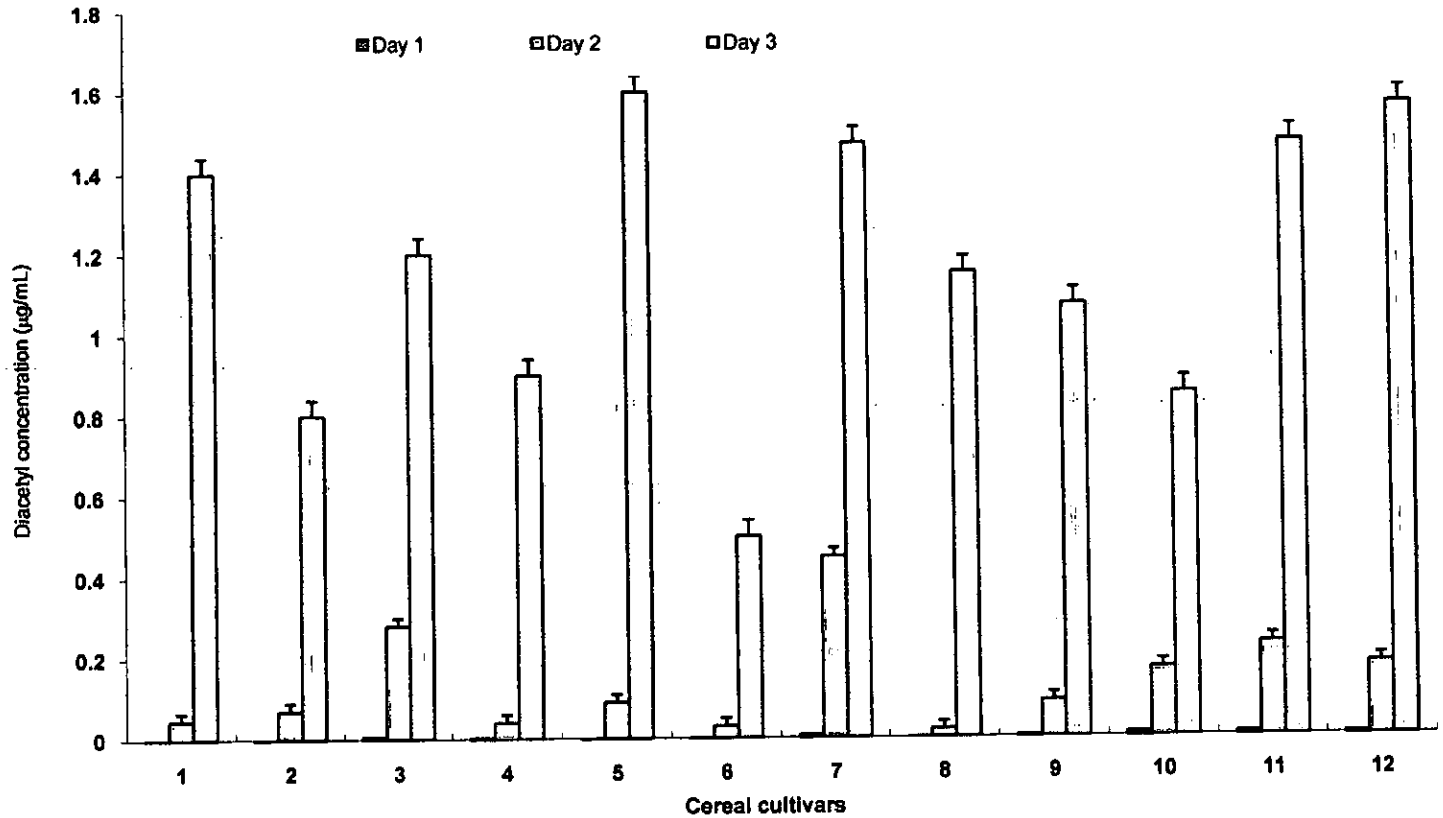

Figure 4.24: Diacetyl concentration in spontaneously- fermented cereal gruels

1D = TZPB-SR, 2D = TZB-SR-SE, 3D = EV8363-SR,QPM, 4D = TZSR-W-1, 5D = TZB-SR, 6D = 8321-21, 7D = SUWAN-1-SR,QPM, 8D = EV8766-SR-Y,QPM, 9D = TZSR-Y-1, 10D = 8321-18, 11D = EV8762-SR, 12D = Sorghum (red)

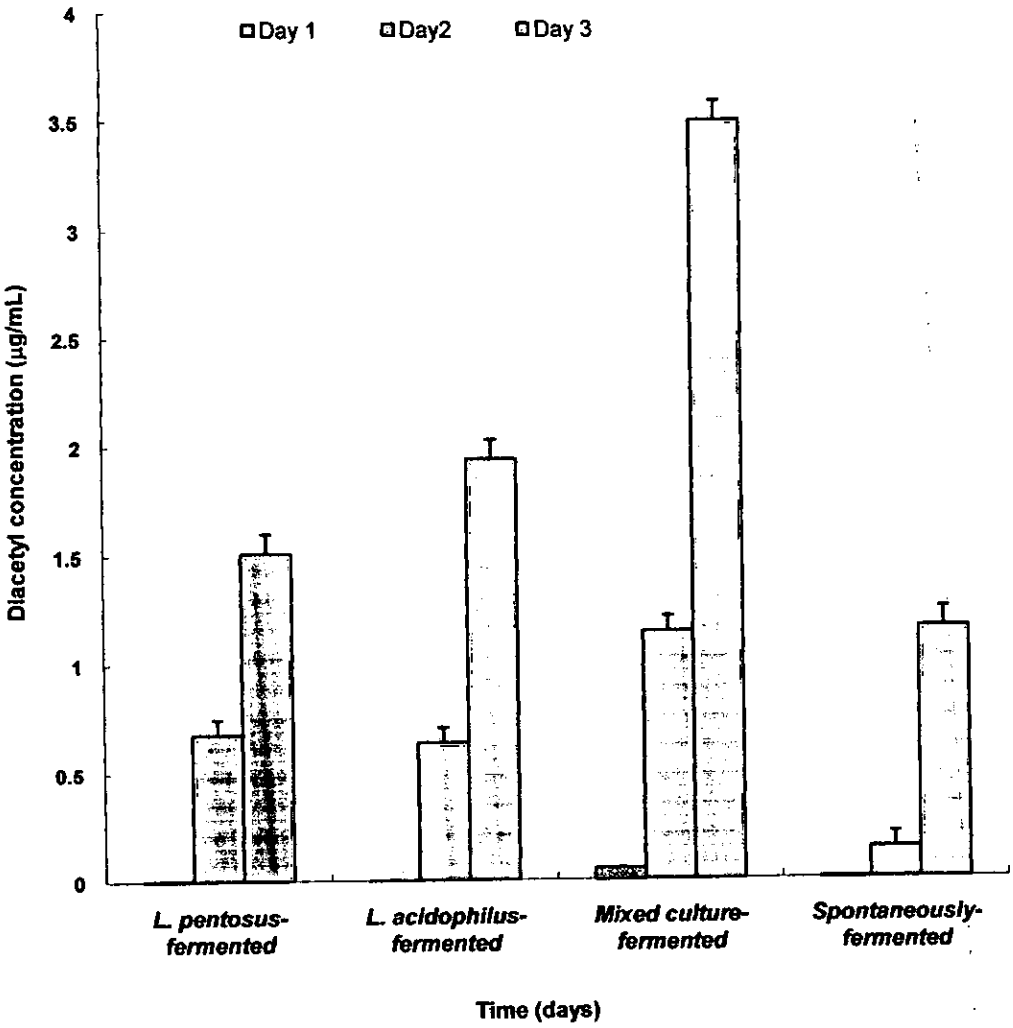

Figure 4.25: Mean total diacetyl concentration in fermented cereal gruels.

4.6: Sensory Evaluation

Organoleptic assessment of the cereal gruels revealed variations in scores for taste, aroma, colour, texture, and overall acceptability. Generally, gruels made from *Lactobacillus* fermentation elicited significantly ($P<0.05$) higher organoleptic scores for each of the sensory attributes investigated. Further analyses between monoculture and mixed culture fermented cereal gruels revealed significant ($P<0.05$) organoleptic differences except for the overall acceptability (Figure 4.26). *L. acidophilus*-fermented gruels had higher scores for aroma than the *L. pentosus* –fermented gruels whereas the colour of *L. pentosus* –fermented gruels had higher organoleptic scores than *L. acidophilus*-fermented. Both *L. acidophilus*-fermented and *L. pentosus* had same organoleptic scores for acceptability and texture. Spontaneously –fermented gruels had the least scores for all parameter assessed.

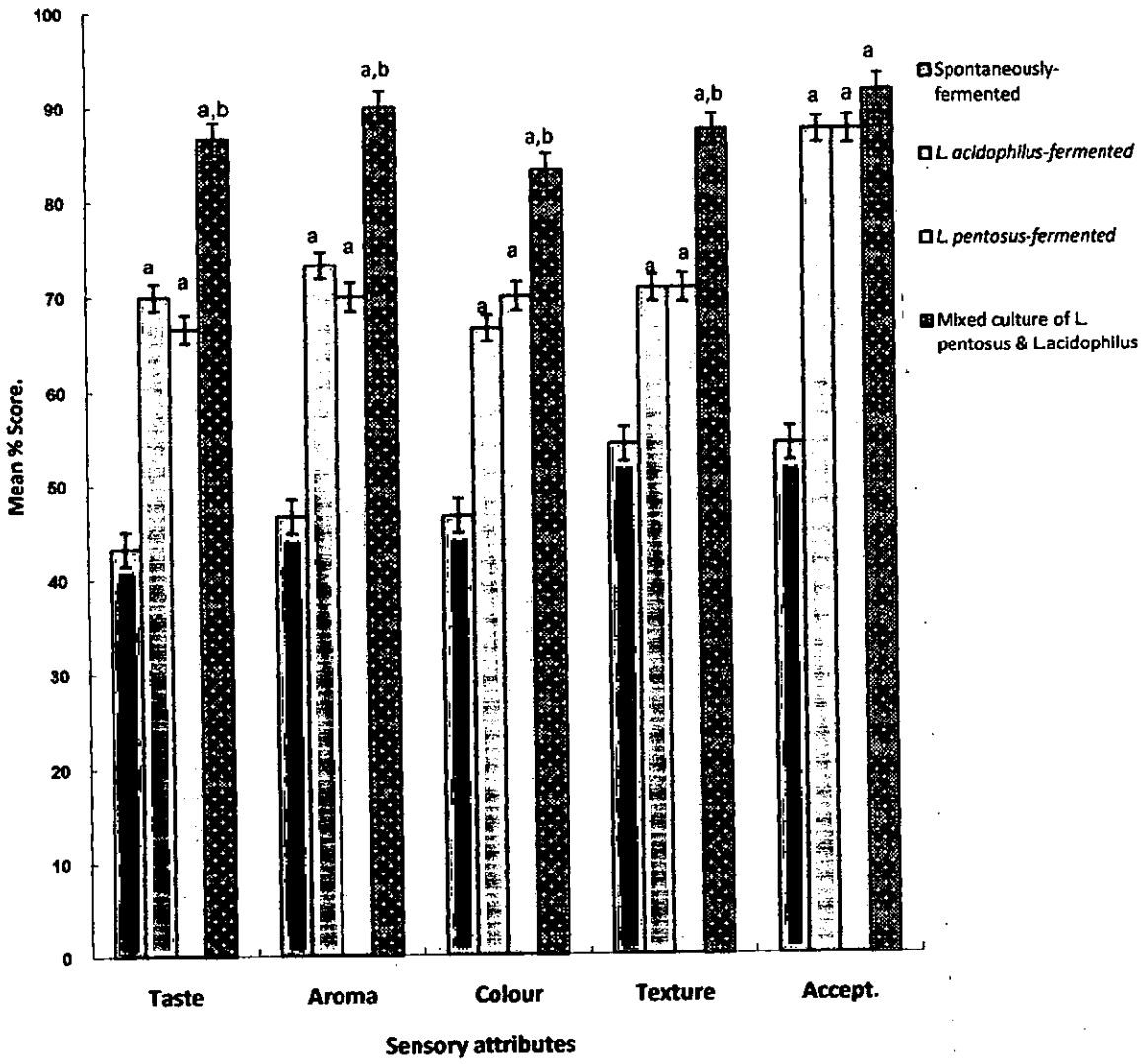

Figure 4.26: Sensory Evaluation of Fermented Cereal Gruel
Each bar represents mean % score of attributes. Vertical lines on bar(s indicate standard error of mean(SEM).[a]$P < 0.05$ compared to spontaneously fermented cereal gruel; [b]$P < 0.05$ (Mixed culture vs.monoculture fermented cereal gruel)

4.7 Titratable acidity and pH

The biochemical analysis of the fermented samples showed the highest total acidity of 15.36 μmol/mL in a mixed culture-fermented sample on day 3 of the fermentation while the lowest value of 0.36 μmol/mL was observed in a spontaneously- fermented sample.

During the fermentation of the tested cereals, pH fell from (5.75 - 6.1) to 3.2 $\pm$ 0.6 in all the slurries. There was decrease in the pH values of the various fermented gruel samples as the fermentation progressed. (Figures 4. 27 - 31).

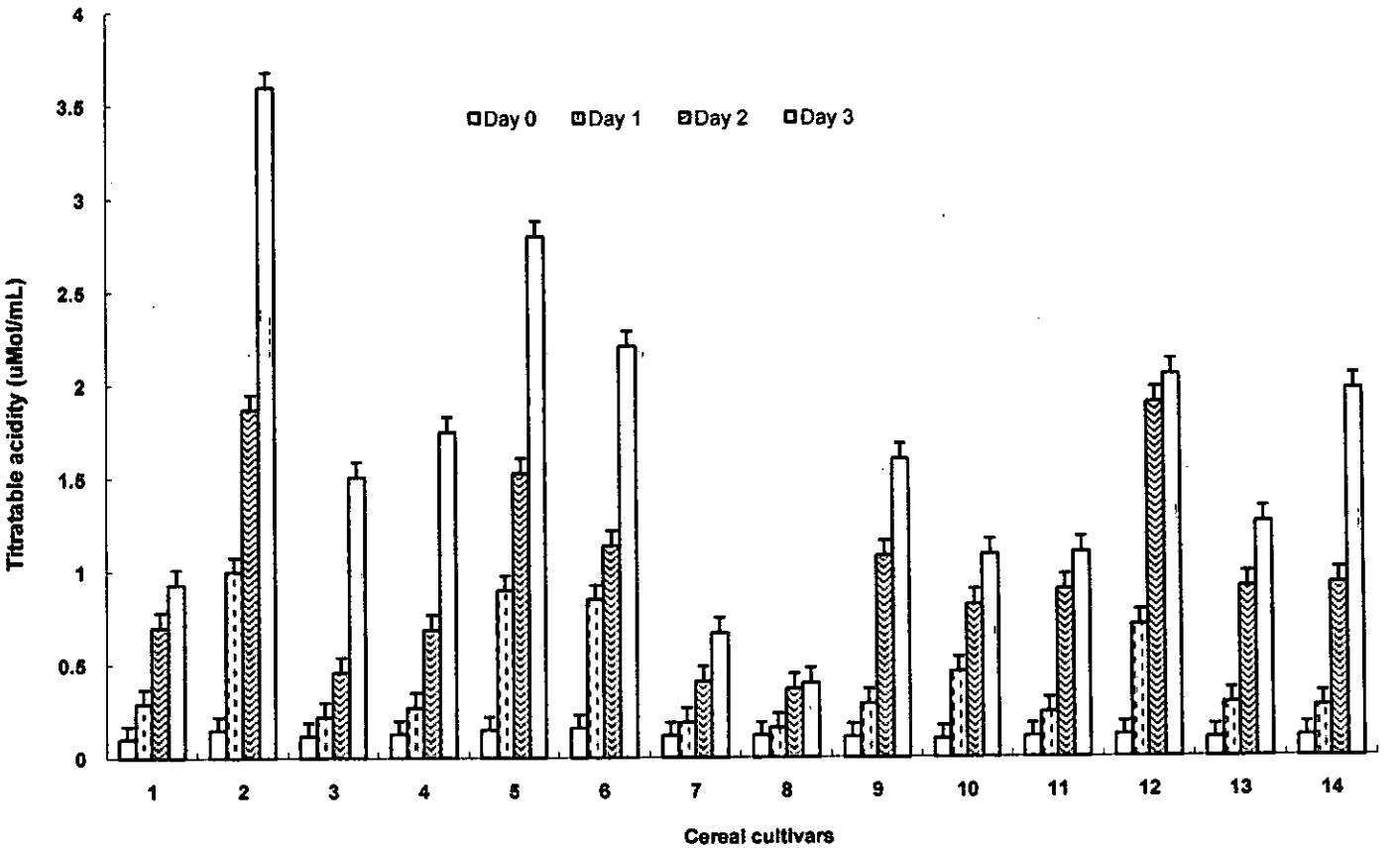

Figure 4.27: Titratable acidity in *L. pentosus*-fermented cereal gruels.

Data expressed as mean ± SD. 1=TZPB-SR,2=TZB-SR-SE,3=EV8363-SR,QPM,4=TZSR-W-1,5=TZB-SR, 6=8321-21,7=SUWAN-1-SR,QPM, 8=EV8766-SR-Y,QPM, 9=TZSR-Y-1, 10=8321-18,11=EV8762-SR,12=Red sorghum,13=Yellow sorghum,14=White sorghum

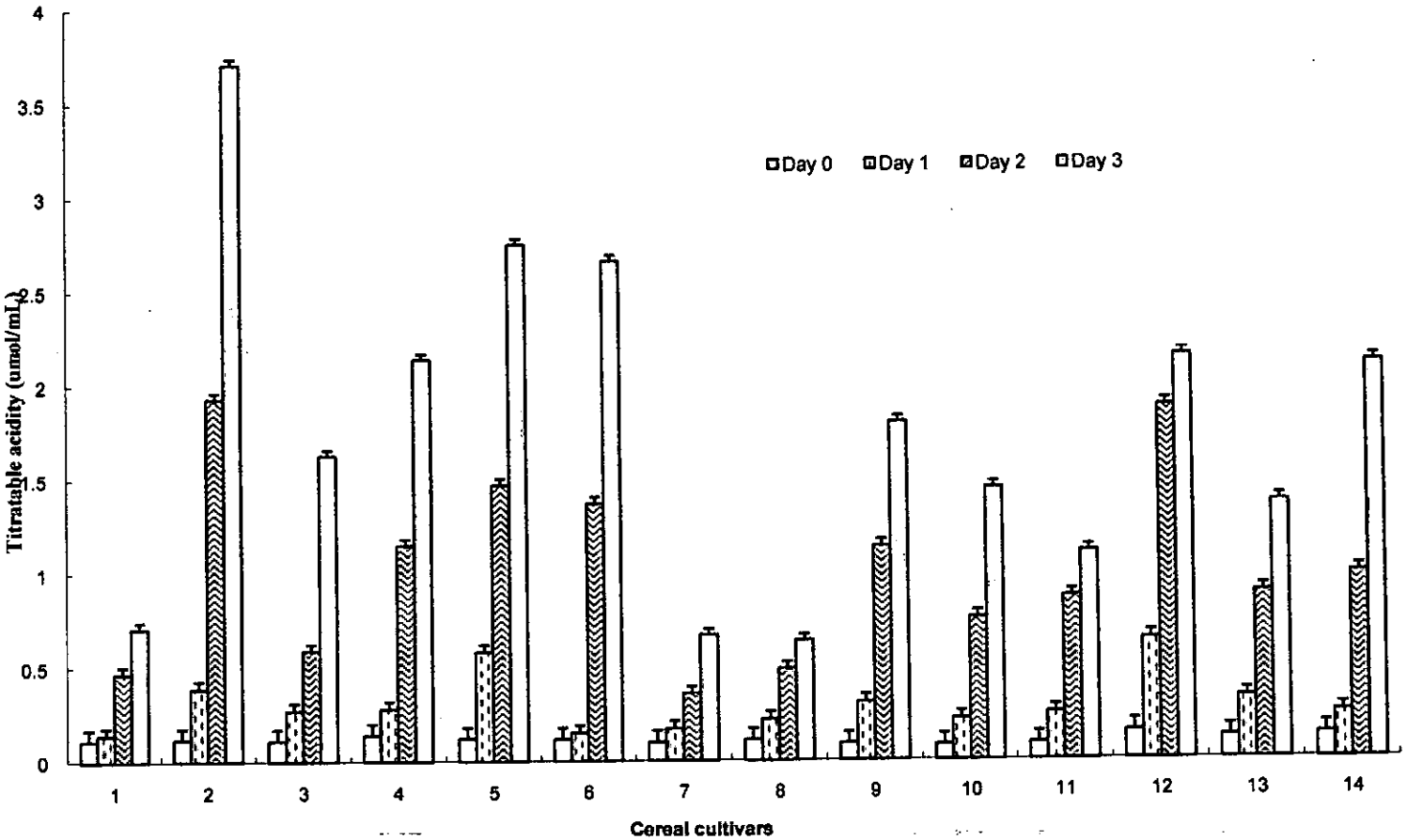

Figure 4.28: Titratable acidity in *L. acidophilus*-fermented cereal gruels.

Data was expressed as mean ± SD. 1=TZPB-SR, 2=TZB-SR-SE,3=EV8363-SR,QPM,4=TZSR-W-1,5=TZB-SR,6=8321-21,7=SUWAN-1-SR,QPM,8=EV8766-SR-Y,QPM,9=TZSR-Y-1,10=8321-18,11=EV8762-SR,12=Red sorghum,13=Yellow sorghum,14=White sorghum

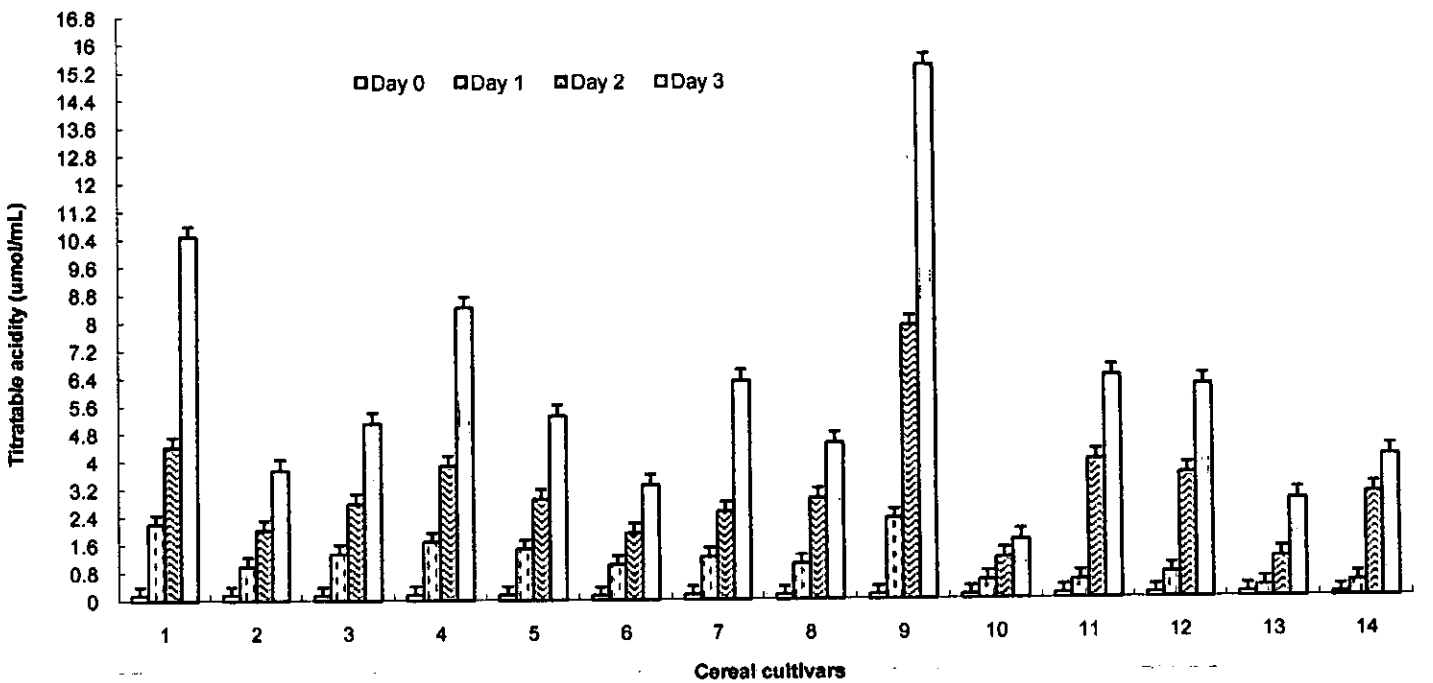

Figure 4.29: Titratable acidity in cereal gruels fermented with mixed culture of *Lactobacillus pentosus acidophilus*. Data was expressed as mean ± SD. 1=TZPB-SR,2=TZB-SR-SE,3=EV8363-SR,QPM, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7=SUWAN-8=EV8766-SR,QPM, 9=TZSR-Y-1, 10=8321-18, 11=EV8762-SR, 12=Red sorghum,13=Yellow sorghum,14=White sorghum

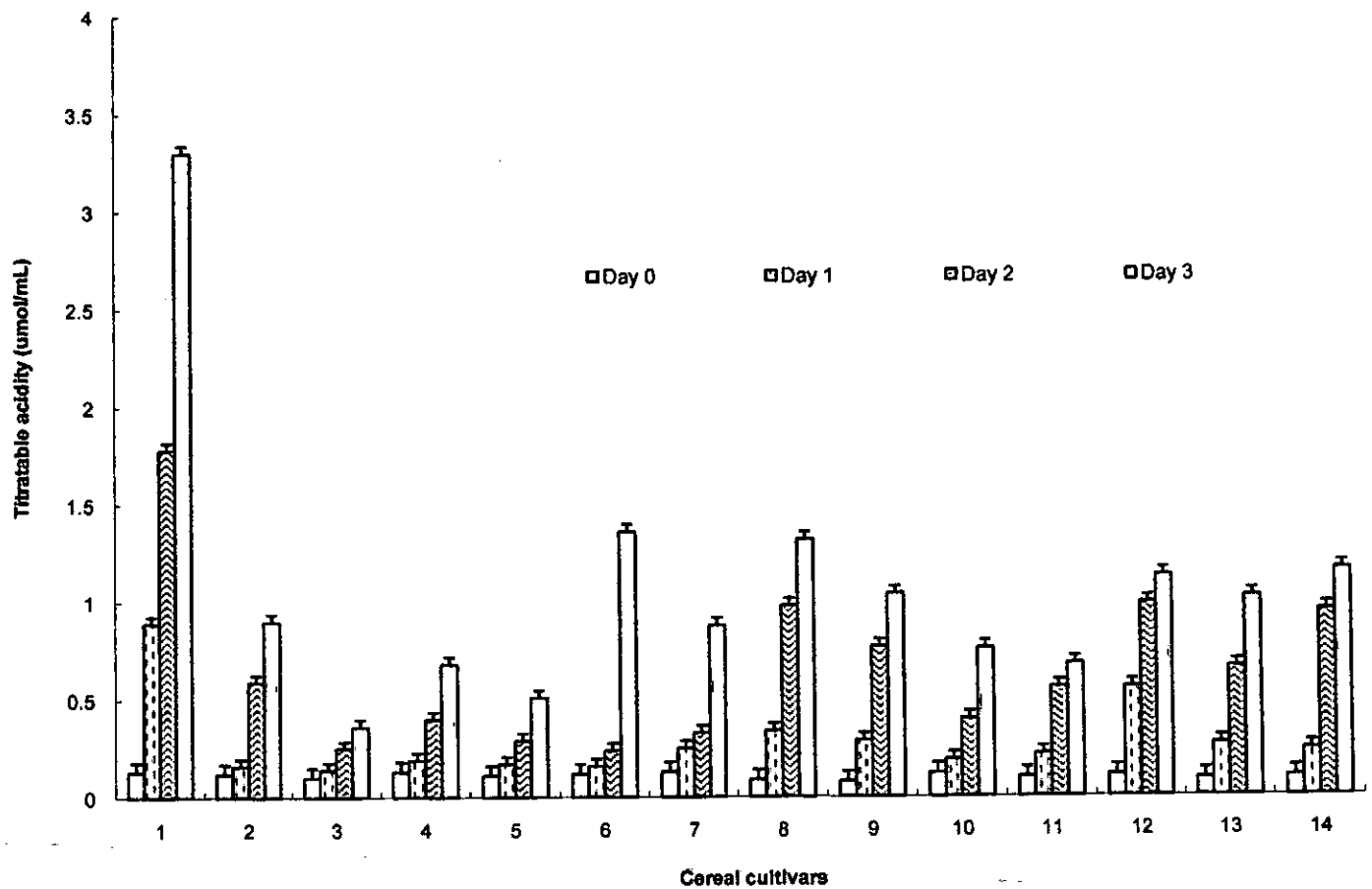

Figure 4.30: Titratable acidity in spontaneously - fermented cereal gruels

Data was expressed as mean ± SD. 1=TZPB-SR,2=TZB-SR-SE,3=EV8363-SR,QPM, 4=TZSR-W-1, 5=TZB-SR, 6=8321-21, 7=SUWAN-1-SR,QPM, 8=EV8766-SR,QPM, 9=TZSR- Y-1, 10=8321-18, 11=EV8762-SR, 12=Red sorghum,13=Yellow sorghum,14=White sorghum

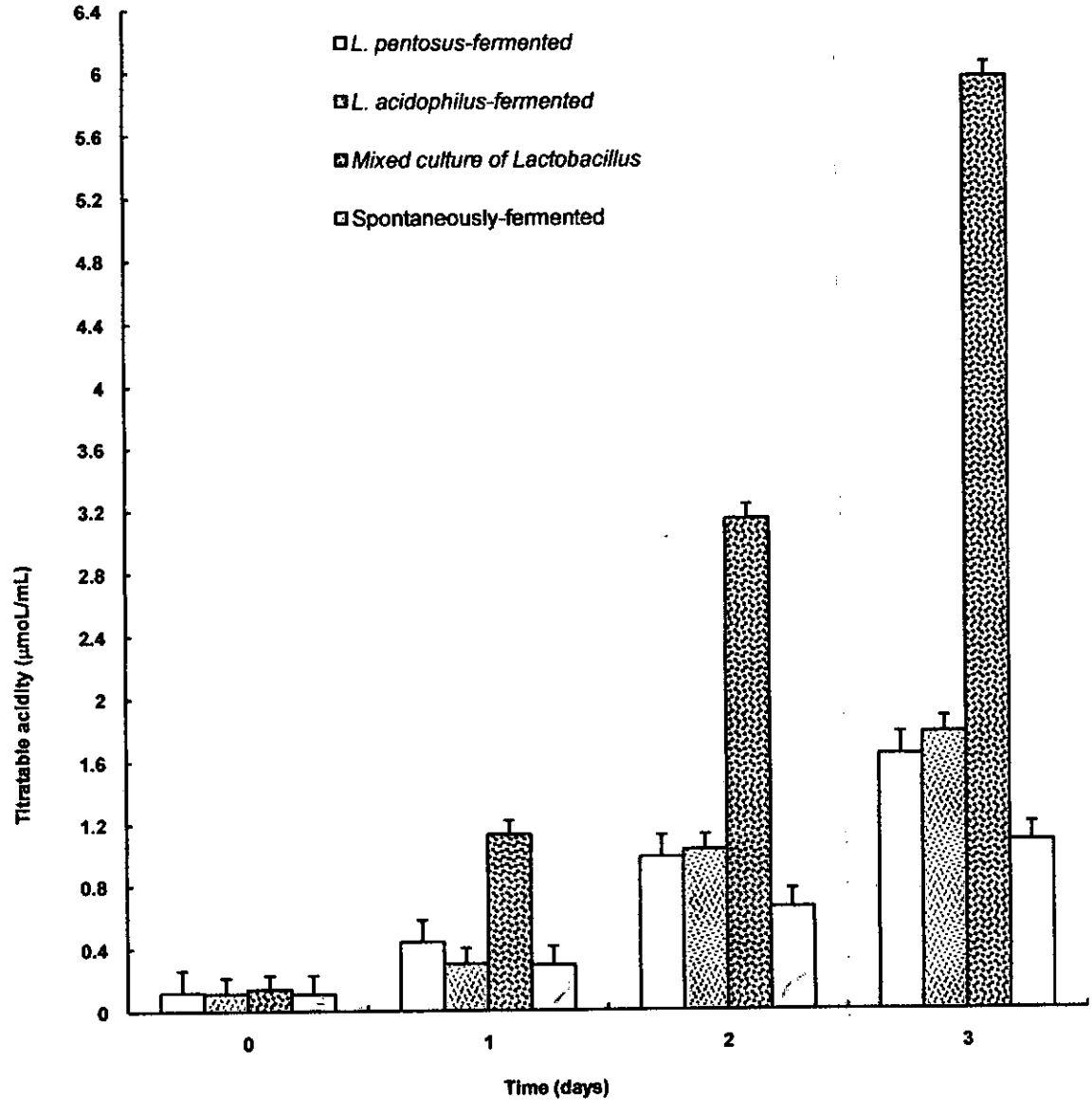

Figure 4.31: Mean titratable acidity in all the fermented cereal gruels during fermentation. Data was expressed as mean ± SD.

Table 4.4 pH changes during fermentation of cereal gruels with *Lactobacillus pentosus*

Cultivar[@]	0	1	2	3	Mean
TZPB-SR	5.95[a]	4.81[a]	3.97	3.65	4.60 (1.03)
TZB-SR-SE	5.90[a]	4.67[a]	4.33[a]	3.40	4.58 (1.03)
EV8363-SR-QPM	5.79[a]	4.60[a]	4.36[a]	3.55	4.58 (0.93)
TZSR-W-1	5.80[a]	4.53[a]	3.91	3.56	4.45 (0.99)
TZB-SR	5.85[a]	4.76[a]	4.00[a]	3.60	4.55 (0.99)
8321-21	5.95[a]	5.03[a]	4.34	3.76	4.77 (0.94)
SUWAN-1 –SR-QPM	5.90[a]	5.40[a]	4.60[a]	3.55	4.86 (1.03)
EV8766-SR-Y-QPM	5.94[a]	5.00[a]	4.17[a]	3.57	4.67 (1.03)
TZSR-Y-1	5.82[a]	4.95[a]	4.60[a]	3.69	4.77 (0.88)
8321-18	5.95[a]	4.77[a]	4.19[a]	3.67	4.65 (0.98)
EV8762-SR	5.97[a]	5.50[a]	4.63[a]	3.74	4.96 (0.98)
RYW-TZ	6.00[a]	5.43[a]	4.24[a]	3.70	4.84 (1.06)
YKT-TZ	5.96[a]	5.44[a]	4.32[a]	3.30	4.76 (1.19)
WHG-TZ	5.85	5.33	4.55	3.60	4.83 (0.98)
Mean	5.90	5.02	4.30	4.71	
SD	(0.07)[a]	(0.34)[a]	(0.24)	(0.99)	

Data presented as mean ± standard deviation from the mean (SD) of three determinations and analyzed by student's t-test (2 – tail)

[@]Cultivars of maize used for fermentation. [a]P< 0.05 (significant pH change).

Table 4.5: pH changes during fermentation of the cereal gruels with *Lactobacillus acidophilus*.

Cultivar	0	1	2	3	Mean
TZPB-SR	5.80[a]	4.70[a]	3.88	3.60	4.50
TZB-SR-SE	5.90[a]	4.72[a]	4.15[a]	3.44	4.55
EV8363-SR –QPM	5.78[a]	4.68[a]	4.20[a]	3.50	4.54
TZSR-W-1	5.83[a]	4.40[a]	3.85	3.40	4.37
TZB-SR	5.81[a]	4.83[a]	3.97	3.49	4.53
8321-21	5.82[a]	4.93[a]	4.23[a]	3.69	4.67
SUWAN-1-SR-QPM	5.75[a]	5.30[a]	4.55[a]	3.66	4.82
EV8766-SR-Y-QPM	5.74[a]	4.78[a]	4.12[a]	3.52	4.54
TZSR-Y-1	5.80[a]	4.95[a]	4.00[a]	3.65	4.60
8321-18	5.95[a]	4.79[a]	4.07[a]	3.56	4.59
EV8762-SR	5.83[a]	5.05[a]	4.49[a]	3.65	4.76
RYW-TZ	5.82[a]	4.96[a]	4.50[a]	3.68	4.74
YKT-TZ	5.90[a]	5.19[a]	4.44[a]	3.25	4.70
WHG-TZ	5.80[a]	5.00[a]	4.33[a]	3.51	4.66
Mean	5.82[a]	4.88[a]	4.20[a]	3.54	4.61
SD	(0.06)	(0.23)	(0.24)	(0.13)	(0.97)

Data presented as mean ± standard deviation from the mean (SD) of three determinations and analyzed by student's t-test (2 – tail)[@]Cultivars of maize used for fermentation. [a]P< 0.05 (significant pH change).

Table 4.6. pH changes during fermentation of cereal gruels with mixed culture of *Lactobacillus acidophilus and L.pentosus*

Cultivar	0	1	2	3	Mean
TZPB-SR	5.77[a]	4.64[a]	4.00[a]	3.45	4.47 (1.00)
TZB-SR-SE	5.87[a]	4.65[a]	4.12[a]	3.35	4.50 (1.06)
EV8363-SR-QPM	5.75[a]	4.39[a]	4.03[a]	3.36	4.38 (1.00)
TZSR-W-1	5.90[a]	4.38[a]	3.70	3.30	4.32 (1.14)
TZB-SR	5.75[a]	4.64[a]	3.96	3.48	4.46 (0.98)
8321-21	5.80[a]	4.85[a]	4.20[a]	3.60	4.61 (0.94)
SUWAN-1-SR-QPM	5.81[a]	5.23[a]	4.44[a]	3.25	4.71 (1.07)
EV8766-SR-Y-QPM	5.81[a]	4.81[a]	3.96	3.40	4.50 (1.05)
TZSR-Y-1	5.80[a]	4.82[a]	4.15[a]	3.56	4.58 (0.96)
8321-18	5.76[a]	4.55[a]	3.96	3.55	4.46 (0.96)
EV8762-SR	5.81[a]	4.90[a]	4.20[a]	3.60	4.63 (0.95)
RYW-TZ	5.78[a]	4.81[a]	4.15[a]	3.40	4.54 (1.01)
YKT-TZ	5.90[a]	5.14[a]	4.39[a]	3.20	4.66 (1.15)
WHG-TZ	5.73[a]	4.96[a]	4.25[a]	3.47	4.60 (0.97)
Mean	5.80[a]	4.77[a]	4.11[a]	3.43	
SD	(0.05)	(0.25)	(0.19)	(0.12)	

Data presented as mean ± standard deviation from the mean (SD) of three determinations and analyzed by student's t-test (2 – tail)

[@]Cultivars of maize used for fermentation. [a]P< 0.05 (significant pH change).

Table 4. 7 pH changes during spontaneous fermentation of cereal gruels

Cultivar	0	1	2	3	Mean
TZPB-SR	5.90[a]	4.85[a]	4.00[a]	3.90	4.66 (0.93)
TZB-SR-SE	6.00[a]	5.40[a]	4.65[a]	4.00	5.01 (0.87)
EV8363-SR-QPM	5.80[a]	5.00[a]	4.35[a]	3.98	4.78 (0.80)
TZSR-W-1	5.99[a]	4.60[a]	4.10[a]	3.95	4.66 (0.93)
TZB-SR	5.95[a]	4.70[a]	4.25[a]	4.00	4.73 (0.87)
8321-21	5.96[a]	5.26[a]	4.50[a]	4.00	4.93 (0.86)
SUWAN-1-SR-QPM	5.90[a]	5.23[a]	4.65[a]	4.00	4.95 (0.81)
EV8766-SR-Y-QPM	5.97[a]	5.30[a]	4.46[a]	3.97	4.93 (0.89)
TZSR-Y-1	6.10[a]	5.05[a]	4.45[a]	3.90	4.88 (0.93)
8321-18	5.98[a]	5.45[a]	4.50[a]	3.99	4.98 (0.90)
EV8762-SR	5.99[a]	5.25[a]	4.67[a]	4.00	4.98 (0.85)
RYW-TZ	5.90[a]	5.35[a]	4.80[a]	4.02	5.02 (0.80)
YKT-TZ	6.00[a]	5.56[a]	4.60[a]	3.94	5.03 (0.93)
WHG-TZ	6.10[a]	5.40[a]	4.70	3.96	5.04 (0.92)
Mean	5.97	5.17	4.48	3.97	
SD	(0.8)	(0.29)	(0.23)	(0.03)	

Data is presented as mean ± standard deviation from the mean (SD) of three determinations and analyzed by student's t-test (2 – tail) [@]Cultivars of maize used for fermentation. [a]P< 0.05 (significant pH change).

4.8 *In Vitro* Studies:

The ability of the *Lactobacillus* species to control growth and survival of the following pathogenic bacteria was investigated in vitro. The pathogenic agents were *Escherichia coli, Shigella, Salmonella, Vibrio cholerae*, and *Staphylococcus aureus*. In all the cereal varieties tested, there was no growth of these diarrhoeagenic bacteria after 4 hours in "ogi" fermented with mixed culture of *Lactobacillus pentosus* and *L. acidophilus* (starter cultures), no growth after 6 hours in *L. acidophilus*-fermented, and the same pattern occurred with the *L. pentosus*-fermented samples except with *E. coli* and *Shigella* which lasted another hour. The control supported the growth of *Vibrio cholerae* for 14 hours, *E. coli* for over 40 hours and all others up to 30 hours (Figures 4.32 – 4.5).

Statistically, all diarrhoeagenic organisms showed same response irrespective of cereal type. These organisms showed differential response to treatment within the first 3 hours in cereals fermented with *L. pentosus* and *L. acidophilus* both singly and mixed and all were dead within 7 hours. However, the organisms responded very differently in the control. *E. coli* was the least affected with cell number reduction by 50%, *Staphylococcus* lost 60% cells, *Shigella* and *Salmonella* had 70% cell death while all *Vibrio* cells died within 16 hours.

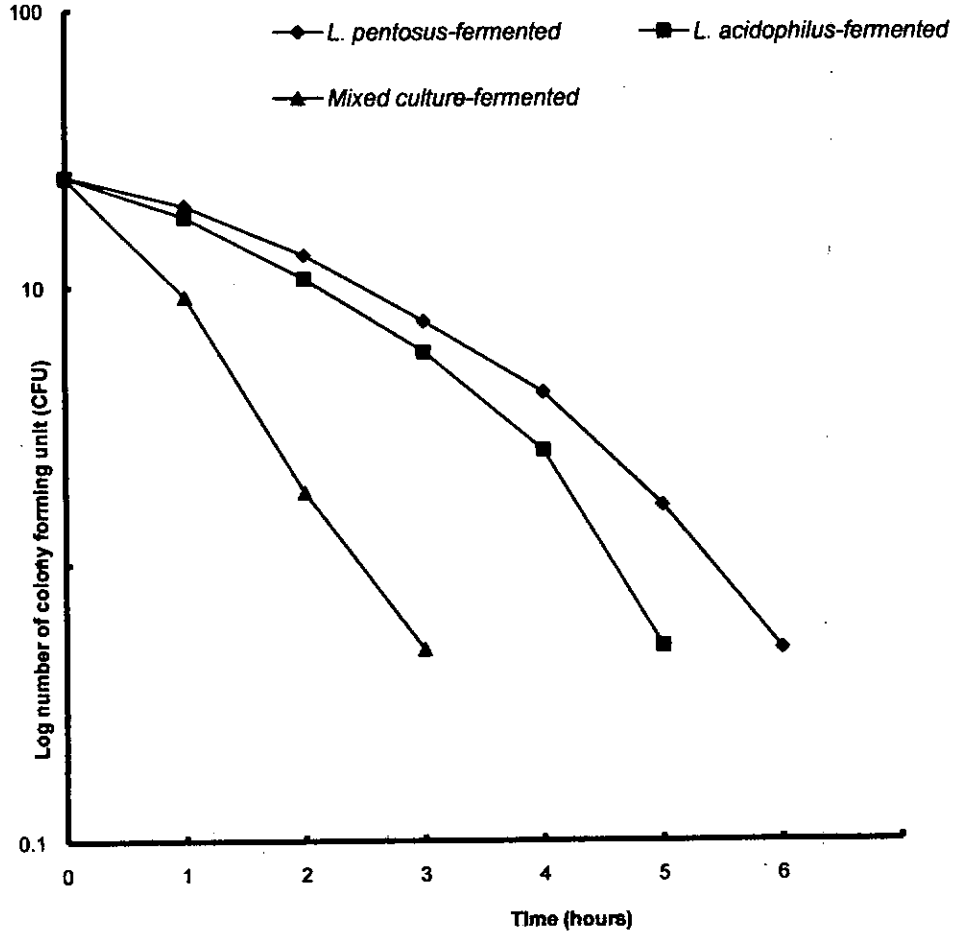

Figure 4.32: Survival of *Vibrio cholerae* AP23622 *Lactobacillus*-fermented cereal gruels.

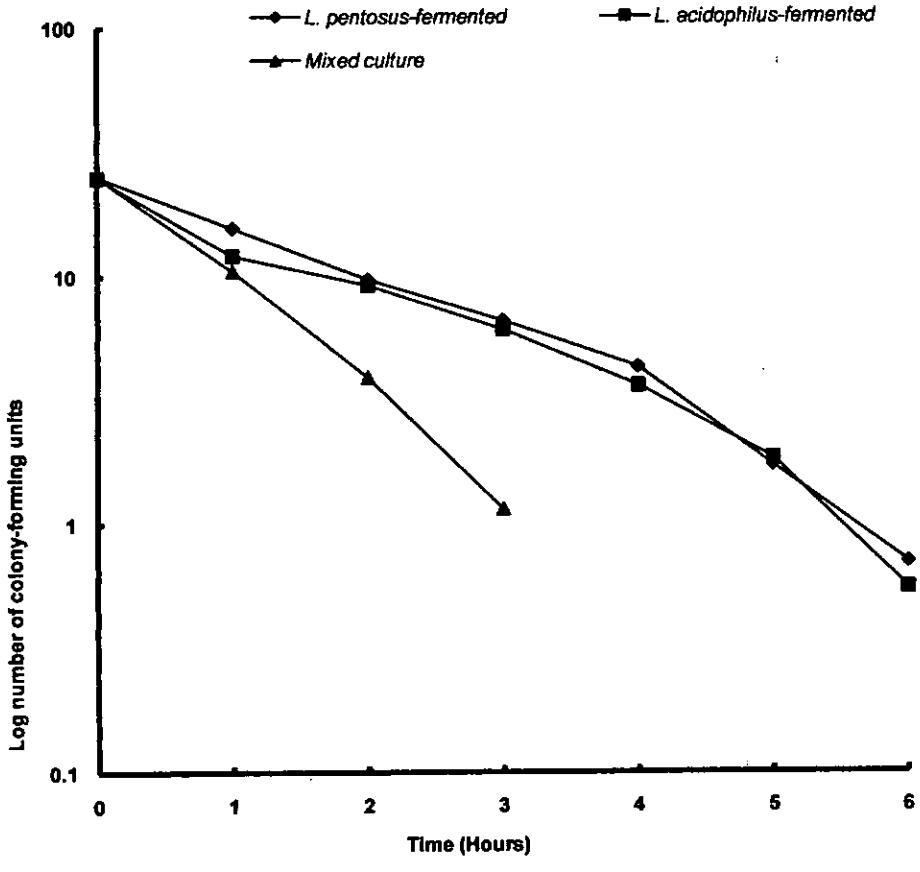

Figure 4.33: Survival of *Salmonella typhi* AP23118 in *Lactobacillus*-fermented cereal gruels.

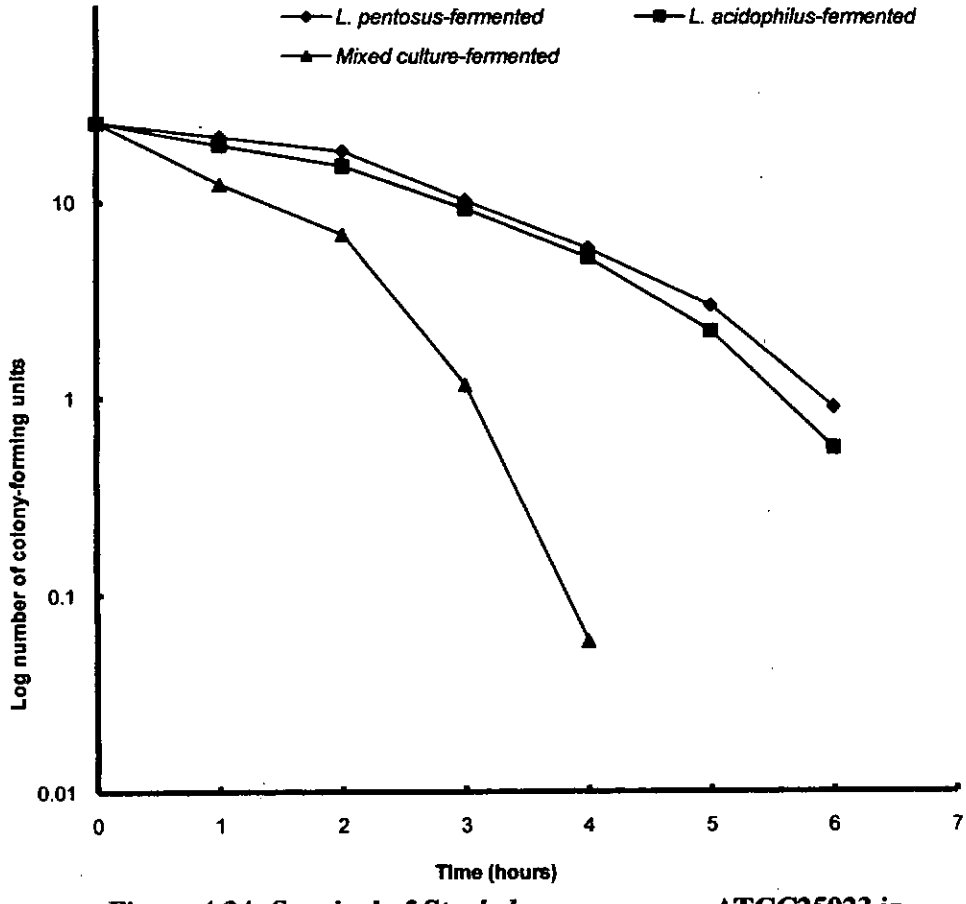

Figure 4.34: Survival of *Staphylococcus aureus* ATCC25923 in *Lactobacillus*-fermented cereal gruels.

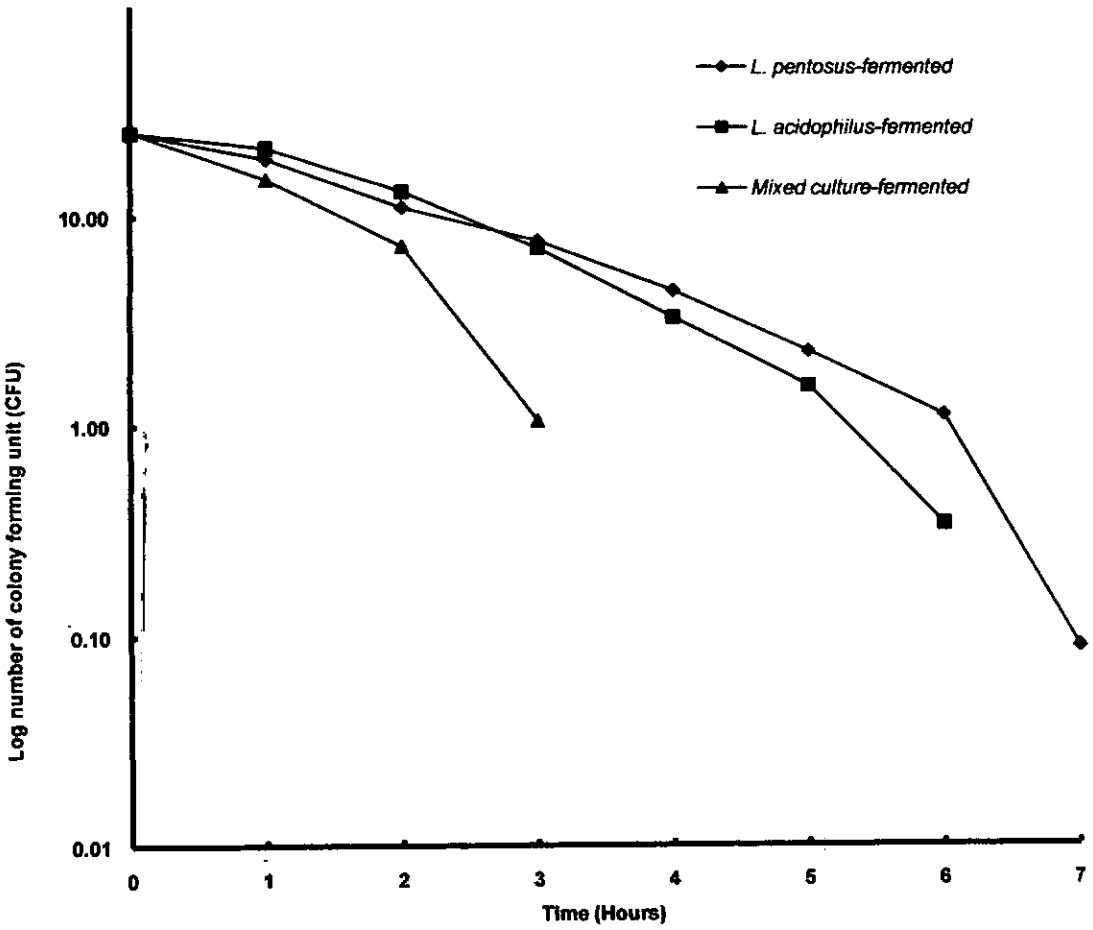

Figure 4.35: Survival *Shigella dysenteriae* AP22433 in *Lactobacillus*-fermented gruels.

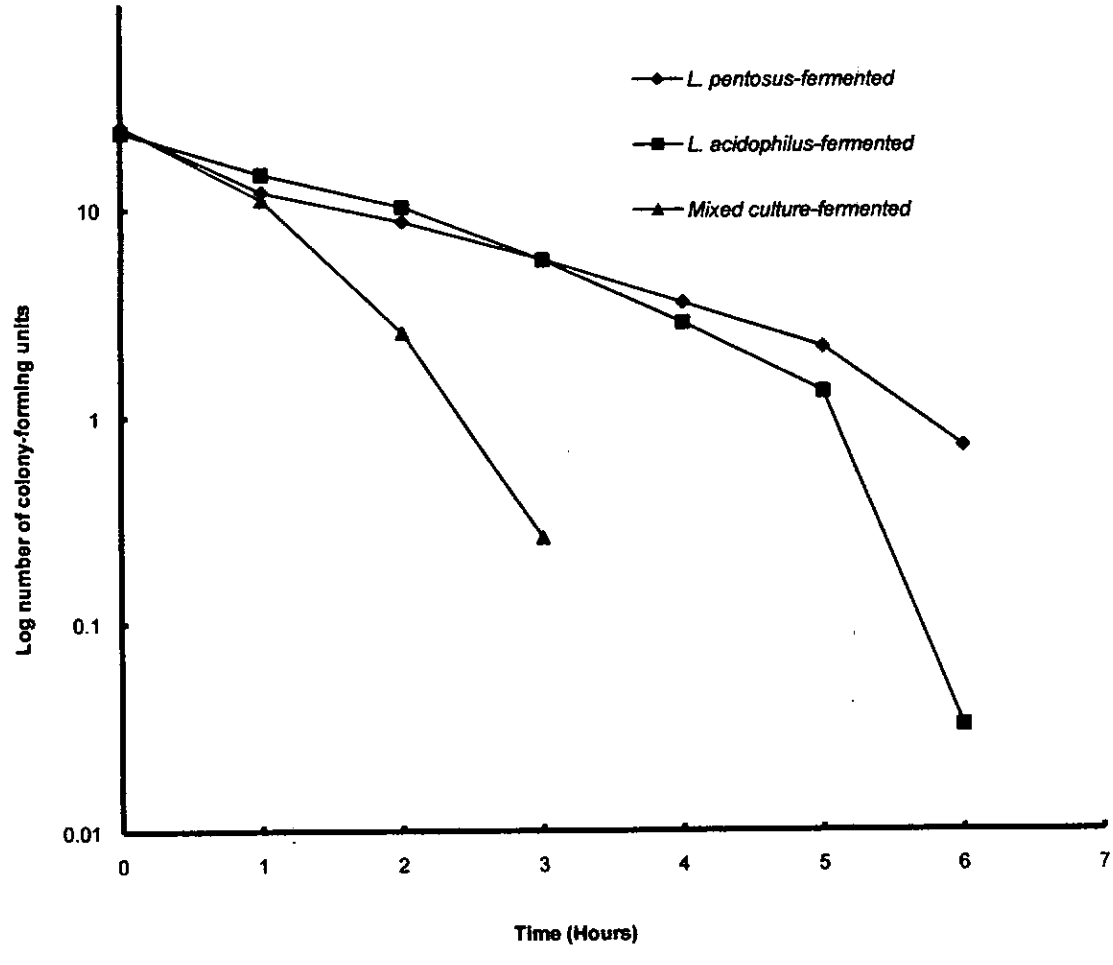

Figure 4.36: Survival of *Escherichia coli* ATCC25922 in *Lactobacillus*-fermented cereal gruels

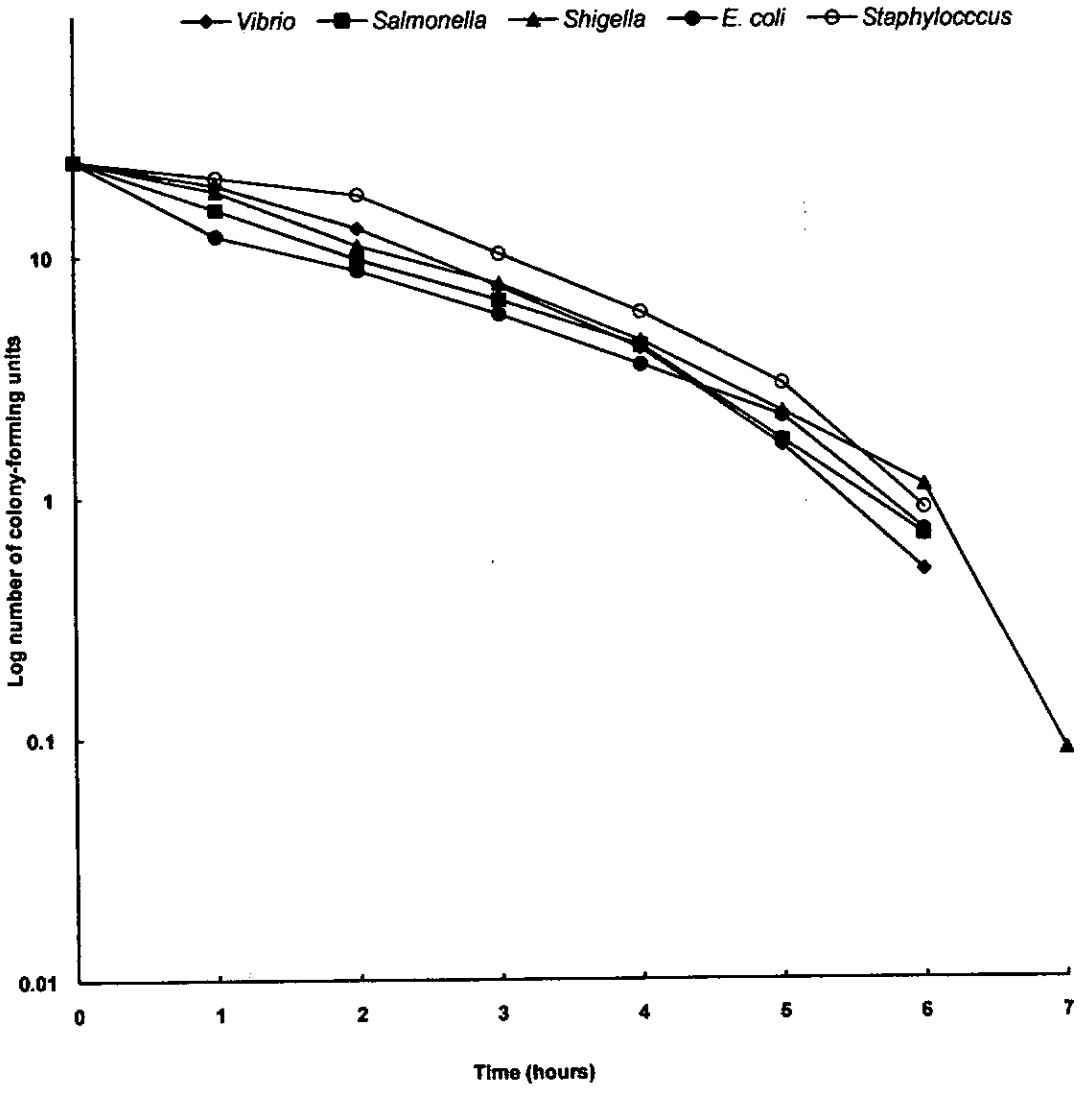

Figure 4.37: Survival of diarrhoeagenic agents in *L. pentosus* fermented cereal gruels

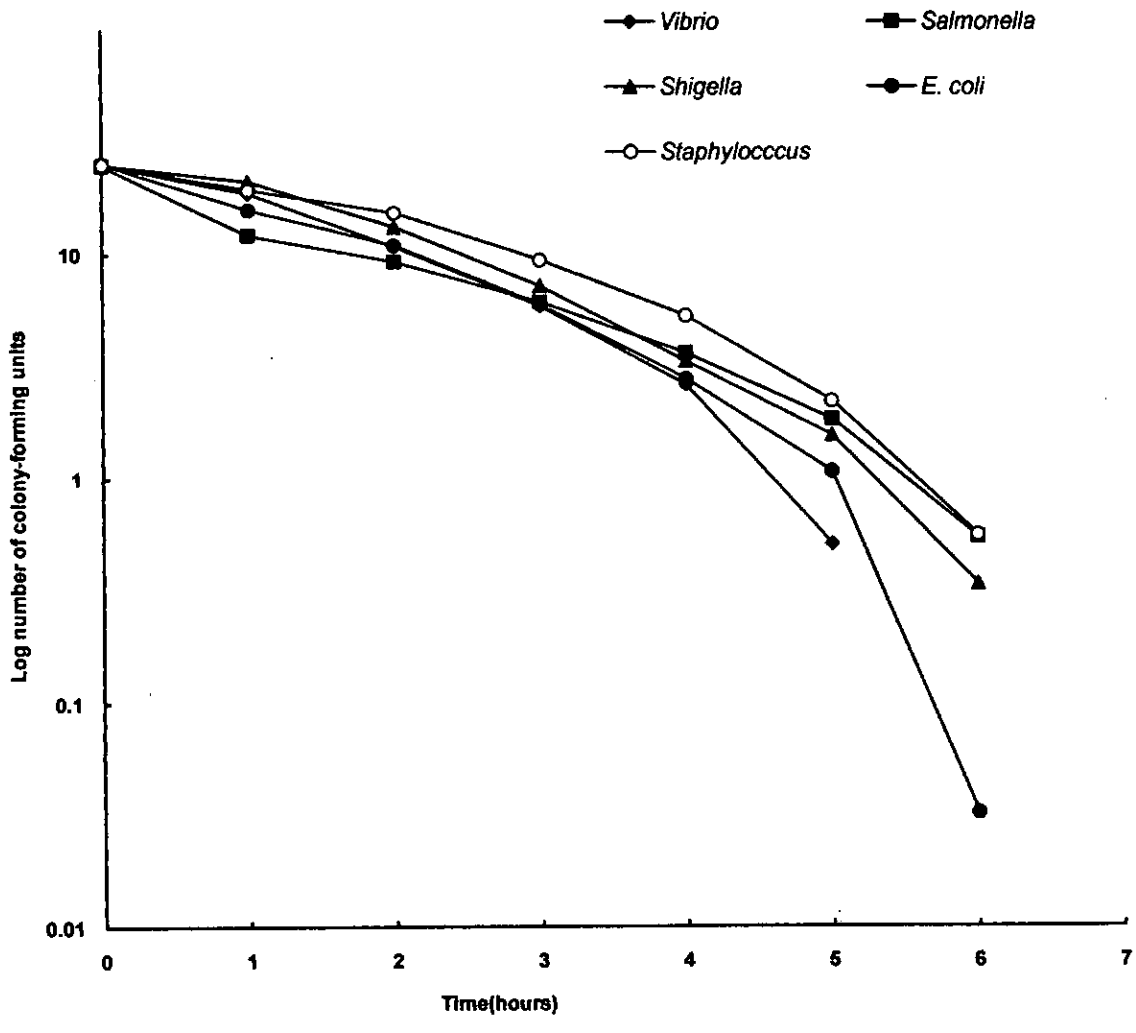

Figure 4.38: Survival of diarrhoeagenic agents in *L. acidophilus* fermented cereal gruels.

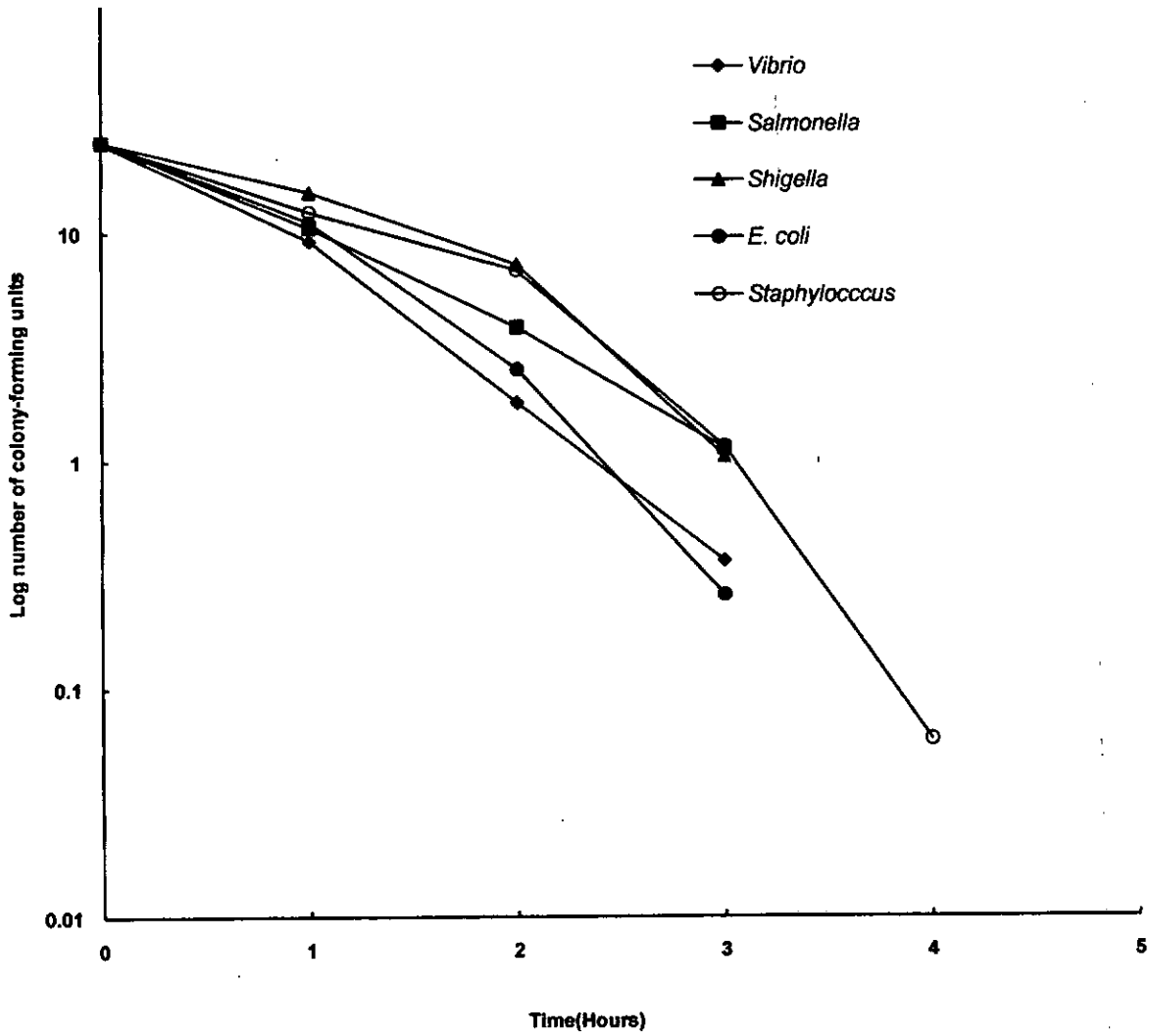

Figure 4.39: Survival of diarrhoeagenic agents in mixed culture of *Lactobacillus*

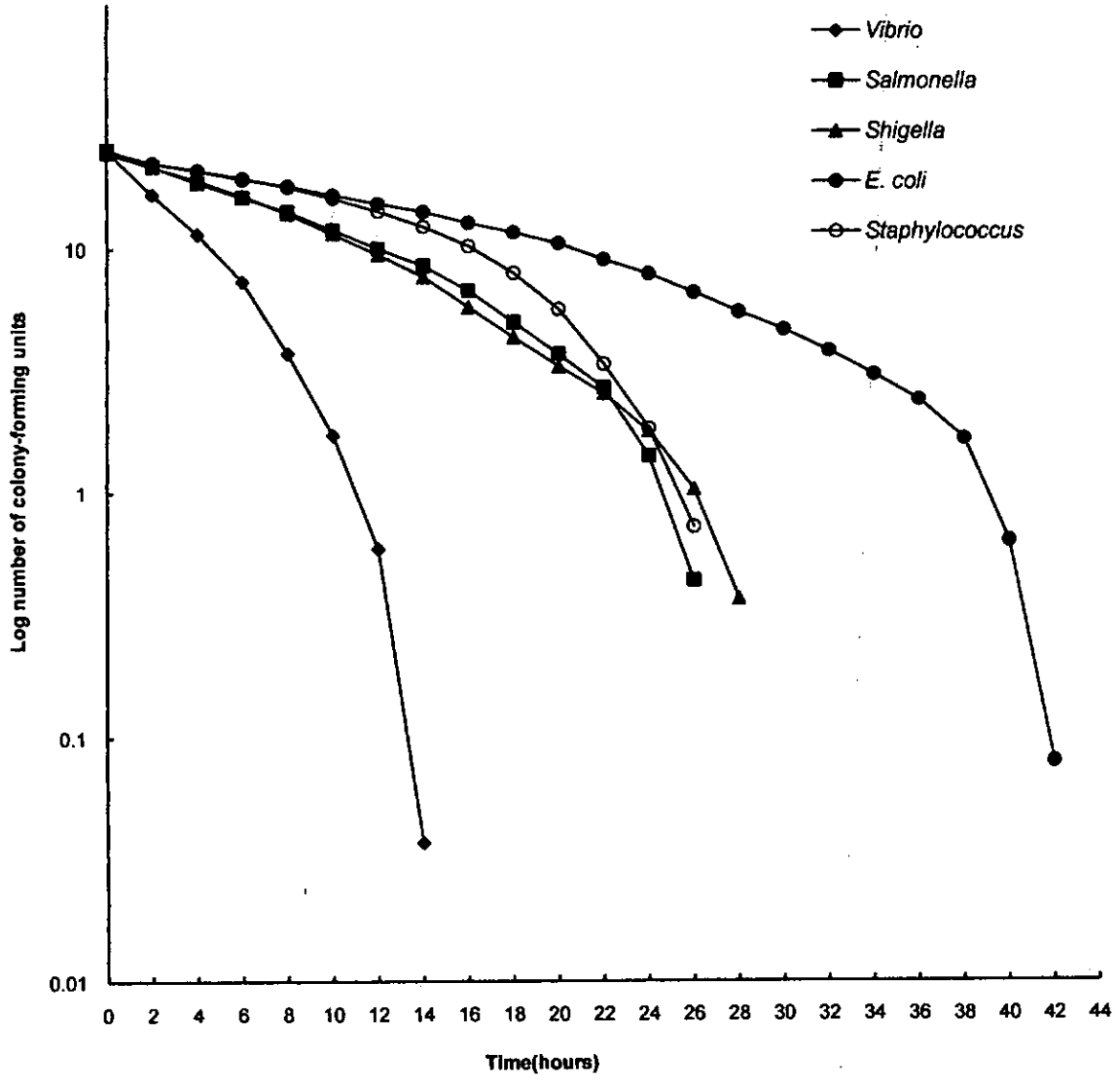

Figure 4.40: Survival of diarrhoeagenic agents in spontaneously - fermented cereal gruels.

4.5 *In- Vivo* Studies

The probiotic nature of the fermenting organisms in the cereal samples was investigated in vivo.

4.5.1 Challenge Test

Infection was manifested by change in the faeces samples from hard pellets to soft or semisolid mass. The infected mice were generally slow moving, showed a withdrawn behaviour and died later, whereas the survivors were recovered fully after 48hours. Survival rates of the experimental animals (mice) challenged with *Shigella dysenteriae* (a pathogenic agent of diarrhoea) and fed with the cooked fermented products were observed. The survival rates of 100%, 90%, and 80% were observed in mice fed with mixed culture samples, *L. acidophilus*, and *L. pentosus* samples respectively after 20 days. The survival rate of mice fed with spontaneously fermented cereal samples were 33.3% while the mice fed on breast milk, which served as controls, had no survivors.

4.5.2 Recovery of *Shigella* cells

The faecal samples of the experimental and control mice were analyzed for the presence of viable *Shigella* cells. The *Shigella* cells recovered in the faeces were counted in both test mice and the controls. It was found that cells were not detected after 4 days, 5 days, and 6 days respectively in mice fed with mixed culture, *L. acidophilus*, and *L. pentosus* respectively. However, high counts of *Shigella* cells were observed up to 9 days though there was recovery of cells until the 15^{th} day in mice fed with spontaneously fermented sample resulting in the death of 66.6% of mice. A geometric increase in the *Shigella* cell count was observed in the control mice and all died within three days. Statistical analysis showed that there was no significant difference in the potency of the starter cultures either single or mixed.

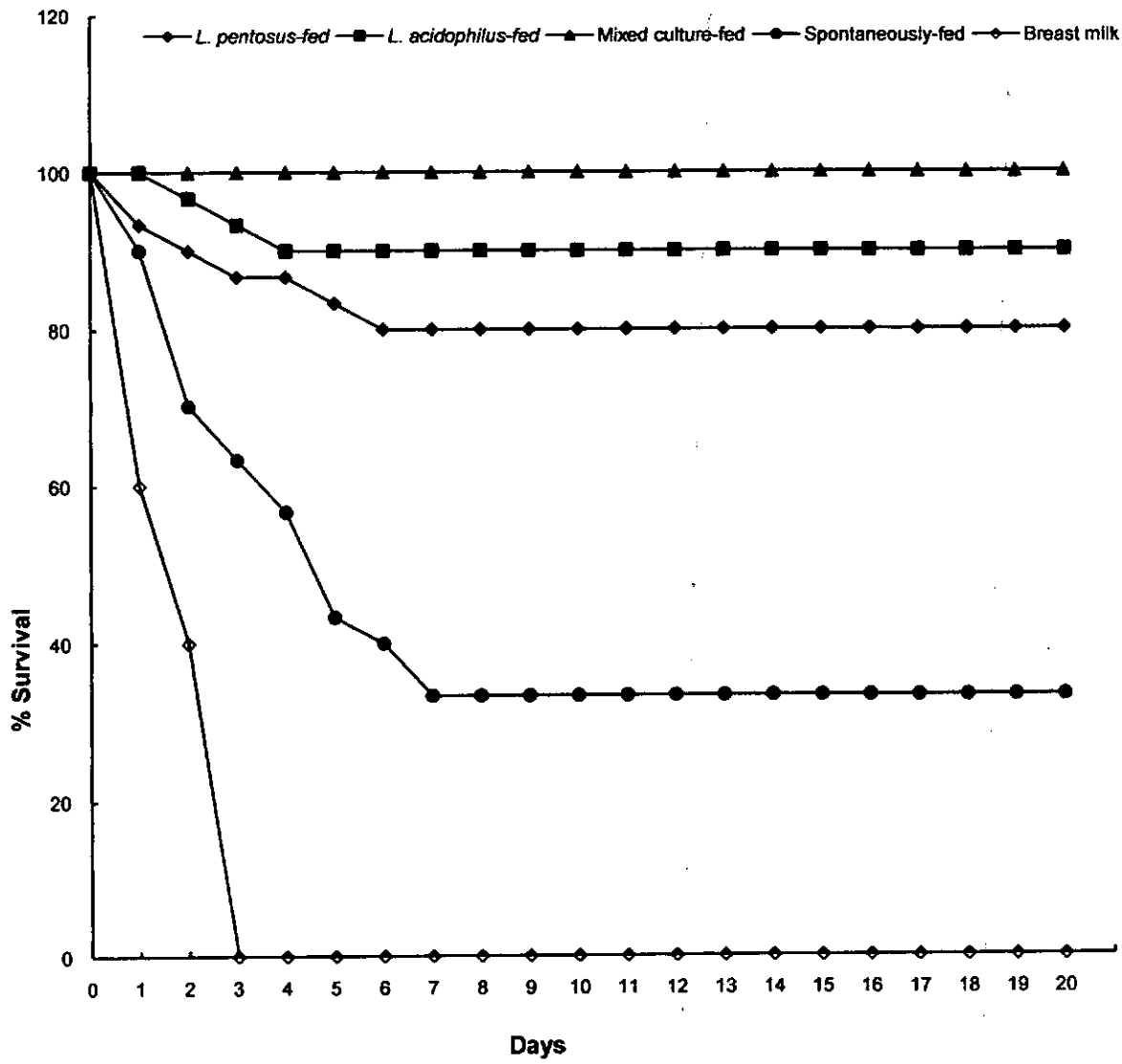

Figure 4.41: Survival rates of mice after *Shigella* Infection

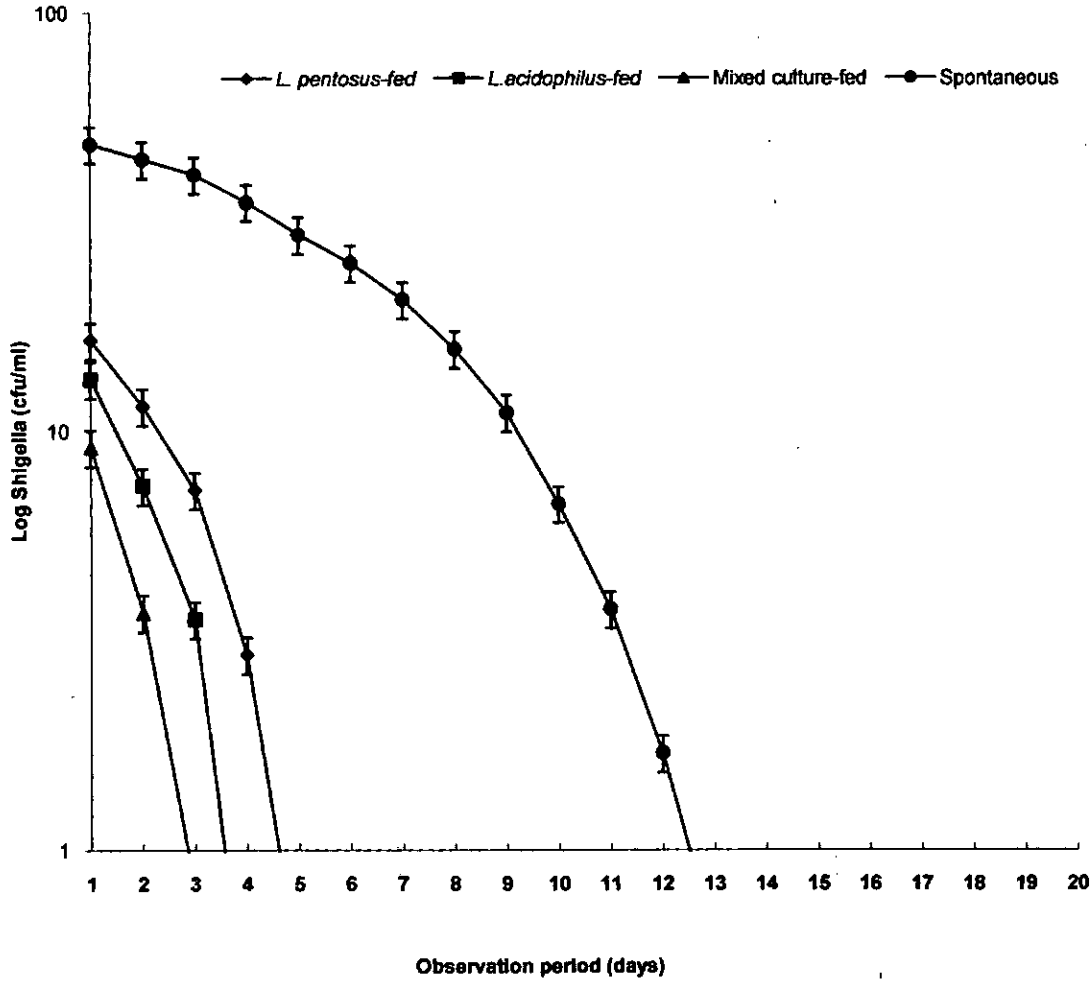

Figure 4. 42: The population of *Shigella* cells in faeces samples of surviving mice.

4.6 Acid and Bile tolerance evaluation

The two strains of *Lactobacillus* grew in all the pH values with the growth being optimum at pH 4 in all conditions. The bile concentration of 0.15% recorded the optimal growth.

4.7 Storage Stability

The flour of the *Lactobacillus* - fermented cereals maintained a pH of 3.70 ± 0.04 throughout the storage period while that of the spontaneously – fermented maintained a pH of 3.90 ± 0.11.

All flours of these fermented cereals exhibited antibacterial activities against the two bacterial test organisms used (*Staphylococcus* sp. and *E. coli*). There was no significant difference in the action of the *Lactobacillus* - fermented gruels on the test organisms, all were eliminated within 7h. However, in the spontaneously – fermented flour

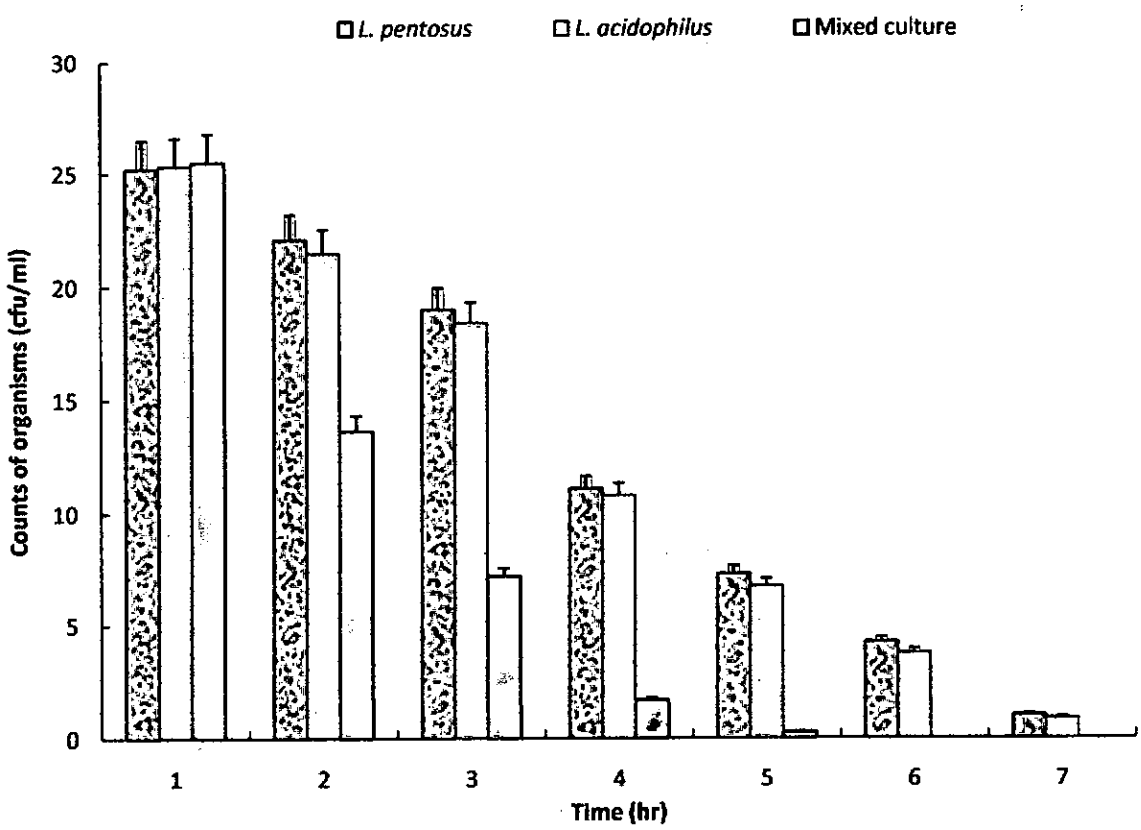

Figure 4.43: Counts of *Staphylococcus* in *Lactobacillus* – fermented cereal flour after storage.

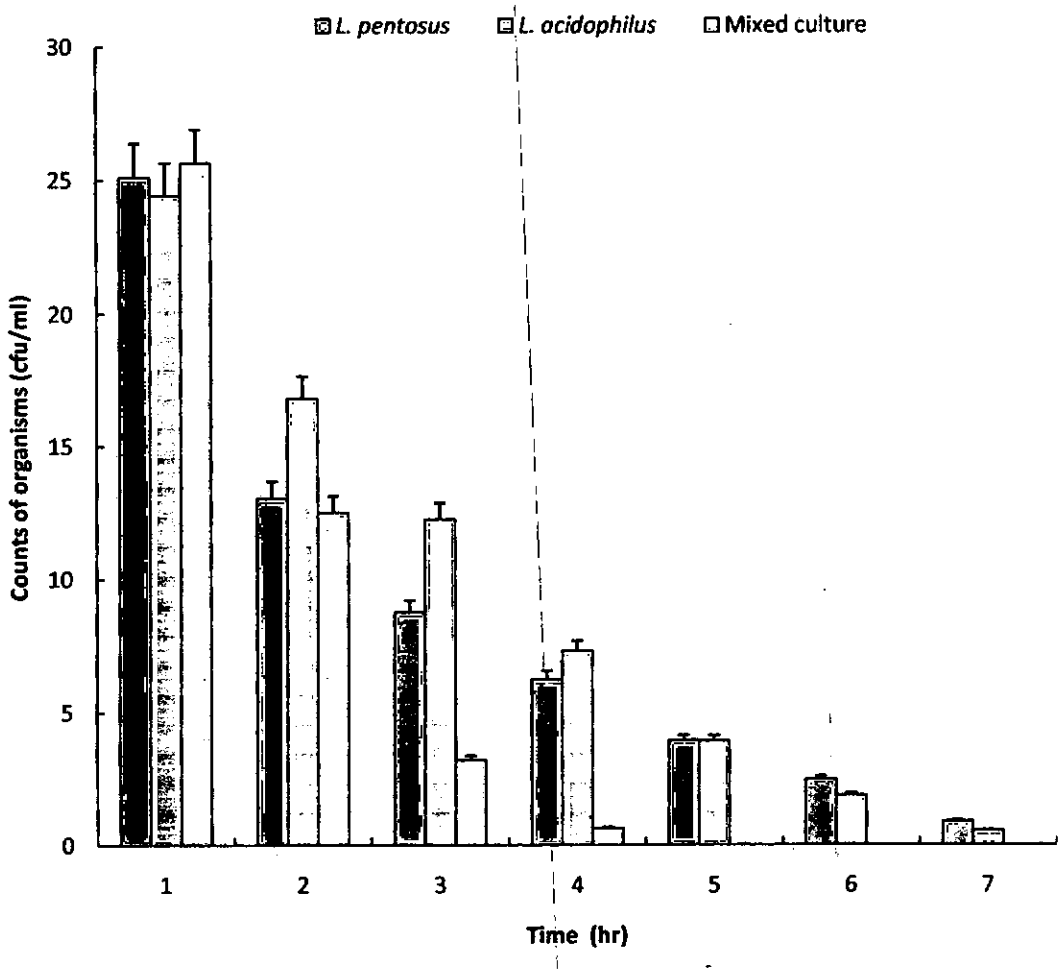

Figure 4.44: Counts of *E. coli* in *Lactobacillus* – fermented cereal flour after storage.

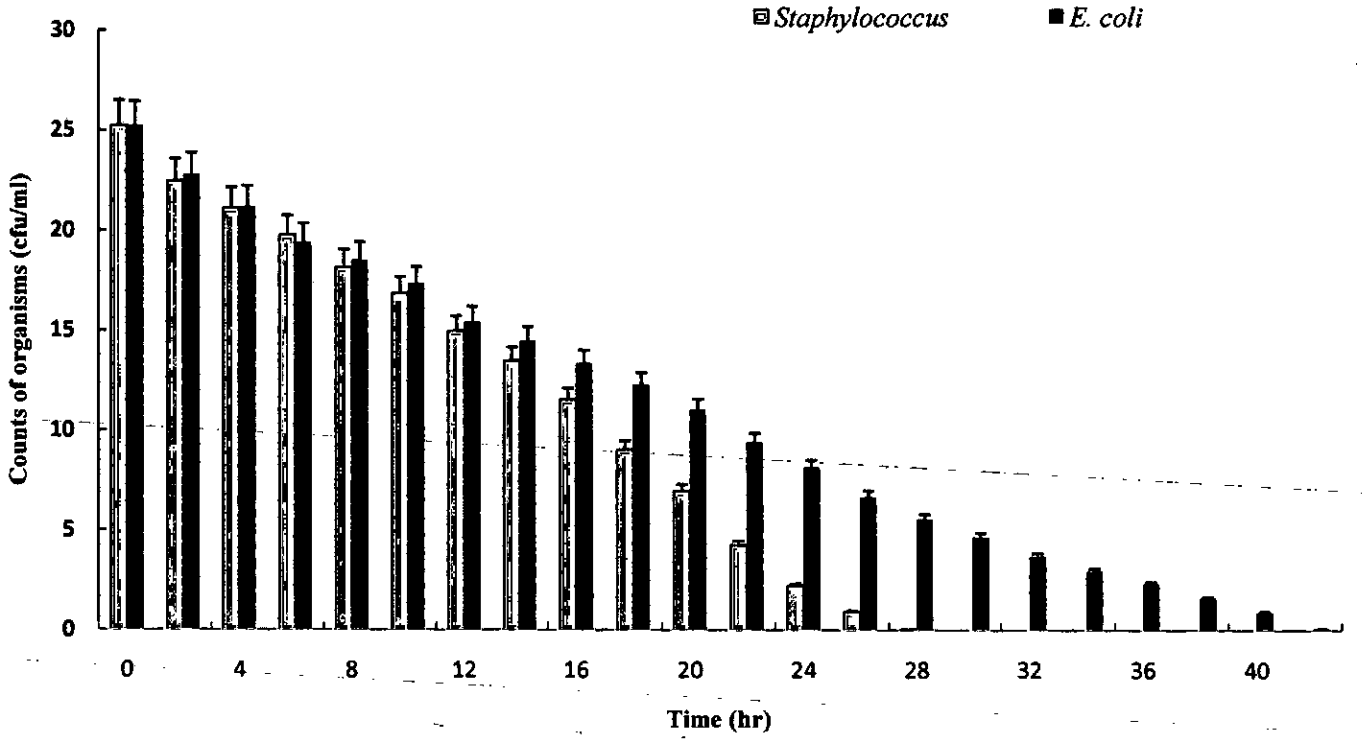

Figure 4.45: Counts of *Staphylococcus aureus* and *Escherichia coli in* spontaneously fermented gruel after storage .

CHAPTER FIVE

DISCUSSION

5.0 DISCUSSION

The antibiotic susceptibility result in this study shows all but one of the diarrhoeagenic bacteria (test organisms) used to be resistant to the commonly used antibiotics in infectious diarrhoea diseases. All the test organisms were resistant to ampicillin and tetracycline, drugs commonly available over the counter in Nigeria and very often used without any prescription. This agrees with the study carried out by Olasupo *et al.* (1999) who identified misuse or abuse of antibiotics as a common phenomenon in developing countries. Oluduro *et al,* (2003) reported a high incidence of multiple antibiotic resistance among members of Enterobacteriaceae.

The microflora of spontaneous cereal fermentation underlies a temporal succession. The surface of cereals and mature intact cereal grains is at the beginning entirely dominated by high numbers of Gram-negative, aerobic, bacteria like Enterobacteriaceae (Muller, 2000). The low count of lactobacilli in the spontaneously fermented gruels was probably due to presence of contaminants, which competed with *Lactobacillus* for the available nutrients. Amusa *et al.* (2005) reported the presence of *B. subtilis, B. cereus, E. coli, P. aeruginosa,* and *S. pyogenes* along with LAB in freshly milled ogi. He reported the presence of these microbes as an indication of possible contamination resulting from the use of well water. The sources of water mostly used in local food processing industries are surface water (wells) which are not usually totally free of microbial contamination. The presence of *E. coli* in water indicates faecal contamination and the presence of other enterobacteriaceae known to be causative agents of foodborne gastroenteritis and bacterial diarrhoea diseases and could become hazardous when ogi is consumed either raw or not properly cooked before consumption. The presence of *Aspergillus* and *Penicillium* in the spontaneously fermented ogi suggests a field to storage and processing route. However, the presence of these fungi is of great public health significance, as some strains of these fungi produce aflatoxins, which have been implicated in cancer in mammals including man. Hence, the use of clean non-infected seeds and starter culture for fermentation be advocated and encouraged.

The ability to degrade available protein in a medium to assimilable amino acids and peptides is essential in the production of fermentation products and implies the

necessity of proteolytic system in any organism used as starter culture in fermentation process. Protein contents have been reported to be higher and more available in sorghum after fermentation than before (Kazanas and Fields, 1981). Of much interest is our observation of higher protein content in the *Lactobacillus* – fermented gruels (*L. pentosus, L. acidophilus*, mixed culture of both) compared with the spontaneously - fermented sample ($P< 0.05$). The results indicate that the fermentation of these cereal cultivars by the tested *Lactobacillus* strains either singly or in combination improves their protein content. This indication is strengthened by the results obtained for the locally fermented cereal gruels, which elicited the lowest protein contents ($P< 0.05$). This finding can be due to contamination. In a related study by Rowland *et al.* (1978) non-lactobacilli isolates were recovered from cereal gruels produced by local fermentation. These organisms, which were recognised as microbial contaminants, include yeast, *Aspergillus, Bacillus,* and coagulase-negative *Staphylococcus aureus.* Apart from competing for available nutrients, with lactobacilli, these organisms are noted for protein utilization for sporulation and modification by toxins. The toxins may also interact with proteins in the gruel via protein-protein or protein-glycoprotein interactions. The nutritional implication of this interaction involves the bioavailability of the macronutrient. In terms of bioavailability, digestibility of proteins and subsequent assimilation of essential amino acids provides formidable indices for evaluating the nutritional quality of foods (Mbata *et al.*, 2006).

In the present study, the *Lactobacillus*-fermented cereal gruels yielded detectable to significant levels of lysine and arginine coupled with higher level of isoleucine when compared with locally fermented gruels and the maize kernels irrespective of soil conditions, in which they were grown, their genetic background and cotyledon architecture (IITA, 1982). Maize and sorghum are cereals poor in lysine (Chavan and Kadam, 1989). The detection of these essential amino acids in the fermented gruels is an indication of nutritional improvement and empowerment by fermentation. Kazanas and Fields (1981), reported that natural lactic fermentation of ground grain sorghum produced significant increases ($p<0.001$) in available lysine/leucine, isoleucine and methionine. Adebawo *et al.* (2000), had previously reported higher lysine content in 'ogi' produced by genetically engineered *L. plantarum.* Mbata *et al.* (2006), reported fermented cereal gruels with higher lysine, arginine and methionine contents under laboratory adapted conditions. Shekib (1994), reported that lysine content increased

significantly in fermented rice even higher than that of FAO/WHO pattern. Lysine and arginine are essential growth factors in foods for growing children and their non-availability has been associated with low-growth for age and stunting in malnourished children. Physiologically, lysine contributes to the active sites of enzymes and helicity of proteins, while arginine serves as a substrate for nitric oxide and polyamine biosyntheses (Mbata *et al.,* 2006). The latter is released in the early phase of inflammatory episode to curtail pathogenesis of infections (Iwalokun *et al.,* 2005). It also plays a major role in smooth muscle relaxation and blood vessel elasticity. Furthermore, the production of the essential amino acids: lysine and tryptophan observed in these fermented cereal gruels can be said to be species-dependent, since higher yields were observed in *L. acidophilus*-fermented gruels compared to other gruels including the mixed culture-fermented 'ogi'. Evidence that *L. acidophilus* improves the amino acid contents of fermented products has been given. Meanwhile in a mixed culture, nutrient competition among the fermenting lactobacilli cannot be ruled out. Tolerability and synergy of actions are also essential factors due to their bacteriocinegenic potentials and cross-metabolic pathways. The degradation and utilization of protein by the starter will usually require the concerted action of proteinases, peptidases, and amino acid and peptide uptake system. This system is believed to be brought into use by these two starter strains and even further enhanced when the two are used in combination. Quality protein maize (QPM) varieties are known to improve protein quality in maize based diets as they have almost double the percentages of lysine and tryptophan compared to normal maize (Edema *et al.,* 2005). The results showed that fermentation with mixed culture of *Lactobacillus* greatly improved the amino acid contents of the fermented cereal cultivars.

The reducing sugar contents were found to increase during the three days of fermentation. This result agrees with that of Sripiya *et al.* (1997) who reported a 13-fold increase in reducing sugar contents when *Eleusine coracara* (finger millet) was subjected to fermentation. Mugula (1991), also reported an increase in reducing sugar during production of tempeh. The increase in reducing sugar content was reported to be due to the activities of amylases (Shekib, 1994).

In the present study, organic acid producing capacity and antimicrobial properties of *L. pentosus* and *L. acidophilus* used as starter cultures during fermentation of cereals for "ogi" production were investigated. It was discovered that the titratable acidity of

the cereals fermented with mixed culture has more than 50% increase over the control and each strain used individually. Production of organic acid by *Lactobacillus* is considered primary regulator of microbial activity in the environment of the product. The very low pH of the environment prevents thriving of acid sensitive pathogens, which cannot therefore exert their effect. *Lactobacillus acidophilus* and *L. pentosus* used in this present study seem to have a very active metabolic pathway that can synthesize diacetyl and acetoin. This is connected with high production of lactic acid and possibly pyruvate which is a key metabolite involved in the synthesis of diacetyl. Diacetyl production could be a way by which these strains eliminate pyruvate, which is highly toxic to cells when they accumulate as the cells enter the stationary phase. Production of diacetyl and consequently acetoin could also be because of the metabolism of citrate through an active citrate permease/citrate lyase, and requires an active diacetyl synthetase, which will convert acetate to diacetyl. It is presumed that these enzyme systems are active in *L. acidophilus* and *L. pentosus*. Organoleptic properties were generally superior compared to when neither of the starters were used. Results suggest that usage of the two starter strains produced new and improved products that are microbiologically safe. The fermentation of a carbohydrate-based cereal by single and mixed cultures of selected *Lactobacillus* spp produced food of improved nutritional quality and organoleptic properties.

Acid and bile tolerance were examined to predict the survival of these *Lactobacillus* starters in the intestinal tracts of live animals. Both starter organisms showed excellent to very good acid tolerance over a period of 24 hours. These starter strains were also tolerant to the various bile conditions. Since bacteria to be used as probiotics are generally delivered to animals and humans through food they should have the ability to resist the stressful conditions in the gastrointestinal tract. These conditions include acid and bile secretions, Based on the results of the acid and bile tolerance tests, these strains show promise with regard to their use as probiotics in live animals because of their ability to survive intestinal conditions.

The overall acceptability of each of the cereal gruels studied by the panellists was observed to be above 50%. Despite its relatively lower organoleptic values, the overall acceptability of the spontaneously fermented cereal gruels was observed to score above 50%. Poverty has been found to further constrain access to food in most developing countries including Nigeria. It has also been found to modulate the perception of consumers on taste, smell, aroma and acceptability (Mbata, *et al.*, 2006).

However, the present study has revealed the appreciable impact which starter culture mediated fermentation had on the organoleptic properties of cereal gruels. Spontaneously fermented cereal gruels not only lack probiotic composition, but also have a questionable safety profile. A study by Black *et al.* (1982) revealed gross contamination of foods fermented spontaneously by food spoilage organisms such as *Bacillus subtilis*, *B. cereus,* and *Salmonella* spp. These organisms have also been reported to cause depletion in organoleptic qualities of fermented foods. Our observations seem to support the susceptibility of spontaneously fermented gruels to food spoilage organisms and pathogens since isolates such as *Staphylococcus aureus,* were isolated in the spontaneously fermented cereal gruel compared to *Lactobacillus* fermented products. Products such as togwa, tefir etc have been reported to improve organoleptic scores and overall acceptability in their respective areas of consumption. Mostly, awareness on the probiotic relevance and nutritional enhancement of defined fermented cereal gruel should be optimized; their consumption should be encouraged and practised.

All the cereal gruels used in this study demonstrated antimicrobial activity against all the diarrhoeagenic bacteria. The gruels produced using the combined *Lactobacillus* starters were the most potent, followed by those produced using the starters individually, while the gruels left to chance inoculation(control) had the least activity against these diarrhoeagenic agents. This finding supports the result from in vitro studies that reported a strongly inhibited growth of diarrhoeagenic agents with the use of lactic acid-fermented cereal-based foods. Mensah *et al.* (1990) reported that fermentation of cereal-based weaning foods reduced gram-negative bacterial contamination from 10^6 to 10^4/g of gruel compared with non-fermented foods. Mensah *et al.* 1991 also reported the inhibition of *Shigella flexneri* and *Escherichia coli* (ETEC) by fermented Ghanaian maize dough, that on examination of these two organisms 8 hours after inoculation half of these tested strains were inhibited. It was reported while studying the viability of enteropathogens in cereal gruels, that *E. coli* (ETEC), *Campylobacter jejuni, S. flexneri,* and *S. typhirimum* were strongly inhibited in sour gruels, but *Staphylococcus* only showed continued reduction in growth. These workers also reported the enteropathogens to remain at the inoculation level or even increased in number in the unfermented control gruels (Kingamkono *et al.,* 1994). Svanberg and Lorri (1994), selected two groups of about 100 Tanzanian children

under five years of age based on the use or non use of fermented gruels. He found out that the mean number of diarrhoea episodes over the study period was 2.1 for children eating fermented gruels compared to 3.5 for those eating non-fermented gruels ($p<$ 001). The health effects exerted by LAB include production of lactic acid and minor amounts of acetic and formic acids. This causes a drop in pH and thereby growth inhibition of food spoilage organisms, killing of certain pathogens, production of antimicrobial compounds and probiotic effects which are all common features of LAB (Svanberg *et al.,* 1992). The role of lactic acid bacteria as probiotics was assessed in the possible control of shigellosis. Mice (neonates) fed with large populations of *Lactobacillus* cells present in their feed were challenged with cells of *Shigella dysenteriae*. The gastrointestinal tract forms a very variable ecosystem with a wide range of different conditions, which naturally affects both the microflora and physiological responses of the host in each particular intestinal location. Thus, the natural microflora of the host forms the background for the action of each probiotics strain, which may survive, colonize, and act in the typical environment of human host. It is necessary therefore that the *Lactobacillus acidophilus* and *L. pentosus* used in this experiment to survive in the physiological environment of the gut to obtain nutritional and therapeutic benefits.

The effect of viable cells of *Lactobacillus* on *Shigella dysenteriae* in the gut of mice was studied. *Shigella dysenteriae* was used because Lampel *et al.* (2001) reported that it could produce the most severe *Shigella* infection. Iwalokun *et al.* (2001) also reported that it manifests as the second highest incidence of Shigellosis in Nigeria.

Weaning mice were used for this experiment because of their low immunity status at such an age and the ecological condition of their gut being similar to that found in human. The double challenge with *Shigella* in an interval of 24 hours was to initiate infection in mice because of the high innate resistance to *Shigella* infection.

However, a significant difference was obtained between the animals previously fed with fermented gruels and the control mice in the resistance and survival rate. A protection of 100% was obtained against *Shigella* infection with the treatment containing mixed *Lactobacillus* culture, 90% with *Lactobacillus acidophilus*, and 80% with the *L. pentosus*. This may be because most successful probiotic strains have been shown to produce antimicrobial substances effective against pathogens or opportunistic pathogens (Kneifel *et al.*, 1999). Clements *et al.* (1981), reported that lactobacilli could inhibit *Escherichia coli*-induced enterotoxin reactions perhaps by

preventing *E. coli* cells from colonizing the jejunum and by producing substances directed against the enterotoxins. It is possible that a similar reaction occurred with the *Lactobacillus*-starter cultures used in this study by inhibiting the shiga toxin produced by *Shigella dysenteriae.* The 33.3% success with the spontaneous-fermented feed was probably because the process allows for succession of various organisms each with its peculiar role. The efficacy expected will be lower than that from the starter cultures. Kingamkono *et al.* (1999) evaluated influence of consumption of togwa (a lactic-acid fermented cereal gruel with pH </= 4 on the presence of faecal enteric bacteria such as *Campylobacter,* enterohaemorrhargic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), *Salmonella,* and *Shigella* in rectal swabs of under 5years healthy children. He concluded that regular consumption of togwa once a day three times a week helped to control intestinal colonization with potential diarrhoea – causing pathogens in young children.

These findings therefore suggest that the use of starter cultures in the preparation of weaning food can control and prevent incidences of Shigellosis in our environment.

6.0 Summary of Findings

This study has shown that the starter culture organisms used in the fermentation of these cereal gruels:

- ❖ Possess the ability to degrade available protein in the medium to assimilable amino acids.
- ❖ Hydrolysed starch in the cereals resulting in the production of reducing sugar thus improving energy value of the product.
- ❖ Enhanced production of organic acids causing a drop in pH. This is considered as a primary regulator of microbial activity in the environment of the product thus inhibiting growth of food spoilage organisms as well as killing of certain pathogens.
- ❖ Produced diacetyl and acetoin thus improving the sensory properties of the products and making them microbiologically safe and acceptable for human consumption.
- ❖ Exhibited probiotic qualities useful in the possible control of shigellosis.
- ❖ Will survive in the intestinal tracts of live animals (when used as probiotic) because of their very good bile and acid tolerance.

It is therefore concluded that use of these strains of *Lactobacillus* species as starter culture in the fermentation of cereal gruel produced a product of increased nutritive and energy values, acceptability, probiotic disposition and longer shelf life.

7.O Contributions to Knowledge

This study has recorded:

- that the nutritional and probiotic potentials of fermented cereal gruels can be improved by controlled fermentation using starter cultures.
- that an improvement in the diacetyl and acetoin contents of fermented cereal gruels could form the basis for improved shelf life, organoleptic properties, and *Lactobacillus* yield.
- that duration, intensity, and severity of diarrhoeal infection as well as recovery can be improved via the mouse diarrhoeal model, via the consumption of cereal gruels specifically fermented by *Lactobacillus.*
- that the readily affordable traditionally weaning infant food can be biologically improved into an acceptable protein rich food that is safe for human consumption.
- that this traditional weaning food will reduce significantly protein deficiency and incidence of diarrhoea in our environment.
- the use of these strains of *Lactobacillus* spp. as starter cultures in the fermentation of cereal gruel produced a product of increased nutritive and energy values, and probiotic disposition.

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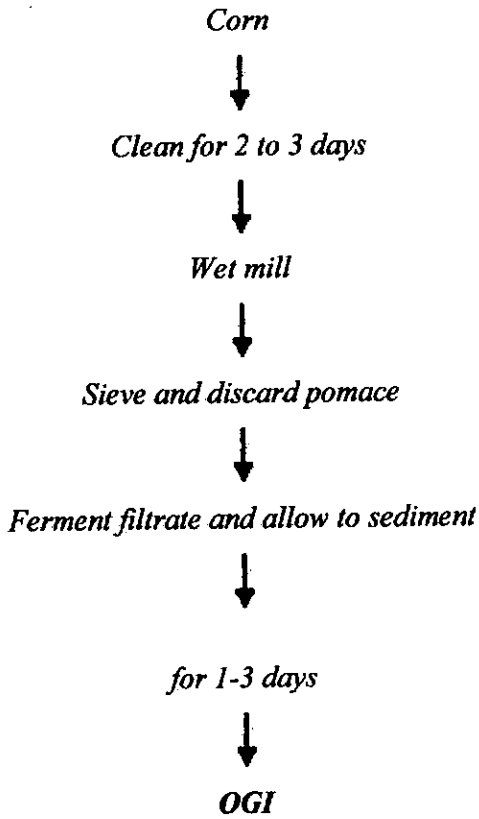
APPENDIX 1



Flow diagram for the preparation of ogi

APPENDIX II

QUESTIONNAIRE
ON
ORGANOLEPTIC ASSESSMENT OF CEREAL GRUELS PREPARED BY CONTROLLED AND UNCONTROLLED FERMENTATION

As a consumer of cereal gruels, I hereby seek your objective assessment of the presented cereal gruels based on the organoleptic attributes indicated below. You are to score hedonically on a scale of 1 to 9 as follows.

9 – like extremely, 7- like, 5- neither like nor dislike, 3- dislike, 1 – dislike extremely

A. PERSONAL DEMOGRAPHY

1. AGE _____ **11. SEX**_____ (M/F)

111. EDUCATION (Tick as appropriate)

i. Primary
ii. Secondary
iii. Tertiary
iv. Illiterate
v. Others (Specify)

B. CONSUMER BEHAVIOUR

I. For how long have you been consuming cereal gruels?
   a. < 1 year b. 1 – 3 years c. 4 – 5years d. > 5 years
II. What is your preference of cereal gruels?
   a. Maize-based b. Millet-based c. Sorghum-based d. All of the above e. Any of the above.
III. How many times do you consume cereal gruel per day?
   a. Once per day b. twice c. Thrice d. As many times as possible

Cereal	TZPB-SR	TZB-SR-SE	EV8363-SRQPM	TZSR-W-1	TZB-SR	8321-21	SUWAN-1-SRQPM	EV8766-SR-Y-QPM	TZSR-Y-1	8321-18	EV8762-SR	RED SORGHUM	YELLOW SORGHUM	WHITE SORGHUM
IV. TASTE														
V. APPEARANCE/ COLOUR														
VI. TEXTURE														
VII. AROMA														
VIII. ACCEPTABILITY														
X. OVERALL ACCEPTABILITY														

Sensory Evaluation of Fermented Gruels

Taste												
No. of Panellists	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**S.D.**
Ogi Type												
Spontaneously-fermented	6	4	6	6	6	4	4	6	4	6	5.2	1.03
L. acidophilus-fermented	10	8	8	8	8	10	8	8	8	8	8.4	0.84
L. pentosus- fermented	8	8	8	8	8	8	8	8	8	8	8	0
Mixed culture of *L. pentosus*& *L.acidophilus*	10	12	10	10	10	10	10	10	12	10	10.4	0.84
Aroma												
No. of Panellists	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**S.D.**
Ogi Type												
Spontaneously-fermented	6	4	6	6	6	6	6	6	6	4	5.6	0.84
L. acidophilus-fermented	10	8	10	8	8	10	8	8	10	8	8.8	1.03
L. pentosus- fermented	10	8	10	6	8	8	8	10	10	6	8.4	1.58
Mixed culture of *L. pentosus*& *L.acidophilus*	12	12	10	10	10	10	12	10	12	10	10.8	1.03
Colour												
No. of Panellists	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**S.D.**
Ogi Type												
Spontaneously-fermented	4	6	6	6	6	6	4	6	6	6	5.6	0.84
L. acidophilus-fermented	8	8	8	8	8	6	8	10	6	10	8	1.33
L. pentosus- fermented	10	8	10	6	8	6	10	10	8	8	8.4	1.58
Mixed culture of *L. pentosus*& *L.acidophilus*	10	12	10	8	10	12	8	10	10	10	10	1.33

Texture

Ogi Type No. of Panelists	1	2	3	4	5	6	7	8	9	10	Mean	S.D.
Spontaneously-fermented	6	8	8	6	6	6	6	6	7	6	6.5	0.93
L. acidophilus-fermented	10	8	8	8	8	8	10	8	9	8	8.5	0.93
L. pentosus- fermented	10	10	8	8	9	7	10	6	8	9	8.5	1.77
Mixed culture of *L. pentosus*& *L.acidophilus*	12	10	10	11	10	10	10	10	11	11	10.5	0.93

Acceptability

Ogi Type No. of Panelists	1	2	3	4	5	6	7	8	9	10	Mean	S.D.
Spontaneously-fermented	8	6	6	6	6	6	8	6	7	6	6.5	0.93
L. acidophilus-fermented	12	12	10	8	10	8	12	12	11	10	10.5	1.77
L. pentosus- fermented	12	10	12	10	10	10	10	10	10	11	10.5	0.93
Mixed culture of *L. pentosus*& *L.acidophilus*	10	11	12	10	12	11	10	12	10	12	11	1.07

	Taste	Aroma	Colour	Textu	Acceptability
Spontaneously-fermented	5.2	5.6	5.6	6.5	6.5
L. acidophilus-fermented	8.4	8.8	8	8.5	10.5
L. pentosus- fermented	8	8.4	8.4	8.5	10.5
Mixed culture of *L. pentosus*& *L.acidophilus*	10.4	10.8	10	10.5	11

Survival Rate of Mice after *Shigella dysenteriae* Infection

Ogi Type	Mice Used	Number of Deaths per day																				% Death	%Survival	Survival
		Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Group1																									
WO1	10	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	70	
WQ1	10	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	90	80
RS1	10	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	80	
Group 11																									
WO2	10	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	80	
WQ2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	90
RS2	10	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	90	
Group 111																									
WO3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	
WQ3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
RS3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	
Group1V																									
WO4	10	0	2	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80	20	
WQ4	10	0	1	2	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	40	33.33
RS4	10	0	1	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	40	
Group V																									
(Control)	10		4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
	10		4	3	3	0	0	0	0	0															

WO
WQ
RS
Group1 - *Lactobacillus pentosus-fermented*
Group 11 - *Lactobacillus acidophilus-fermented*
Group 111 - Mixed culture of 1&11
Group 1V - Spontaneously-fermented
Group V - Milk

APPENDIX III

Mueller Hinton Agar (M-H Agar)

Composition:	Gram/Litre
Beef infusion solids	4.0
Starch	1.5
Casein hydrolysate	17.5
Agar	15

Final pH 7.4 +/- 0.2 at 37°C

Directions:

Approximately38 g was suspended in 1 litre of distilled water, brought to boil to dissolve the medium completely and sterilized

by autoclaving at 121°C for 15 minutes.

Staphylococcus Selective Agar

Composition	Gram/Litre
Pancreatic Digest of Casein.	10.0
Yeast Extract	2.5
Gelatin	30.0
D-Mannitol	10 .0
Sodium Chloride	75.0
Dipotassium phosphate	5.0.
Lactose	2.0
Agar	15

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Directions

Approximately 1491 g of the medium was suspended in 1 litre of purified water.

It was heated to boil with frequent agitation for one minute to completely dissolve the medium.

It was then autoclaved at 121°C for 15 minutes.

Nutrient Agar

Composition

	Gram/litre
Peptone from meat	3.45
Peptone from casein	3.45
Sodium chloride	5.1
Agar	13.0

Directions

Approximately 25g was suspended in 1 litre of distilled water, and autoclaved for15 min at 121°C.

pH: 7.5 ± 0.2 at 25°C.

Mac Conkey Agar .

Composition	Gram/Litre
Peptone	20.0
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	12.0

Final pH 7.4 +/- 0.2 at 37°C

Directions:

Approximately 45 g was suspended in 1 litre of distilled water and heated until completely dissolved, and sterilized by autoclaving at 121°C for 15 minutes.

De Mann Rogosa Sharpe (MRS) Agar

Composition

Ingredients	Gram/Litre
Universal peptone	10.0
Meat extract	5.0
Yeast extract	5.0
D(+) Glucose	20.0
Dipotassium hydrogen phosphate	2.0
Diammonium hydrogen citrate	2.0
Sodium acetate	5.0
Magnesium sulphate	0.1
Manganous sulphate	0.05
Agar	12.0

Directions:

Approximately 61 g of powder was dissolved in one litre distilled water and 1ml Tween 80 (Fluka No. 93780) was added and autoclaved at 121°C for 15 minutes.

Phosphate buffer solution pH 7.0

To 50 ml 0.1 molar potassium dihydrogen phosphate was added 29.1 ml of 0.1 molar NaOH.

Alternatively :

Approximately 1.20g of sodium dihydrogen phosphate and 0.885g of disodium hydrogen phosphate were dissolved in 1 litre volume distilled water.

Nelson's Reagents

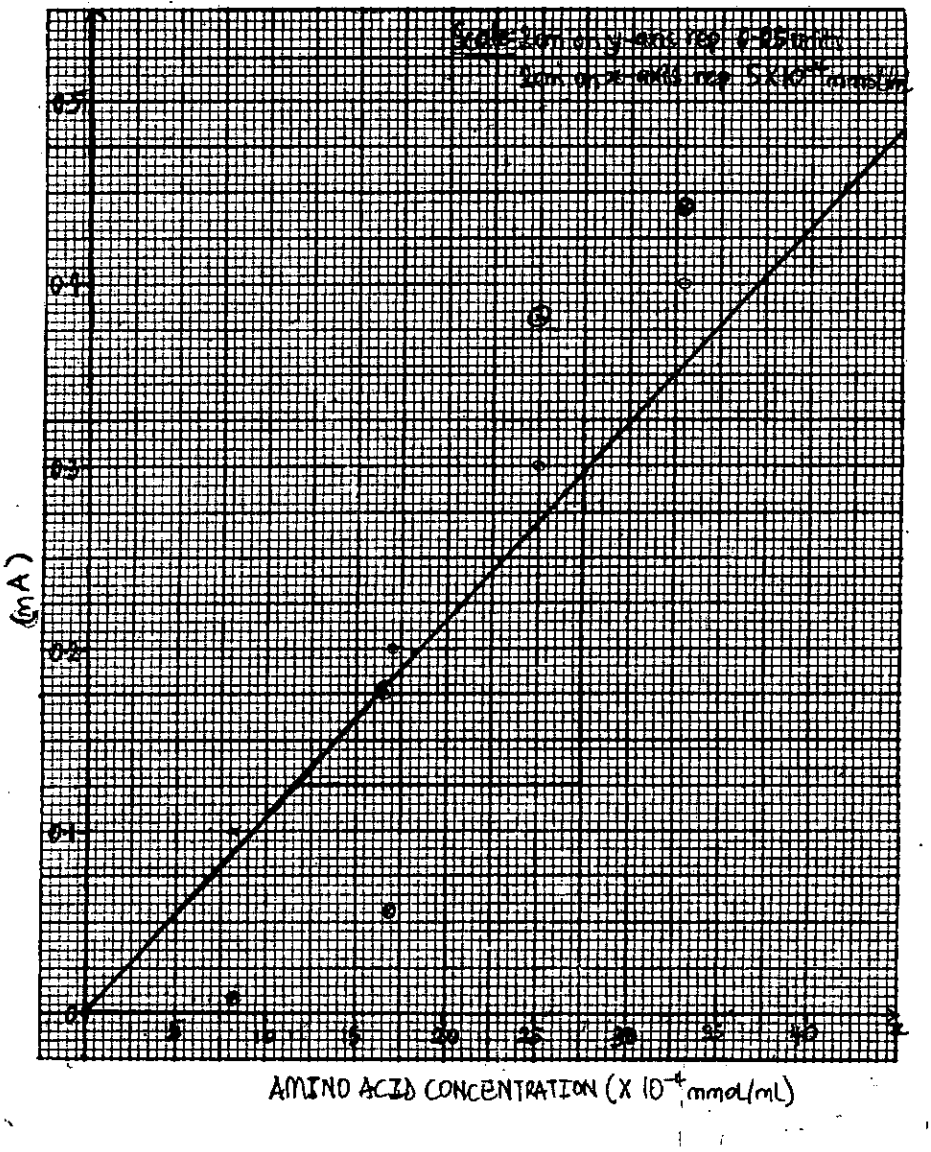
A

Approximately 2g each of sodium carbonate and sodium bicarbonate, 2.5g of anhydrous potassium sodium tartarate, 20g of anhydrous sodium sulphate were dissolved in 80 ml distilled water and made up to 100ml.

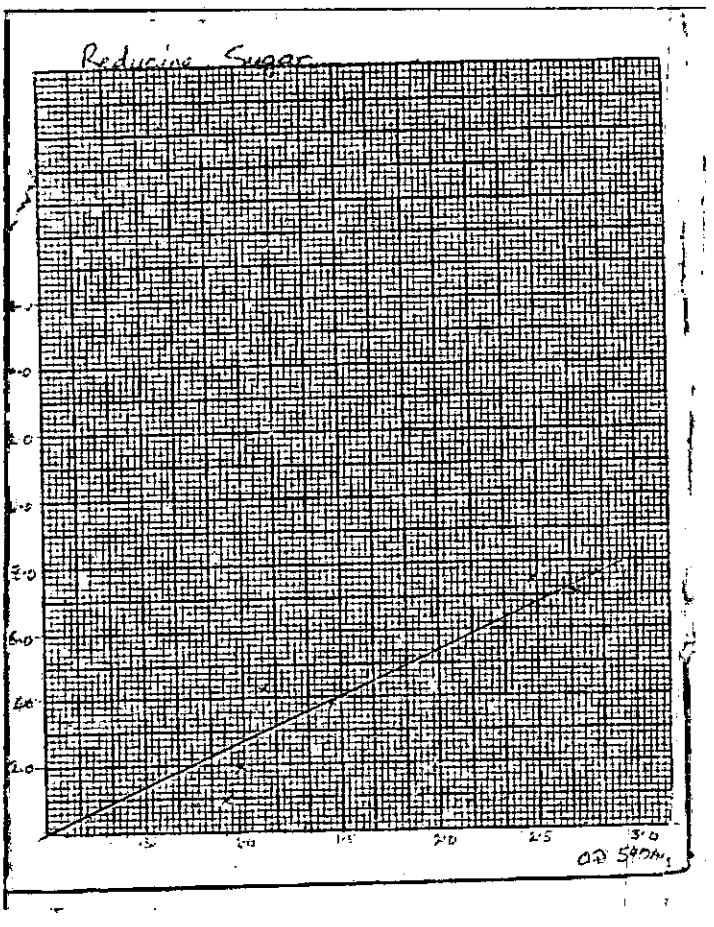
B

Approximately 15g copper sulphate was dissolved in small volume of distilled water, one drop of sulphuric acid was added and made up to 100ml.

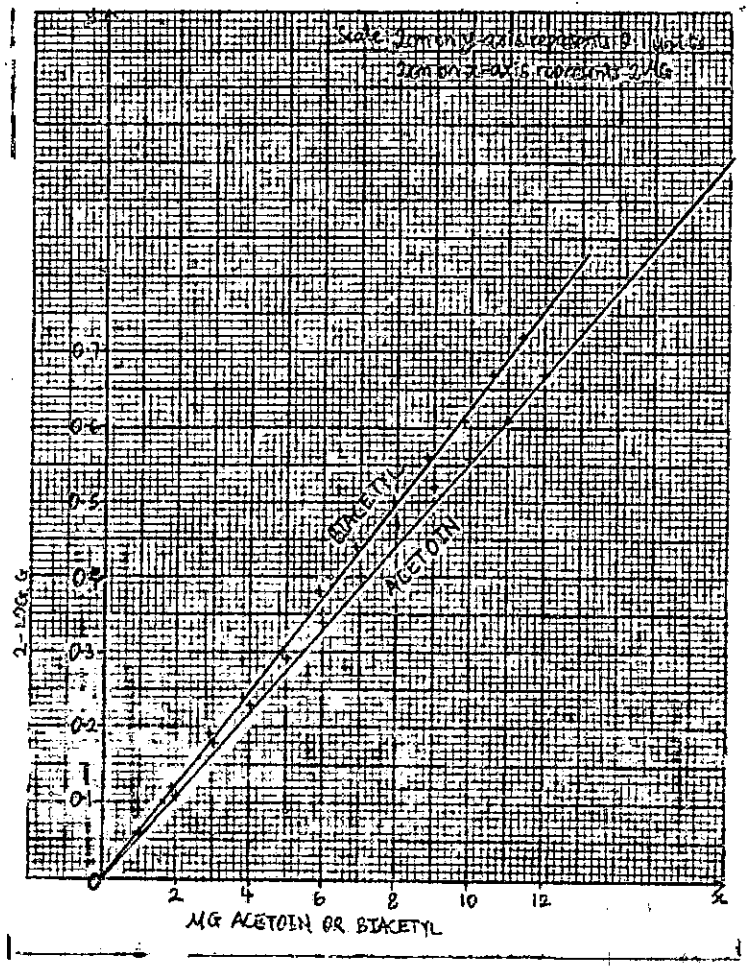
APPENDIX IV



Calibration Curve of Amino acid



Calibration Curve of Reducing Sugar



Calibration Curve of Acetoin and Diacetyl