

Fermentation of a finger millet-dairy composite gruel

by

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ABSTRACT

FERMENTATION OF A FINGER MILLET-DAIRY COMPOSITE GRUEL

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Fermentation is widely used in Africa to preserve foods where modern methods of preservation are not available. Fortification of cereals, which are the foods that are widely fermented together with high protein foods such as soyabeans and milk would improve their protein content and quality.

The fermentation of a finger millet-dairy (skim milk) composite gruel was investigated. This was done to determine what cultures can be used to ferment both the lactose and starch components of the gruels to reduce the pH and produce a safe product. Three starter cultures namely: YC380 and V2, which were developed to ferment dairy products, and JC which was developed to ferment cereal slurries were studied.

The three starter cultures seemed to prefer thermophilic conditions, i.e. an incubation temperature of 45°C. Composite gruels with a pH of 4.5 or below were produced after fermentation at 45°C for 5 and 4 h with starter cultures YC380 and V2, respectively. This is an advantage since rapid acid production at higher temperatures, over a short period of time, reduces the risk of growth of microbial contaminants.

Gruels with a thick consistency were obtained when an incubation temperature of 45°C was used and when the proportion of skim milk was high. Gruels that had low

proportions of skim milk and had been stored at 7°C had high firmness and consistency. This was probably due to retrogradation of the starch in the gruels. Starter culture JC produced an undesirable coagulum. Syneresis was low when the proportion of skim milk was low since with increasing proportions of finger millet gruel the starch probably acted as stabiliser.

The lactose content of the unfermented skim milk and skim milk fermented with starter cultures YC380 and V2 was 5.4%, 4.7% and 4.5% respectively. The lactose content of the unfermented composite gruels and those fermented with starter cultures YC380 and V2 was 2.8%, 1.9% and 2% respectively. Lactose intolerant individuals are the ones who are most likely to benefit from this decrease due to compositing and fermentation. The energy content of the gruels made with finger millet gruel only was 0.9 MJ/kg. The energy content of the unfermented composite gruels and those fermented with starter cultures YC380 and V2 was increased to 1.3 MJ/kg, 1.3 MJ/kg and 1.4 MJ/kg respectively.

The protein content of the gruels that were prepared using finger millet only was 0.4%. The protein content of the unfermented composite gruels and those fermented with starter cultures YC380 and V2 was increased to 2%, 1.9% and 1.8% respectively. The lysine content of the unfermented gruels and those that were fermented with starter cultures YC380 and V2 was 19 mg/g crude protein (CP), 34 mg/g CP and 34 mg/g CP respectively. The lysine content of the unfermented composite gruels and those fermented with starter culture V2 was 54 mg/g CP, 68 mg/g CP and 70 mg/g CP. The increase in the lysine content of the fermented gruels may have been a result of the proteolytic activity and transamination by the bacterial starter cultures.

The importance of nutrients is in terms of their contribution to nutritional requirements. The composite gruels contributed 3% and the gruels with finger millet only contributed 2% to the recommended dietary energy requirements for infants. The contribution to the recommended dietary protein requirements for infants was 3.9% for those gruels that were prepared with finger millet gruel only and 13.9% for the composite gruels. Fermented composite gruels can be produced with skim milk,

finger millet and starter cultures such as YC380 and V2 but not with starter culture JC. Fermentation reduced the pH of the gruels to 4.5 or below. Most pathogenic and spoilage micro-organisms do not grow below this pH.



UITTREKSEL

FERMENTASIE VAN 'N DUN PAP BESTAANDE UIT 'N BABALA-SUIWELSAMESTELLING

deur

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Fermentasie word algemeen in Afrika gebruik om voedsel te preserveer waar moderne metodes van preserving nie beskikbaar is nie. Fortifisering van grane, die voedsels wat algemeen gefermenteer word, met proteïenryke voedsels soos sojabone en melk, sal hulle proteïeninhoud en gehalte verbeter.

Die fermentasie van 'n dun pap bestaande uit 'n babala-suiwel (afgeroomde melk) samestelling is bestudeer ten einde geskikte kulture te vind wat gebruik kan word om die laktose sowel as die styselkomponente van die dun pappe te fermenteer en die pH te verlaag ten einde 'n veilige produk te verseker. Drie suurselkulture, naamlik YC380 en V2, wat albei ontwikkel is om suiwelprodukte te fermenteer, en JC, wat ontwikkel is om graanpappe te fermenteer, is bestudeer.

Die drie suurselkulture verkies blykbaar termofiele kondisies, naamlik 'n inkubasietemperatuur van 45°C. Saamgestelde pappe met 'n pH van 4.5 of laer is

verkry na fermentasie by 45°C vir 5 en 4 h met suurselkulture YC380 en V2, onderskeidelik. Dit is 'n voordeel aangesien vinnige suurproduksie by hoë temperature oor 'n kort tydperk die risiko van die groei van mikrobiese kontaminante verminder.

Pappe met 'n dik konsistensie is verkry wanneer 'n inkubasietemperatuur van 45°C gebruik is en die verhouding van afgeroomde melk hoog was. Pappe wat lae proporsies afgeroomde melk bevat het en wat by 7°C opgeberg is, was baie dik en stewig. Dit was moontlik die gevolg van die retrogradering van die stysel in die pappe. Suurselkultuur JC het 'n onaanvaarbare koagulum geproduseer. Sinerese was laag wanneer die proporsie afgeroomde melk laag was want met toenemende proporsies babalap het die stysel waarskynlik as stabiliseerder opgetree.

Die laktose-inhoud van die ongefermenteerde afgeroomde melk en die afgeroomde melk wat met suurselkulture YC380 en V2 gefermenteer is, was onderskeidelik 5.4%, 4.7% en 4.5%. Die laktose-inhoud van die ongefermenteerde saamgestelde pappe en dié wat gefermenteer is met suurselkulture YC380 en V2, was onderskeidelik 2.8%, 1.9% en 2%. Laktose-onverdraagsame individue sal waarskynlik meeste voordeel trek uit die verlaagde laktose-inhoud as gevolg van samestelling en fermentasie. Die energie-inhoud van die pappe wat met babala alleen berei is, was 0.9 MJ/kg. Die energie-inhoud van die ongefermenteerde saamgestelde pappe en dié wat gefermenteer is met kulture YC380 en V2 is verhoog na 1.3 MJ/kg, 1.3 MJ/kg en 1.4 MJ/kg onderskeidelik.

Die proteïëninhoud van die pappe wat van babala alleen berei is, was 0.4%. Die proteïëninhoud van die ongefermenteerde saamgestelde pappe en dié wat gefermenteer is met kulture YC380 en V2, is verhoog na 2%, 1.9% en 1.8% onderskeidelik. Die lisieninhoud van die ongefermenteerde pappe en dié wat gefermenteer is met kulture YC380 en V2, was onderskeidelik 19 mg/g ruproteïen (RP), 34 mg/g RP en 34 mg/g RP. Die lisieninhoud van die ongefermenteerde saamgestelde pappe en dié wat gefermenteer is met suurselkultuur V2, was 54 mg/g RP, 68 mg/g RP en 70 mg/g RP.

Die toename in die lisieninhoud van die gefermenteerde pappe kan moontlik die gevolg wees van die proteolitiese aktiwiteit en transaminasie deur die bakteriese suurselkulture.

Die belangrikheid van nutriënte lê in hulle bydrae tot voedingsvereistes. Die saamgestelde pappe het 3% bygedra en die pappe met babala alleen het slegs 2% bygedra tot die aanbevole dieetenergiebehoefte van kleuters. Die bydrae tot die aanbevole dieetproteïenvereistes van kleuters was 3.9% in die geval van pappe wat met babala alleen berei is, en 13.9% in die geval van saamgestelde pappe. Gefermenteerde saamgestelde pappe kan geproduseer word met afgeroomde melk, babala en suurselkulture YC380 en V2, maar nie met suurselkultuur JC nie. Fermentasie het die pH van die pappe verlaag na 4.5 of laer. Meeste patogene en bederwende mikro-organismes kan nie groei by hierdie lae pH-waardes nie.



I declare that the thesis submitted for the PhD degree at the University of Pretoria, has not been previously submitted by me for a degree at any other university or institution of higher education.

P. N. gocha
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Dedicated to my family (Yona, Yona Menon and Takudzwa Bakili)

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CHAPTER 1

INTRODUCTION

Fermented foods and beverages constitute a major portion of peoples' diets all over the world and provide 20-40% of the total food supply (Campbell-Platt, 1994). Apart from providing variety to foods, fermented foods have the advantage of prolonged shelf life due to organic acids such as lactic acid, acetic acid and other acids produced during fermentation which lower the pH, thus inhibiting the growth of spoilage micro-organisms (Fields, Ahmed & Smith, 1981; Sanni, 1993; Byaruhanga, Bester & Watson, 1999). The lactic acid bacteria used in starter cultures may also have other beneficial effects which include viricidal effects, antitumour effects, bacteriostatic and bactericidal effects against several bacteria including enteric pathogens (Nout, 1994). *Lactobacillus* species play a significant role in most of the fermented cereals and cassava roots in Africa (Reddy & Pierson, 1994).

Preparation procedures for most products are still traditional arts and the fermentation is, by and large, uncontrolled. Starter cultures are not normally used and therefore variations in the quality and stability of the products are often observed. These technological problems in the manufacture of traditional fermented foods need to be addressed in order to reduce losses due to wasteful and inefficient fermentation pathways, poor quality and unstable shelf-life of products (Odunfa & Oyewole, 1998).

Most traditional fermented and unfermented foods are based on cereals such as millets, sorghum and maize. In a review by Taylor & Dewar (2001), these foods can be divided into two groups. The first group includes liquid to liquid-like beverages, gruels and porridges while the second group includes solid pancakes and flat breads. The former exhibit a continuum in consistency, from thin liquids such as *pito* beer of Nigeria through viscous liquids such as African (opaque) beer and gruels such as *mohoto oa mabela* of Lesotho to porridges such as *ting* of Botswana. What

characterises these products is that they have undergone some form of lactic acid fermentation and therefore they are to a greater or lesser degree sour in taste.

An example of a fermented beverage/food that is popular in many parts of Southern Africa, including Zimbabwe and South Africa, is *mahewu*, *amahewu*, also known as *maheu*, *magou* or *mageu*. As is the case with most cereal-based foods, *mahewu* has a low protein content (7-9% on dry basis) and the proteins are of a low biological value. It is also a poor source of calcium, sodium, niacin, vitamins A and C (Schweigart & Fellingham, 1963; Ashworth, 1982). Because of the low protein quality and the high moisture content of most cereal-based foods, protein-energy malnutrition is a major problem in Africa (Odunfa & Adeyele, 1985). In Tanzania, as in most countries in Eastern, Central and Southern Africa, it was estimated that about 50% of young children were chronically malnourished and 7-10% of these were severely malnourished (Lorri & Svanberg, 1995). It is important to note that the foods that are generally used for weaning are cereal-based gruels (Nout, Haustvast, van der Haar & Rombouts, 1988).

The nutritional value of fermented foods is therefore an important factor that needs to be addressed. If a fermented cereal product such as *mahewu*, which is already acceptable, could be fortified, it might become a more valuable source of nutrients particularly for pre-school children, children of school going age, pregnant women, nursing women and people recovering from illnesses. Methods of fortifying *mahewu* that have been suggested include use of milk, whey, sour milk products, skim milk powder, whey protein, soya flour, food yeast or fish flour (Hesseltine, 1983). One litre of such a fortified product could produce about 30% of the daily assimilable protein requirements for an adult male as well as 20 – 30% of the requirement for nicotinamide and thiamine (Schweigart & de Wit, 1959).

In addition to the nutritional benefits that are associated with fermented foods, they also offer good keeping quality as a result of the acidity which is produced by lactic acid bacteria (pH < 4.5) (Mensah, 1997). It has been estimated that over three million

children under the age of five years die annually as a result of diarrhoeal diseases and that 70 % of all diarrhoeal diseases are due to the consumption of food that is contaminated (Nout & Motarjemi, 1997). The sources of contamination include polluted water, flies, dirty utensils and pots, food handlers and cross-contamination during food preparation. One of the major factors leading to food contamination is time-temperature abuse during preparation which, in turn, leads to survival and/or growth of pathogens as well as the production of toxins (Adams & Nicolaides, 1997). If the basic rules for safe food production (e.g. using safe water, washing hands thoroughly and keeping food preparing premises meticulously clean) were to be observed, the contamination, growth and survival of pathogens in foods would decrease. This in turn would reduce the incidence of diarrhoeal diseases. This application of basic rules for safe food preparation is not always possible in communities where safe water supplies are inadequate, where fuel for hot-holding or thorough heating are in short supply and where time to prepare food properly prior to each meal is not available. Lactic acid fermentation needs to be considered seriously as an alternative to conventional methods of preservation such as dehydration or refrigeration.

In this study, a cereal-based traditional gruel from finger millet was combined with milk and fermented using starter cultures under controlled conditions. The traditional product from which the composite gruel was prepared is referred to as *mahewu* or *maheu* among the *shona* and *manyika* speaking people and *amahewu* by the *ndebele* speaking people in Zimbabwe. Traditionally, *mahewu* is prepared by boiling a maize, sorghum or finger millet porridge of about 8-10% solids into which, on cooling, a handful of malt made from finger millet or sorghum is mixed. The malt serves as a source of inoculum of lactic acid bacteria. The α -amylase enzyme in the malt probably also serves to thin the gruel by facilitating the breakdown of starch to dextrins since the gruel tends to retrograde and hence solidify upon cooling. Another source of inoculum could be the walls of the clay pots that are used as the fermentation vessels. Although this beverage is most popular during the hot summer

months as a refreshing drink and thirst-quencher, it is boiled and served hot in winter by the *korekore* people in the Mashonaland province in central Zimbabwe.

1.1 Aim of the project

To investigate the processes involved in the preparation of a cereal-dairy composite gruel based on finger millet and skim milk and to study its microbiological, nutrient and physico-chemical characteristics.

1.2 Objectives of the project

1. To determine the effect of microbiological cultures and fermentation conditions (temperature of incubation and period of incubation) on the acidity, pH and lactic acid bacterial count in the gruels.
2. To assess the effects of varying the proportions of finger millet to skim milk on the pH, consistency, whey syneresis and the firmness of the gruels.
3. To assess the effects of varying the proportions of finger millet to skim milk on the energy, starch, protein and lysine content of the gruels.

1.3 Justification

1. The problems of quality, especially the poor nutritional quality and the low biological value of the cereal proteins can be addressed through fortification with a high protein product such as milk. The nutritional value and physico-chemical properties of such supplementary foods need to be investigated.
2. Problems in the microbiological aspects of fermentation need to be investigated to prevent wasteful fermentation pathways through proper selection of starter cultures and the creation of optimal conditions for lactic acid fermentation.

CHAPTER 2

LITERATURE REVIEW

In this review, the metabolic process known as fermentation will be defined. The biochemical processes that occur during, as well as the end-products of fermentation that are important in food preservation will be looked at. The organisms involved in fermentation and how they alter food constituents will also be discussed. Examples of some popular traditional African fermented foods, their nutritional properties as well as how food products such as cereals and legumes or dairy products can be combined to improve the nutrient profiles of these foods will be looked at. The effects of fermentation on anti-nutritional factors and the health benefits of fermented foods will be reviewed. The manufacture of yoghurt, *mahewu* and *kishk* will be reviewed. An attempt will be made to show the work that has been done to change the production of some fermented foods from traditional processes to large scale manufacturing where the microbial and nutritional quality of the product and the biochemical processes occurring during fermentation can be controlled.

2.1 The role of the lactic acid fermentation in food preservation

2.1.1 Historical background

The origins of fermented foods in the diets of humans date back many thousands of years and usually predate the existence of written records of their production and consumption (Campbell-Platt, 1987).

Ancient Egyptians kept cows for their milk which was consumed as such or processed into other products. There are many drawings that illustrate the milking of cows and milk processing. It is believed that Egyptian fermented milks were some of the oldest known dairy products in the world (Abou-Donia, 1984).

In Europe, Asia and Africa, from time immemorial, sour milk was known as being more stable and advantageous than fresh milk. Fermentation preserves the high quality nutrients present in a form that has a longer shelf-life (Oberman & Libudzisz, 1998).

2.1.2 Fermentation

Metabolism can be defined as the sum total of all chemical transformations that occur in cells (Stanier, Adelberg & Ingraham 1980). Fermentation is a metabolic process in which carbohydrates and related compounds are oxidised with the release of energy in the absence of any external electron acceptors (Jay, 1978). According to Volk & Wheeler (1984), in fermentation electrons are not passed through an electron transport chain and oxidative phosphorylation does not occur. For example many micro-organisms convert sugars to pyruvic acid and NADH is formed. NADH must pass its acquired electrons on to some acceptor if the organism is to continue to metabolise. This is accomplished by using pyruvic acid or some other product formed from pyruvic acid as a final electron acceptor.

Lactic acid bacteria are perhaps the most widespread of desirable micro-organisms in food fermentations. They are found in fermented cereal products, milks, cheeses and fermented meats (Campbell-Platt, 1987). Lactic acid bacteria convert the available carbohydrate to organic acids and lower the pH of the food. These acids as well as other flavour compounds which include diacetyl, acetaldehyde and acetoin contribute to the desired taste and flavour of the food (Pederson, 1971). The low pH that is created also makes the food unfavourable for the proliferation of spoilage and pathogenic bacteria (Djien, 1982).

2.1.3 Biochemistry of the lactic acid fermentation

Lactic acid fermentations can be divided into two broad categories distinguishable by the products formed from glucose (Stanier *et al.*, 1980). These are referred to as homofermentation and heterofermentation.

Homofermenters convert glucose to glucose -1,6- diphosphate using the Embden Meyerhof (EM) pathway (Dirar & Collins, 1972). The enzyme aldolase cleaves fructose-1,6-diphosphate between C₃ and C₄ to give the phosphate esters dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate. The reaction favours the production of the glyceraldehyde isomer at equilibrium. The end product in this fermentation pathway is lactic acid (de Vries & Stouthamer, 1968; Doelle, 1975) (Figure 1a).

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



The homolactic acid fermentation pathway is important in the dairy industry. It is the pathway responsible for souring milk and is used in the production of yoghurt, cottage cheese and cream cheeses (Atlas, 1995).

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

The ethanol and the CO₂ come from the glycolytic portion of the pathway. There are two possible ways by which ethanol is formed (Caldwell, 1995). Acetaldehyde formed by the cleavage of pyruvate by pyruvate decarboxylase is reduced in the presence of alcohol dehydrogenase to form ethanol. Ethanol can also be formed by a combination of acetyl coA reduction to acetaldehyde followed by reduction of acetaldehyde by ethanol dehydrogenase.

Lactate is formed by direct pyruvate reduction with lactate dehydrogenase. Formic acid and acetyl coA are produced by the action of pyruvate-ferredoxin on pyruvate. Acetyl coA is converted to free acetic acid. Vinegars are characterised by a significant acetic acid content. While vinegar is not a food of major importance, in

most traditional African diets, it plays an important role as a highly effective food preservative. Formate is converted to CO₂ and hydrogen by the joint operation of formic dehydrogenase and hydrogenase (Volk & Wheeler, 1984).

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



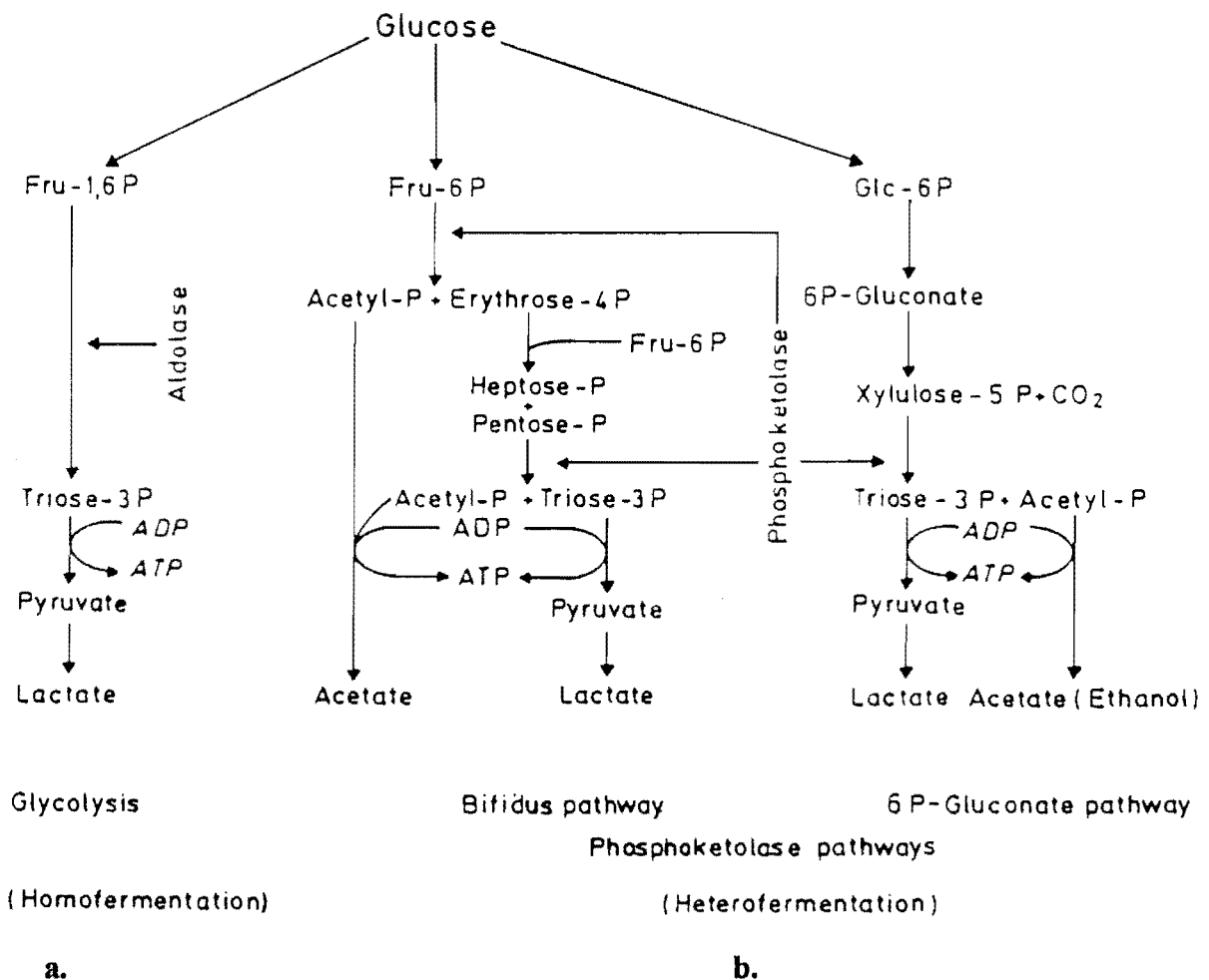


Figure 1. The pathway for glucose dissimilation by homofermentative and heterofermentative bacteria (Kandler, 1983)

Mixed acid fermentation is a third type of fermentation that is carried out by members of the family *Enterobacteriaceae* that includes *Escherichia coli* and members of the genera *Salmonella* and *Shigella* (Prescott, Harley & Klein, 1993). These bacteria ferment glucose by the EM pathway to form pyruvate which is converted to succinate, ethanol, lactate, CO₂ and H₂ (Figure 2). According to Atlas (1995), succinate is formed by the carboxylation of phosphoenol pyruvate by phosphoenol pyruvate carboxylase to produce oxaloacetate. The oxaloacetate is converted to

succinate by the combined actions of malate dehydrogenase, fumarase and fumarate reductase. The remaining products of mixed acid fermentation arise from pyruvate metabolism. Lactate is formed by the direct reduction of pyruvate with lactate dehydrogenase. Formate and acetyl coA are produced by the action of pyruvate ferredoxin oxidoreductase. Acetyl coA is converted to free acetic acid with the intermediate formation of acetyl phosphate by the actions of phosphotransacetylase and acetyl kinase. This allows the coA bond energy of acetyl coA to generate ATP. A portion of acetyl coA is converted to ethanol by the actions of aldehyde and alcohol dehydrogenases. Formate is converted to CO₂ and H₂ by the joint operation of formic dehydrogenase and hydrogenase

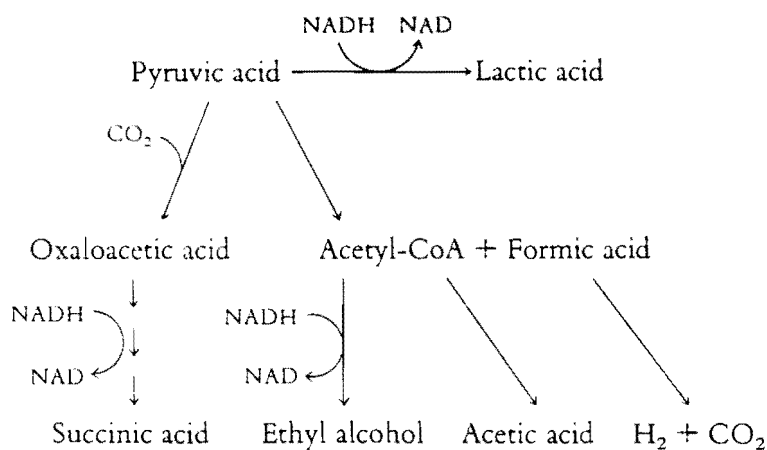


Figure 2. Mixed acid fermentation by some enteric bacteria (Prescott, Harley & Klein, 1993)

2.1.4 Micro-organisms involved in the lactic acid fermentation

The lactic acid bacteria are rod-shaped or spherical micro-organisms. Their name derives from the fact that ATP is synthesised through fermentations of carbohydrates, which yield lactic acid as a major and sometimes the sole end-product (Stanier *et al.*, 1980).

Lactic acid bacteria are unable to synthesise ATP by respiratory means, a reflection of their inability to produce cytochromes and other haem-containing enzymes (Prescott *et al.*, 1993). Lactic acid bacteria are unable to mediate the decomposition of hydrogen peroxide according to the following reaction:



Another distinctive physiological feature of lactic acid bacteria is their tolerance of acid. Although the spherical lactic acid bacteria can initiate growth in neutral or alkaline pH, most of the rod-shaped forms cannot grow in media with an initial pH greater than 6. Growth of all lactic acid bacteria continues until the pH has fallen to a value of 5 or less (Jay, 1992).

The taxonomy of lactic acid bacteria is in a state of flux but the principle genera are *Lactobacillus*, *Pediococcus*, *Streptococcus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Lactosphaera*, *Oenococcus*, *Weissella*, *Tetragenococcus* and *Vagococcus* (Berkeley *et al.* 1994; Adams & Nicolaides, 1997; Jay, 1998)

Lactobacilli are Gram positive, catalase negative rods that often occur in long chains. They can also be almost coccoid. They grow poorly in air but better under reduced oxygen tension. Although those in foods are typically microaerophilic, many true anaerobes also exist. They are chemoorganotrophic and require complex media. Their metabolism is fermentative and at least one of the carbon end-products is lactate. Lactobacilli are common in dairy products and are rarely pathogenic. Their optimum growth temperature is 30 to 40°C.

All members of the genera *Pediococcus* and *Streptococcus* are homofermentative, Gram positive, catalase negative cocci. *Pediococci* divide in two planes at right angles to produce tetrads of cells but sometimes only pairs of cells are seen. Single cells are rare and chains are not formed. They are facultative anaerobes although some strains

are inhibited on incubation in air. They are chemoorganotrophic and require nutritionally rich media and a fermentable carbohydrate (mainly mono- and disaccharides). Glucose is fermented with the production of acid but no gas. The major product is DL or L(+) lactate. The optimum growth temperature is 25–40°C.

Streptococci divide in one plane to produce chains (Stanier *et al.*, 1980). The cells are spherical or ovoid, occurring in chains or pairs. Some species are encapsulated. They are facultatively anaerobic chemoorganotrophs which require nutritionally rich media for growth and sometimes 5% carbon dioxide. Growth is generally restricted to a temperature of 25–45°C (optimum 37°C).

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

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

Enterococci were once a sub-group of the genus *Streptococcus*. The cells are spherical or ovoid and occur in pairs or short chains. They are sometimes mobile by means of scanty flagella. They lack obvious capsules, are facultative anaerobic and are chemoorganotrophs with fermentative metabolism. A wide range of

carbohydrates, including lactose, are fermented with the production of mainly L(+)-lactic acid. Gas is not produced. They are Gram positive and most can grow at 10°C and 45°C with an optimum temperature of 37°C.

Lactococci are Gram positive, catalase negative, non-motile spherical or ovoid cells that occur singly, in pairs or in chains. Endospores are not formed. They are non-motile and without capsules. They are facultative anaerobes which can grow at 10°C but not at 45°C (optimum temperature 30°C). They are chemoorganotrophs with fermentative metabolism. A number of carbohydrates are fermented with the production of L(+) -lactic acid but no gas. Their nutritional requirements are complex.

Vagococci are Gram positive, non-sporing, spheres, ovals or short rods which occur singly, in pairs or in short chains. Some motile by peritrichous flagella. They are catalase negative and chemoorganotrophic with a fermentative metabolism. They produce acid but no gas from a number of carbohydrates. Glucose fermentation yields mainly L(+)-lactate. They can grow at 10°C but not at 45°C (optimum temperature 25-35°C).

Lactosphaera, Weissella and Oenococci are heterofermenters. Weissella produce gas from carbohydrates. DL-lactate is the main product from glucose fermentation (Jay, 1998). Weissella were formerly classified as *Leuconostoc confusus* and *Leuconostoc paramesenteroides* (Schleifer & Ludwig, 1995). Oenococci were formerly classified as *Leuconostoc oenos* (Dicks, Dellaglio & Collins, 1995). The reclassification of lactic acid bacteria has been made possible by the development of modern taxonomic tools based on immunoassays, PCR techniques and DNA hybridisation methods (Ehrman, Ludwig & Scheifer, 1994).

Lactic acid bacteria differ with respect to the isomers of lactic acid that they produce. This is determined by the specificity of the lactic dehydrogenases which mediate pyruvate reduction. Some species contain only D-lactic dehydrogenase and hence

form the L-isomer. Some species contain two lactic dehydrogenases of differing stereospecificity and form racemic lactic acid (Stanier *et al.*, 1980).

The ability to convert carbohydrates to lactic acid, acetic acid, alcohol and carbon dioxide with only minor changes in the other food components has made this group of micro-organisms extremely important in the preservation of food (Pederson, 1971; Snoep & de Mattos, 1997). There is little calorific change in the conversion of carbohydrates to lactic acid and very little loss of total nutritive value. The lactic acid produced during fermentation is effective in inhibiting the growth of other bacteria that may decompose the food or make it poisonous (Adams & Hall, 1988; Jeppesen & Huss, 1993; Leisner, Greer, Dilts & Stiles, 1995).

2.1.5 Factors controlling fermentation

A number of intrinsic and extrinsic factors influence the intensity and particular type of fermentation (Tomkins, Alnwick & Haggerty, 1988). Intrinsic parameters are an inherent part of the food or beverage and include pH, water activity, oxidation–reduction potential (Eh) and nutrient content (Jay, 1978).

The pH of a solution describes the hydrogen ion concentration $[H^+]$ (Conn, Stumpf, Bruening & Doi, 1987). Bacterial growth rates are greatly influenced by pH values and the effects are mainly based on the nature of proteins (White, Handler & Smith, 1967). Charge interactions within the amino acids of a polypeptide chain strongly influence the secondary and tertiary structure and folding of a protein (Ludescher, 1996). This change in shape of the active site of enzymes affects their function. Enzymes are normally inactive at very high and very low pH values (Atlas, 1995).

Most micro-organisms grow best at pH values around 7 (6.6–7.5). Lactic acid bacteria will grow at low pH ($< pH 4$) and through the production of lactic acid the pH is lowered further (Tomkins *et al.*, 1988). Many bacteria, particularly the spoilage bacteria and pathogenic bacteria, do not grow at such a low pH. *Listeria monocytogenes* is regarded as showing a poor ability to survive and grow at acid pH

and it is considered that it usually dies at a pH lower than 5.6 (George, Lund & Brocklehurst, 1988; Sorrells, Enigl & Hatfield, 1989). This has important consequences with regard to the shelf-life and safety of fermented foods and beverages.

All bacteria require water for growth and reproduction. Water is an essential solvent and is needed for all biochemical reactions in living systems (Conn, Stumpf, Bruening & Doi, 1987). The availability of water has a marked influence on bacterial growth rates (Jay, 1992). The water requirements of micro-organisms are defined in terms of water activity (a_w) in the environment. This parameter is defined by the ratio of the water vapour pressure of the food substrate to the vapour pressure of pure water at the same temperature i.e. $a_w = p/p_o$, where p = the vapour pressure of the solution and p_o = the vapour pressure of the solvent (usually water) (Atlas, 1995). The concept is related to relative humidity, R.H., in the following way: Relative humidity = $100 \times a_w$. The a_w of most fresh foods is 0.99. Most spoilage bacteria do not grow below a_w of 0.91. With respect to food-poisoning bacteria, *Staphylococcus aureus* has been found to grow at water activities as between 0.83 and 0.86 (Farber, Coates & Daley, 1992) while *Clostridium botulinum* does not grow at water activities below 0.95 (Gaze, 1992; Jay, 1992).

Micro-organisms display varying degrees of sensitivity to the oxidation-reduction potential (O/R, Eh) of their growth medium. The Eh of a substrate is referred to as the ease with which the substrate loses or gains electrons. A substance that readily takes up electrons is a good oxidising agent while one that readily gives up electrons is a good reducing agent (Jay, 1992). The more highly oxidised a substance is, the more positive will be its Eh and the more highly reduced a substance, the more negative will be its electrical potential. Aerobic micro-organisms such as those belonging to the genus *Bacillus* require positive Eh values (oxidised) for growth while anaerobic bacteria such as those belonging to the genus *Clostridium* require negative Eh values (reduced). Some aerobic micro-organisms grow better under slightly reduced

conditions and are often referred to as microaerophilics (Jay, 1992). Examples of microaerophilic bacteria are lactobacilli and streptococci.

To grow, micro-organisms must draw from the environment all the nutrients that they require for the synthesis of their cell materials and for the generation of energy. Water accounts for 80 to 90 % of the total weight of cells and is therefore always the major essential nutrient in quantitative terms (Stanier *et al.*, 1980). Micro-organisms also require carbon as a source of energy. Micro-organisms that are of importance in fermented foods get their carbon from organic nutrients. The nitrogen and sulphur requirements are often met by organic nutrients containing amino acids, proteins, or products of complex protein degradation such as peptones. Growth factors are a group of nutrients that the micro-organisms cannot synthesise. They include amino acids as constituents of proteins, purines and pyrimidines required as constituents of nucleic acids and vitamins (Frazier, 1967). Vitamins are a diverse collection of organic compounds which form parts of the prosthetic groups or active centres of certain enzymes.

Extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their environment (Jay, 1992). Temperature is an important factor. Low temperatures reduce membrane fluidity and hence restrict transport of essential nutrients. Such temperatures also slow down enzyme reactions (Bronck, Madigan, Martinko & Parker, 1994). Below the minimum growth temperature, metabolic processes are too low to meet the requirements of the cell. Within the growth range for a particular micro-organism, there is an optimal growth temperature at which enzyme reaction rates are at their peak, the highest rate of reproduction occurs and growth rate is maximal (Atlas, 1995). The micro-organisms reproduce with the shortest doubling time (McKane & Kandel, 1996). Mesophilic lactic acid bacteria tend to grow best between 10 and 40°C with an optimum around 30°C and they include *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* as well as *Leuconostoc mesenteroides* subsp. *cremoris* (Oberman & Libudzisz, 1998). Thermophilic lactic acid bacteria such as those that are used to produce yoghurt are

represented by the two species *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. Some thermophilic species such as *Lactobacillus leichmanii* (*delbrueckii*), which is homofermentative, will grow at temperatures of between 48-50°C (Van der Walt, 1956). Raising the temperature above the maximum usually kills the microbial cells by denaturing protein and irreversibly damaging molecules essential to the cell's survival (Bronck *et al.*, 1994).

These various factors, particularly in traditional fermented cereal products where a spontaneous fermentation is often relied upon, result in a sequence of different micro-organisms responsible for the fermentation. Many lactic fermentations are initiated by spherical bacteria such as *Leuconostoc mesenteroides* and *Pediococcus acidilactici*. The rod-shaped bacteria take over and lower the pH to around pH 3.6 (Tomkins *et al.*, 1988). Then yeasts may grow in the final product and spoil it. Alternatively, in alcoholic beverages, the initial fermentation would be alcoholic due to yeasts, followed by a bacterial lactic acid fermentation which would ultimately spoil the product. The complexity of such systems poses great challenges when attempting to elucidate the micro-organisms of importance in a particular product.

2.2 The effect of fermentation on food safety

Diarrhoeal diseases are among the major causes of death in most developing countries and also a major factor in malnutrition in young children (UNICEF, 1988). In Africa cereal gruels and porridges are generally used as weaning food for infants. It can be assumed that a large part of the diarrhoeal diseases are food-borne and it has been confirmed that cereal-based weaning foods and water are important sources of pathogens (Mathur & Reddy, 1983).

The bacteria that are most common aetiological agents include enterotoxinogenic *Escherichia coli*, *Campylobacter*, *Shigella* and *Vibrio cholerae* (Fernandes, Shahani & Amer, 1987). Lactic acid bacteria in fermented gruels significantly suppress the growth of some food-borne pathogens (Svanberg, Sjogren, Lorri, Svennerholm & Kaijser, 1992). The lactic and acetic acids produced when the gruels ferment lower

the pH to less than pH 4.5. This strongly inhibits the pathogenic bacteria which do not grow at such low pH (Jay, 1992). The inhibition of the Gram positive *Staphylococcus aureus* is probably due to the formation of bacteriocins during the lactic acid fermentation as well as the effect of low pH (Svanberg *et al.*, 1992). The use of an inoculum, obtained from a previous gruel, has been found to be more effective than spontaneous fermentation in inhibiting the growth of enteropathogenic bacteria and this appears to be the result of the more rapid decrease in pH (Kingamkono *et al.*, 1995). On the other hand, the emergence of acid resistance in some enteropathogens such as *E. coli* 0157:H7 whereby food products such as yoghurt have been vehicles of infection means that the potential to enhance food safety should be assessed in the light of the total manufacturing process (Adams & Nicolaides, 1997).

A further advantage of fermentation, in addition to inhibiting the growth of pathogenic bacteria, is that it also slows down the rate of microbial spoilage of food (World Health Organisation, 1996). For the maximum benefits, fermented foods must be consumed without heating since such a treatment might eliminate viable bacteria in the product that may be beneficial (Hargrove & Alford, 1980). The importance of hygiene before and during fermentation cannot, therefore, be underestimated.

2.2.1 Fermentation end-products and food preservation

Organic acids

Lactic acid fermentation is characterised by the accumulation of organic acids, primarily lactic and acetic acids, and the accompanying reduction in pH (Berry, Liewen, Mandigo & Hutkins, 1990). Acid production is an efficient tool for inhibiting pathogenic and spoilage bacteria since organic acids produced during fermentation have broad antibacterial activities (Kociubinski, Perez, Anon & Antoni, 1996). Lactic acid, generated *in situ*, is traditionally used for improving food safety and shelf-life (Adams & Hall, 1988). Levels and proportions of organic acids produced depend on the species of the micro-organism involved, the chemical composition of

the culture environment and the physical conditions encountered during fermentation (Fields, Ahmed, & Smith, 1981; Lindgren & Dobrogosz, 1990; Sanni, 1993).

The preservative action of acids may be partly due to the depression of internal (cytoplasmic) pH (Russell, 1992). Undissociated acid molecules are lipophilic and pass readily through the plasma membrane by diffusion. In the cytoplasm (approximately pH 7) acid molecules dissociate into charged anions and protons (Salmond, Roll & Booth, 1984). These cannot pass across the lipid bilayer and accumulate in the cytoplasm thus reducing the pH. The acidified cytoplasm in turn inhibits metabolism, in particular the enzymes of metabolism (Krebs, Wiggins & Stubbs, 1983; Stratford & Anslow, 1998).

A principal target for many antimicrobial compounds is the plasma membrane and an alternative mechanism which may explain how acids act as preservatives is that they eliminate the proton motive gradient (Eklund, 1985). The selective permeability of the plasma membrane to protons allows the cells to create a pH gradient and an electrical potential which together form the proton motive force (Eklund, 1980). In the chemiosmotic theory, the energy contained in the proton motive force is then used to drive the uptake of essential nutrients such as amino acids (Bracey, Holyoak & Coote, 1998). The accumulation of charged acid particles in the cytoplasm disrupts the proton motive force and prevents uptake of amino acids (Freese, Sheu & Galliers, 1973).

Bacteriocins

Lactic acid bacteria are well-known for their production of antimicrobial proteins or peptides collectively known as bacteriocins (Gross & Morell, 1971; Kociubinski *et al.*, 1996). These protein complexes (protein aggregates, lipocarbohydrate proteins, glycoproteins) are active against Gram positive bacteria and normally known to display a narrow range of inhibitory activity that affects closely related species within *Lactobacillaceae* (Klaenhammer, 1988; Klaenhammer, 1993). Bacteriocins have been

isolated from fermented milk and dairy products (Litopoulou-Tzanetaki, 1987) and *mahewu* (Visser, Holzapfel, Bezuidenhout & Kotze, 1986)

The ability of many bacteriocins to inhibit some food-borne pathogens makes them attractive as potential food preservation agents. The best characterised bacteriocin produced by lactic acid bacteria is nisin. Nisin is produced by *Lactococcus lactis* subsp. *lactis* and has been available commercially in concentrated form since 1959 (Coventry, Gordon, Wilcock, Harmark, Davidson, Hickey, Hillier & Wan, 1997). Most micro-organisms require an intact plasma membrane (Bracey *et al.*, 1998). Nisin is strongly attracted to phospholipids in bacterial and liposomal membranes. Cationic nisin molecules initially interact by electrostatic attractions with anionic membrane phospholipids. They reorient themselves in the membrane such that they form non-selective pores (Muriana, 1996). The net result is that nisin makes the cytoplasmic membrane permeable which causes the release of accumulated amino acids from the cells as well as membrane vesicles of sensitive bacteria by leakage (Barrena-Gonzalez, Huot & Petitdemange, 1996; Muriana 1996).

Nisin exhibits broad spectrum inhibitory activity against Gram positive bacteria, including spore-forming bacteria (Klaenhammer, 1988). It inactivates thermophilic spoilage microorganisms in canned goods (Stevens, Sheldon, Klapes & Klaenhammer, 1991). Nisin and pediocin, a bacteriocin produced by *Pediococcus* species, have been shown to be effective in controlling *Listeria monocytogenes* in white pickled cheese, skim milk, yoghurt and other foods (Schaack & Marth, 1988; Abdulla, Davidson & Christen, 1993; Green, Dick, Bruggeman, van Damme & Chikindas, 1997; Ming, Webber, Ayres & Sandinè, 1997).

2.3 The effect of fermentation on the nutritive value of foods

Food fermentations are very complex processes since they normally involve the interaction between plant or animal tissue and a group of microorganisms. This means that any changes that occur during fermentation will depend on the available nutrients and nutrient precursors in the raw materials, the metabolic activities of the

microorganisms responsible for the fermentation and any possible interactions of these elements (McFeeters, 1988).

2.3.1 Effect of fermentation on proteins

Changes in the nutritive value of proteins as a result of fermentation are particularly important in cereals and legumes. The proteins in cereals such as sorghum and millets are seriously deficient in the amino acid lysine and are, generally, poorly digestible in their cooked form (Klopfenstein & Hosoney, 1995). These sources of protein are therefore often of lower nutritional quality compared to animal products yet they tend to be the major dietary sources of protein for people with marginal or sub-marginal protein intake (McFeeters, 1988).

Fermentation processes that consistently improve the quality of protein or availability of protein from cereals and legumes could have a positive impact on the diets of many people. Conversely, any fermentation that results in the unnecessary loss of protein content and quality could particularly have a negative effect (Van Veen & Steinkraus, 1970).

Changes produced by fermentation are limited both in time and extent to which microorganisms are allowed to grow. Fermentation is believed to increase total protein content (Kazanas & Fields, 1981; Umoh & Fields, 1981). This is due to the decrease in starch and sugars as a result of hydrolysis by bacterial enzymes with the formation of volatile products. With homofermentative bacteria some of the products formed are lactic acid with small amounts of acetic acid and carbon dioxide. With heterofermentative bacteria other volatile products of fermentation are acetic acid, ethanol, lactic acid and carbon dioxide (Frazier 1967). This leads to changes in proportions of nutrient components. It is believed that the increase in protein content at the expense of starch is beneficial to consumers who need a higher protein intake (Steinkraus, 1994).

The total amino acid composition of yoghurt and other fermented milk products does not differ substantially from that of the milk from which they originate (Fernandes, Chandan & Shahani, 1992). During fermentation some lactic acid bacteria utilise milk proteins as a nitrogen source to ensure their growth. *Lactobacillus helveticus*, in particular, is recognised as possessing efficient protease and peptidase activities with respect to milk proteins (Fernandez, Bhowmik & Steele, 1994). The protein efficiency ratio of milk proteins is already very high and it was found that the bioavailability of yoghurt proteins, as measured using rats, was not improved significantly above that of milk.

Fermentation was found to increase the free amino acids as well as the quantity of the essential amino acids lysine, tryptophan and methionine (Umoh & Fields, 1981; Chavan & Kadam, 1989; Steinkraus, 1994). Contrary to the above-mentioned, results in fermentation studies done with foods of higher nutritional value such as milk, lysine decreased by as much as 40% when skim milk was fermented with *Lactobacillus acidophilus* (Rao, Pulusani & Rao, 1982). On the other hand fermentation studies with *tempeh*, a fermented soyabean product made with *Rhizopus oryzae* or *Rhizopus arrhizus*, showed little change in either methionine or lysine (Kao & Robinson, 1978).

2.3.2 Effect of fermentation on vitamins

According to McFeeters (1988) fermentations may result in changes in vitamin content by several mechanisms which include:

1. Synthesis of vitamins by the fermenting micro-organisms.
2. Loss of vitamins by metabolism of fermenting micro-organisms.
3. Loss of vitamins by metabolism of the fermenting food.
4. Loss of vitamins by chemical reactions not directly related to fermentation.
5. Increase/decrease in stability of vitamins due to pH changes.
6. Soaking or cooking losses associated with preparation of product prior to or after fermentation

During the manufacture of yoghurt, heat treatment of the milk causes losses in the amount of vitamin B₁₂ (Rasic & Panic, 1961). Some lactic acid bacteria require B vitamins for growth while several lactic acid bacteria are capable of synthesising them. *Lactobacillus delbrueckii* subsp. *bulgaricus* require folic acid (Deeth & Tamime, 1981; Gilliland, 1990). Vitamins which increase during the manufacture of yoghurt are niacin and folic acid because they are actively synthesised by *Streptococcus salivarius* subsp. *thermophilus* during the manufacture of yoghurt. *Lactobacillus acidophilus* was found to increase folic acid levels in skim milk (Deeth & Tamime, 1981; Friend, Fiedler & Shahani, 1983).

Fermentation of cowpea flour using, *Rhizopus microsporus* var *oligosporus*, was found to improve the content of folacin, niacin and riboflavin (Prinyawiwatkul, Eitenmiller, Beuchat, McWatters & Phillips, 1996). During the fermentation of sorghum using *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus fermenti* as test micro-organisms, an increase in niacin, thiamin and riboflavin was observed (Kazanas & Fields, 1981). Improvements in vitamin content, particularly the B vitamins, with fermentation are important and are due to microbial biosynthesis of the vitamins. This is important in foods such as polished rice which is deficient in Vitamin B₁ (Steinkraus, 1988).

2.3.3 The effect of fermentation on anti-nutrients

Raw ingredients such as cereals, legumes and tubers that are used to prepare fermented foods contain significant amounts of antinutritional and toxic components such as phytates, oxalates, tannins, cyanogenic glycosides, saponins, lectins and inhibitors of enzymes such as α -amylase, trypsin and chymotrypsin (Reddy & Pierson, 1994).

Polyphenols

Phenolic compounds have been divided into three categories: phenolic acids, flavonoids and tannins (Serna-Saldivar & Rooney, 1991). Phenolic acids are derivatives of benzoic acid or cinnamic acid. They may occur as free acids, soluble

esters or insoluble esters in cereals and are concentrated in the outer layers of the grain (Harn, Rooney & Earp, 1984). Flavonoids consist of two units: a C₆-C₃ fragment from cinnamic acid and a C₆ fragment from malonyl-coenzyme A (Serna-Saldivar & Rooney, 1991). Tannins are oligomers of flavan-3-ols and flavan-3,4-diols that are joined by a carbon-carbon bond between the C₄ of one unit and C₆ or C₈ of another (Reddy & Pierson, 1994). Tannins are able to precipitate alkaloids, gelatin and other proteins. Tannins are characteristic of the chemical defense of plants and act as barriers to predators such as insects and birds which may feed on such plants (Haslam, 1989).

Many millets test positive for traces of tannins but only finger millet contains condensed (non-hydrolysable) tannins chemically known as proanthocyanidins (reviewed by Serna-Saldivar & Rooney, 1991). White varieties of finger millet have been found to contain tannin levels that are lower than those of brown ones (Ramachandra, Virupaksha & Shadaksharaswamy, 1977).

A relationship between tannin levels and *in vitro* digestibility in some finger millet varieties has been established. Tannins in cereals can bind dietary proteins with the formation of indigestible protein-tannin complexes (Reddy & Pierson, 1994). Proteins interact with tannins by means of hydrogen bonding, hydrophobic interaction, electrostatic attraction and covalent bonding (Butler, Riedl, Lebryk and Blytt, 1984). In addition, dietary tannin could interfere with the digestion by inhibiting digestive enzymes. Phenols inhibit many enzymes *in vitro* including digestive enzymes such as trypsin and α -amylase (Chibber, Mertz & Axtell, 1980; Haslam, 1989).

Fermentation on its own does not lead to a decrease in the amount of tannins. It has been observed, however, that a combination of soaking, sprouting and fermentation reduces the level of assayable tannins probably due to polymerisation (Dhankher & Chauhan, 1987a; Khetarpaul & Chauhan, 1990a).

Enzyme inhibitors

Enzyme inhibitors that specifically inhibit trypsin and chymotrypsin have been identified in sorghum, Japanese millet and pearl millet (Klopfenstein & Hosoney, 1991; Serna-Saldivar & Rooney, 1991). Enzyme inhibitors are more prevalent in legume seeds which tend to contain various protease inhibitors. The peanut trypsin-chymotrypsin inhibitor complex was isolated from peanuts. The complex shows weak antichymotryptic activity while trypsin is rapidly inactivated. Peanut trypsin-chymotrypsin inhibitor possesses two reactive sites for trypsin and one reactive site for chymotrypsin (Ikenaka & Norioka, 1983).

One of the factors limiting the consumption and utilisation of cowpeas is the presence of protease inhibitors (Prinyawiwatkul *et al.*, 1996). As is the case with peanut inhibitors, the protease inhibitors in cowpeas are associated with trypsin. This prevents the complete utilisation of the proteins in cowpeas. Fermentation has been found to be ineffective in reducing trypsin inhibitor activity on its own although soaking and boiling are effective (Reddy & Pierson, 1994).

Phytates

Phytic acid (myoinositol 1,2,3,4,5,6 hexakis dihydrogen phosphate) occurs primarily as a salt of monovalent and divalent cations in discrete regions of cereal grains, legumes, some tubers and roots (Ologhobo & Fetuga, 1984; Reddy & Pierson, 1994).

The presence of high concentrations of phytic acid in cereals and legumes is of nutritional concern because of its ability to reduce the bioavailability of minerals, particularly divalent cations including zinc, calcium, iron and magnesium (McFeeters, 1988). Phytates also interact with enzymes such as trypsin, pepsin, α -amylase and β -galactosidase resulting in a decrease of their activity.

Fermentation of pearl millet has been shown to reduce phytic acid concentrations. When a traditional Indian food *rabadi*, which is made from pearl millet flour and buttermilk, phytic acid contents decreased (Dhanker & Chauhan, 1987a).

Cyanogenic glycosides

Cyanogenic glycosides are widely distributed in beans and tubers such as cassava. Cassava is an important staple food for about 500 million people in developing countries and bitter varieties are potentially toxic because of their cyanide content. In West Africa cassava is processed into foods such as *gari* and *fufu*. Cassava is one of the few and by far the most important of human food crops in which the content of cyanide creates nutritional problems (Cooke & Coursey, 1981). Acid hydrolysis of cyanogenic glycosides yields hydrocyanic acid (HCN) which is a potent respiratory inhibitor. HCN inhibits cytochrome oxidase, a terminal respiratory catalyst. The glycoside found in cassava is linamarin (Hosel, 1981). The human body is capable of cyanide detoxification but the mechanisms involved increase the requirement for sulphur-containing amino acids. Thiocyanate is one of the detoxification products and it inhibits iodine absorption. This promotes goitre, which is one of the common ailments in developing countries (Narsted & Muller, 1983; Balagopalan, Padmaja, Nanda & Moorthy, 1988).

A combination of grating, fermenting and roasting is required for the effective removal of cyanogenic glucosides from cassava (Nout & Matorjemi, 1997).

In sorghum, although cyanogenic glycosides occur in most varieties their quantities depend on variety and environment. The main cyanogenic glycoside, dhurrin, occurs mainly in the leaves of developing sorghum plants (Serna-Saldivar & Rooney, 1991). In the Sudan, the high incidence of goitre has been found to be associated with a goitrogen called thioamide which occurs in pearl millet. Fermentation of grain has not been found to be effective in reducing the levels or activity of goitrogens (Klopfenstein & Hosney, 1991).

2.4 Apparent health benefits of fermented foods

2.4.1 Improved lactose utilisation

Lactose intolerance describes a situation in which an individual lacks adequate ability to digest lactose. This inability is for the most part due to an insufficient amount of the enzyme β -galactosidase in the small intestine. The usual symptoms associated with this problem include cramps, flatulence and diarrhoea following the consumption of milk products (Fuller, 1989).

Lactose-intolerant individuals can consume certain fermented dairy products without harmful effects. Where beneficial effects have been found, they have been attributed to the reduced level of lactose in the fermented product and to the production of β -galactosidase by the fermenting micro-organisms following ingestion of the products (Blanc, 1984). The bacteria used to make yoghurt contain the enzyme β -galactosidase which can improve lactose utilisation by lactose-intolerant individuals. Being intracellular, β -galactosidase of the yoghurt starter culture bacteria seems to be able to survive passage through the stomach to reach the small intestine (McFeeters, 1988).

2.4.2 Hypocholesterolemic activity

Risks of heart attacks in hypercholesterolemic individuals can be significantly reduced by lowering their plasma cholesterol (Fuller, 1989). It has also been postulated that cholesterol is lowered due to a factor produced or enhanced by the action of the starter culture bacteria during fermentation (Jay, 1992). The factor may lead to decreased synthesis of cholesterol and removal of cholesterol or its precursors from the gastrointestinal tract. The factor may also inhibit cholesterol synthesis in the body (Danielson, Peo, Shahani, Lewis, Whalen & Amer, 1989; Gilliland, 1990; Akalin, Gonc & Duzel, 1997). In another study done with rats, the hypocholesterolemic effects of *Lactobacillus gasseri* were established and they were attributed to the ability of the culture to suppress the reabsorption of bile acids into

the enterohepatic circulation and to enhance the excretion of acidic steroids in faeces of hypercholesterolemic rats (Usman & Hosono, 2000).

2.4.3 Antagonistic actions towards enteric pathogens

Most lactic acid bacteria will exert antagonistic action towards pathogens *in vitro*. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* have received the most attention in their inhibitory activity toward the commonly known food-borne pathogens (Fuller, 1989). Both micro-organisms have been shown to be both preventative and therapeutic in controlling intestinal infections through administration of milk containing one or both micro-organisms. The exact mechanism whereby dietary cultures of lactobacilli may inhibit intestinal pathogens is not clear. It is likely that the acids produced by the micro-organisms may be involved in such antagonistic action (Fuller, 1986). Other studies, however, seem to suggest that neither *Lactobacillus* nor *Bifidobacterium* have an effect on intestinal infections (Fuller, 1989).

2.4.4 Anti-cancer effects

Intestinal microflora may be involved in such intestinal diseases as colon carcinogenesis (Ling, Korpela, Mykkanen, Salminen & Hanninen, 1994; Fonden, Mogensten, Tanaka & Salminen, 2000). Anaerobes such as *Peptostreptococcus* and *Clostridium* as well as *E. coli* produce high amounts of β -glucuronidase and nitroreductase, enzymes which increase the rate of conversion of indirectly acting carcinogens into proximal carcinogens (Cole, Fuller, Mallet & Rowland, 1985). β -glucuronidase influences the enterohepatic circulation of carcinogenic conjugates and nitroreductase enhances the formation of reactive N-nitroso and N-hydroxy intermediates thereby converting aromatic nitro-compounds into potentially harmful amines (Ling *et al.*, 1994).

There is interest in understanding how the colonic flora can be modified through diet. Diet influences the activities of these enzymes in the intestinal tract (Goldin & Gorbach, 1984). Among the food products studied as potential modifiers of the

colonic microflora, dairy products containing viable organisms have received the most attention (Marteau, Pochart, Flourie, Pellier, Santos, Desjieux & Ramboud, 1990; Jay, 1992; Sreekumar & Hosono, 2000). Results by Ling *et al.* (1994) confirm a reduction in faecal β -glucuronidase and nitroreductase activities after ingestion of *Lactobacillus*. They suggested that the partial replacement of the flora in the gastrointestinal tract by *Lactobacillus* could reduce the levels of these enzymes.

2.5 Fortification of fermented foods

Although cereals contribute significantly to the nutritional requirements of a large population in sub-Saharan Africa, their nutritive value is low (Plahar, Leung & Coon, 1983). *Mahewu*, a fermented maize-based beverage, has a low protein content of 7-9% on dry basis (Schweigart & Fellingham, 1963). One litre of mahewu containing 10% solids produces about 4000 kJ, derived from the carbohydrate in the maize meal (Schweigart, Van Bergen, Weichers & de Wit, 1960). If consumed by people on a fully adequate diet, the nutritional value of *mahewu* would be of little importance and there would be no need for fortification. Where malnutrition is prevalent, fortification of mahewu would help to alleviate the problem (Schweigart & Fellingham, 1963). Methods of fortifying *mahewu* that have been suggested include the use of milk, whey, sour milk products, skim milk powder, whey protein, soya flour, food yeast, blood or fish flour (Bates, Wu & Murphy, 1974; Green, Lawhon, Cater & Mattil, 1976; Hesseltine, 1983; Serna-Saldivar, Canett, Vargas, Gonzalez, Bedolla & Medina, 1988). One litre of fortified *mahewu* would then provide about 30% of the daily assimilable protein requirement of an adult human male and between 20% and 30% of the requirement for thiamine and nicotinamide (Schweigart & de Wit, 1960).

Cereal proteins are deficient in some essential amino acids, particularly lysine (Virupaksha & Sastry, 1968; Nche, Nout & Rombouts, 1994). To overcome the problems of protein energy malnutrition, fortification of the commonly used cereal products with inexpensive protein is needed (Del Valle & Perez-Villasenor, 1974). The complementary effect of amino acids from one protein on the nutritive value of another is recognised (Wang & Hesseltine, 1981). An economical and practical way

of improving protein quality and quantity is the combined use of complementary proteins. Vegetable protein sources such as legumes and oil seeds (Bressani, Murillo & Elias, 1974; McPherson & Ou, 1976) are high in lysine, an essential limiting amino acid in most cereals (Shekib, Zoual, Youssef & Mohamed, 1986; Akpapunam & Sefa-Dedeh, 1995). Cereal grains, on the other hand, have adequate amounts of sulphur-containing amino acids while legume proteins are deficient in these amino acids (Wang & Hesseltine, 1981).

Milk can also be used to complement the proteins in cereal grains. An example of a product in which this has been successfully done is *kishk*, a popular fermented food in parts of the Middle East, which is made by mixing boiled, dried and ground wheat grains with fermented milk (Hafez & Hamada, 1984). Dried *kishk* can have up to 23% protein and can also have as much lysine as 310 mg/g nitrogen compared to the Food and Agriculture Organisation provisional pattern of 270 mg/g nitrogen (El-Gendy, 1983). Combining the two proteins in the proper proportions results in a mixture that is nutritionally superior to each one alone (El-Sadek, Zawahry, Mahmoud & El-Motteleb, 1984). The high protein content of *kishk* and the complementary effect which the milk proteins exert on the lysine deficient wheat may make this product comparable to milk in protein nutritional quality (Hamad & Fields, 1982; Hafez & Hamada, 1984).

Fortification of maize meal with soya flour (Plahar *et al.*, 1983) was found to improve protein quality of fermented maize dough foods in Ghana. The fortified flour was found to be as acceptable as the traditional maize dough.

In Nigeria, inclusion of cashew nut meal, locust bean meal and sesame oil meal in maize-based flour was found to improve the total amino acid profile of fermented gruels that are commonly used as weaning foods (Ekpenyong *et al.*, 1977).

In Ghana the supplementation of maize with cowpea during the preparation of the fermented maize dumpling *kenkey* was found to improve the protein content of the

product. The product also compared well with the traditional product in terms of its sensory characteristics (Nche *et al.*, 1994). Similar studies with a weaning food developed from maize and cowpea again confirmed that fortification improved the protein content of the product (Akpapunam & Sefa-Dedeh, 1995).

Other projects carried out include the fortification of sorghum with green gram which was found to increase the proteins, free amino acids, soluble proteins and *in vitro* digestibility of the protein (Chavan & Kadam, 1989).

In the following section, three examples of fermented products will be discussed as follows: *Mahewu* as an example of a fermented food produced from cereals, yoghurt as an example of a fermented product from milk and, lastly, *kishk* a fermented cereal-milk composite. The discussion will focus on the biochemical and microbiological processes that are involved in the traditional and industrial processing of these products.

2.6 Mahewu - An example of a cereal fermented beverage

2.6.1 Background

In southern Africa, *mahewu* is a traditional fermented beverage that is prepared using maize. It contains little or no alcohol, has a pH of about 3.5 and is popular among the black people of southern Africa (Holzapfel, 1989).

2.6.2 Traditional processing of *mahewu*

Traditionally, *mahewu* is prepared by boiling a thin maize porridge containing 8 to 10% solids. Cooking of the maize leads to gelatinisation of the starch. Gelatinisation is characterised by swelling of the starch granules, leaching of the starch components (especially the amylose), increase in the viscosity of the porridge and increased susceptibility to enzymatic digestion (Kent & Evers, 1994; Lii, Tsai & Tseng, 1996). The porridge is allowed to cool to room temperature (25-30°C). In South Africa, a small quantity of wheat flour or bran (2-5% of the maize meal) is added and mixed

thoroughly into the porridge. In Zimbabwe a handful of malt made from finger millet or sorghum is added instead of the wheat flour or bran. The wheat flour, bran or malt acts as a source of inoculum and as a source of enzymes (Van der Merwe, Schweigart & Cachia, 1964), particularly α -amylase which produces a small amount of maltose which is utilised in the fermentation. The enzyme partially hydrolyses the gelatinised starch making the gruel softer, or even liquefying it. This offers the possibility of making a porridge of acceptable viscosity but with a higher energy density.

The inoculated mixture is left to ferment in a warm place. In Zimbabwe, fermentation is generally carried out in clay pots where the residue in the pot from previous fermentations provides the inoculum for fermentation. The lactic acid bacteria consist of mesophilic strains that are capable of rapid growth in the porridge. Sugars derived from the enzymatic hydrolysis of starch are fermented to lactic acid and other metabolic products such as carbon dioxide, acetic acid or ethanol (Holzapfel, 1989).

The main micro-organisms in native *mahewu* are *Leuconostoc mesenteroides* and *Lactobacillus brevis*. The traditional spontaneous souring process is not suitable for large scale production of *mahewu* because it is too slow and proceeds too irregularly. The development of other types of undesirable bacteria can produce secondary fermentations, the products of which (e.g. acetic acid or butyric acid) influence the taste negatively. *Mahewu* should have a pH of around 3.3, a titratable acidity of 0.4 to 0.5% (Schweigart & De Wit, 1960) and contain very little alcohol [a maximum of 0.25% (w/w)] (South African Bureau of Standards, 1990).

2.6.3 Industrial production of *mahewu*

In South Africa the making of *mahewu* has been industrialised to meet the demand by the increasing urban market of black people. A flow diagram of a modern processing plant is shown in Figure 5. The porridge is prepared by first mixing 8% of maize meal with water at ambient temperature and then pumping the mixture into stainless steel pots where it is heated at 85 to 90°C for 20 to 30 min. Heating not only gelatinises the starch making it more susceptible to enzymatic hydrolysis, it also kills any micro-

organisms in the maize meal that may compete with the starter culture and spoil the product (Holzapfel, 1989).

The porridge is cooled to 50°C before it is transferred to the bioreactors where 1 to 2% sugar and 0.1 to 0.2% wheat flour are added followed by 7 to 12% of the thermophilic *Lactobacillus* starter culture (an adapted pure culture of *Lactobacillus delbrueckii*) (Schweigart, 1970). The sugar provides a readily hydrolysable source of energy for the starter culture. Starch has to be broken down into a mixture of maltose and dextrans by bacterial amylases and then into glucose which means the energy to bacteria is supplied more slowly. During the next 17 to 29 h, the temperature gradually falls to about 30°C and the pH reaches 3.4 to 3.8. When the desired pH has been attained, about 2% sugar (based on the total volume) is added as a sweetener. A sharper decline in pH and culture activity is experienced in winter when the final temperature ranges from 3.5 to 3.9 compared to 3.1 to 3.6 in summer (Holzapfel, 1989).

Initial starter culture preparation occurs in the pre-fermenter where porridge at 50°C is inoculated with 10% of an active culture. Within 18 to 24 h at 30°C, the pH gradually drops and reaches a range of 3 to 3.4.

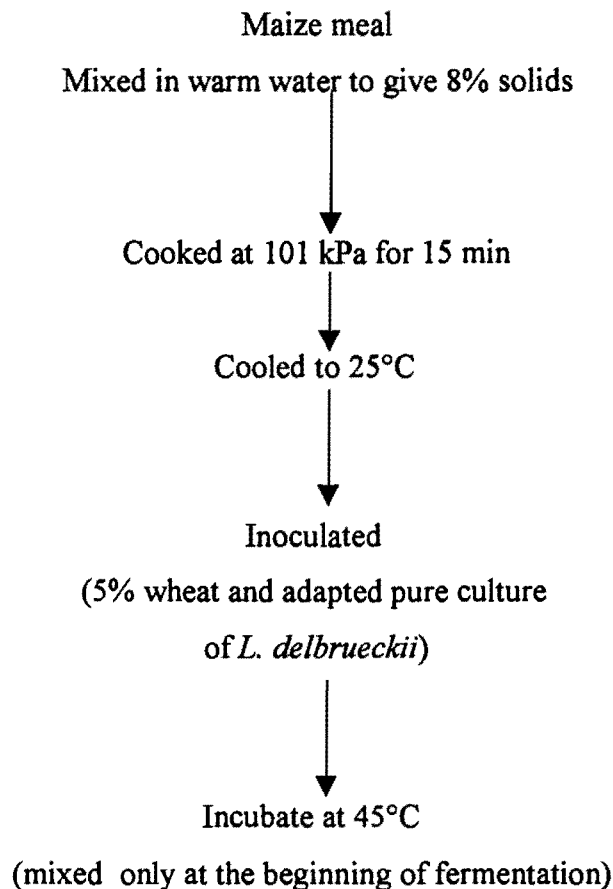


Figure 3. Summary for the improved *mahewu* fermentation process (Schweigart & Fellingham, 1963)

Lactobacillus delbrueckii is the micro-organism of choice because its optimum temperature is high and at this temperature the development of undesirable micro-organisms is suppressed. This starter culture is also known to produce large quantities of lactic acid with few by-products, thus giving a relatively pure lactic acid flavour. It remains active until a low pH has been attained and it produces lactic acid by the fermentation of glucose, maltose, sucrose, fructose, galactose and dextrans (Holzapfel, 1989). Fermentation is improved by the addition of bran and high quality proteins also improve the activity of lactic-acid bacteria. To maintain the high rate of acid production, the addition of buffering salts such as CaHPO_4 may be necessary. The

degree of sourness required depends on individual taste but is usually on average 0.4-0.5% titratable acidity, calculated as lactic acid, at which the average pH is 3.5 (Schweigart & Fellingham, 1963).

2.6.4 The microbiology of industrial *mahewu* production

During the early 1950s when the first attempts were made to produce *mahewu* on a large scale, the problems that were encountered included the development of undesirable micro-organisms including yeasts, *E. coli* and even clostridia due to the mesophilic conditions (30°C) under which fermentation occurred, irregular unpredictable fermentation due to contamination leading to the production of volatile fatty acids such as acetic and butyric acids and off-flavours (Holzapfel, 1989).

Pure cultures of *Lactobacillus acidophilus*, *L. bulgaricus*, *L. delbrueckii* and *Streptococcus lactis* have been adapted to the maize meal substrate at 51°C (*Streptococcus lactis* at lower temperatures). Factors that have been found to have a stimulatory effect on the starter cultures are buffer salts in the form of KH_2PO_4 and proteins such as yeast extract, soya and whey powder (Schweigart & Fellingham, 1963).

The most important micro-organisms that can cause the spoilage of *mahewu* are yeasts. The major spoilage yeasts are those belonging to the *Pichia* spp. (Holzapfel, 1989). Yeasts will ferment carbohydrates to form ethanol. *Acetobacter liquefaciens* was found to be another major spoilage micro-organism. This micro-organism converts lactic acid into acetic acid leading to off-odours. It also causes discolouration of the product (Holzapfel, 1989).

2.6.5 Biochemical changes during the processing of *mahewu*

The main product of homofermentative *Lactobacillus* cultures is lactic acid which has very little flavour and aroma but has a distinct, refreshing sour taste. In traditionally processed *mahewu* where heterofermentation occurs, in addition to lactic acid, acetic acid and butyric acids are formed which contribute to the flavour and aroma of the product (Schweigart, 1970).

Maize meal has very little buffering capacity and the pH quickly drops to less than 3.5. The addition of buffering salts such as CaHPO_4 and protein rich supplements means that the buffering capacity of *mahewu* will be improved and the micro-organisms can produce more acid (Schweigart & Fellingham, 1963).

Sugar is added at a rate of between 1 and 2% in the modern procedure for making *mahewu*. The sugar is readily fermented and provides energy for the starter culture. In the traditionally processed product, fermentation sugars are derived from the wheat flour enzymes hydrolysing the starch in the maize porridge (Schweigart *et al.*, 1960).

2.6.6 The role of starch in fermentation of gruels

In order to appreciate the relationship between viscosity and starch in fermented gruels it is important to look briefly at the structure of starch granules.

Cereals store energy in the form of starch. In addition to its nutritional value, starch is important because of its effect on the physical properties of many foods (Hoseney, 1994). α -D-glucose is the building block of starch and the polymerisation of glucose in starch results in two types of polymers. According to Thomas & Artwell, (1999) amylose is considered to be essentially a linear polymer composed almost entirely of α -1,4-linked D-glucose. Amylopectin is a branched polymer that is much larger than amylose. It is composed of α -1,4-linked glucose segments connected by α -1,6-linkages. Amylopectin has two distinct populations of chain lengths. The smaller chains are thought to be in such close proximity that they interact strongly resulting in crystalline regions that are extensive and arranged regularly with respect to each other throughout the starch granule. Compared with the crystalline areas, amorphous regions are generally degraded more easily by acid and enzymes. While the location of amylose within the granule remains unknown, amylose might be an important component of the amorphous areas (Kent & Evers, 1994).

The increase in viscosity that occurs when a suspension of starch in water is heated is a result of the starch taking up water and swelling substantially. The heat disrupts the hydrogen bonds that hold the polymer chains. It is believed that initial swelling occurs in the amorphous regions of the granule where hydrogen bonds are less numerous and polymers are more susceptible to dissolution (Thomas & Artwell, 1999). With continued heating and swelling, the granule becomes distorted and soluble starch is released into the solution. The soluble starch and the continued uptake of water by the remnants of starch granules are responsible for the increase in viscosity (Hoseney, 1994).

Cooked starch suspensions undergo firming and syneresis with cooling. The process is known as retrogradation. Retrogradation refers to changes that occur in gelatinised starch from an initially amorphous state to a more ordered or crystalline state (Gudmundsson, 1994).

According to Klucinec & Thompson (1999), during retrogradation amylose may form double helical associations of 40-70 glucose units whereas amylopectin forms shorter double helices than amylose due to the restrictions imposed by the branching structure of the molecules and the chain lengths of the branches. Double helices may associate and organise into crystallites and gelation results.

The starch gel is made up of a small amount of solid material which holds a large amount of water and can be visualised as starch chains with layers of water molecules attached by hydrogen bonding (Hoseney, 1994). As the paste is cooled the starch chains become less energetic and the hydrogen bonds become stronger giving a firmer gel.

The viscosity of foods containing starch depends not only on the temperature but also on the presence of such constituents as food acids (Whistler & Daniel, 1985). Acidic conditions have a significant impact on the viscosity and texture of starch gels. Native (i.e. unmodified) starches are typically unstable under acid conditions and tend to

undergo viscosity breakdown at low pH (Lineback & Inglett, 1982). Acid is believed to penetrate the amorphous parts and to hydrolyse the glucosidic bonds. The acid cannot penetrate the crystalline areas and they remain intact (Thomas & Artwell, 1999). The effect of low pH is therefore a reduction in molecular weight of the starch molecules and hence the viscosity of the gels (Hoseney, 1994).

Although a reduction in the viscosity would be desirable in the production of gruels for feeding infants, research on the viscosity reducing effects of fermentation have yielded inconsistent results. Westby & Gallat (1991) found that fermentation before cooking had little effect on the viscosity of sorghum porridges. Mbugua, Ledford & Steinkraus (1983) also concluded that the acidification resulting from the fermentation of *uji* (a thin fermented gruel made from sorghum, maize or millets and popular in East Africa) was not sufficient to reduce the viscosity of the gruel.

On the other hand, work by Lorri & Svanberg (1993) showed a reduction in the viscosity of gruels. They found that the viscosity of sorghum porridge was reduced considerably when the pH was lowered to pH 3.6-3.8 during fermentation with a culture of *Lactobacillus plantarum*. As a result the amount of flour required to produce a porridge of semi-liquid consistency was increased to 14-17% compared to 10-14% in the unfermented porridge.

To sum up, it appears as if the effect of reduced pH on viscosity depends on the method of fermentation and the type of micro-organism responsible.

2.7 Yoghurt - An example of a fermented dairy food

2.7.1 Background

Yoghurt is a traditional food in the Balkans and the Middle East but its popularity has spread throughout the world (Tamime & Deeth, 1980). Yoghurt can be manufactured using milk from cows, sheep, goats, buffalo and camels.

2.7.2 The traditional procedure for making yoghurt

The procedure for making yoghurt has evolved from the traditional fermentation which was carried out under ambient conditions (Figure 3). It involved the successive inoculations of the starter culture. The bacteria whose growth was encouraged under these conditions were *Leuconostoc cremoris*, *Lactobacillus casei* and *Lactobacillus plantarum* (Odunfa & Oyewole, 1998). These were natural contaminating micro-organisms present in the milk or residing in the cracks and crevices of the milk containers (Thunell & Sandine, 1985). One of the set-backs of this procedure was that the low incubation temperatures (ambient) resulted in slow acidification of milk. This led to undesirable side-effects such as whey syneresis that adversely affected the quality of the yoghurt. Another set-back was that the procedure provided no control over the level of lactic acid produced during the manufacture (Tamime & Deeth, 1980).

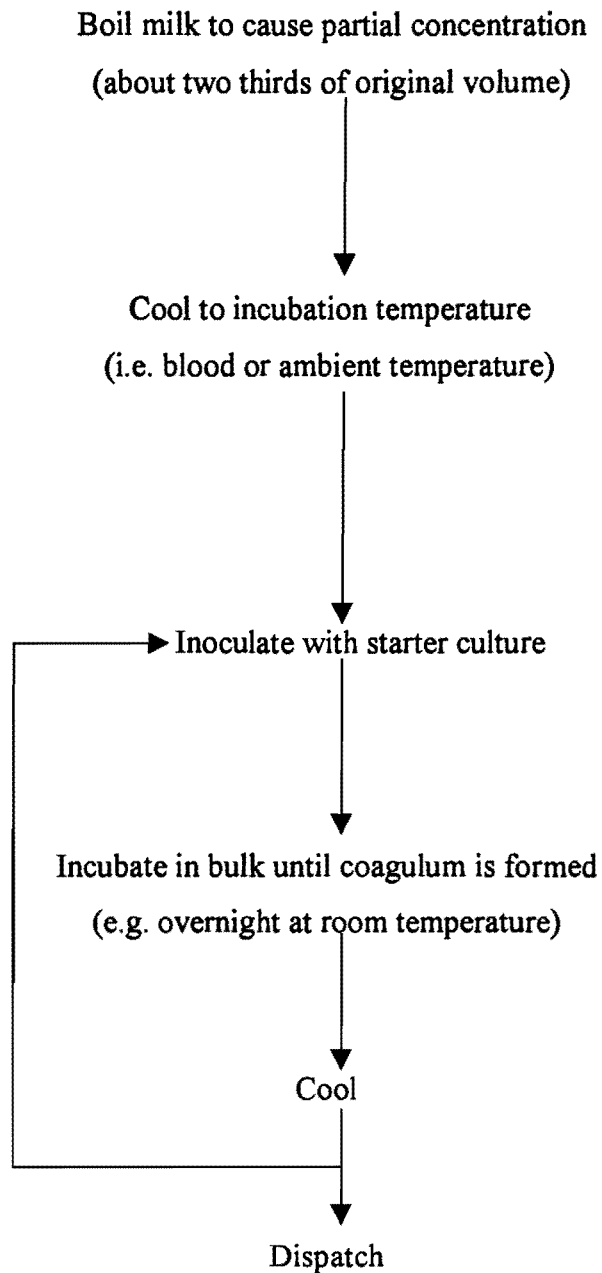


Figure 4. The traditional procedure for making yoghurt (Tamime & Robinson, 1985)

2.7.3 The modern process for making yoghurt

Yoghurt is a coagulated milk product obtained by lactic acid fermentation of milk, with or without additives (whole milk powder, skim milk powder or whey powder) through the action of *Lactobacillus delbruckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (Sharma & Prasad, 1986). In the modern large and small-scale manufacture of yoghurt, the process involves a number of distinct steps (Figure 4).

Increasing the total solids content of milk increases the firmness and viscosity of yoghurt. It is often considered that unless the solids content of milk is increased to 16-18%, the yoghurt gel will be weak and prone to syneresis (Tamime & Deeth, 1980). The solids content of milk can be increased by addition of skim-milk powder, whey protein concentrate or sodium caseinate (Tamime & Robinson, 1985).

Yoghurts can have fat contents ranging from 0.5 to 10%. Fat gives the perception of creaminess and improves the mouth feel of the yoghurt products. Homogenisation of milk for yoghurt manufacture prevents fat separation during storage, improves consistency, increases whiteness and reduces whey separation (Lampert, 1964).

Heat treatment of milk is considered to be one of the most important parameters affecting the texture of acid-milk gels such as yoghurt. Optimum conditions for heating milk are a temperature of 80-85°C with a holding time of 30 min. The objectives of heating are:

1. To eliminate any micro-organisms that might compete with the starter culture to be added later and/or lead to spoilage of the retail product.

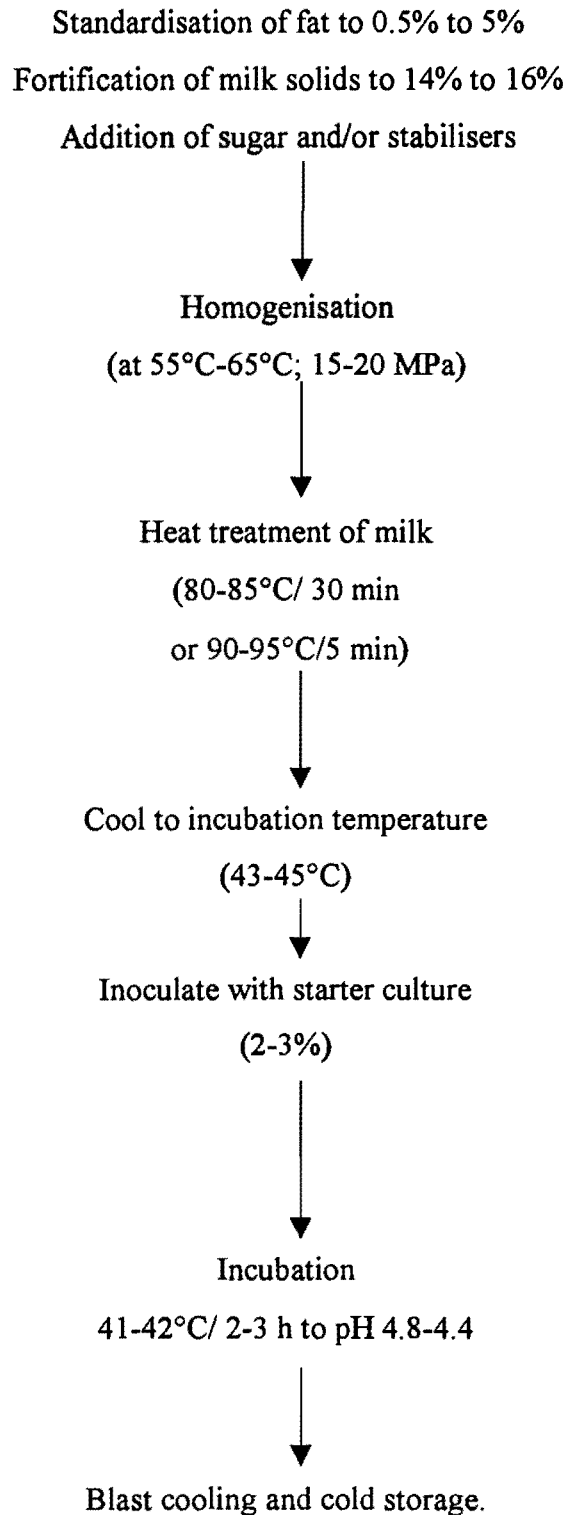


Figure 5. Flow diagram for the manufacture of set-style plain yoghurt (adapted from Deeth & Tamime, 1981)

2. To induce chemical changes in milk such as expulsion of oxygen and release of free amino acids that encourage the rapid development of starter micro-organisms.
3. To modify the milk proteins in such a way that the physico-chemical properties of the yoghurt are altered. Milk is cooled to 42-43°C prior to inoculation (Lampert, 1964; Tamime & Deeth, 1980).

During the process of fermentation, live bacteria are involved and both the cells and enzymes remain active in the final product (Rosenthal, 1991). The characteristics of the coagulum formed are determined largely by the behaviour of the proteins present in the milk. The incubation temperatures of yoghurt are usually in the range of 40-45°C. The final pH of most yoghurts varies from 4.0 - 4.6. After the pH of the yoghurt has decreased to the desired level, the gel is cooled to less than 10°C (Van den Berg, 1988).

The two cultures commonly used in the manufacture of yoghurt are *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (Singh & Sharma, 1982; Malaton & Sandine, 1986). They are both essential for proper development of flavour and aroma. Lactic streptococci are nutritionally fastidious and require an exogenous supply of pre-formed leucine, valine, methionine, arginine, histidine, glutamic acid and in some cases phenylalanine, proline and cystine (Akpemado & Bracquart, 1983; Novak, Cocaign-Bosquet, Lindley & Loubiere, 1997). Free amino acid concentrations in milk are too low to support the starter growth required in the manufacture of fermented milk products (Law & Sharpe, 1978). The amino acids essential for *Streptococcus salivarius* subsp. *thermophilus*, are liberated by the *Lactobacillus* (Rajagopal & Sandine; Abu-Tarboush, 1996). Formic acid produced by *Streptococcus thermophilus* during fermentation is believed to stimulate acid production by *Lactobacillus delbrueckii* subsp. *bulgaricus* (Bottazzi, Battistotti & Vescovo, 1971).

2.7.4 Biochemical changes that occur during yoghurt fermentation

In milk, lactose is the only carbohydrate available for the production of energy by lactic acid bacteria. The initial step by *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* is the transport of lactose through the cell membranes before utilisation by the intracellular enzyme systems. Lactose is hydrolysed inside the bacterial cell by the enzyme β -galactosidase to glucose and galactose. Glucose is metabolised through the Embden Meyerhof Pathway to pyruvic acid which in turn is converted to lactic acid by the action of the enzyme lactose dehydrogenase. The metabolism of galactose is not clear. It accumulates in yoghurt and is not catabolised to any great extent (Pederson, 1971).

The two main roles of the starter culture during the manufacture of yoghurt are the production of lactic acid and development of flavour in the product. The major flavour components are the carbonyl compounds, acetone, acetoin and diacetyl. The presence of acetaldehyde is also important for good yoghurt flavour. Other compounds associated with flavour enhancement in yoghurt are volatile fatty acids and amino acids (Marth, 1974).

The lactic acid helps to destabilise the casein micelle and this leads to coagulation of the milk protein and formation of the yoghurt gel. The lactic acid also gives the sharp taste to yoghurt and contributes towards the typical aromatic flavour (Herrington, 1948).

2.7.5 The role of protein in the formation of the coagulum

The proteins of milk can be divided into two broad categories namely caseins and serum proteins. Caseins are defined as phosphoproteins which precipitate at pH 4.7 while serum proteins are soluble under these conditions (Walstra & Jenness, 1984). Cows' milk contains about 30-35 g protein per litre of which 80% is present as casein micelles (McMahon & Brown, 1984).

The caseins form aggregates, known as micelles, of up to 680 nm in diameter with a very wide size distribution (Griffin & Anderson, 1983). In cows' milk, casein micelles occur in colloidal dispersion. They are highly hydrated, sponge-like and can bind about 3.7 g water/g protein. Very little of the water is bound to the protein. The remainder is occluded within the micelle and moves with the micelle (Fox, 1989).

The caseins consist of four principal proteins which are α_{s1} -, α_{s2} -, β - and κ - caseins (Dalgleish, 1982; Swaisgood, 1989). Caseins have entirely different polypeptide chains and this accounts for the different properties which they exhibit and the different roles that they have during the manufacture of fermented dairy products (Swaisgood, 1989). All caseins are phosphorylated to varying extents (Snoeren, Van Narkwijk, Van Montfort, 1980).

During the fermentation of milk, the colloidal calcium phosphate in the casein micelles progressively solubilises and aggregation of the casein occurs as the isoelectric point (pH 4.6) is approached (Mulvihill & Fox, 1990; Tamime & Robinson, 1999). According to Mulvihill & Fox (1989), the κ - and β -caseins from the micelles gradually diffuse into the aqueous phase of the milk. A size-determining frame-work of α_{s2} -caseins remains intact. As the pH decreases, the released caseins become positively charged and are reabsorbed on the surface of the negatively charged α_{s2} -caseins leading to the formation of particles that are different from the original micelles in milk. As the pH decreases further the charge on the α_{s2} -caseins decreases and the particles aggregate into chains and clusters to form the final network. Denatured β -lactoglobulin associates with κ -casein and filamentous appendages are formed on the surface of the micelles. This complex is probably formed by disulphide bonding (Davis, Shankar, Brooker & Hobbs, 1978) and it is believed to protect the micelles from excessive fusion during fermentation (Dannenburg & Kessler, 1988). At a molecular level, an acid-set gel is held together by hydrophobic forces between casein molecules along with some specific interactions provided by hydrogen bonds and ionic interactions (Johnston, Austin & Murphy, 1993).

2.8 *Kishk* - An example of a cereal-dairy composite food

2.8.1 Background

Kishk is a very popular fermented wheat-milk composite common in Egypt and other Middle East countries such Syria, Lebanon and Jordan (Odunfa, 1985). In Turkey it is known as *tarhana* and in Iraq as *kushuk*. It is made by mixing fermented buttermilk with boiled, dried and ground wheat grains (Hafez & Hamada, 1984). It consists of small, round or irregular pieces, yellowish brown in colour, which have a rough surface and hard texture. When moistened, *kishk* becomes white and breaks up as it absorbs moisture. It is used to make a refreshing drink when reconstituted with water. Dried *kishk* is shelf-stable and can be stored for up to three years under ambient conditions in open clay jars (Abou-Donia, 1984). The low moisture content (less than 10%) and low pH of 4.2 of the final product are a safeguard against the growth of pathogenic micro-organisms (Hamad & Fields, 1982).

2.8.2 The traditional process of making *kishk*

The first stage is the preparation of the sour buttermilk, known as *laban zeer*, by processing milk directly into butter. In winter, the buttermilk is used to make cheese. In summer, when temperatures are high, milk becomes contaminated and bacterial loads are high. This leads to the milk coagulating before the butter and cheese have been produced (Tamime & Robinson, 1978). The coagulated milk is stored in earthenware pots. The walls of the pots are porous and thus the moisture evaporates from the buttermilk. The sour buttermilk thickens and salt is added to taste (Abou-Donia, 1984). The pH of the buttermilk drops to 3.5-3.8 while the corresponding titratable acidity ranges from 1.3 to 1.6%. Acetoin is not formed during fermentation (Figure 6)(El-Gendy, 1983).

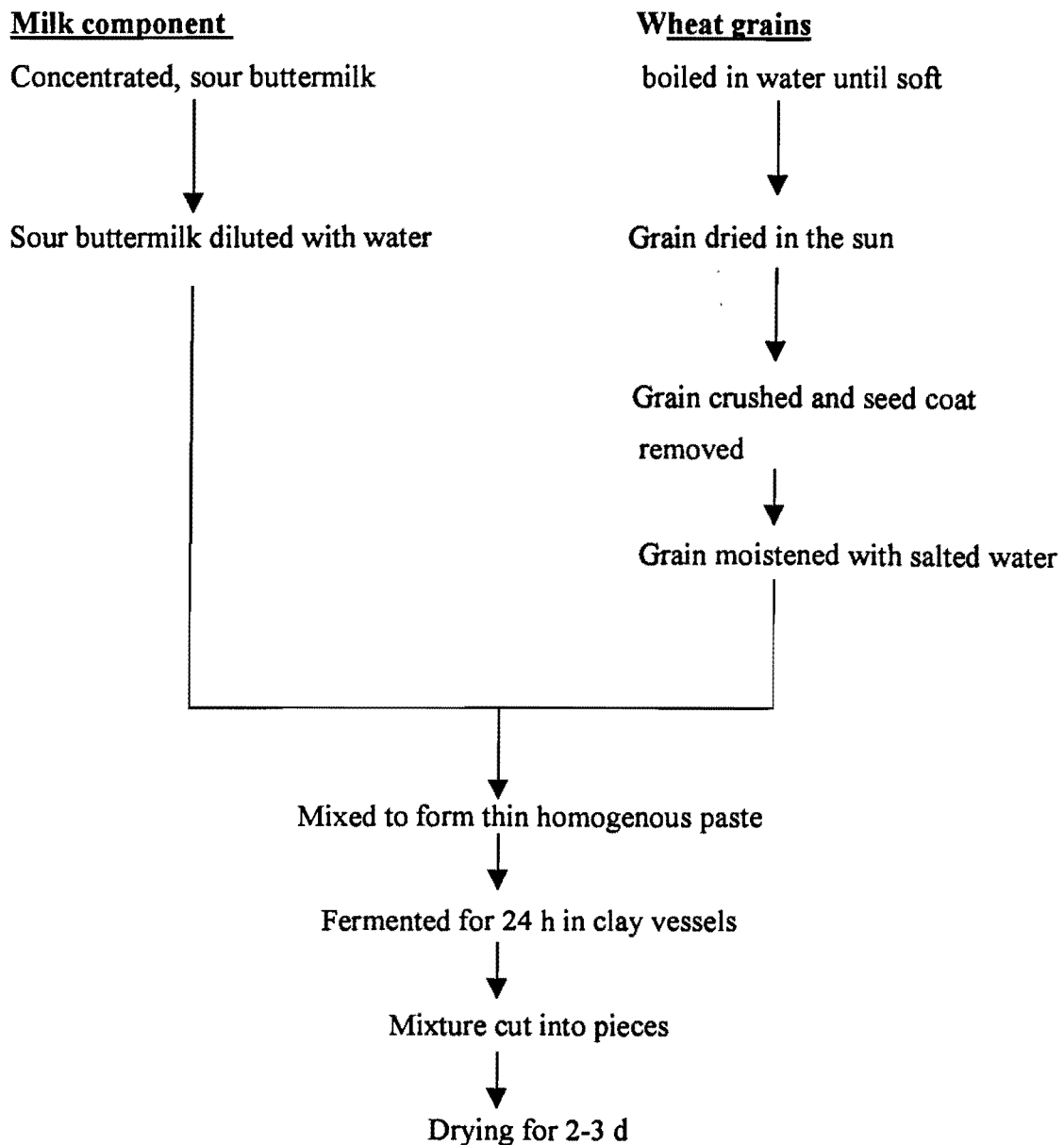


Figure 6. Traditional process for making *kishk* (El-Gendy, 1983)

The second stage involves the preparation of the wheat grains. Fresh wheat grains are boiled until they are soft. Cooking gelatinises the starch. The cooked grains are spread on mats, dried in the sun and ground. After removal of the seed coat, the wheat grains are placed in a large earthenware container and moistened with slightly salted boiling water (El-Gendy, 1983).

The fermented buttermilk is diluted with raw milk or water and added to the wheat grain until a thin homogenous paste of creamy consistency is obtained. The earthenware container is covered with heavy wool cloth and left for 24 h. The mixture of wheat and milk ferments and its volume increases. At the end of fermentation the pH will have dropped from 4.7 to 4.2 and the titratable acidity will be 1.9%. The fermented mixture is thoroughly mixed, divided into small round or irregularly shaped pieces and dried in the sun on straw mats for 2-3 d (El-Gendy, 1983).

2.8.3 The microbiology of traditionally manufactured *kishk*

When the mixture of fermented buttermilk and wheat grains is left for 24 h, it contains a dense population of *Bacillus subtilis* and *Bacillus megaterium*. Although cooking the wheat grains reduces the microbial flora, the population of *Bacillus* species develops during drying. *B. subtilis* produces diastase which increases the reducing sugar content of the mix. The fermented buttermilk is a source of homo- and heterofermentative bacteria. Examples of these bacteria include *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus brevis*. The reducing sugars provide the energy source for the development of lactic acid bacteria. As acidity develops, the *Bacillus* spp. as well as other bacteria are completely inhibited by the high acidity (Abou-Donia, 1984).

Yeast counts increase during fermentation. They produce alcohol and carbon dioxide. The gas is responsible for the increase in the volume of *kishk* during fermentation. Yeasts also synthesise B-vitamins.

2.8.4 The manufacture of a modern '*kishk*-like' product

A *kishk*-type product has been developed using a starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at 45°C. Use of a thermophilic starter culture leads to more rapid acid production which suppresses the growth of spoilage and potentially pathogenic bacteria. The procedure used is summarised in Figure 7. The milk is heated for 30 min at 85°C to kill micro-organisms that might compete with the starter culture during fermentation and then



cooled to a temperature at which inoculation can be safely done without destroying the culture. Cooking of the wheat under pressure ensures complete gelatinisation of starch. The protein content of this product compared with that of the traditional product is lower (17% compared to 24%). The carbohydrate content is higher for the *kishk*-like product at 71% compared to 60% in the traditional product (Robinson & Tamime, 1985). The main disadvantage of the *kishk*-like product is that it has lower levels of lysine and threonine . This may be attributed to the process of roller drying that the product goes through to remove moisture.

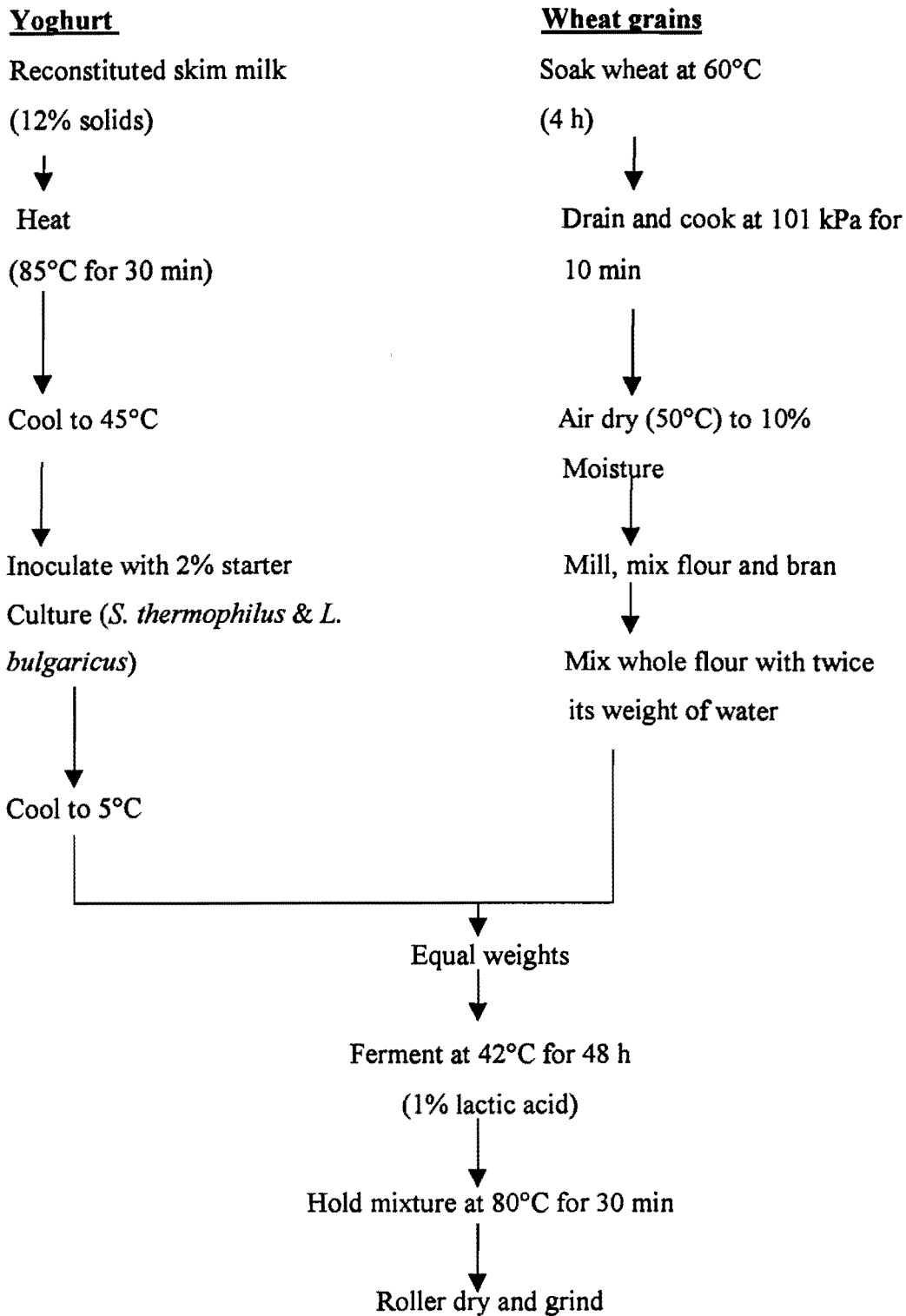


Figure 7. Flow diagram of a method for the manufacture of a *kishk*-like product (Robinson & Tamime, 1985)

2.9 Concluding remarks

Cereals are known to be low in proteins and deficient in essential amino acids especially lysine. Fortification with high protein sources can be used to overcome this problem. Fortification has been successfully used to improve the protein and amino acid content of fermented foods made with sorghum and maize. Some of the high protein sources that have been used include cowpea, groundnuts, soyabeans, locust bean meal and cashew nut meal. While the use of milk, skim-milk powder, whey protein and sour milk products has been suggested for fortifying *mahewu*, little is known about fortification of finger millet with dairy products and how fortification influences the nutrient content.

The lactic acid produced during fermentation lowers the pH of the fermented gruels to 4.5 or lower. This inhibits the growth of pathogenic bacteria that have been implicated in diarrhoeal diseases in infants and children. Fermentation also slows down the rate of microbial spoilage. It has been observed that the use of starter cultures to ferment composites does not lead to a pH decrease to 4.5 and lower especially when one of the ingredients is milk. The buffering capacity of milk proteins slows down the decrease in pH. There is a need to determine what cultures can be used to ferment both the lactose and starch in cereal composites to sufficiently reduce the pH and produce a safe product. It is important to establish the optimal conditions at which pH reduction occurs and temperature is one of the most important factors. Low incubation temperatures increase the risk of contamination with spoilage and pathogenic bacteria. Time of incubation is another factor and prolonged incubation can lead to over-acidification.

At the same time, the effect of reducing pH on some of the physico-chemical characteristics of the fermented products (e.g. viscosity, consistency and firmness which might affect acceptability of the product) need also to be determined. Firmness influences the amount of solids that can be incorporated into the gruels. This affects the nutritional value of the gruels. Fermentation that leads to an increase in viscosity

of gruels limits their nutrient content while fermentation that leads to an increase in the solids content has the potential to improve the nutrient content of the gruels.

CHAPTER 3

MATERIALS AND METHODS

3. 1 Sample preparation

3. 1. 1 Cereal and skim milk samples

Traditionally processed finger millet (*Eleusine coracana*) meal was purchased from rural farmers in Manicaland, a province in the Eastern Highlands in Zimbabwe. The traditional process of making finger millet meal involved roasting of the grain to loosen the pericarp and to develop flavour. The pericarp was removed by pounding with the traditional mortar and pestle. This was followed by winnowing to separate the loose pericarp from the clean grain. The clean grain was then milled using hammer milling. The finger millet meal was stored in sealed plastic bags at 10°C until required. Skim-milk powder was sourced in South Africa and stored under cool dry conditions until required.

Table 1 shows the proximate composition of the finger millet meal and the skim-milk powder.

Table 1. Proximate composition of finger millet meal and skim-milk powder*

Component (g/ 100 g DM)	Finger millet	Skim-milk Powder
Protein	8.9	32
Crude fat	3.3	1
Ash	3.1	8
Starch	66.2	nd
Lactose	nd	48
Crude fibre	6.1	nd

*based on analysis

nd - not detected

3. 1. 2 Preparation of cereal-dairy composite gruels

Skim-milk powder was reconstituted, according to the manufacturer's instructions. One part skim-milk powder was added to nine parts water and stirred until completely dispersed. The finger millet gruel was prepared by cooking five parts of finger millet meal in 95 parts of water at low heat for 10 min while stirring continuously. Proportions of finger millet greater than 5% gave a gruel that solidified on cooling. The reconstituted skim milk was added to the gruel to give composites containing 100%, 90%, 80%, 70%, 60%, 50% and 0% (skim milk only) finger millet gruel by volume. The unfermented gruels were sterilised by autoclaving for 15 min at 121°C.

3. 2 Starter cultures

Three starter cultures were used for fermentation. YC380 (Chr. Hansen, Denmark) and Joghurt V2 (Wiesby, Denmark) were commercial yoghurt type starter cultures that were supplied by Darleon CC. in South Africa. They are often used in the dairy

industry in South Africa for the manufacture of yoghurt. According to the suppliers, they contain strains of the bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* subsp. *salivarius*. Both cultures are mixed cultures with Gram positive cocci and rods. The third starter culture, JC, was a mixed strain culture developed in our laboratory over a period of time to ferment raw cereal slurries at 25°C. Table 2 summaries the characteristics of the three starter cultures

Table 2. Some characteristics of starter cultures YC380, V2 and JC

	YC380	V2	JC
Description	A mixture of rods and cocci.	A mixture of rods and cocci.	Predominantly rods with some in chain formation, some cocci, occurring in clusters.
Growth on agar	Grew on acidified MRS agar as round, whitish colonies	Grew on Rogosa agar as pin-head round, whitish colonies	Grew on acidified MRS agar as round, whitish colonies
Gram reaction	+ ve	+ ve	+ ve
Catalase reaction	- ve	- ve	- ve

3. 3 Starter culture propagation

Bacterial starter cultures YC380 and V2 were each added to sterilised, reconstituted skim milk at a rate of 1 g of starter culture per 99 ml of skim milk. This was done under aseptic conditions. The mixture was thoroughly mixed and incubated at 45°C for 3 h. The fermented products were cooled to 5°C. These preliminary yoghurt cultures were subcultured into sterilised skim milk at a rate of 1 ml of culture per 99 ml of skim milk. After thorough mixing they were incubated at 45°C for 3 h and then

cooled to 5°C. Subculturing was repeated twice leading to batches of yoghurt culture, hereafter referred to as Starter Cultures. These were stored in the refrigerator.

The starter culture JC was prepared by mixing 50 g dehulled sorghum meal and 64 ml of water at 30°C into a slurry which was then fermented at 25°C until the pH dropped to 3.6 . The culture was maintained by inoculating a mixture of 50 g of dehulled sorghum meal and 64 ml of water with 5 ml of the fermented slurry.

3. 4 Gruel fermentation

Initially, the inoculated samples were incubated at 10°C, 25°C, 30°C, 37°C and 45°C for 10 h. All analyses were carried out in triplicate. In the second phase of the experiment the optimum time of incubation was determined. This was measured as the time that it took for the pH of inoculated samples to fall to 4.6 . Sterile samples were inoculated with bacterial starter cultures YC380 and V2 at an inoculation rate of 2% (v/v) under aseptic conditions. The samples were incubated at 37°C and 45°C. At 1 h intervals the pH of the samples was determined. The control samples were not inoculated.

3. 5 Gruel inoculation

Inoculation of gruel samples was carried out aseptically at room temperature and each mixed by shaking the bottle. Gruel samples that had been measured to give a volume of 98 ml were inoculated with 2 ml of starter culture to give an inoculation rate of 2%. The samples were thoroughly mixed to ensure even distribution of the inoculum.

3. 6 Freeze-drying

Gruel samples that were used for the determination of crude fat, crude fibre, amino acids and energy were freeze-dried (Specht Scientific, Model no. SJ-FD-5/PC, Asslar, Germany). The freeze-dried samples were milled using a Waring blender and stored in sample bottles at 10°C away from light until required.

3. 7 Physicochemical analyses

3. 7. 1 pH and titratable acidity

Acidity was determined by the potentiometric procedure (International Dairy Federation, 1991) by weighing 10 g of the sample into small beakers, adding 10 ml of distilled water and titrating with 0.1M sodium hydroxide to a pH of 8.3. Acidity was expressed as % lactic acid. The pH was determined using a combined glass electrode connected to a Mettler DL-25 pH meter (Mettler-Toledo AG, Greifensee, Switzerland) by immersing the electrode directly into the gruel sample.

3. 7. 2 Syneresis

The % syneresis (v/m) was determined by a modification of the centrifugation procedure described by Harwarkar & Kalab (1983). Samples of the fermented gruel, which had been incubated at 30°C, 37°C and 45°C, were chilled to 4°C for 24 h and then centrifuged at 1000 x g for 20 min. The lower temperatures of incubation (10°C and 25°C) that were used for pH determination were left out because the gruels fermented at these temperatures did not attain the desired pH of 4.5 or lower. The volume of serum (cm³) was expressed as a percentage of the mass (g) of the sample centrifuged.

3. 7. 3 Consistency

The consistency of the fermented gruel incubated at 37°C and 45°C was determined as the distance flowed, in centimetres, within a time period of 30 s using a Bostwick consistometer (Gould, 1974). This instrument is used widely by the dairy industry in South Africa for quality control.

3. 7. 4 Gruel firmness

Gruel firmness was measured as the maximum force required to penetrate undisturbed gruel in glass containers using a texture analyser (TA-XT2; Stable Microsystems, Surrey, England) and a cylindrical probe which had a base diameter of

20 mm (Hess, Roberts & Ziegler, 1997). The containers were half filled and had a maximum capacity of 55 cm³, a base diameter of 35 mm and a height of 70 mm.

3. 8 Proximate analyses

3. 8. 1 Moisture

A commonly used procedure for determining the moisture content of a food product is based on the separation of water from the solids and its measurement as the resulting loss in weight or by measurement of the amount of water lost (Joslyn, 1970). The accurate determination of moisture is difficult because of the problem of completely separating all the water from the food product without completely causing its decomposition with concomitant production of water which would be included in the determination (Joslyn, 1970). The loss of volatile constituents from the food is another factor. A modification of the oven drying method was used (AOAC, 1980a).

A sample of known mass was dried in an oven at 100°C for 3 h. This was followed by cooling in a desiccator to room temperature and the weighing of the cooled sample. The moisture content was determined as loss in moisture using the following formula:

$$\% \text{ Moisture} = \frac{A \times 100}{B}$$

Where A was moisture loss in grams and B was the original weight of the sample.

3. 8. 2 Ash

Ash was determined as the residue that remained after all the moisture had been removed and the organic material (including fats, proteins, carbohydrates, vitamins and organic acids) had been combusted at a temperature not less than 550°C to carbon dioxide and oxides of nitrogen (James, 1995). Ash content was calculated as follows:

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

3. 8. 3 Fat

Fat in skim milk was determined using a modification of the Babcock test (Davis, 1959). Milk fat exists in milk as an emulsion of the oil-in-water type. In the modified Babcock procedure, sulphuric acid and alcohol are added to coagulate the protein and to remove the phospholipid layer around the fat globule. This breaks the emulsion and separates the fat allowing it to coalesce (Joslyn, 1970). Hot water is added to raise the liberated fat layer above the water layer.

To 17.6 ml of milk, 4 ml iso-butyl alcohol was added. This was followed by thorough mixing and addition of 17.5 ml of sulphuric acid (specific gravity 1.82-1.83). The mixed sample was centrifuged for 6 min in a Babcock centrifuge to separate the fat from the water component of the emulsion. The flask was filled with hot water and centrifuged for a further 14 min. The contents of the flask were rapidly mixed and centrifuged for 10 min after which a reading was taken.

3. 8. 4 Crude fat

Fats, oils and fatty acids are characterised by their extreme insolubility in water, very slight solubility in alcohol and by the readiness with which they are dissolved by ethyl ether, petroleum ether, carbon disulphide and carbon tetrachloride (Joslyn, 1970). The Soxhlet apparatus is constructed to permit the passage of the vapours of the solvents into the condenser by a separate tube and return of the condensed solvent after having stood in contact with the sample, to the evaporating flask by an external siphon. The advantage of the process lies in freeing the sample entirely from the rise in temperature resulting from contact with the hot vapours of the solvent (Joslyn, 1970).

Crude fat was determined on freeze-dried gruels using a modification of the AOAC procedure (AOAC, 1980b). Samples were milled using a Waring blender.

Approximately 4 g of thoroughly mixed sample was weighed onto filter paper. The filter paper was folded and inserted into an extraction thimble. Fat-free cotton wool was used to plug the extraction thimble. Extraction was carried out for 4 h on a Soxhlet extraction unit using petroleum ether (boiling point 40-60°C). The extract was dried at 100°C for 30 min, cooled and weighed. Crude fat was then calculated as follows:

$$\% \text{ Crude fat} = \frac{(\text{weight of flask + fat}) - \text{weight of flask}}{\text{weight of sample (g)}} \times 100$$

3. 8. 5 Crude protein

The Kjeldahl procedure for the determination of nitrogen in biological materials is characterised by the use of boiling, concentrated sulphuric acid to effect the oxidative destruction of the organic matter of the sample. The acid also reduces organic nitrogen to ammonia. The process is facilitated by the use of a catalyst. The ammonia is retained in the acid digest as ammonium bisulphate (Chang, 1994). The digest is made alkaline using sodium hydroxide and the ammonia is distilled off. The ammonia is measured by titration (Lillevik, 1970).

The traditional method of estimating the protein content of a food is to multiply its content of nitrogen by a suitable conversion factor. This factor will vary according to the nitrogen content of the particular proteins and can vary from 12 to 30%. The factor of 6.25 is generally used and it would apply to a protein containing 16% nitrogen (Lillevik, 1970) For dairy products, a factor of 6.38 is used (Pomeranz & Meloan, 1994).

Samples were analysed for crude protein using a Kjeldahl method. Approximately 0.5 g sample was weighed accurately into a digestion flask. One Kjeltab (Thompson & Capper, Cheshire, England), consisting of 100 parts K₂SO₄, 6 parts CuSO₄.5H₂O and 2 parts selenium was added. To this, 20 ml of concentrated H₂SO₄ was added. Samples were digested for approximately 2 h using a Büchi 430 Digester (Büchi,

Flavil, Switzerland). Distillation of ammonia, reaction with boric acid and titration with standard HCl (0.1M) were done with a Büchi 322 Distillation Unit (Büchi, Flavil, Switzerland). The crude protein content was calculated as follows:

$$\% \text{ Protein} = \frac{(\text{ml std NaOH} \times \text{M of NaOH}) \times \text{factor} \times 1.4007}{\text{weight of sample (g)}}$$

One of the products that was analysed was a composite containing cereal and milk hence the factor that was used was 6.38 .

3. 8. 6 Crude fibre

The hot sulphuric acid and sodium hydroxide used in the determination of crude fibre precipitates protein, hydrolyse the protein into smaller molecules that are removed through washing. The reagents also hydrolyse starch and pectin into smaller molecules that are soluble and can be removed by washing and remove mineral matter that is not bound in the cell wall (Woodman, 1941). The acetone wash helps to remove water that is not bound to the cellulose prior to drying.

Fibre was determined using a Fibertec System M (1020 Hot extractor, Tecator AB, Hoganas, Sweden). A ground sample (2 g) was weighed into a crucible that had been left in a muffle furnace at 500°C for 1 h and then cooled. The first extraction was carried out for 30 min using 150 ml of pre-heated sulphuric acid (0.128M). The sample was rinsed three times with hot water and then filtered. The second extraction was carried out for 30 min using 150 ml of hot potassium hydroxide (0.128M). The sample was washed with hot water and filtered, washed three times with acetone and dried at 100°C overnight. The dried sample was ashed in a muffle furnace at 500°C for 3 h and then cooled in a desiccator followed by weighing.

3. 8. 7 Energy

The energy (measured as heat of combustion) content was measured on 0.5 g freeze-dried sample. A waterless bomb calorimeter was used (dds, model no. CP 500,

Midrand, South Africa). The sample was completely combusted in the presence of oxygen at a pressure of 3000 kPa.

3. 8. 8 Lactose

A sample (1 g) was weighed into a 100 ml volumetric flask, 60 ml of water was added followed by 5 ml of Carrez I solution (potassium hexacyanoferrate (II) 85 mol/l $K_4[Fe(CN)_6] \cdot 3H_2O$) and 5 ml of Carrez II solution (zinc sulphate, 250 mmol/l $ZnSO_4 \cdot 7H_2O$). The contents of the flask were thoroughly shaken and the pH was adjusted to 7.5-8.5 with 0.1 mol/l NaOH. The contents of the flask were again mixed and the volumetric flask filled up to the mark with distilled water. The solution was filtered and the clear filtrate was stored at 7°C.

A lactose/ D-galactose enzymatic kit (Boehringer, Mannheim, Germany) was used for the determination of lactose. Lactose was hydrolysed to D-glucose and D-galactose at pH 6.6 by the enzyme β -galactosidase in the presence of water. D-galactose was converted by nicotinamide adenine dinucleotide (NAD) to D-galactonic acid in the presence of the enzyme β -galactose dehydrogenase. The absorbance of the sample was then read at 340 nm against a reagent blank. The concentrations of lactose and D-galactose in the sample were calculated using the following formula:

$$c = \frac{V \times MW}{\epsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

Where:

c = Lactose calculated as lactose monohydrate / D-galactose

ΔA = Absorbance of the sample, less the absorbance of the blank

V = final volume (ml)

v = sample volume (ml)

MW = molecular weight of lactose monohydrate / D-galactose

d = light path (cm)

ϵ = extinction coefficient of NADH at 340 nm (= 6.3)

The amount of lactose was determined as the difference between the concentrations of lactose and D-galactose.

3. 8. 9 Total Starch

A total starch assay kit (α -amylase/amyloglucosidase method, AA/AMG 9/97, Megazyme International Ireland Limited, Wicklow, Ireland) was used to determine total starch. The analysis included solubilisation of the starch with dimethyl sulphoxide, hydrolysis with thermostable α -amylase and hydrolysis with amyloglucosidase. The glucose formed was then determined using a glucose oxidase/oxidase reagent (GOPOD). The absorbance was read at 510 nm. The total starch was calculated using the following formula:

$$\% \text{ Starch} = \frac{\Delta E \times F \times 90}{W}$$

Where ΔE was the absorbance read against a reagent blank, F was the conversion from absorbance to μg glucose, 90 the adjustment from free glucose to anhydro glucose (as occurs in starch) and W was the weight of the sample.

3. 8. 10 Amino acids

High performance liquid chromatography uses high pressure to force a solution containing the compounds to be separated rapidly through resin held in a strong metal tube (McDonald, Edwards, Greenhalgh & Morgan, 1995). For the separation of the amino acids, the first stage is the hydrolysis of the proteins using a strong mineral acid such as hydrochloric acid (White, Handler & Smith, 1964). The products of hydrolysis are ammonia and free amino acids. Phenol is added to the acid during hydrolysis as an oxygen scavenger to minimise the destruction of labile amino acids such as cysteine and arginine. Reaction with phenylisothiocyanate produces amino acid derivatives which can be detected in the ultraviolet region below 250 nm (Chiou, 1988). The sample is then dissolved in a solvent to obtain all the amino acids present in the food sample in a liquid phase (Macrae, 1985).

Amino acid analysis was done using the Pico.tag[®] method (Bidlingmeyer, Cohen and Tarvin, 1984). Approximately 10-20 mg of defatted freeze-dried sample was put into a hydrolysis flask into which 1 ml 6M HCl and 1% phenol were added. The flask was evacuated and flushed with nitrogen to remove oxygen and then sealed under vacuum. The sample was left at 110°C for 24 h. After cooling the sample was made up to 5 ml and 25 µl were taken to dry.

Ten microlitres of a mixture containing methanol, water and triethylamine in the ratio 2:2:1 was added to the sample. The sample was mixed, left to dry and 20 µl of a mixture containing methanol, water, triethylamine and phenylisothiocyanate was added to the dried sample. The flask was left at room temperature for 20 min and the sample dried under vacuum to remove excess reagent. The dried sample was dissolved in 200 µl of a solution consisting of 710 mg Na₂HPO₄ made up to 1 litre with water and the pH adjusted to 7.40 using 10% H₃PO₄ and 5% acetonitrile.

Separation of amino acids was done using a reverse phase column (Pico.tag column for hydrolysate amino acid analysis, 3.9 mm x 15 cm). Two pumps were used to create a gradient for optimum separation. The monitor was set at 254 nm.

3. 9 Microbiological tests

3. 9. 1 The Gram stain

A heat-fixed smear was prepared from 18-24 h cultures of YC380, V2 and JC. The smear was stained with crystal violet for two minutes. The crystal violet solution was washed off the smear using Gram's iodine solution and the iodine was allowed to react for one minute. The smear was blotted dry and washed with 95% ethanol until no dye could be detected in the ethanol. The slide was rinsed under running water and counter-stained with dilute carbol fuchsin for ten seconds. The slide was washed with water, blotted and examined using light microscopy (Harrigan, 1998). The crystal violet, Gram's iodine and carbol fuchsin were prepared according to the procedures described by Harrigan (1998).

3.9.2 Catalase reaction

The catalase test is used to identify strains of bacteria that possess the enzyme catalase and can break down hydrogen peroxide to water and oxygen (Harrigan, 1998). The catalase test was carried out on 24 h old cultures of YC380, V2 and JC that had been enriched in nutrient broth. The nutrient broth was prepared according to the manufacturer's instructions (Biolab Merck, Midrand, South Africa). Five millilitres of starter culture were added to 1 ml of freshly prepared 3% hydrogen peroxide in a clean test-tube. The mixture was observed for the formation of bubbles (Harrigan, 1998).

3.9.3 Enumeration of lactic acid bacteria

Sterile peptone water at a concentration of 0.1% was used as the diluent of choice (Houghtby, Maturin, Koenig & Messer, 1992). Using sterile pipettes and following the procedure outlined in Figure 8, serial dilutions of the samples were prepared and plated up to a dilution of 10^{-7} .

MRS (de Man, Rogosa, Sharpe) agar (Merck, Darmstadt, Germany) and acidified Rogosa agar (Merck, Darmstadt, Germany) were used for the enumeration of lactic acid bacteria in the fermented product. The agar media were prepared according to the manufacturers' instructions.

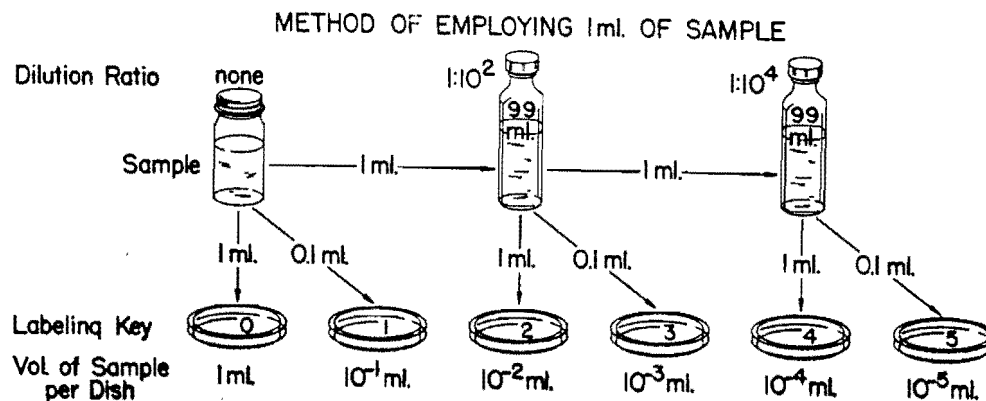


Figure 8. Examples for preparing dilutions (Houghtby, Maturin, Koenig & Messer, 1992)

Molten agar at approximately 45°C was added to petri dishes in which the diluted samples had been added. The agar in the petri dish was thoroughly mixed with the sample and left to cool and solidify before incubation.

The petri dishes were incubated at 37°C for 3 d in an inverted position in sealed anaerobic jars. The anaerobic conditions were created by using Anaerocult A® (Merck, Darmstadt, Germany). The strips of anaerocult were activated according to the manufacturer's instructions.

3. 10 Statistical analysis

The experimental design was a completely random design with the following variables: bacterial starter culture, temperature of incubation, temperature of storage, proportion of finger millet gruel and period of incubation. SAS (1982) was used to analyse the data based on the following model:

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_m + (ab)_{ij} + (bc)_{jk} + (ad)_{im} + (bd)_{jm} + (cd)_{km} + (abc)_{ijk} + (abcd)_{ijklm} + \varepsilon_{ijklm}$$

Where:

Y_{ijklm} = the response variable

μ = the overall mean

a_i = change from the mean value due to the bacterial starter culture (i=1 to 3)

b_j = change from the mean value due to temperature (j=1 to 5)

c_k = change from the mean value due to proportion of finger millet gruel to skim milk (k= 1 to 7)

d_m = change from the mean value due to the effect of storage temperature (m= 1 to 2)

$(ab)_{ij}$, $(bc)_{jk}$, $(ad)_{im}$, $(bd)_{jm}$, $(cd)_{km}$, $(abc)_{ijk}$ and $(abcd)_{ijklm}$ = the effects of interaction and

ε_{ijklm} = the random residual term.



The least squares analysis was used to obtain the least squares estimates of differences between sub units for comparison purposes. A probability level of $p = 0.05$ was used to test the significance of the results.



CHAPTER 4

RESULTS

4.1 Effect of incubation temperature, type of bacterial starter culture and proportion of finger millet gruel on the lactic acid bacteria count of fermented gruels

The lactic acid bacteria count was significantly affected by the proportion of finger millet and the incubation temperature but not by the bacterial starter culture ($p < 0.05$) (Table 3).

For both starter cultures YC380 and V2 in general, there was a slight increase in the bacterial count, as the temperature of incubation was increased from 37°C to 45°C.

The bacterial count generally decreased as the proportion of finger millet gruel increased (i.e. as the proportion of milk decreased).

Table 3. Effect of proportion of finger millet gruel relative to skim milk, temperature of incubation and type of bacterial starter culture on the lactic acid bacterial count of finger millet-skim milk composite gruels

Proportion of finger millet in gruel (%)	Bacterial starter culture			
	YC380		V2	
	Incubation temperature (°C)			
	37	45	37	45
	Lactic acid bacteria count (log₁₀)			
0	8.3	8.5	8.7	8.9
	0	(±0.2)	(±0.1)	(±0.1)
50	7.7	7.6	8.5	8.7
	0	0	(±0.1)	0
100	6.6	6.6	7.3	7.5
	(±0.1)	0	0	0

Figures in brackets are the standard deviation of the mean

4.2 Effect of incubation temperature, type of bacterial starter culture and proportion of finger millet gruel on the physico-chemical characteristics of the fermented gruels

pH

The production of acid was significantly ($p < 0.05$) affected by the temperature of incubation, the proportion of finger millet gruel and the type of bacterial starter culture (Figures 9a-9d).

The lowest pH in the control (no inoculum) was 6.1 (Figure 9a). The gruels that were fermented at 10 and 25°C (lower temperatures) did not achieve a pH of 4.5 or lower, regardless of the proportion of finger millet gruel or starter culture (Figures 9b-9c). A decrease in pH to 4.5 or lower occurred when the gruels were incubated at 30, 37 and 45°C (higher temperatures) (Figures 9b-9c).

Starter culture YC380 produced a pH of 4.5 or lower when the proportions of finger millet gruel in the product were between 0 and 90%, i.e. only when skim milk was present (Figure 9b). The pH was not lowered sufficiently when the proportion of finger millet gruel was 100%. Starter culture V2 effectively lowered the pH of the product to 4.5 or lower at all proportions of finger millet gruel (Figure 9c). Culture JC was more effective in reducing the pH when the proportions of finger millet gruel in the product were between 50 and 100% (Figure 9d). It was not effective when the proportion of finger millet gruel was 0% (skim milk only).

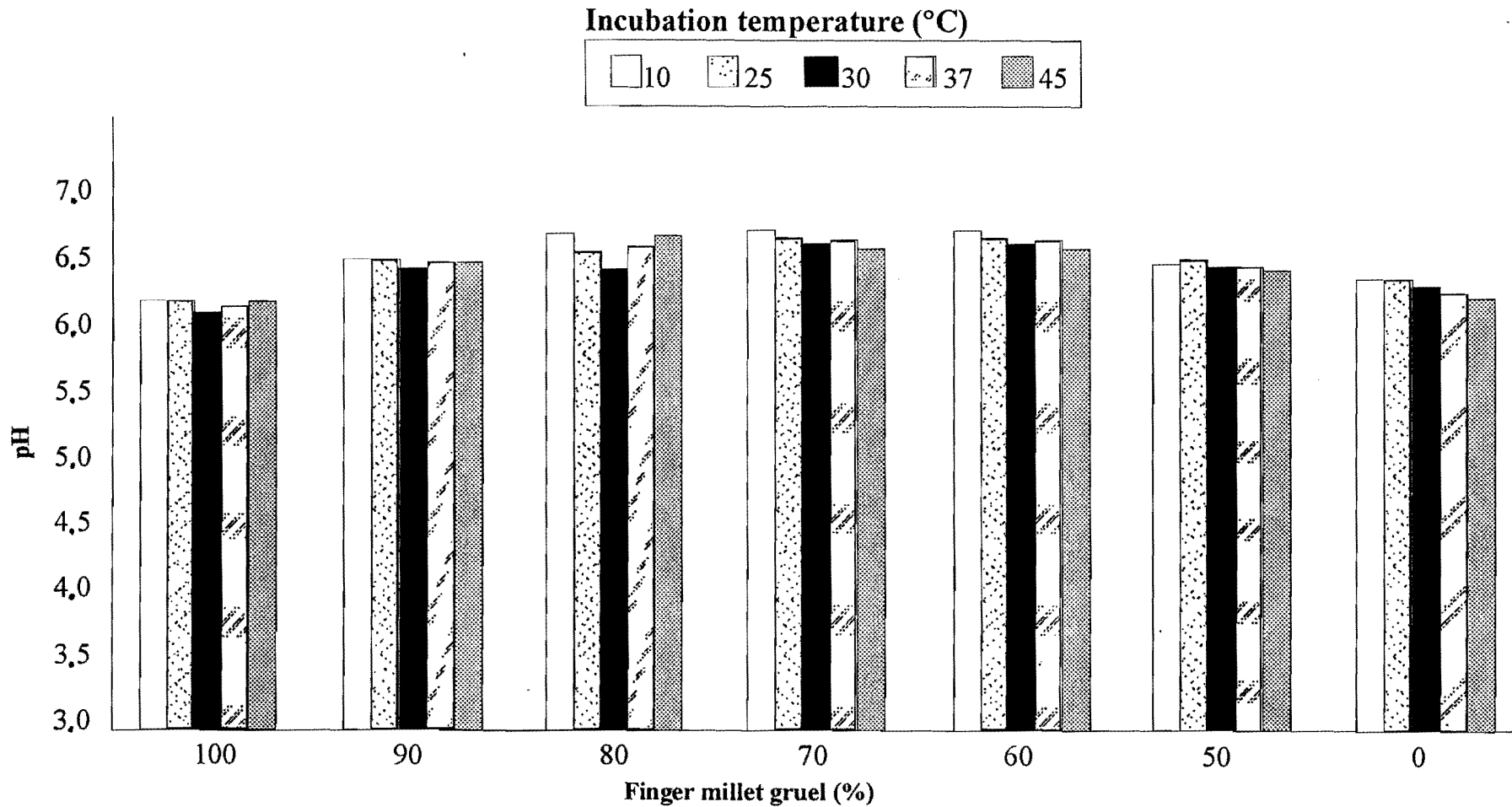


Figure 9a: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the pH of unfermented gruels (Control)

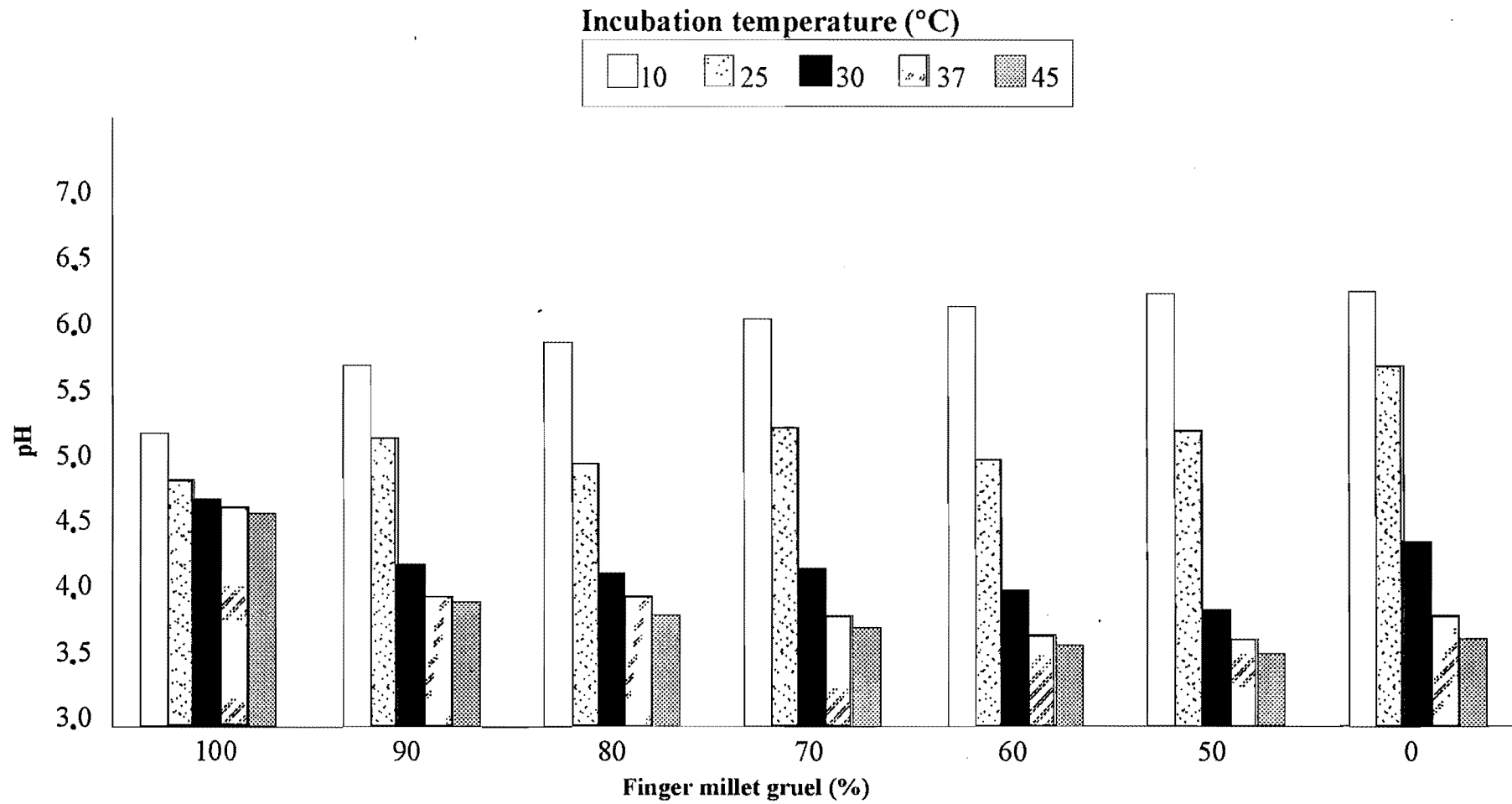


Figure 9b: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the pH of gruels fermented with starter culture YC380

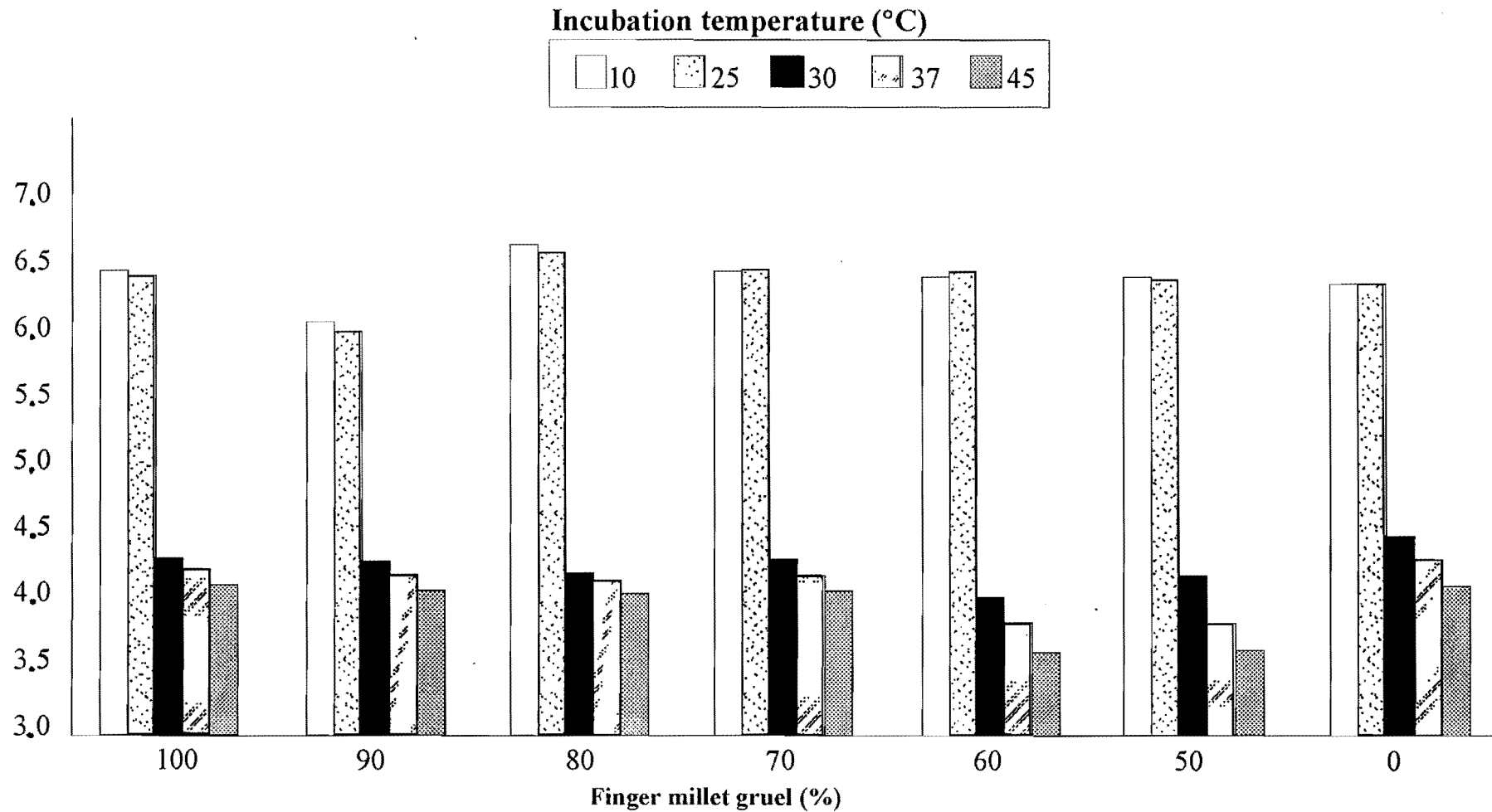


Figure 9c: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the pH of gruels fermented with starter culture V2

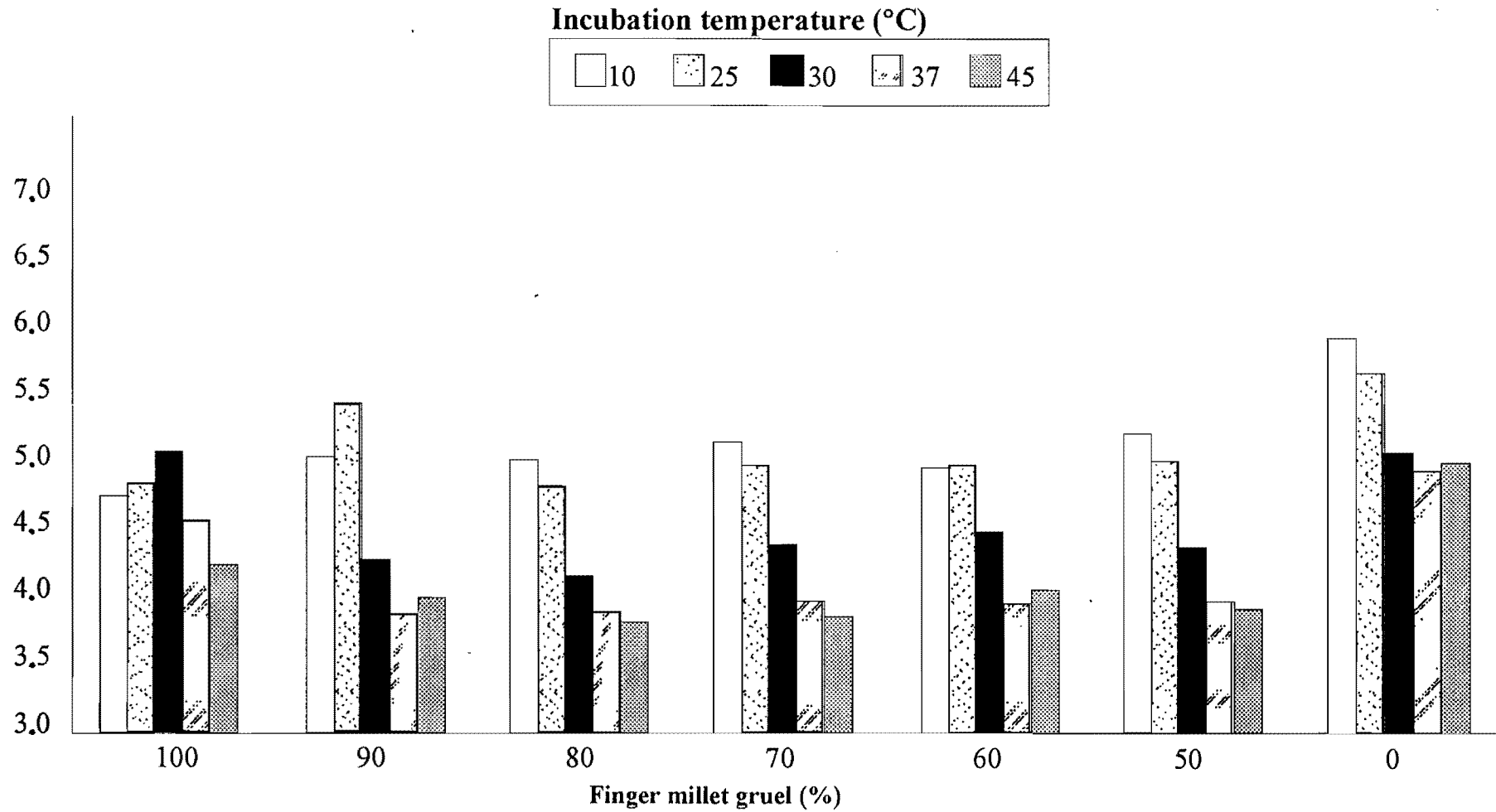


Figure 9d: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the pH of gruels fermented with starter culture JC

Optimisation of incubation time

The time that the gruels took to attain a pH of 4.5 or lower was significantly ($p < 0.05$) affected by incubation temperature, starter culture and proportion of finger millet gruel.

The effects of incubation temperature and proportion of finger millet gruel were similar to those in the experiment in which the incubation period was fixed. The differences were that firstly, the pH of the fermented gruels after 10 h of incubation was lower compared to gruels that were fermented for 6 h. Secondly, the pH of gruels that were fermented at 37°C was not reduced to 4.5 or lower after 6 h of incubation.

The lowest pH in the control (not inoculated) was 5.86 in the product fermented at 37°C and 5.75 in the product fermented at 45°C (Figures 10a and 10d).

Starter cultures YC380 and V2 did not reduce the pH of finger millet gruels after 6 h when the incubation temperature was 37°C (Figures 10b and 10c).

It took 5 h to reduce the pH of the fermented gruels to 4.5 or lower with starter culture YC 380 and 4 h with starter culture V2, but only in gruels where the proportion of finger millet gruel was between 0 and 90% (Figures 10e and 10f).

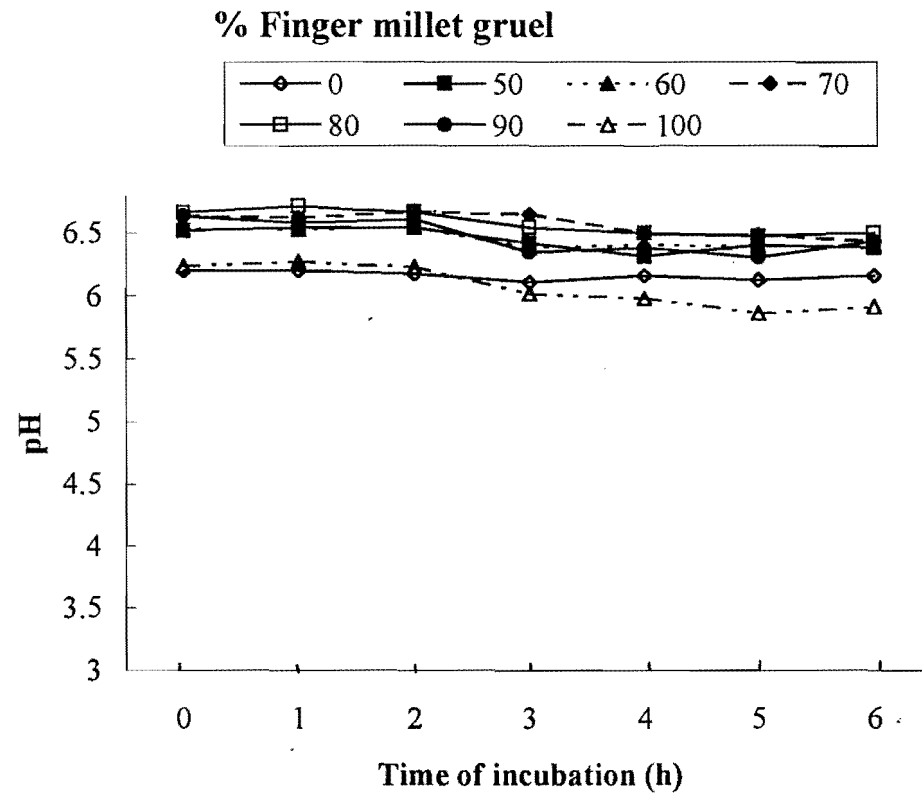


Figure 10a: Effect of the time of incubation and the proportion of finger millet gruel relative to skim milk on the pH of unfermented gruels (control) at 37°C

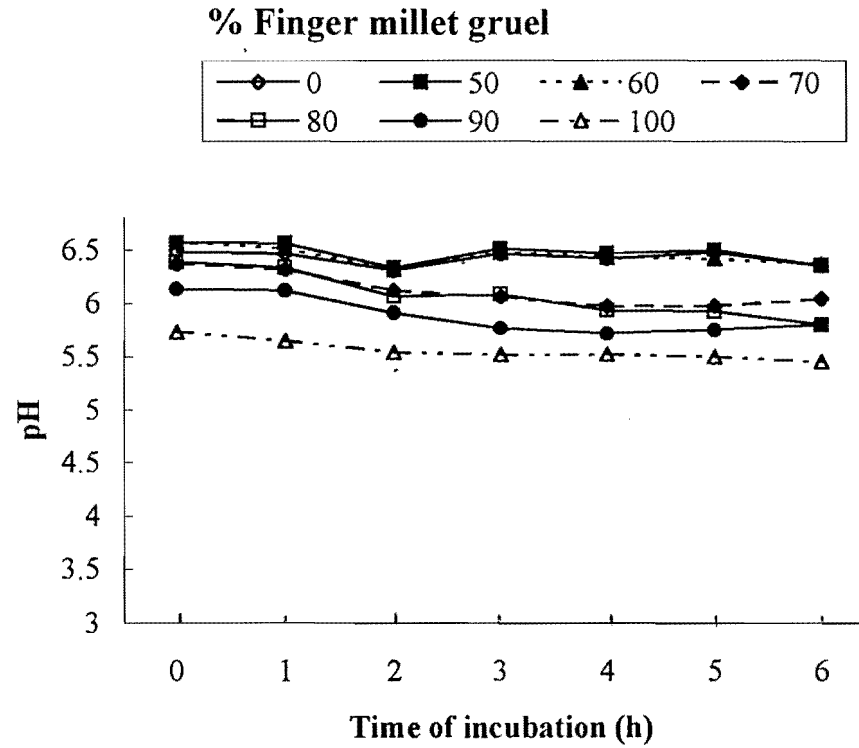


Figure 10b: Effect of the time of incubation and the proportion of finger millet gruel relative to skim milk on the pH of gruels fermented with starter culture YC380 at 37°C

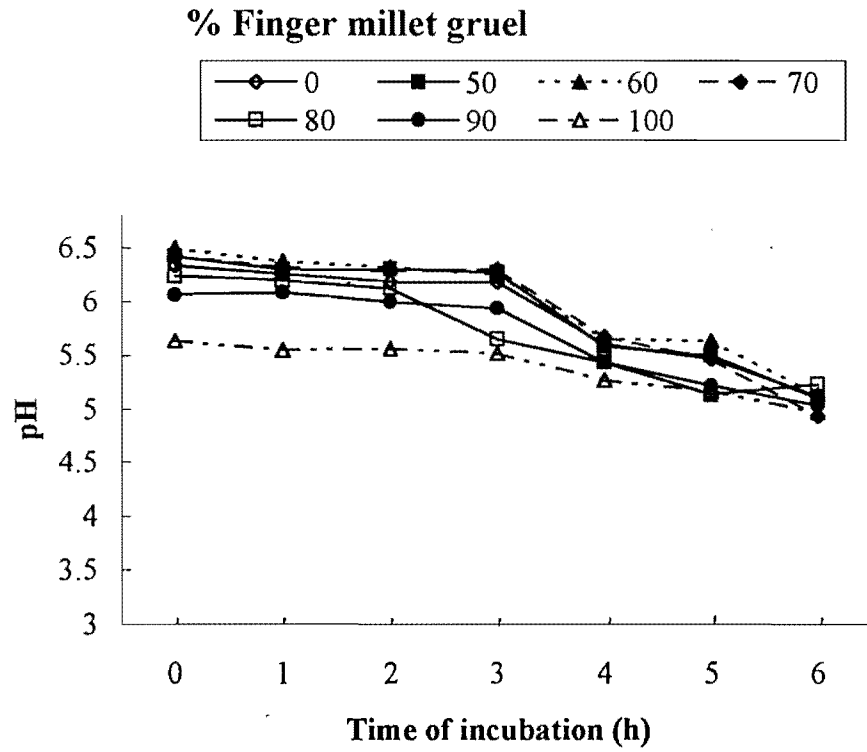


Figure 10c: Effect of the time of incubation and the proportion of finger millet gruel relative to skim milk on the pH of gruels fermented with starter culture V2 at 37°C

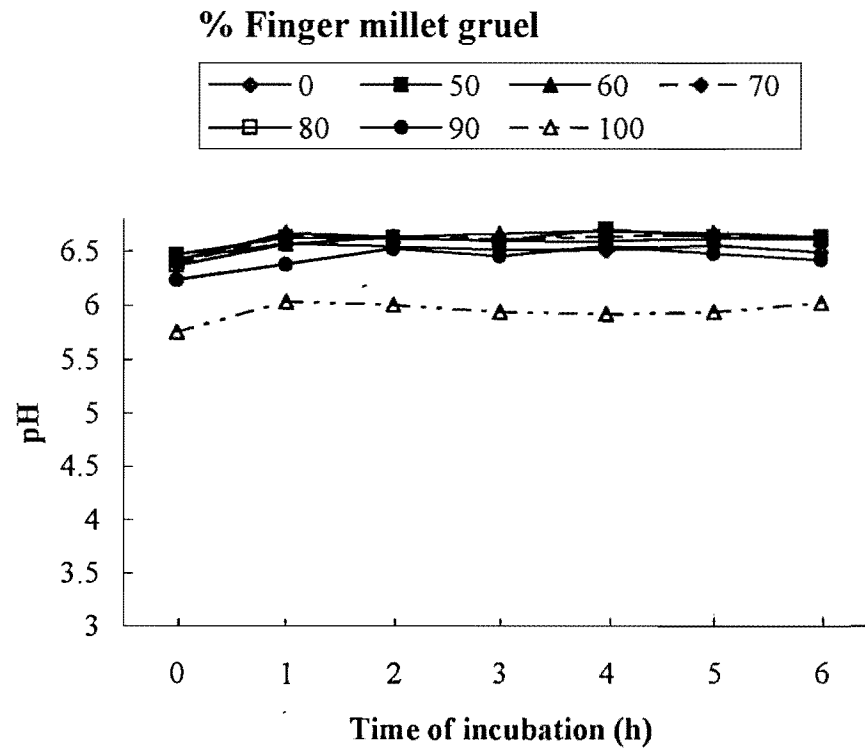


Figure 10d: Effect of the time of incubation and the proportion of finger millet gruel relative to skim milk on the pH of unfermented gruels (control) at 45°C

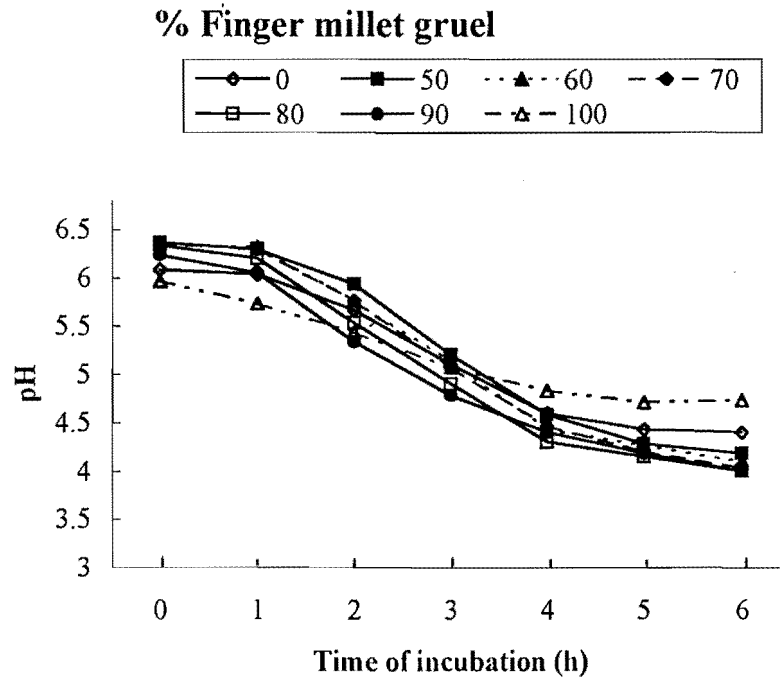


Figure 10e: Effect of the time of incubation and the proportion of finger millet gruel relative to skim milk on the pH of gruels fermented with starter culture YC380 at 45°C

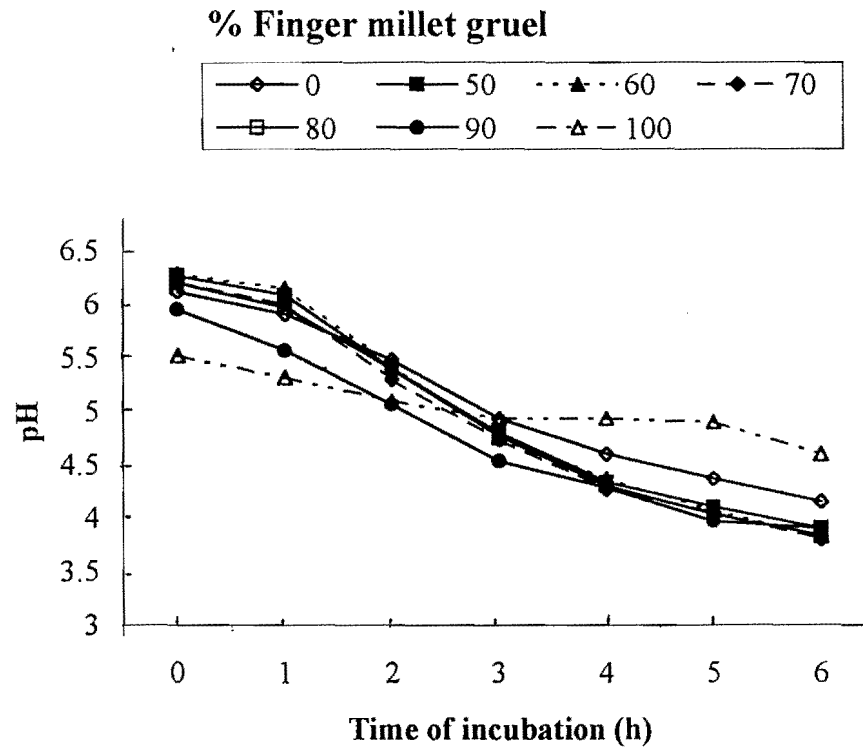


Figure 10f: Effect of the time of incubation and the proportion of finger millet gruel relative to skim milk on the pH of gruels fermented with starter culture V2 at 45°C

Titrateable acidity

Titrateable acidity was significantly affected by the proportion of finger millet gruel and the type of the bacterial starter culture used ($p < 0.05$). The unfermented milk (control, not inoculated) had an acidity of 0.17% (Figure 11). The unfermented finger millet gruel (control, not inoculated) had a titrateable acidity of 0.06%.

Acidity increased as the proportion of finger millet decreased.

Generally, the gruels that were fermented with starter culture V2 developed higher acidity levels compared to those fermented with starter culture YC380. The highest titrateable acidity for the fermented finger millet and skim milk composite gruel was 0.6% and it was obtained when starter culture V2 was used.

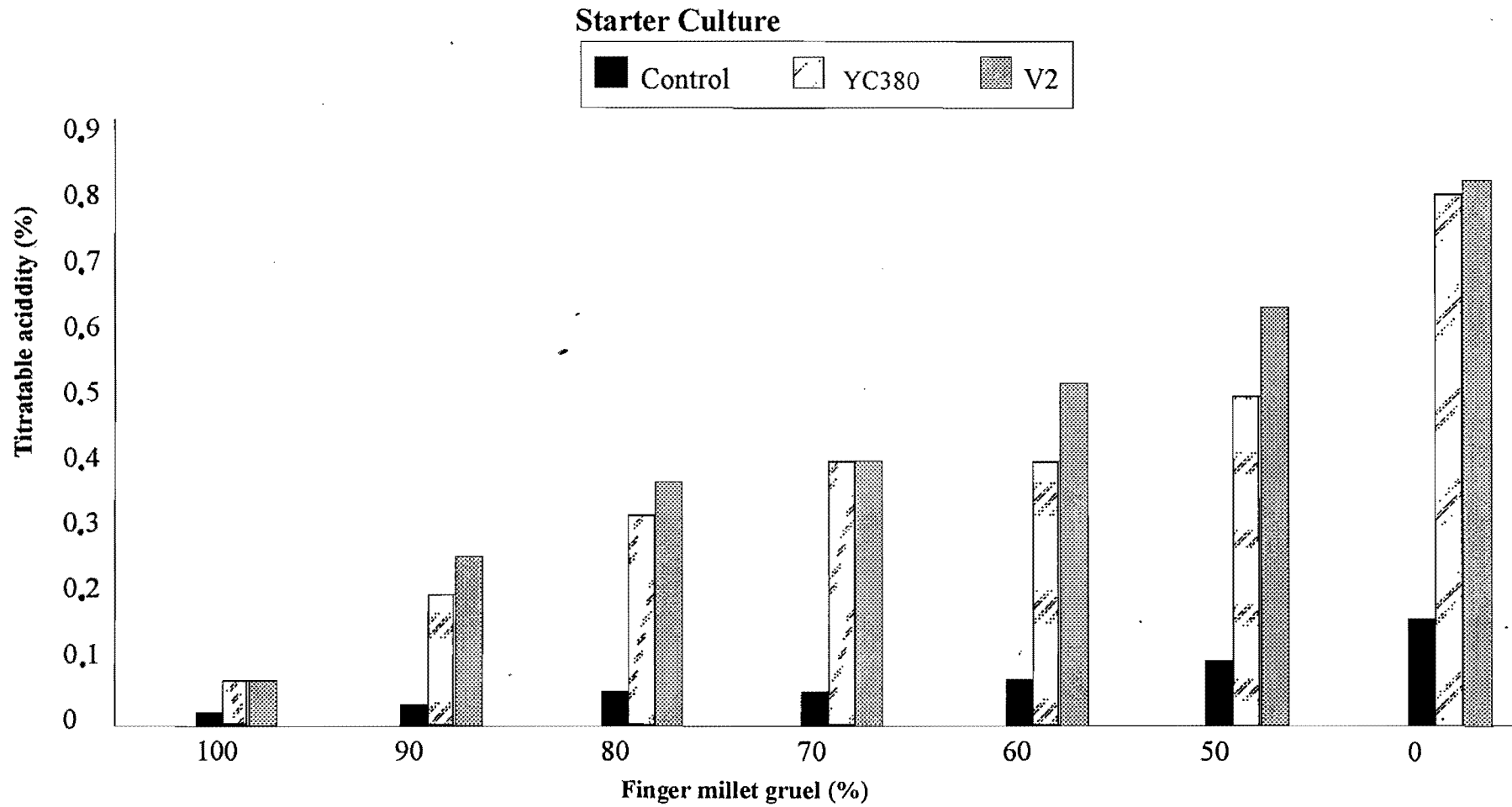


Figure 11: Effect of proportion of finger millet gruel relative to skim milk on the titratable acidity of fermented gruels incubated at 45°C

Syneresis

Syneresis (wheying off) was significantly ($p < 0.05$) influenced by the proportion of finger millet gruel in the composite gruel and the temperature of incubation (Figures 12a and 12b). The type of bacterial culture did not significantly affect syneresis. While there was a significant incubation temperature effect, a trend could not be established between syneresis and increasing/decreasing temperature of incubation. The effect of temperature could not be separated from the effect of proportions of finger millet gruel. Syneresis increased as the proportion of finger millet gruel in the composite decreased. Syneresis was highest when the proportions of finger millet gruel were between 0 and 60%, i.e. when the proportion of skim milk was relatively high.

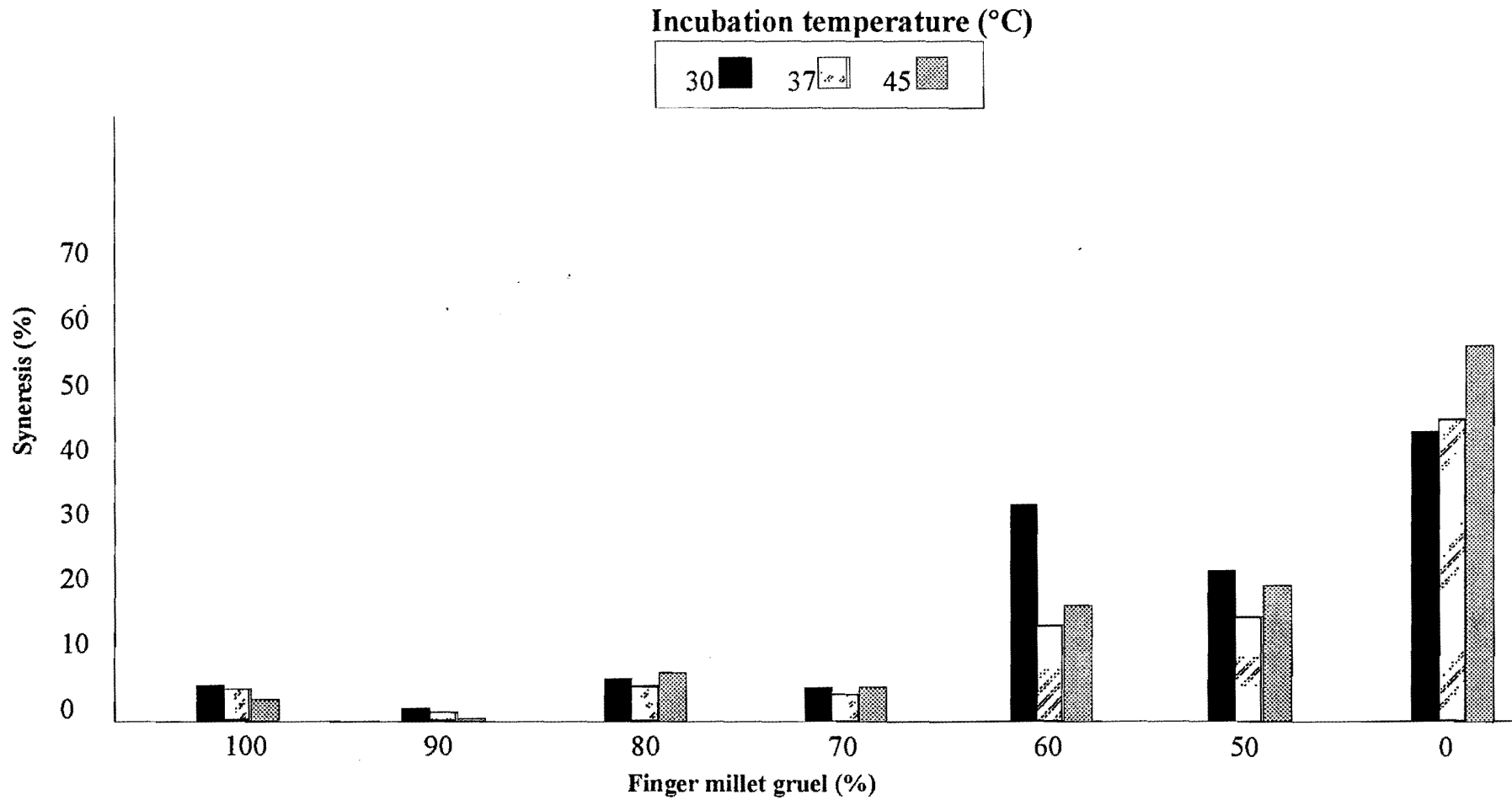


Figure 12a: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on whey syneresis of the gruels fermented with starter culture YC380

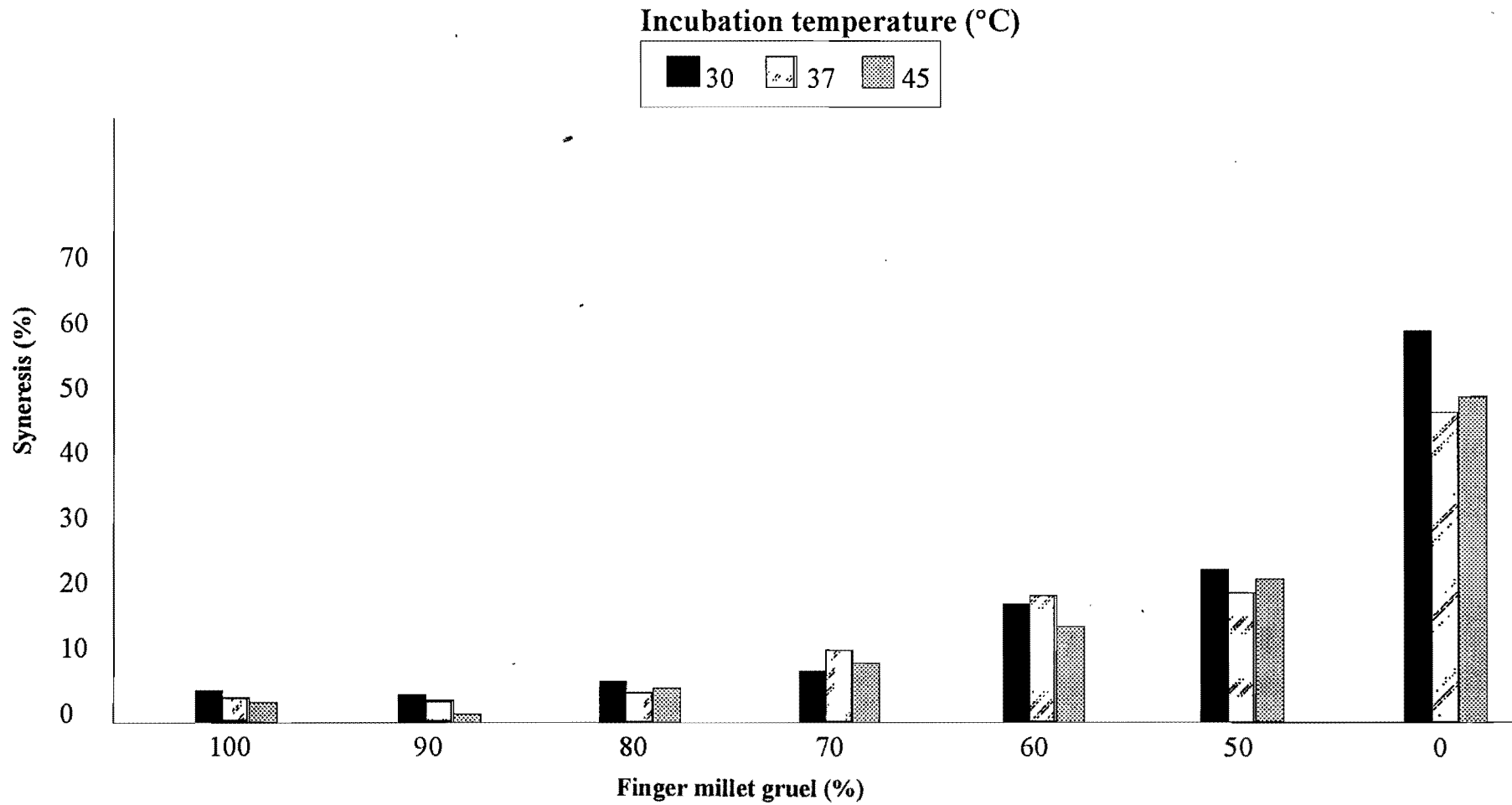


Figure 12b: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on whey syneresis of the gruels fermented with starter culture V2

Consistency

The proportion of finger millet gruel in the composite, the type of bacterial starter culture, the incubation temperature and storage temperature significantly ($p < 0.05$) affected the consistency of the fermented product (Figures 13a-13d).

The highest (thickest) consistencies were obtained when the proportion of finger millet gruel in the gruels was 0% (when there was skim milk only) (Figures 13a-13d). No trend could be established between increasing or decreasing proportions of finger millet gruel and consistency when the storage temperature of gruels was 7°C (Figures 13a and 13c).

Generally the highest consistencies were obtained when an incubation temperature of 45°C, followed by storage at 7°C was used and when the proportions of finger millet gruel were between 0 and 50% (Figure 13a). At 37°C, the highest consistency was obtained when the gruel was fermented using starter culture V2, at a proportion of finger millet of 90% with storage at 7°C (Figure 13c).

The highest consistency was observed when the gruels were stored at 7°C (Figure 13a). Generally, storage at 25°C produced gruels that had lower (thinner) consistency compared to those stored at 7°C (Figures 13a-13d).

Generally, the gruels that were prepared using starter culture YC380 had the higher consistency when the storage temperature was 7°C compared to gruels that were prepared using starter culture V2 at the same storage temperature (Figures 13a and 13c). The thinnest consistencies were observed when the gruels were inoculated with starter culture YC380, stored at 7°C and when the finger millet proportions were between 80 and 100% (Figure 13b).

Visual examination of the fermented products that were obtained when starter cultures YC380 and V2 were used showed differences in consistency and smoothness. The product that was produced with starter culture YC380, had a grainy



consistency while the product produced with starter culture V2 had a smooth and slimy consistency.

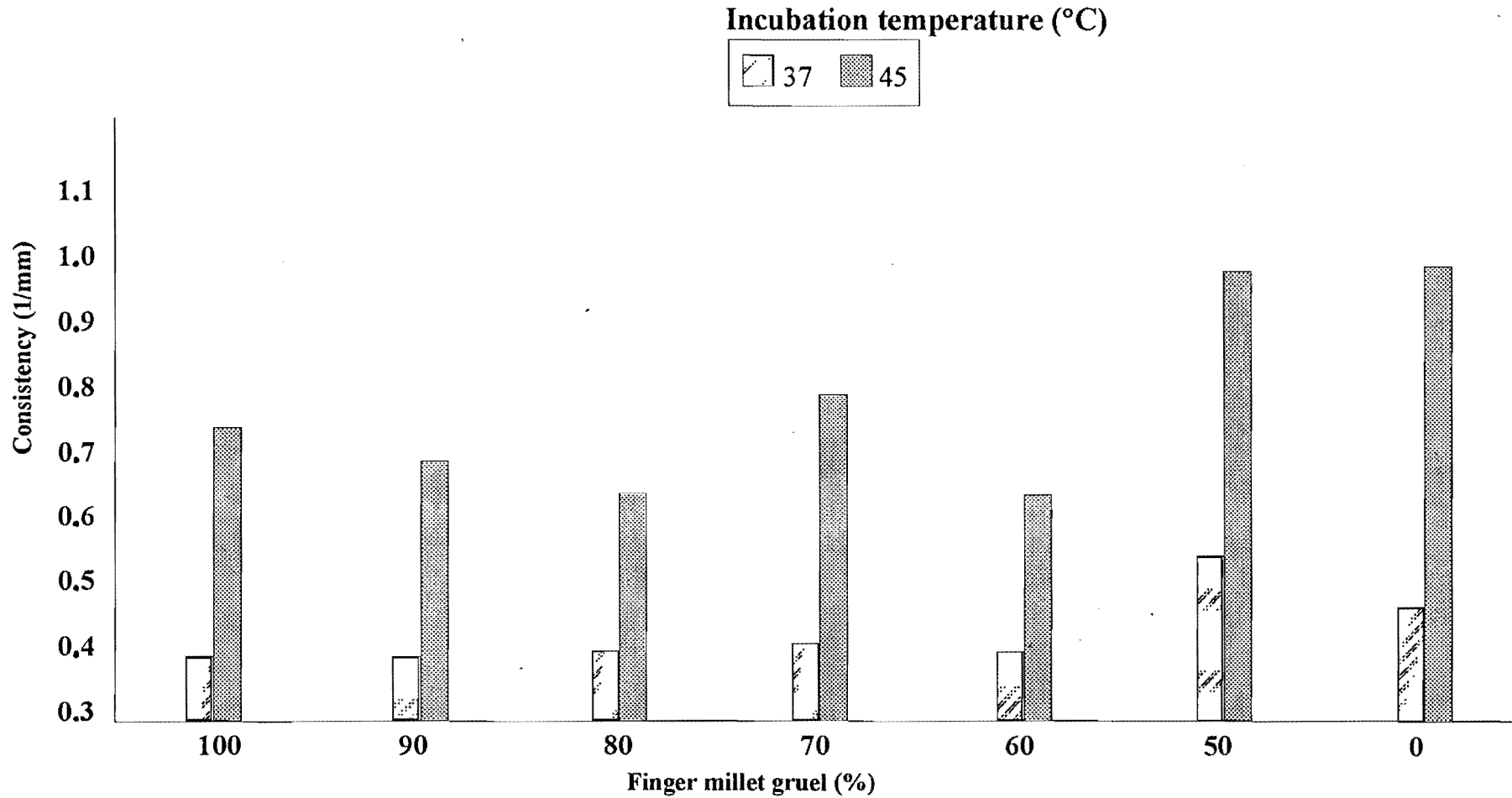


Figure 13a: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the consistency of the gruels fermented with starter culture YC380 and then stored at 7°C

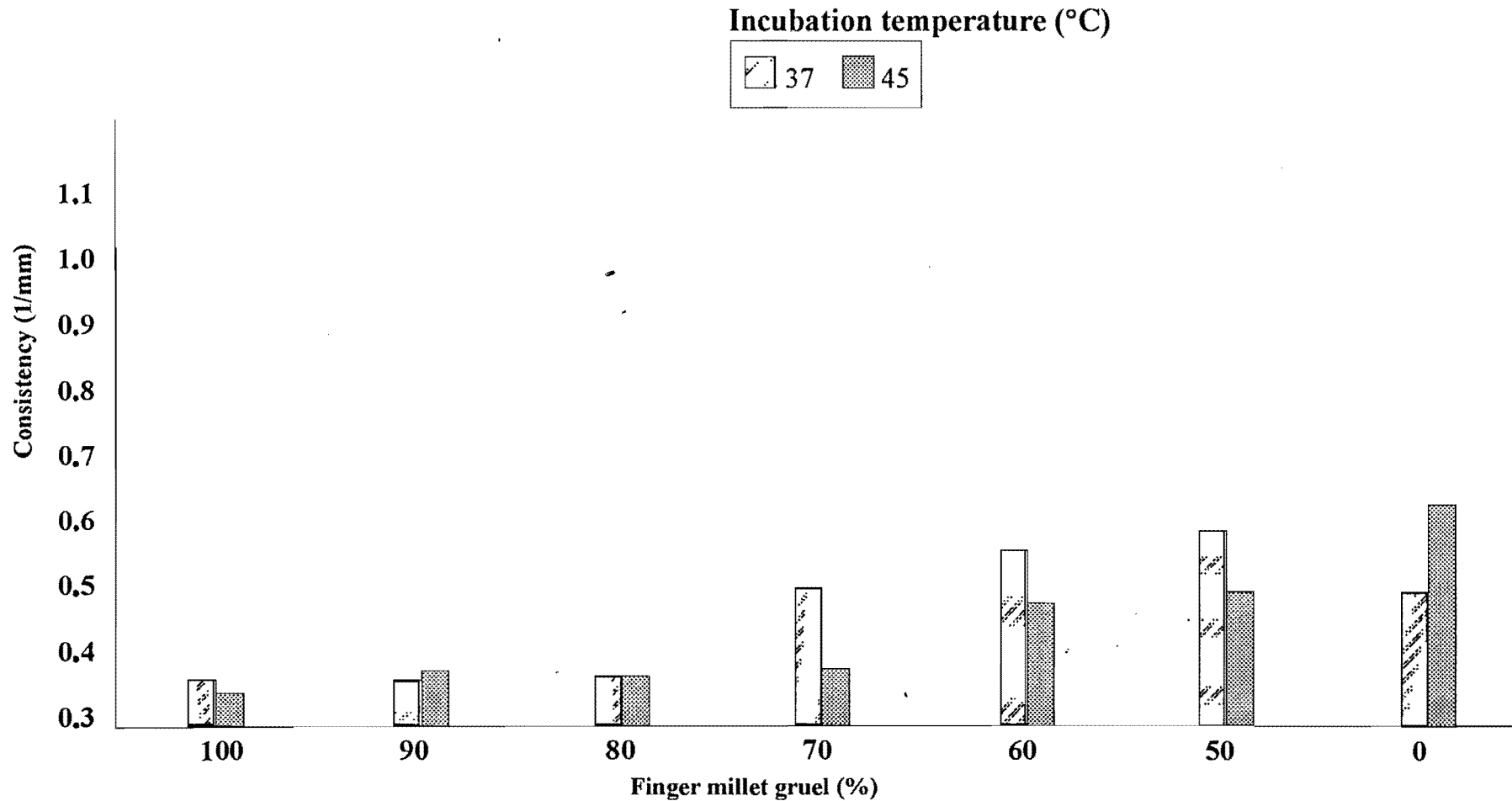


Figure 13b: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the consistency of the gruels fermented with starter culture YC380 and then stored at 25°C

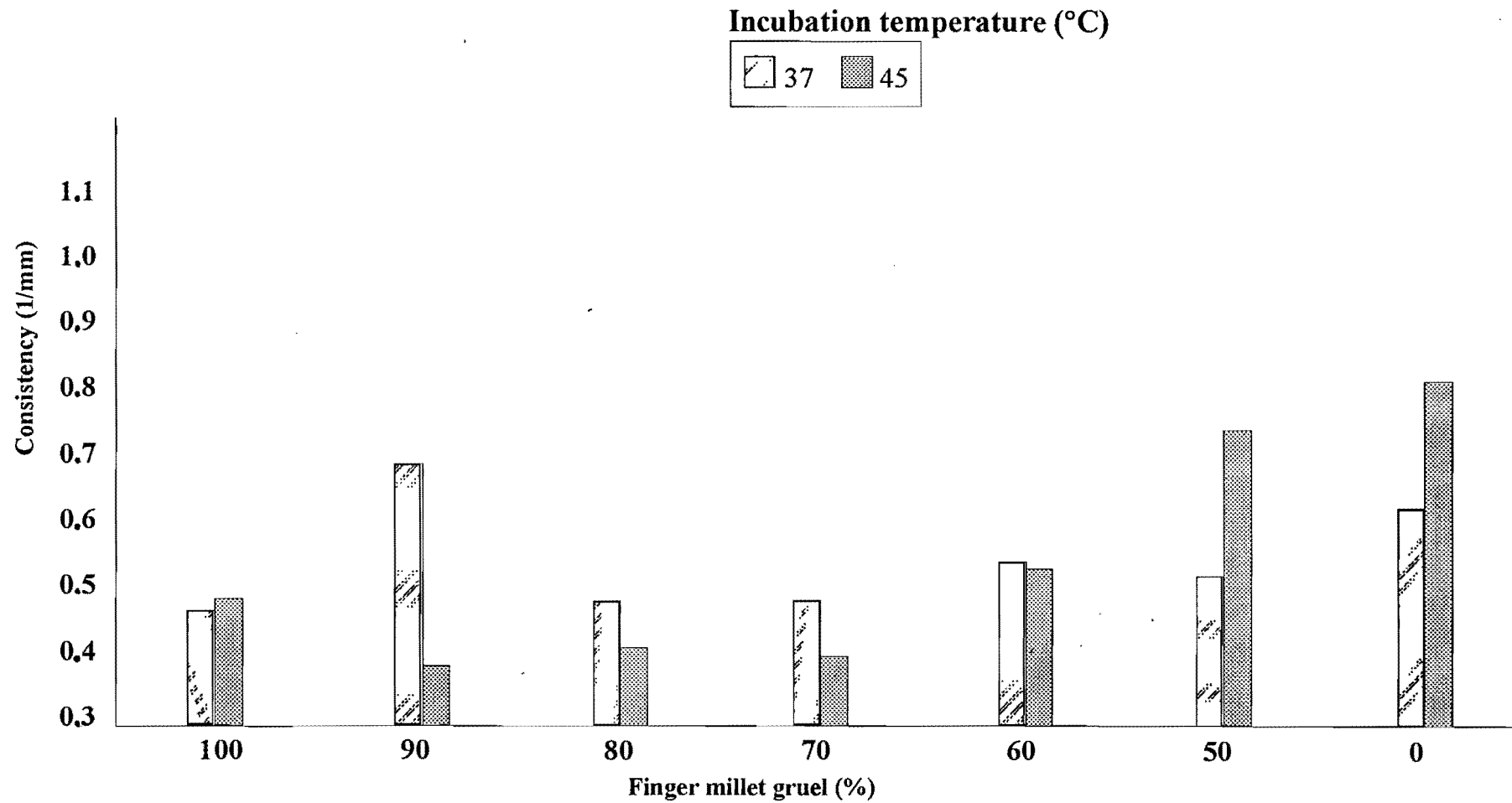


Figure 13c: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the consistency of the gruels fermented with starter culture V2 and then stored at 7°C

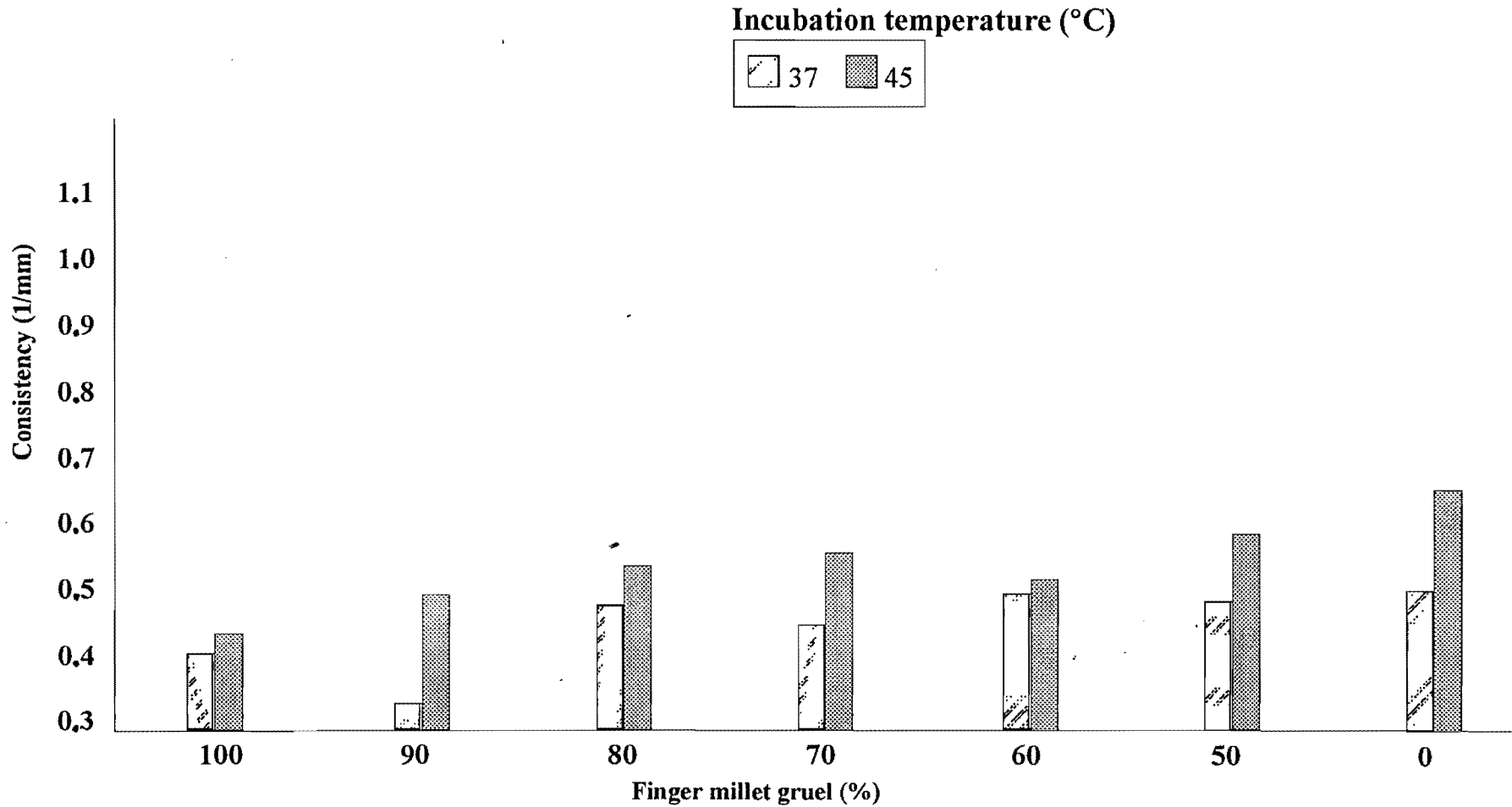


Figure 13d: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the consistency of the gruels fermented with starter culture V2 and then stored at 25°C

Firmness

The proportion of finger millet gruel in the fermented gruel, the type of bacterial starter culture, the incubation temperature and the storage temperature significantly ($p < 0.05$) affected gruel firmness.

Although no trend could be established between increasing or decreasing the proportion of finger millet gruel and firmness of gruel generally, the highest firmness was obtained when the proportion of finger millet gruel was 0% (Figures 14a, 14b and 14d). The exception was a high gruel firmness that was observed when the proportion of finger millet gruel was 90% (Figure 14c).

No relationship could be established between the type of bacterial starter culture and the firmness of the gruel (Figures 14a-14d). The effect of type of bacterial starter culture on firmness appeared to be dependent on the proportion of finger millet in the gruel. Starter culture YC380 produced gruels that had the highest firmness when the proportion of finger millet gruel was 0% (when skim milk only was present) (Figures 14a-14b). The gruels that were produced using starter culture V2 had high firmness values when the proportion of finger millet gruel was 90% (Figure 12c).

No trend could be established between incubation temperature and gruel firmness (Figures 14a - 14d). The effect of incubation temperature on firmness appeared to be related to the proportion of finger millet gruel. Generally, the higher firmness values that were obtained when the incubation temperature was 45°C occurred when the proportion of finger millet gruel was 0% (when skim milk only was present) (Figures 14a-14d). On the other hand, relatively high firmness values were also obtained when gruels were incubated at 37°C and when the proportion of finger millet gruel was 90% (Figure 14c) and 100% (Figure 14d).

Generally, a storage temperature of 7°C produced gruels that were firmer than those stored at 25°C (Figure 14a and 14c). Storage at 25°C produced gruels that were

relatively firmer when starter culture V2 was used and when the proportions of finger millet gruel were from 80% and 90%.

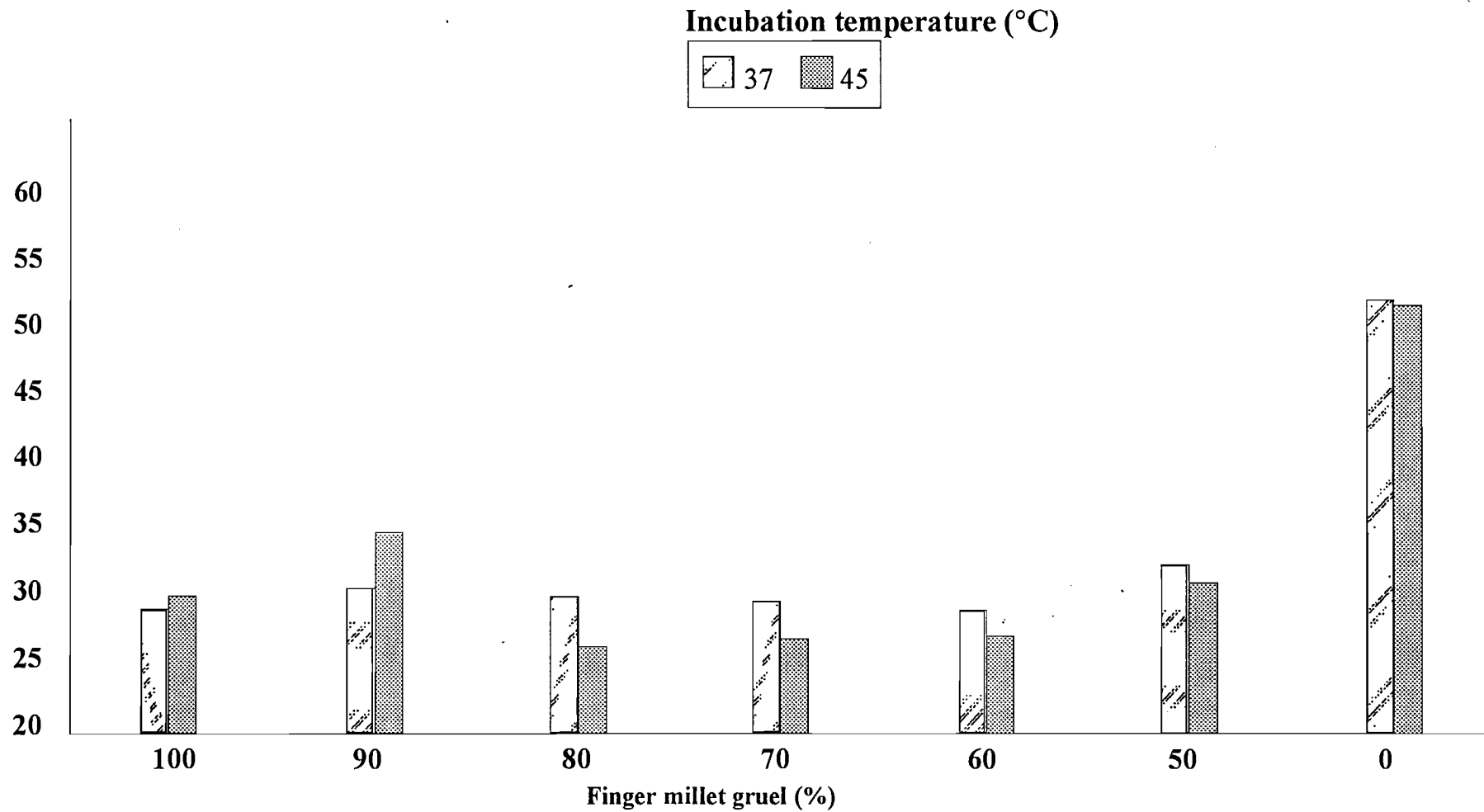


Figure 14a: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the firmness of the gruels fermented with starter culture YC380 and then stored at 7°C

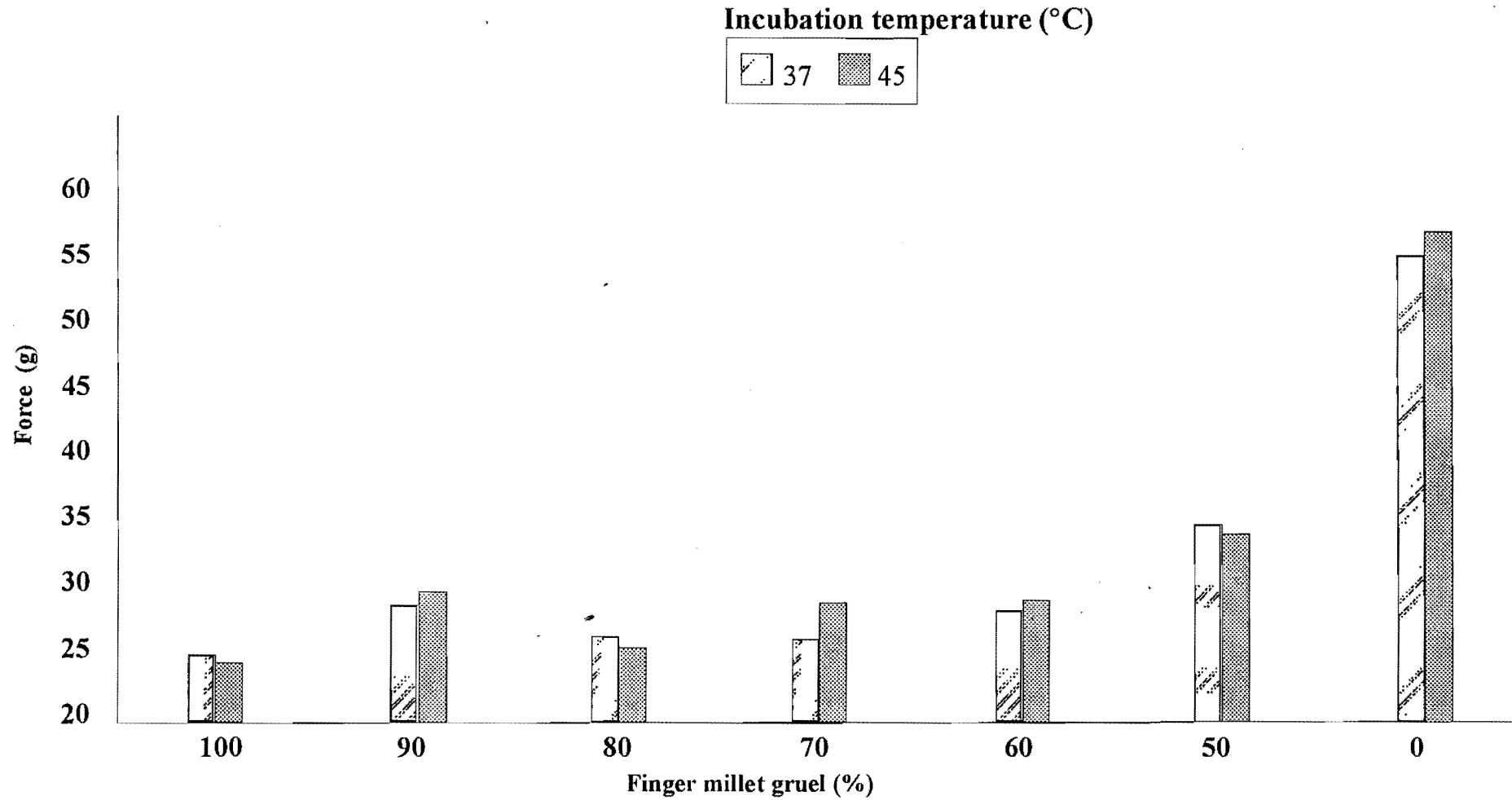


Figure 14b: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the firmness of the gruels fermented with starter culture YC380 and then stored at 25°C

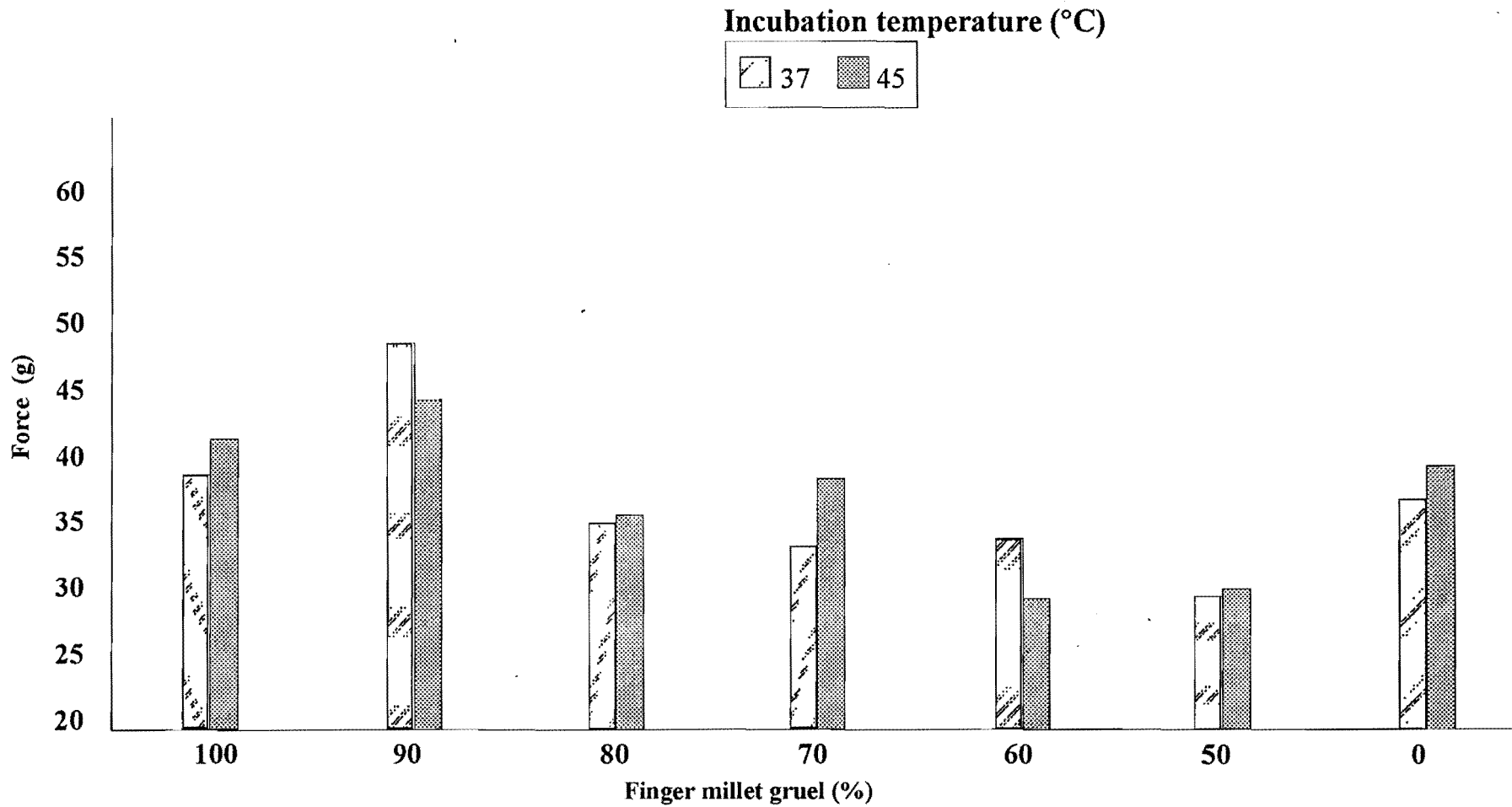


Figure 14c: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the firmness of the gruels fermented with starter culture V2 and then stored at 7°C

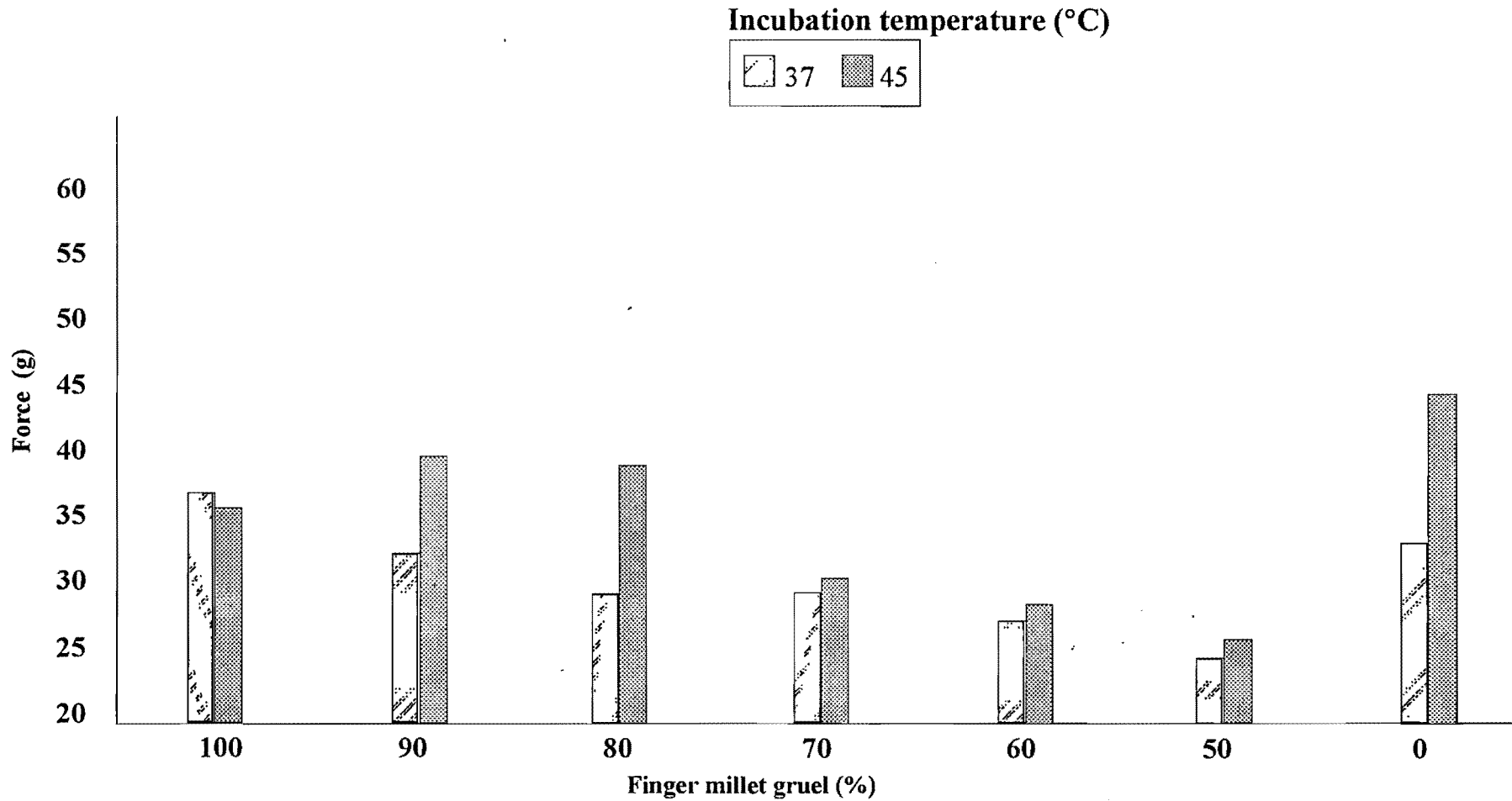


Figure 14d: Effect of the temperature of incubation and the proportion of finger millet gruel relative to skim milk on the firmness of the gruels fermented with starter culture V2 and then stored at 25°C

4.3 Effect of type of bacterial starter culture and proportion of finger millet gruel on the proximate composition of the fermented composite gruels

The proportion of finger millet gruel and the type of bacterial starter culture both significantly affected the proximate composition of the fermented finger millet-dairy composite ($p < 0.05$) (Tables 4 to 6). Generally, the effect of changing the proportion of finger millet gruel was more apparent than the effect of type of bacterial starter culture.

Dry matter

The dry matter content was highest in the unfermented skim milk and lowest in the unfermented finger millet gruel. The unfermented 50% finger millet and skim milk composite gruel had a dry matter content that was between that of finger millet and skim milk (Table 4). The decrease in dry matter with decreasing finger millet proportions was observed with the gruels that were fermented with starter cultures YC380 and V2 (Tables 5 to 6).

The use of bacteria starter cultures YC380 and V2 did not appear to significantly affect the dry matter content of the fermented gruels (Tables 5 and 6 respectively).

Crude protein

The crude protein content was lowest in the unfermented finger millet gruel and highest in the unfermented skim milk. The unfermented 50% finger millet and skim milk composite gruel had a protein content that was almost three times that of the finger millet gruel (Table 4). This increase in crude protein content with decreasing proportions of finger millet was also observed with the gruels that were fermented using starter cultures YC380 and V2 (Tables 5 to 6).

The use of bacteria starter cultures YC380 and V2 did not appear to have a significant effect on the crude protein content of the fermented gruels (Tables 5 to 6 respectively).

Crude fibre

As the proportion of finger millet in the unfermented gruel decreased to 50% in the unfermented gruel, there was no observed change in the crude fibre content (Table 4). This was also observed in the Gruels that were fermented with starter cultures YC380 and V2 (Tables 5 to 6 respectively).

The bacteria starter cultures YC380 and V2 did not appear to significantly affect the crude fibre content of the fermented Gruels (Tables 5 to 6 respectively).

Crude fat

The unfermented skim milk had a lower crude fat content compared to the unfermented finger millet gruel and the unfermented skim milk and finger millet composite gruel (Table 4). The crude fat content of the Gruels that were fermented with starter cultures YC380 and V2 was similar to that of the unfermented Gruels (Tables 5 and 6 respectively).

The bacteria starter cultures YC380 and V2 did not appear to influence the crude fat content of the fermented Gruels (Tables 5 and 6 respectively).

Lactose

The lactose content of the 50% unfermented finger millet and skim milk composite gruel was lower than that of the skim milk (Table 4). The decrease in lactose with decreasing proportions of finger millet gruel was also observed in the skim milk and the composite Gruels that were fermented using starter cultures YC380 and V2 (Tables 5 and 6).

Fermenting the composite gruel and the skim milk with starter cultures YC380 and V2 significantly reduced the lactose content of the fermented Gruels (Tables 5 and 6 respectively).

Starch

The starch content of the unfermented finger millet gruel was higher than that of the 50% finger millet and skim milk composite gruel (Table 4). The increase in starch with increasing proportions of finger millet gruel was also observed with the gruels that were fermented with starter cultures YC380 and V2 (Tables 5 and 6).

The use of bacterial starter cultures YC380 and V2 did not appear to significantly affect the starch content of the fermented gruels (Tables 5 and 6 respectively).

Ash

Ash content was highest in the unfermented skim milk and lowest in the unfermented finger millet gruel. The unfermented 50% finger millet and skim milk gruel had an ash content between that of the finger millet and the skim milk (Table 4). The increase in ash content with decreasing proportions of finger millet gruel was also observed when the gruels were fermented with starter cultures YC380 and V2 (Tables 5 and 6).

The ash content of the gruels was not significantly affected by the use of starter cultures YC380 and V2 (Tables 5 and 6 respectively).

Energy

Energy content was highest in the unfermented skim milk and lowest in the unfermented finger millet gruel. The unfermented 50% finger millet and skim milk gruel had an energy content between that of the finger millet and the skim milk (Table 4). The increase in energy content with decreasing proportions of finger millet gruel was also observed when the gruels were fermented with starter cultures YC380 and V2 (Tables 5 and 6).

The energy content of the gruels was not significantly affected by starter cultures YC380 and V2 (Tables 5 and 6 respectively).

Table 4. Effect of the proportion of finger millet gruel relative to skim milk on the proximate composition of a finger millet-dairy composite gruel (Control: not fermented)

Component (g/ 100 g sample)	Proportion of finger millet gruel (%)		
	<u>100</u>	<u>50</u>	<u>0</u>
Moisture	95.1 ±0.2	92.6 ±0.6	89.6 ±0.2
Dry matter	4.9 ±0.2	7.4 ±0.6	10.4 ±0.2
Crude protein	0.4 (8.9) 0 (±0.3)	2.0 (24.8) ±0.1 (±0.8)	3.5 (33.3) ±0.1 (±0.5)
Crude fibre	0.3 (6.1) 0 (±0.3)	0.3 (4.7) 0 (±0.1)	0
Crude fat	0.1 (3.3) 0 (±0.1)	0.2 (2.5) 0 (±0.1)	0.2 (2.0) 0 (±0.1)
Lactose	0	2.8 (34.9) ±0.3 (±2.8)	5.4 (51.9) ±0.5 (±5.0)
Starch	3.3 (66.2) ±0.2 (±6.2)	1.9 (25.3) 0 (±2.1)	0
Ash	0.2 (3.1) 0 (±0.1)	0.4 (5.4) ±0.1 (±1.1)	0.7 (7.1) ±0.1 (±0.5)
Energy (MJ/kg)	0.9 (17.4) ±0.1 (±1.2)	1.3 (17.4) ±0.1 (±1.1)	1.8 (17.4) ±0.1 (±1.3)

Figures in brackets represent the proximate composition on dry basis
± represents the standard deviation

Table 5. Effect of the proportion of finger millet gruel relative to skim milk on the proximate composition of a finger millet - skim milk composite gruel fermented with starter culture YC380

Component (g/ 100 g sample)	Proportion of finger millet gruel (%)		
	<u>100</u>	<u>50</u>	<u>0</u>
Moisture	94.9	92.6	89.8
	0	0	0
Dry Matter	5.1	7.4	10.2
	0	0	0
Crude protein	0.4 (7.7)	1.9 (26.2)	3.5 (34.6)
	0 (±0.2)	±0.1 (0)	±0.1 (±0.5)
Crude fibre	0.3 (6.4)	0.3 (3.5)	0
	0 (0)	0 (0)	
Crude fat	0.2 (3.2)	0.2 (2.6)	0.1 (1.2)
	0 (±0.1)	0 (0)	0 (0)
Lactose	0	1.9 (29.9)	4.7 (45.9)
		±0.2 (±3.0)	±0.2 (±2.1)
Starch	3.4 (66.2)	1.6 (24.6)	0
	±0.5 (±4.80)	0 (±2.5)	
Ash	0.1 (2.1)	0.6 (7.7)	0.7 (9.0)
	0 (±0.2)	0 (±0.1)	±0.2 (±0.2)
Energy (MJ/kg)	0.9 (17.6)	1.3 (17.7)	1.8 (17.3)
	±0.1 (±1.4)	±0.1 (±1.3)	±0.2 (±1.6)

Figures in brackets represent the proximate composition on dry basis

± represents the standard deviation of the mean

Table 6. Effect of the proportion of finger millet gruel relative to skim milk on the proximate composition of a finger millet- skim milk composite gruel fermented with starter culture V2

Component (g/ 100 g sample)	Proportion of finger millet gruel (%)		
	<u>100</u>	<u>50</u>	<u>0</u>
Moisture	94.7 ±0.1	92.2 ±0.9	80.1 0
Dry matter	5.3 ±0.1	7.8 ±0.9	9.9 0
Crude protein	0.4 (7.2) ±0.2 (±0.2)	1.8 (23.2) 0 (±0.1)	3.3 (33.8) ±0.1 (±4.1)
Crude fibre	0.3 (6.5) 0 (±0.1)	0.4 (4.5) 0 (±0.2)	0
Crude fat	0.2 (3.2) 0 (±0.1)	0.2 (2.2) 0 (±0.2)	0.1 (1.2) 0 (±0.1)
Lactose	0	2.0 (30.6) ±0.1 (±1.2)	4.5 (45.3) ±0.4 (±3.9)
Starch	3.4 (63.3) ±0.3 (±10.7)	2.2 (27.8) ±0.2 (±3.3)	0
Ash	0.1 (2.3) 0 (±0.3)	0.4 (5.7) 0 (±0.1)	0.9 (8.6) 0 (0)
Energy (MJ/kg)	0.9 (17.9) ±0.2 (±1.7)	1.4 (17.6) ±0.4 (±1.5)	1.8 (18) ±0.2 (±1.3)

Figures in brackets represent the proximate composition on wet basis

± represents the standard deviation of the mean

4.4 Contribution of nutrient components to energy in composite gruels

The protein to energy (P-E) ratios of the gruels increased with decreasing proportions of finger millet (Table 7).

Cultures YC380 and V2 did not appear to have much effect on the P-E ratios.

The percentage contribution of fat (% fat kJ) to the total energy of gruels decreased with decrease in the proportion of finger millet gruel.

Bacteria starter cultures YC380 and V2 did not appear to affect the contribution of fat to energy when the gruels had finger millet (i.e. finger millet gruels and 50% finger millet and skim millet composite gruels).

A decrease in the percentage contribution of carbohydrate energy to total energy was observed as the proportion of finger millet in gruels decreased.

Starter cultures YC380 and V2 appeared to reduce the contribution of the carbohydrates to total energy when the gruels contained skim milk (i.e. skim milk only and 50% finger millet and skim milk composite gruels).

Table 7. The contribution of nutrient components to the energy content of fermented finger millet-skim milk composite gruels¹

Component	Bacterial starter culture								
	Control (no inoculum)			YC380			V2		
	Proportion of finger millet gruel (%)								
	100	50	0	100	50	0	100	50	0
Protein energy (kJ/100 g)	6.8 (151)	34 (422)	59.5 (566)	6.8 (131)	32.3 (445)	59.5 (566)	6.8 (122)	30.6 (394)	56.1 (574)
% Protein energy ²	7.6	26.2	33.1	7.5	24.8	33.1	7.6	21.9	31.2
Fat energy (kJ/100 g)	7.6 (125)	7.6 (95)	3.8 (46)	7.6 (122)	7.6 (99)	3.8 (46)	7.6 (122)	7.6 (84)	3.8 (46)
% Fat energy	8.4	5.8	4.2	8.4	5.8	4.2	8.4	5.4	2.1
Carbohydrate ³ energy (kJ/100 g)	56.1 (1125)	79.9 (1023)	91.8 (882)	57.8 (1125)	59.5 (926)	79.9 (780)	57.8 (1076)	78.2 (992)	76.5 (770)
% Carbohydrate energy	62.3	61.5	51.0	64.2	57.8	44.4	64.2	55.9	42.5

¹ Energy calculated using 17 kJ/g for protein, 17 kJ/g for carbohydrates and 38 kJ/g for fat,

² Protein- energy ratio, ³ Lactose and starch, Figures in brackets represent the energy content on dry basis

4.5 Contribution of energy in gruels to daily requirements

The contribution of 100 g of the gruels to daily energy requirements increased with increased proportions of skim milk in the gruels. The contribution of the gruels to energy requirements was highest for infants and lowest for nursing women (Table 8).

Bacteria starter cultures YC380 and V2 did not appear to influence the contribution of the gruels to energy requirements.

Table 8. The contribution (%) of the energy content of fermented finger millet-skim milk composite gruels (per 100 g) to daily energy requirements

	Control (no inoculum)			Starter culture YC380				V2		Energy RDA (kJ/ day) ⁴
	100	50	0	Proportion of finger millet gruel (%)				50	0	
				100	50	0	100			
				Contribution per day (%)						
Woman ¹	1.0	1.4	1.9	1.0	1.4	1.9	1.0	1.5	1.9	9360
Pregnant Woman ¹	0.9	1.3	1.8	0.9	1.3	1.8	0.9	1.4	1.8	9990
Nursing woman ¹	0.8	1.1	1.6	0.8	1.1	1.6	0.8	1.2	1.6	11 557
Man ²	0.8	1.1	1.5	0.8	1.1	1.5	0.8	1.2	1.5	11 630
Infant ³	2.0	3.0	4.1	2.0	3.0	4.1	2.0	3.2	4.1	4395

¹A rural 25 year- old woman in a developing country with a weight of 50 kg and a height of 1.6 m, ² A 25 year old male subsistence farmer with a height of 1.61 m and a weight of 58 kg, ³ A 12 month old male infant ⁴ (World Health Organisation 1985)



4.6 Contribution of protein in gruels to daily requirements

The contribution of 100 g of the gruels to daily protein requirements increased with increased proportions of skim milk in the gruels. The contribution of the gruels to protein requirements was highest for infants and lowest for nursing women (Table 9).

Starter cultures YC380 and V2 did not appear to influence the contribution of the gruels to protein requirements.

Table 9. The contribution (%) of the protein content of fermented finger millet-skim milk composite gruels (per 100 g) to daily protein requirements

	Control (no inoculum)			Starter culture YC380 Proportion of finger millet gruel (%)				V2		Protein RDA (g / day) ⁴
	100	50	0	100	50	0	100	50	0	
	Contribution per day (%)									
Woman ¹	1.0	5.0	8.8	1.0	4.8	8.8	1.0	4.5	8.3	39.7
Pregnant woman ¹	0.9	4.7	8.1	0.9	4.4	8.1	0.9	4.2	7.7	43.0
Nursing woman ¹	0.8	4.0	7.1	0.8	3.8	7.1	0.8	3.6	6.7	49.6
Man ²	0.8	4.2	7.4	0.8	4.0	7.4	0.8	3.8	7.0	47.3
Infant ³	3.9	19.3	33.8	3.9	18.4	33.8	3.9	17.4	31.9	10.4

¹25-50 years old and weighing 63 kg, ²25-50 years old and weighing 75 kg, ³A 12 month old infant ⁴World Health Organisation (1985)

4.7 The effect of the proportion of finger millet gruel and the type of bacterial starter culture on the lysine content of the fermented composite gruels

Lysine content of the fermented composite gruels

The lysine content of the gruels was improved by a decrease in the proportion of finger millet gruel (i.e. the increase in the proportion of skim milk) (Table 10). The increase in lysine content with increasing proportions of skim milk was also observed when the gruels were fermented with starter cultures YC380 and V2.

The use of starter cultures YC380 and V2 significantly improved the lysine content of the fermented finger millet gruel and the 50% finger millet and skim milk composite gruel.

Table 10. Effect of proportion of finger millet gruel and bacterial starter culture on the lysine content (mg/ 100 g of gruel) of finger millet - skim milk composite gruels

	Bacterial Starter culture									Milk ^{1,3}	Finger millet ^{2,3}
	Control			YC380			V2				
	Proportion of finger millet (%)										
	100	50	0	100	50	0	100	50	0		
Amino acid											
Lysine	8	108	268	14	128	272	14	126	268		
	(19)	(54)	(77)	(34)	(68)	(78)	(34)	(70)	(77)	(74)	(26-55)

¹ Rosenthal (1991), ² Serna-Saldivar & Rooney (1995)

Figures in brackets represent lysine content in mg per g crude protein

Contribution of Gruels to Lysine Requirements

The lysine quality of the Gruels increased with increased proportions of skim milk in the Gruels (Table 11).

Starter cultures YC380 increased the contribution of lysine to requirements based on ideal patterns. Bacterial starter culture V2 increased the contribution of lysine to requirements in the finger millet Gruels, 50% finger millet and skim milk Gruels but not in skim milk.

The contribution of the protein in the Gruel to lysine requirements was highest for adults and lowest for infants.

For adults, the protein of all Gruels met the requirements for lysine.

For infants, the protein in the 50% finger millet and skim milk composite Gruels met the requirements for lysine when starter cultures YC380 and V2 were used for fermentation.

Table 11. The lysine content of finger millet-skim milk composite gruel protein expressed as a percentage of its quantity in an ideal pattern

	Bacterial Starter Culture									Ideal pattern ¹ (mg / g protein)
	Control (no inoculum)			YC380			V2			
	Proportion of finger millet gruel (%)									
	100	50	0	100	50	0	100	50	0	
Infants (12 months)	29	82	116	52	102	118	52	106	117	66
Preschool children (2-5 years)	33	93	133	59	117	134	59	121	133	58
School children (10-12 years)	43	123	175	77	155	177	77	159	174	44
Adults	119	339	478	213	422	486	213	441	484	16

¹World Health Organisation (1985)

CHAPTER 5

DISCUSSION

During the preparation of the finger millet and skim milk composite gruels, decisions had to be made on the choice of raw materials and how the mixing of the finger millet and skim milk would be done. These will be looked at before the results are discussed.

Skim milk was used instead of full fat milk because it is more shelf-stable when stored under ambient conditions. Full fat milk tends to be more susceptible to both oxidative and hydrolytic rancidity (Walstra & Jenness, 1984). The hot tropical climates that are experienced in many parts of Africa are associated with high ambient temperatures. The development of off-flavours as a result of oxidative rancidity increases with increasing temperature. In addition to being shelf-stable, skim milk has most of the nutritional benefits of full fat milk. Reconstituted skim milk was used to simulate liquid skim milk. Given that the gruels would thicken upon cooling, the use of skim milk in its reconstituted form helped to ensure more thorough mixing of the composites in comparison to using the skim milk in powder form.

The preparation of the composite gruels was done by substituting a portion of the finger millet gruel with the same amount of reconstituted skim milk. According to Roehrig (1991), this preparation procedure makes it possible to look at the impact of exchanging nutrients from one food for those of another. It is important to note that finger millet gruel was being replaced by milk, a product that has a higher protein biological value.

The reduction in pH to 4.5 or lower that occurred when starter cultures JC, YC380 and V2 were used for fermentation is of significance for food safety. The reduction in pH was therefore considered to be the first criterion on which the choice of starter culture and conditions of incubation were based. Rapid acid production is essential for lowering pH

because it is only when the pH is lowered to 4.5 or lower that inhibition of the growth of pathogenic micro-organisms occurs (Daeschel & Fleming, 1984). Some of the bacteria that are inhibited include enteropathogenic strains of *Escherichia coli* (Khedkar, Dave & Sannabhadti, 1990; Sable, Pons, Gendron-Gaillard & Cottenceau, 2000) which are associated with diarrhoeal diseases in infants (Nout & Motarjemi, 1997; Hounhouigan, Nout, Nago, Houben & Rombouts, 1999), *Staphylococcus aureus* and *Salmonella typhimurium* (Schaack & Marth, 1988). Fermentation also slows down the rate of microbial spoilage (Nout, Hautvast, van der Haar, Marks & Rombouts, 1988; Nout, Rombouts & Havelaar, 1989). Coliforms are an example of spoilage micro-organisms that are known to cause flavour defects and are suppressed by lowering the pH (Mbugua, Ledford & Steinkraus, 1984).

It is important to note that no significant reduction in pH was observed when no starter culture was used which shows the need to add a culture to the gruels in order to achieve the desired results. When starter culture JC was used for fermentation, the reduction in pH to 4.5 or lower occurred only when finger millet was present in the gruels (i.e. when the proportions of finger millet were between 50 % and 100 %) and this can be related to the purpose for which this culture was selected. It was selected through fermentation of cereal slurries and back-slopping was the procedure that was used. The process of back-slopping involves the repetitive use of previously fermented cereal slurries as starter cultures (Nout, 1991; Nout & Rombouts, 1992). As fermentation progresses, a succession of naturally occurring micro-organisms results in the development of a population that is dominated by lactic acid bacteria (Agati, Guyot, Morlon-Guyot, Talamond & Hounhouigan, 1998). Thus a population of lactic acid bacteria which is capable of breaking down the available carbohydrates efficiently is established. In cereals the available carbohydrates tend to be starch (amylopectin and amylose), and the products of starch hydrolysis which include maltose, maltodextrins and glucose (Khetarpaul & Chauhan, 1990b). This is probably the reason why the starter culture JC was effective in reducing the pH of the composite gruels only when finger millet was present. The advantages of back-slopping compared to natural fermentation are that the slow natural process is accelerated by the inoculum that is enriched with acid-producing strains of

lactic acid bacteria and the consumption of fermentable carbohydrates by aerobes and enterobacteriaceae is inhibited by the immediate dominance of lactic acid bacteria (Nout, Rombouts & Hautvast, 1989).

Starter culture YC380 was developed to ferment dairy products and as a result the pH of the fermented gruels was reduced to 4.5 or below only when milk was present in the gruels. The reduction in the lactose content that was observed when the starter cultures YC380 and (to some extent V2) were used indicates that the reduction in pH to 4.5 and lower was, primarily, a result of the fermentation of lactose in the skim milk component of the composite. This preference for lactose as a source of energy may also be supported by the decrease in the bacterial count that occurred when the proportion of finger millet increased and particularly in the gruels that contained little or no skim milk. As the proportion of finger millet decreased (i.e. as the proportion of skim milk increased) more lactose was available to the lactic acid bacteria for conversion to lactic acid and this resulted in a lower pH. The decrease in pH with increased proportions of skim milk can be compared with the decrease in pH with increasing proportions of milk solids that was observed by Muir & Tamime (1993) as well as Thomopolous, Tzia & Milkas (1993) during the manufacture of yoghurt. They observed that the final pH of yoghurt decreased as the content of milk solids increased.

Starter culture V2, which was also developed to ferment dairy products, reduced the pH of the fermented gruels to 4.5 or lower even when milk was not present. Since the results do not show a significant reduction in the starch content of the gruels, the reduction in pH in the gruels that had finger millet only is probably not due to starch break-down. As is the case with other cereals, finger millet contains some soluble sugars (Serna-Saldivar & Rooney, 1995) and it has been observed that when the cereals are cooked these sugars can be fermented by bacteria (Khetarpaul & Chauhan, 1990b). In the absence of lactose (i. e. in the gruels with finger millet only) starter culture V2 may have utilised these sugars as an energy source. When transferred to a different environment, some bacteria are capable of synthesising new enzymes to metabolise the nutrients (McKane & Kandel, 1996). Putting this in the context of this study, starter culture V2 may be one of those

micro-organisms that are able to adapt to different nutritional environments such as milk and finger millet gruel. Starter culture YC380 on the other hand may be the type of micro-organism that takes long to adapt or may not adapt at all in a new environment.

The decrease in the pH of the fermented gruels with increased temperature of incubation that was observed in this study indicates that starter cultures YC380 and V2 prefer thermophilic conditions. Although the term thermophilic should be reserved for micro-organisms whose optimum growth temperature lies between 55°C and 75°C (Tamime & Robinson, 1988), in the dairy industry starter cultures that are used for yoghurt manufacture are selected for optimal acid production where the temperatures of incubation range from 37°C to 45°C and are referred to as thermophilic starter cultures (Tamime & Robinson, 1999). The decrease in pH with increasing temperatures of incubation would therefore be expected. Within the temperature range where bacteria show minimum growth and maximum growth, a temperature rise increases growth rate. The reason is that a rise in temperature increases the rate of enzyme-catalysed reactions and because the rate of each reaction increases metabolism as well as acid production are more active at higher temperatures of incubation (Prescott *et al.*, 1984). The decrease in the pH of the fermented gruels with increased temperature of incubation agrees with work by Cooke, Twiddy & Reilley (1987) in which they evaluated acid production at 15°C, 25°C, 30°C and 37°C in yoghurt. They observed that the rate of pH decrease was lower at lower temperatures of incubation. Starter culture JC, which was selected to ferment cereal slurries under mesophilic conditions (25°C), also seemed to perform better when the temperature of incubation was high. In the dairy industry, mesophilic bacteria can be defined as those that grow well between 20°C and 30°C (Tamime & Robinson, 1988). Based on this definition it would appear as if bacteria are capable of growing over a wide range of temperatures and there is therefore a considerable degree of overlapping between conditions that can be defined as either mesophilic or thermophilic. Thus a starter culture such as JC which was selected under mesophilic conditions may have a wide range of temperatures in which it would be capable of producing lactic acid. This range may overlap between mesophilic and thermophilic conditions. Secondly, the process of back-slopping that was used to produce starter culture JC leads to the

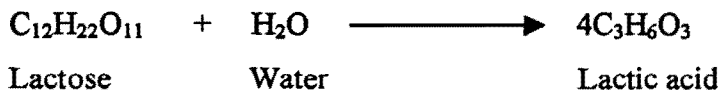
concentration of a population of lactic acid bacteria (Daeschel, Andersson & Fleming, 1987) and not the formation of pure cultures. Some of these bacteria may be thermophilic while others may be mesophilic and this might explain why starter culture JC developed for fermentation under mesophilic conditions was also good at producing lactic acid when the temperature of incubation was increased.

Regarding the optimum conditions of incubation, although the results show that it is not possible to produce fermented gruels with a pH of 4.5 or lower using a lower incubation temperature (30°C or 37°C) after incubation period of 6 h, this can still be done successfully using a longer incubation period of at least 10 h. This is referred to as long fermentation (Tamime & Robinson, 1999). Fermented gruels can also be prepared using a higher incubation temperature (45°C) for a shorter period of time (4-5 h) and this is known as short fermentation (Tamime & Robinson, 1999). The choice of incubation conditions is therefore important because they directly influence acid development rate. When low temperatures of incubation are used for fermentation, the rate of acid development is reduced and this can promote the growth of undesirable micro-organisms during processing (Hemme, Schmal & Auclair, 1981). High standards of hygiene have to be maintained to avoid contamination. For the purposes of this study, incubation of gruels at 45°C was preferred because of enhanced acid production which is effective at suppressing the growth of spoilage and pathogenic bacteria.

As soon as it is drawn from a cow, milk will show an acid reaction with phenolphthalein as an indicator. This is due to the presence of protein, phosphates, CO₂ and citrates (Modler, Larmond, Lin, Froehlich & Emmons, 1983). The titratable acidity of fresh milk ranges from 0.13 to 0.17% and it is referred to as apparent titratable acidity (Newlander & Atherton, 1964). The increase in the proportion of skim milk (i.e. the reduction in the proportion of finger millet gruel) which led to an increase in the titratable acidity of the gruels can be compared with the increase in titratable acidity that has been observed in the manufacture of yoghurt when the proportion of milk solids is increased (Thomopolous *et al.*, 1993). Milk has a higher protein content compared to finger millet and would be expected to have a higher titratable acidity. The amino groups which are

basic and the carboxyl groups which are acidic cause the milk system to resist changes in reaction when acids or bases are added and hence act as buffers (Creighton, 1984).

The increase in acidity that was observed when starter cultures YC380 and V2 were used to ferment gruels was probably due to the fermentation of lactose by the action of the bacteria with the formation of lactic acid by the following reaction:



In addition to their ability to reduce the pH to 4.5 or lower, the starter cultures and incubation conditions were also selected on the basis of the consistency of the gruels that were formed during fermentation. In the presence of milk, the coagulum that was formed when starter cultures YC380 and V2 were used resembled, in appearance, the coagulum that is formed during yoghurt manufacture. This coagulum is produced when the milk protein is partially denatured in the presence of lactic acid and it has been described as a 'soft clot' (Tamime & Robinson, 1999). It has been suggested that such denatured protein is nutritionally superior compared to the protein in unfermented milk because the denaturation uncoils intrastructural linkages and makes the protein more accessible for attachment by enzymes in the digestive tract (Alm, 1981; Rasic, 1987a). On the other hand, when starter culture JC was used the coagulum that was formed had a hard rubbery consistency and it completely separated from the aqueous phase. A possible explanation could be that heterolactic fermentation may have occurred and that lactic acid may not have been the only end-product of the fermentation of the gruels when starter culture JC was used. Some heterofermentative bacteria produce ethanol (Dellaglio 1988a; Hounhouigan, Nout, Houben, & Rombouts, 1993). Addition of ethanol to milk may cause it to coagulate (Fox & Mulvihill, 1990). Denaturation of the protein results from the changes in the secondary, tertiary and/or quaternary structure through disruption of covalent interactions (Brown, 1987). The interactions include hydrogen bonds and these are interrupted by the presence of the -OH group on the ethanol molecule. According to Walstra & Jenness (1984), the effects of low pH and the presence of ethanol on the

stability of the casein micelle are additive. In other words, while the gel that is formed in the presence of lactic acid only consists of casein micelle aggregates with water droplets dispersed within this network, the presence of ethanol leads to the separation of the coagulum from the aqueous phase. In the absence of ethanol, the separation of the coagulum from the whey phase would occur if the pH of the product falls to less than 3.8 (Walstra, van Dijk & Geurts, 1985).

The firmness and consistency of fermented products containing milk can be related to the quantity as well as the type of milk proteins present in the product (Mortensen, 1983; Rohm, 1989; Barrantes, Tamime & Sword, 1994; Mlecko, Achremowicz & Foegeding, 1994). The general increase in the firmness and consistency of gruels with increasing proportions of skim milk (i.e. decreasing proportions of finger millet) that was observed may be partly explained in terms of the increasing proportion of milk proteins, particularly the caseins (Schkoda, Hechler & Hinrichs, 2001a). During fermentation, the acid produced causes the casein to become unstable and coagulation occurs with the formation of a gel in which casein is the main component (Fox & Mulvihill, 1990). The formation of a gel can therefore be considered to be a result of the interactions between casein micelles. Since the firmness and consistency of the gruels depends on the formation of the gel, the proportion of skim milk present in the gruels is important.

In order to understand how increased proportions of skim milk affect the consistency of fermented composite gruels it is important to briefly look at the structure of acid-set milk gels. During fermentation, each casein micelle associates with β -lactoglobulin to form filamentous appendages that project from the surface of the micelle (Davis, Shankar, Brooker & Hobbs, 1978). As the pH decreases during fermentation, casein particles fuse to give larger particles and this leads to coagulation. The appendages form a protective barrier around the micelles and prevent them from excessive fusion as the pH decreases (Dannenburg & Kessler, 1988). At a molecular level, hydrophobic bonding, van der Waals attractions, electrostatic interactions and probably hydrogen bonding contribute to the conformation of the casein micelles (Mulvihill & Fox, 1990; Johnston, Austin & Murphy, 1993). It has been suggested that with increasing proportions of skim milk, more

casein micelles are observed per unit volume of the product and this gives rise to more compact clusters and appendages of casein micelles (Kalab & Harwarkar, 1974; Schkoda, Hechler & Hinrichs, 2001b). This compact structure offers more resistance to a penetrating probe during firmness measurement. The increase in the proportion of skim milk can also be considered as an increase in the space occupied by milk proteins and this has the effect of restricting the mobility of free water (Lankes, Ozer & Robinson, 1998). The net effect is an increase in the viscosity of the gruels and this was observed in this study when the proportion of skim milk in the gruels increased.

The changes in the proportion of skim milk are likely to have only partly explained the firmness and consistency of the gruels because some gruels that had low proportions of or no skim milk had high firmness and consistency values. Retrogradation of starch in the finger millet component of the fermented gruels may have played an important role in the firmness of the gruels. When gels that contain starch cool, the crystallisation of amylose causes the gel to slowly increase in rigidity (Kent & Evers, 1994). The gruels therefore become firmer.

The quality of fermented products that contain milk as determined by viscosity, consistency and smoothness depends in part on acid development which, in turn, is related to incubation temperature (Ying, Duitschaeffer & Buteau, 1990). Gruels with a thick consistency were obtained when an incubation temperature of 45°C was used compared to 37°C. According to Tamime & Robinson (1999), in the manufacture of yoghurt a coagulum of desired firmness is obtained by incubating the inoculated milk at 40-45°C. Lankes *et al.* (1998) observed a decrease in the firmness and consistency of yoghurt when the incubation temperature was lowered to 30°C from 42°C. They attributed this to a decrease in the activity of the thermophilic starter culture which resulted in a slower rate of acidification.

The increase in firmness and viscosity of gruels when the proportion of finger millet was 0% and when the storage temperature was 7°C suggests that there might be a relationship between firmness and the storage temperature. During the ageing of yoghurt and

regardless of storage temperature, bonds between casein molecules continue to be formed. It has been observed that gel firmness increases when a storage temperature of 4°C is used compared to storage at 30°C (Mulvihill & Fox, 1990) and they have attributed the increase to either an increase in the number of bonds between casein micelles or an increase in the strength of the bonds. Some composite gruels that had relatively high proportions of finger millet gruel also had high consistency values when the storage temperature was 7°C. Again, retrogradation of starch in the finger millet component of the gruels cannot be overlooked and may have played an important role in the consistency and the firmness of these gruels. Compared to storage at room temperature, storage of starch gels at low temperatures (but above -5°C) increases retrogradation and hence firmness (Gudmundsson, 1994). This may be the reason why the composite gruels with high proportions of finger millet also had high firmness values.

Changes in the consistency and viscosity are of significance. Increasing the firmness and viscosity imparts texture and mouthfeel to the fermented product and this is important if the fermented products are to be consumed by adults. On the other hand, fermentation that leads to an increase in the viscosity of fermented products may not be desirable in gruels that are prepared for infants as weaning foods. Infants have difficulties swallowing stiff gruels and their capacity to chew is poor. Viscosity also affects the quantity of solids that can be incorporated into gruels. The nutritional implications of this limitation will be discussed later.

The smooth, slimy consistency that was observed when gruels containing milk were fermented using starter culture V2 compared to a more grainy consistency when starter culture YC380 was used suggests that one or both of the bacterial strains in the the starter culture V2 may have been capable of producing exopolysaccharides during fermentation. Exopolysaccharides are mucous substances produced by some strains of yoghurt starter cultures (Dellaglio, 1988b; Petry, Furlan, Crepeau, Cerning & Desmazeaud, 2000). Such starter cultures can play beneficial roles in the rheology behaviour and the texture of the fermented milks by preventing gel fracture and wheying-off and by increasing viscosity (Wacher-Rodarte, Gavan, Farres, Gallardo, Marshall & Garcia-Garibay, 1993; Bouzar,

Cerning & Desmazeaud, 1996). Quantitatively, it was difficult to show the effect of using starter culture YC380 compared to starter culture V2 (which might produce exopolysaccharides), on the viscosity and consistency of the fermented gruels. This is probably because of the confounding effects of finger millet gruel.

According to Lucey & Singh (1998) syneresis can be defined as the contraction of a gel and this occurs concomitantly with expulsion of liquid (whey separation). Both the skim milk and the finger millet components of the gruels contributed to syneresis. In milk the liquid phase is immobilised in a three dimensional matrix composed of casein micelles that are linked to each other (Harwarkar & Kalab, 1983). The liquid occurs as finely dispersed droplets and it can be released from the gel by external forces such as centrifugation (Dannenberg & Kessler, 1988). External forces cause shrinkage of the network as it rearranges itself into a more compact configuration (Walstra, Van Dijk & Geurts, 1985). During the preparation of the gruels, the starch structures took-up some water and as the gel cooled, the amylose chains in the starch (which have a tendency to interact strongly with each other through hydrogen bonding) forced water out of the gel (Hoseney, 1994; Lorri & Svanberg, 1995).

There are at least two ways of looking at why a decrease in the proportion of finger millet (i.e. an increase in the proportion of skim milk) led to an increase in syneresis. Since skim milk contributed more to syneresis of gruels compared to the finger millet, a decrease in the skim milk might have led to a decrease in syneresis. The presence of starch in the fermented gruels might have played an important role in reducing syneresis. Starch may have acted as a stabiliser in the fermented gruels by immobilising the aqueous phase in the milk protein network (Modler & Kalab, 1983; Modler, Larmond, Lin, Froehlich & Emmons, 1983). It may be worth noting that starch (Tamime & Robinson, 1999) and modified starches (Bassett, 1983; Chen & Ramaswamy, 1999; Tamime & Robinson, 1999) are used as stabilisers in commercially-produced yoghurts. As is the case with most carbohydrates, starch is made up of repeating glycosyl units which on average possess three hydroxyl groups. Each glycosyl unit can bind one or more molecules of water. The motions of the water molecules that bind the carbohydrate

molecules are retarded and yet the same molecules are still able to exchange freely and rapidly with bulk water molecules in the capillaries of the gel. It is the bulk water that can be removed by syneresis (Whistler & BeMiller, 1997).

Having established the optimal conditions that were needed to ferment the Gruels successfully and having looked at the characteristics of the Gruels produced, the next stage was to look at the nutrient profiles of the Gruels.

The nutrient that is most likely to have been limiting in the Gruels that were prepared in this study is the energy. Energy requirements have been defined as the amounts needed to maintain health, growth and an appropriate level of physical activity (World Health Organisation (FAO), 1985). The energy requirements of infants are particularly critical because of their rapid growth (Roberts & Young, 1988). If 1 l of Gruel is taken to be equivalent to 1 kg, an adult male who is getting 40% of his energy requirements from the fermented finger millet Gruels or the 50% finger millet and skim milk composite Gruels would need to consume 5 l, or 3.7 l respectively, to meet such requirements. On the other hand an infant consuming 250 ml of either finger millet Gruel or the 50% finger millet and skim milk composite Gruels in four feedings per day would, respectively, meet 50% and 73% of its requirements for energy assuming that the Gruels provide 40% of the daily requirements for energy with the remainder coming from breast milk (assumptions adapted from Wanink, van Vliet & Nout, 1994). Thus, although reducing the proportion of finger millet in the Gruels improved their contribution to energy requirements, the problem of low energy density that is characteristic of the cereal-based Gruels that are commonly used as weaning foods (Nout, Haustvast, van der Haar, Marks & Rombouts, 1988) was apparent. During preliminary research that was carried out, it was found that the swelling of starch that occurred during the cooking of the Gruels led to an increase in viscosity and any attempts to increase the solids content of the Gruels only led to the formation of a stiff product. Such a product was obtained when more than 5% finger millet flour was used. The resulting thick Gruel was difficult to handle and to inoculate. In studies done by Mbugua *et al.* (1984), the amount of maize flour needed to make *uji* (a thin fermented Gruel consumed in East Africa), was limited to 7% for the same reasons.

A thick gruel would also be unsuitable for infant feeding because they have difficulties swallowing (Nout, 1993). The low solids content of fermented and unfermented gruels needs to be addressed because the water content of foods is a critical determinant of energy density. According to the World Health Organisation (1985) the energy density is a measure of the energy content of a given amount of food (per 100 g or per g). Energy density as opposed to macronutrient content of foods is currently believed to be the key factor in the regulation of energy intake (Drewnowski, 1998). If this is the case, then under conditions where food is not restricted, people tend to consume a constant weight of food as opposed to a constant quantity of energy (Roberts, Pi-Sunyer, Dreher, Hahn, Hill, Kleinman, Peters, Ravussin, Rolls, Yetley & Booth, 1998). It can be concluded that the consumption of foods that contain a lower amount of energy per unit weight may contribute to an overall lower energy intake (McCrorry, Fuss, Saltzman & Roberts, 2000). The following example may help to put the relationship between energy density, water content and the bulk of gruels into perspective. If a thick gruel containing 30% flour has an energy density of 5 kJ/g, then a thin gruel from the same type of flour at a concentration of 5% will have an energy density of 0.8 kJ/g (comparable with the energy content of the finger millet only gruels in this study). On the other hand, breast milk has an average energy density of 3 kJ/g (Lorri & Svanberg, 1995). It is clear, therefore, that water will increase the weight or volume of food and thus decrease its energy content when the total energy of a food is held constant. This is of concern in the formulation of weaning foods for infants where energy intake is limited by difficulties in chewing and swallowing, small stomach capacity (Nout, Hautvast, van der Haar, Marks & Rombouts, 1988) and low frequency of feeding compared with the amount of energy and nutrients derived from the meals (Lorri & Svanberg, 1995).

Reducing the viscosity of the finger millet component of the composite gruels could make it possible to increase the flour content and hence nutrient content of the gruels. The use of malt (germinated cereal grain) (Malleshi & Desikachar, 1979; Marero, Payema, Aguinaldo & Homma, 1988; Marero, Payumo, Librando, Lainez, Gopez & Homma) assuming that it does not lead to dry matter losses (Malleshi & Desikachar, 1986) is seen as one of the more promising ways of reducing the paste viscosity gruels.

The enzyme α -amylase that is produced by the germinating grain during malting reduces the bulk of starchy foods by breaking down some of the starch into dextrans and simple sugars (Asiedu, Nilsen, Lie & Lied, 1993). Flour prepared from the germinated seeds can therefore be used in greater amounts to give the same viscosity as flour from ungerminated grain (Malleshi, Daodu & Chandrasekhar, 1989; Mtebe, Ndabikunze, Bangu & Mwemezi, 1993). Thus the use of malted flour would improve the nutrient and energy density of the gruels. Practically, the maximum dry matter that could be included in the gruels that were prepared in this study was 5% (for the finger millet gruels) and 7.5% (for the 50% finger millet and skim milk composite gruels). With the use of malt to reduce the viscosity of the gruels it would be possible to double the dry matter content of the gruels and if the gruels still supplied 40% of the requirements for energy for infants and adults, then for infants the contribution to daily energy requirements (World Health Organisation, 1985) would increase to 100% for the finger millet gruels and 146% for the 50% finger millet and skim milk composite gruels. Adult males would need to consume 2.5 l of the finger millet gruel and almost 1.9 l of the 50% finger millet and skim milk composite gruels. Reducing the viscosity of the gruels would have made it possible to increase the contribution of starch to the energy content of the gruels.

It is important to realise that the energy values quoted in this study and their contribution to energy requirements were possibly over-estimates based on gross energy. They represented the total energy that can be released by the complete combustion of a food-stuff. They included energy from the combustion of the dietary fibre component of the gruels which is not completely available to the body as a nutrient (Gurr, 1984).

Because skim milk was used, it was observed that decreasing the proportion of finger millet led to a decrease in the contribution of fat to the total energy supplied by the gruels. It is essential that a diet provides an adequate source of energy for children because low levels of dietary energy often lead to the utilisation of dietary and tissue nitrogen as a source of energy (Robinson & Tamime, 1999). Because fat contains 37.6 kJ/g compared with 16.7 kJ/g for carbohydrate and protein, foods with fat will have a high energy density and the inclusion of even small portions of foods that are high in fat

can be expected to significantly improve the energy content of gruels such as those that were prepared in this study. It has been shown that supplementation of energy-restricted diets with oil significantly improves nitrogen balance, weight gain and it reduces the loss of nitrogen in urine (Nomani, Forbes, Mossahebi, Salaita, Loth-Haglin, Harvey & Brooks, 2000). Examples of high fat products that are commonly added to gruels include peanut butter and margarine. While the inclusion of fats and oils might improve the energy content of gruels meant for consumption for adults, the use of the same gruels for infant feeding has to be done with care. Intestinal digestion of fat in infants is limited by the amounts of digestive enzymes produced by the pancreas and the bile salts produced by the liver since these tissues are still relatively immature (Gurr, 1981).

It is difficult to tell from the results whether any qualitative changes occurred in the fat content as a result of fermentation. Any changes due to hydrolysis of the fats during fermentation would be of interest since they may influence the sensory properties of the fermented foods (Chavan & Kadam, 1989). Since skim milk, which contains low levels of fat, was used and bacterial starter cultures that are used to make yoghurt are weakly lipolytic (Tamime & Deeth, 1980), a small degree of hydrolysis of the milk fat would be expected and it might contribute to the flavour of the fermented product (Walstra & Jenness, 1984).

Because starch is such an important source of dietary energy (Achinewhu, 1986), the substitution of some of the skim milk with finger millet gruel in the 50% finger millet and skim milk composite is important. It means that those individuals who cannot utilise lactose in dairy products can still get some of the nutritional benefits of consuming dairy products. Thus they will not completely eliminate dairy products from their diets.

There are two ways of looking at why there were no significant changes that were observed in the starch content of gruels as a result of fermentation with starter cultures YC380 and V2. Firstly, and as stated earlier, the bacterial starter cultures that were used for fermentation were selected to ferment lactose as the energy-yielding substrate during the manufacture of yoghurt and may lack the ability to synthesise the amylolytic

enzymes required to break down the starch in the gruels. Secondly, the procedure that was used to determine starch involved enzymatic hydrolysis of the starch using α -amylase and amyloglucosidase followed by the specific measurement of glucose using glucose oxidase (Pomeranz & Meloan, 1994). This means that any changes due to partial hydrolysis of the starch would not be detected. If a culture could be found that would ferment both lactose and starch it would be beneficial in reducing the viscosity of the gruels and in reducing the starch content. It has been suggested that the partial hydrolysis of starch improves the contribution of starch to dietary energy (Graham, Maclean, Morales, Hamaker, Kirleis, Mertz & Axtell, 1986; Dhanker & Chauhan, 1987b). The formation of oligosaccharides by partial hydrolysis of the starch would reduce the starch content of the gruels, particularly the amylose (Khertapaul & Chauhan, 1990b). When foods containing starch are cooked and cooled, the amylose fraction associates to form resistant starch (Knudsen & Munck, 1985) which is not digestible (Knudsen, Kirleis, Eggum & Munck, 1988). Because of the important role of cereal grains in providing energy in the diets of many people (Nyman, Siljestrom, Pederson, Knudsen, Asp, Johansson & Eggum, 1985) any improvements to the contribution of starch to energy needs as a result of fermentation would be desirable.

Any effects resulting from the reduction in pH as a result of fermentation on the viscosity of starch were not apparent. If any reduction in viscosity did occur, the effect was masked by the increase in viscosity that occurred as a result of the coagulation of the milk protein. While Nout (1994) reported that fermenting cereals on their own reduced the viscosity of the gruels, Wanink *et al.* (1994) observed that fermenting cereals in the presence of a high protein source such as legumes (which can be compared with the skim milk that was used in this study) increased the viscosity of the composite gruels.

One of the useful changes resulting from bacterial fermentation and a decrease in the proportion of skim milk in this study was the decrease in the lactose content in the gruels that contained milk. This reduction in the amount of lactose in dairy products is important for lactose-intolerant individuals. The high nutritive value of milk makes it attractive in community-based supplementary feeding programmes. One of the issues that was raised

in the introduction is the need to develop nutritional supplementary foods for infants and children of school-going age using fermentation and fortification with milk. It is important to look at how lactose-intolerance may influence the utilisation of such foods. Studies done in South Africa (Garza, 1979) and the United States (Leveille, 1979) showed that the prevalence of lactose-intolerance among black people is quite high. The studies also suggest that lactose-intolerance has a genetic basis. It has been observed, however, that diets which include fermented dairy products are effective in relieving the symptoms associated with lactose-intolerance (Goodenough & Kleyn, 1976; Kim & Gilliland, 1983; Speck, 1983). According to Gilliland (1985b), lactose-intolerant individuals are deficient in the enzyme β -D-galactosidase (lactase) which is responsible for catalysing the hydrolysis of lactose into glucose and galactose. As a result, lactose passes unchanged from the small intestine to the colon where osmotic equilibrium is disrupted. Water is drawn into the colon and diarrhoea results. Some of the lactose is fermented by the natural bacteria in the gastro-intestinal tract. This results in gas formation (hydrogen) and consequently cramping and bloating occur. Most lactose-intolerant individuals tend to eliminate milk, a rich source of protein and calcium, from their diet (Shahani & Chandan, 1979). Calcium is required for calcification of bone and lactose-intolerant individuals often suffer from osteoporosis (Martin & Mazir, 1985). Lactose-intolerant individuals have been found to tolerate yoghurt but not milk. Yoghurt contains reduced amounts of lactose and studies done by Kelly (1984) showed that the average lactose content of yoghurt decreased from 8.5 % to 5.8 % during fermentation. It is important to note that the lactose is never completely fermented (Rasic, 1987b). The second reason why lactose-intolerant individuals can consume fermented dairy products is that the lactase enzyme that is present in yoghurt survives passage through the stomach and contributes towards digestion of lactose (Kim & Gilliland, 1983). The lactase activity gained from bacterial lactase makes up for the lack of endogenous lactase. Substantial amounts of lactase are bound to bacterial cell walls and gastric digestion helps to release the enzyme (Kilara & Shahani, 1976; Gilliland & Kim, 1983).

The dietary fibre content of the composite gruels came from the finger millet component. Cereal grains are a rich source of dietary fibre (Serna-Saldivar & Rooney, 1995). Crude fibre was determined in this study and it refers to the material that is indigestible in acid

and alkali. This definition, which is based on the method of analysis, limits crude fibre to cellulose, insoluble forms of hemicellulose and lignin (Coffey, Bell & Henderson, 1995). Crude fibre underestimates the fibre content by excluding soluble hemicelluloses (e.g. β -glucans) which are important in human nutrition (Doehlert, Zhang & Moore, 1997). Dietary fibre is a more inclusive term and refers, in general, to plant polysaccharides and lignin which are not digested by endogenous enzymes in the upper gastro-intestinal tract of man (Theander & Westerlund, 1986; Bach Knudsen, Kirleis, Eggum & Munck, 1988). In adults the consumption of dietary fibre is inversely related to cardio-vascular disease (Anderson, Deakins, Floore, Smith & Whitis, 1990), colon cancer (Hillman, Peters, Fisher & Pomare, 1983; Karpinnen, Liukkonen, Aura, Forssell & Poutanen, 2000) and diabetes (National Research Council, 1993), and is therefore recommended. Dietary fibre also forms a bulky mass and speeds-up transit time through the gastro-intestinal tract because of its bulk (Eastwood, Robertson, Brydon & MacDonald, 1983; Kelsay 1999). This alleviates symptoms that are associated with constipation.

While there are nutritional benefits that are observed when fibre is consumed, the level of dietary fibre in gruels may be of concern when energy density is low. This is important with reference to the finger millet gruels. The inclusion of skim milk in the 50 % composite gruels helps to reduce the fibre content. Dietary fibre affects energy density in a number of ways. Fibre may reduce the digestion of starch by accelerating the rate of passage through the intestine (Southgate & Durnin, 1970) which decreases the time available for digestion (Harris, Tasman-Jones & Fergusson, 2000). It has also been observed that an increase in dietary fibre is associated with a change in the other dietary constituents, particularly energy (National Research Council, 1993). This is related to the ability of fibre to add bulk and weight to food. Thus, for a given weight or volume of food, fibre can displace the energy provided by other nutrients. If people consume a constant weight of food rather than a constant quantity of energy (Hill, Melanson & Wyatt, 2000; Ludwig, 2000) then increasing the proportion of dietary fibre leads to lower energy intake (Burton-Freeman, 2000). This is particularly important in infant nutrition where small stomach capacity limits the intake of energy and other macro-nutrients.

Milk supplies all the dietary minerals (ash) that are required by the body except iron (Walstra & Jenness, 1984) and in this study skim milk contributed more to the mineral (ash) content of the composite gruels than the finger millet gruel. Finger millet is one of the few cereals that is not deficient in calcium and is a good source of iron (Serna-Saldivar, 1995). Blending finger millet and skim milk would therefore provide a better balance of dietary minerals than if the two components were utilised separately. This is also important for the mineral calcium which is more readily absorbed in the presence of lactose (reviewed by Oberman & Libudzisz, 1998).

Studies that were done using sorghum (Kazanas & Fields, 1981), sorghum and legume blends (Chavan & Kadam, 1989) and milk (reviewed by Oberman & Libudzisz, 1998) did not show any significant effect of fermentation on the total mineral content. While the results in this study also show that fermentation may not have a significant effect on fermentation an important reason for the continued use of this process is that it improves the bioavailability of calcium, phosphorus and other minerals that bind to phytates (reviewed by Klopfenstein & Hosene, 1995). Fermentation enhances the activity of the enzyme phytase (Dhanker & Chauhan, 1987a).

The results suggest that fermentation did not lead to any significant changes in the protein content of the gruels and this can be related to the procedure which was used to determine the protein. The Kjeldahl method, which was used to determine the crude protein of the gruels, estimates the total nitrogen content of a food and then the nitrogen content is converted to protein using a conversion factor. The assumption is that all the nitrogen in the food is present as protein. Since there was no smell that was detected in the fermented gruels, which might have indicated loss of nitrogen through volatilisation as ammonia, it is highly likely that the amount of nitrogen that was present in the unfermented gruels remained unchanged even after the gruels had been fermented.

While changes in the total nitrogen content may not have occurred as a result of fermentation, the changes in the lysine content as a result of fermentation indicate that some proteolysis of the protein did occur and this would be expected. The bacterial

starter cultures that are used in the manufacture of yoghurt, such as YC380 and V2, tend to utilise proteolytic enzyme systems as a means of making protein and peptide nitrogen available from the milk protein (Law & Kolstad, 1983). Proteolysis of the proteins in the milk leads to the formation of free amino acids which the bacterial cell utilise for cell growth (Miller & Kandler, 1967; Graham *et al.*, 1986). Pre-formed amino acids are, therefore, a requirement for lactic acid bacteria. It may be that the careful selection of starter cultures such as YC380 and V2 which bring about desired changes in fermenting milk has led to the development of proteolytic systems in the bacteria that efficiently break down milk protein to produce free amino acids and the desired organoleptic properties (Law & Kolstad, 1983; Thomas & Pritchard, 1987). This may be another reason (besides the low levels of an energy source in the form of lactose) why bacterial growth may have been low, as reflected by bacterial counts, especially in gruels that contained finger millet gruel only.

Transamination may also have occurred and it could have led to an increase in the lysine content of the gruels with finger millet only and the 50% finger millet and skim milk composite gruels. During transamination the amino group of glutamate is exchanged for an α -keto acid group leading to the formation of a new amino acid (from the α -keto group) and the regeneration of α -ketoglutaric acid from glutamate (Jakubke, Jeschkeit, Cotterrell & Ulbrich, 1977; Brock, Madigan, Martinko & Parker, 1994). Enzymes called transaminases (aminotransferases) are required for this process (McKane & Kandel, 1996). Transamination would provide a way of adjusting the level of lysine to meet the particular requirements of the starter cultures since the lysine content of the gruels might not correspond precisely to the requirements of the bacterial starter cultures.

The protein content of finger millet flour was higher compared to the values that were obtained by Virupaksha, Ramachandra & Nagaraju (1975). They obtained values ranging from 3.49% to 6.33% using endosperm. The difference is likely to be a reflection of the effects of decortication which reduces protein content as a result of degerming (reviewed by Serna-Saldivar & Rooney, 1995).

From a nutritional point of view, the inclusion of skim milk in the finger millet gruels improved the protein content of the 50% finger millet and skim milk composite gruels and their contribution to infant requirements. Infants and pre-school children, who are weaned on bulky cereal-based gruels, risk succumbing to protein deficiency because they constitute the human group that has the highest protein requirements (Walker, 1983). It is therefore important for children to be weaned with a supplement that is of good nutritional quality. Not only do they require protein to support optimal growth, they also require protein for maintenance and mental development. Protein deficiency in the early years of life can lead to stunting and poor brain growth (Mosha & Svanberg, 1990) from which the children are not likely to recover even when nutrition improves (Smart, Massey, Nash & Tonkiss, 1987; Schroeder, Martorell, Rivera, Ruel & Habicht, 1995). Growth tends to dominate the body's requirements for proteins and as a result they require more protein per kg of body weight compared to adults. Infants require protein to foster the well-being of the body and part of this protein is required for the maintenance of the body's defense against invasion by micro-organisms. An infant consuming 250 ml of finger millet gruel and is offered the gruel four times a day would consume only 4 g of protein which constitutes 38% of its daily protein requirements. If the infant consumed the 50% finger millet and skim milk composite gruel (fermented or unfermented) using the same feeding regime, the gruels would more than meet the infant's protein requirements for the day.

Assuming that the gruels supply 60% of the daily protein requirements of an adult man, he would need to consume 7 l of finger millet gruel or approximately 1.5 l of the 50% finger millet and skim milk composite gruels. Given that adults do not have the limitation of small stomach capacity that infants have, they are more efficient at recycling amino acids and that maintenance functions dominate their requirements for proteins, they should be able to meet their requirements for protein from the gruels if they consume enough to meet energy requirements.

The lysine content of the finger millet gruels was lower than the values that have been quoted in literature for finger millet meal. This might have been due to the heat treatment

that was used to sterilise the gruels. Thermal processing affects the availability of lysine through a chemical reaction which occurs between the carbonyl group of a reducing sugar and a primary amino group in the lysine (Gogus, Bozkurt & Eren, 1998). The ϵ -amino group is particularly reactive and once it binds to a reducing sugar it is no longer available as a nutrient (Tsao, Frey & Harper, 1978). Deficiency symptoms that have been observed in studies carried out with animals when lysine is not adequately supplied in the diet include cessation of growth, reduced appetite and emaciation (McWard, Becker, Norton, Terrill & Jensen, 1959).

The use of skim milk to fortify finger millet gruels improved the lysine content of the 50% finger millet and skim milk composite gruels to such an extent that the pattern for requirements for lysine for infants was met. The higher lysine content of the composite gruel compared to that of the finger millet on its own may be a reflection of the complementary effect that one protein can have on the quality of another. Fortification on its own is often recommended as a way of improving the lysine content of cereals (Tsao, Frey & Harper, 1978). Work done using milk protein to fortify wheat and triticale has shown that the quality of protein in the composites improves (Maccoll, 1987). Fortification of wheat with soyabeans and milk (Cheng, Gomez, Bergen, Lee, Monckenberg & Chichester, 1978) as well as wheat, maize and triticale with chickpeas (Del Angel & Sotelo, 1982) without fermentation improved the quality of the protein in the composites. The fortification of cereals with legumes and oilseeds is important in areas where milk is not available or where it is only available seasonally.

Improvements in the lysine content have also been observed when wheat is fermented with milk to make *kishk* (El-Sadek, Zawahry, Mahmoud & El-Motteleb, 1958) and when sorghum has been fermented with milk and soyabeans to make *ogi* (Oyeleke, Morton & Bender, 1985).

Cereals can also be blended with legumes and/or oilseeds such as soyabeans, black gram beans, chickpea, cowpea and peanut and successfully fermented to improve their lysine

content (Beuchat & Nail, 1978; Chavan, Chavan & Kadam, 1988; Chavan & Kadam, 1989; Nche, Nout & Rombouts, 1994) when milk is not available.

The primary function of dietary proteins is to supply a mixture of amino acids in the correct proportions for the synthesis and maintenance of tissue protein. The quality of a protein not only depends on its quantity in a food but on the amino acid composition (Hamad & Fields, 1979) as well as how closely the amino acids meet the specific needs for maintenance, growth and tissue repair. In infants the major specific need is for growth. As stated elsewhere, lysine is likely to be the most limiting amino acid in the gruels that were produced. From the point of view of making the lysine content of the gruels resemble a pattern that has been approved for infants, the use of skim milk to fortify the gruels was successful. It is important to note that the digestibility of the gruels that were prepared in this study may be influenced by chemical reactions which occurred during cooking (e.g. Maillard reactions) which interfere with the release of amino acids from proteins by enzymatic processes. It is therefore necessary to make an adjustment for digestibility when translating requirements for reference proteins to safe levels of intake of ordinary mixtures of dietary proteins (World Health Organisation, 1985).

To sum up, when individual nutrients contribute to the body's requirements they do not do so in isolation. One of the most important relationships between nutrients is the protein-energy ratio. Although both the finger millet gruels and the 50% finger millet and skim milk composite gruels had protein-energy ratios that were lower than the 42-55 that has been recommended for cereal-based weaning foods (Walker, 1990) it is important to note that the protein-energy ratio for the composite gruels was higher. The protein-energy ratio is a measure of how much the protein calories contribute to the total energy of the food. Protein synthesis and break-down are energy-dependent and thus are sensitive to dietary energy deprivation. A restriction in energy in the diet is associated with an increase in the catabolism of body protein in an effort to meet the energy deficiency. This restriction in energy would be observed if gruels such as the finger millet gruels that were prepared in this study were used as the sole source of energy during weaning.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

It was possible to produce fermented finger millet-dairy composite gruels with a pH of 4.5 or lower using yoghurt-type bacterial starter cultures such as V2 and YC380. It has been shown by many workers that most pathogenic as well as spoilage micro-organisms are unable to grow when the pH of the environment is 4.5 or lower. From a food safety point of view, these results are of significance particularly in those areas where methods of preservation such as refrigeration are not readily available. The fermented gruels can be stored under ambient conditions and used as refreshment beverages for adults or supplementary foods for infants as and when required with minimal risk of diarrhoeal diseases provided hygienic procedures are observed during the preparation and storage of the fermented gruels. The availability of such a microbiologically safe product would also contribute positively towards the nutrition of infants who are particularly susceptible to diarrhoeal diseases and yet require frequent feeding. The use of starter culture JC, which was developed to ferment cereals, was not successful in all the gruels that contained milk since the type of coagulum formed was not desirable.

Generally, the starter cultures that were used to produce the fermented composites seemed to prefer thermophilic conditions. A higher temperature of incubation may be desirable since it leads to rapid acid production which minimises the risk of proliferation of spoilage and particularly pathogenic micro-organisms. When starter culture JC was used for fermentation, the reduction in pH to 4.5 or lower occurred only when finger millet was present.

Increasing the proportion of skim milk in the gruels increased the viscosity and the firmness of the gruels. It was also observed that some of the gruels that had low proportions of skim milk, or no skim milk at all, had high consistency and firmness

values. This may have been due to retrogradation of starch, especially when the gruels were stored at 7°C.

Generally, gruels with a thick consistency were obtained when an incubation temperature of 45°C was used compared to 37°C. In the manufacture of yoghurt, an incubation temperature of 40 to 45°C is preferred since the rate of acidification is optimal for a product of the desired consistency and firmness.

The composite gruels that were obtained when starter culture V2 was used for fermentation had a smooth, slimy consistency while those that were obtained when starter culture YC380 was used had a grainy consistency. It is known that some bacterial starter cultures that are used for yoghurt manufacture produce mucous substances called exopolysaccharides which may increase the viscosity of the product. Starter culture V2 may be such a culture. One of the problems that is encountered in the production of fermented products containing milk is whey separation. If starter culture V2 is one of these starter cultures that produce exopolysaccharides, the need to add stabilisers to prevent whey separation may be minimised.

Syneresis was observed in the composite gruels that had higher proportions of skim milk. Since milk was the component that contributed more to syneresis compared to the finger millet gruel, reducing its proportion in the composite gruels may therefore have led to a decrease in syneresis. The presence of starch in the gruels may also have played an important role in reducing syneresis with starch acting as a stabiliser. Starch is sometimes used as a stabiliser in the manufacture of yoghurt.

During the preparation of the gruels, the amount of finger millet flour that could be used was limited to 5%. Attempts to use more flour resulted in a thick gruel that quickly solidified upon cooling. From a nutritional point of view, the water content of gruels is important since it contributes to the poor nutritional quality of cereal-based gruels by reducing their energy and nutrient densities. While replacing some of the finger millet gruel with skim milk markedly improved the energy content of the composite gruels, this

was not sufficient to meet all the daily energy requirements for an infant or an adult. In situations such as weaning where infants require a gruel with high energy or even for adults who might need a high energy refreshment beverage or snack, consumption of the composite gruel as opposed to the gruel that has cereal only would be preferred from a nutritional point of view. Ways that have been suggested of improving the contribution of gruels to daily energy requirements would be the addition of malt (power flour) and/or vegetable oil to the fermented gruels.

A product with decreased fat content, as was present in the composite gruels with increasing proportions of skim milk, might be favoured by adults who are interested in reducing the amount of fat in their diet. No significant changes were observed in the amount of fat as a result of fermentation. It is important to note that a limited degree of lipolysis would be desirable as it contributes towards the flavour of fermented products.

The replacement of some of the finger millet gruel with skim milk in the composite gruels and the use of lactic acid bacterial starter cultures YC380 and V2 led to a decrease in the lactose content of the gruels. It is generally accepted that many individuals tend to eliminate dairy products, which are a valuable source of calcium from their diets as a result of lactose intolerance. On the other hand many workers have shown that if the lactose content of dairy products is reduced through fermentation or even enzymatic hydrolysis, lactose-intolerant individuals will be able to benefit from the consumption of such products without experiencing the discomfort associated with the symptoms of lactose intolerance.

The proportion of starch decreased as some of the finger millet gruel was replaced with skim milk. The use of bacterial starter cultures did not significantly affect the starch content of the gruels. Since starch is such an important source of dietary energy, changes as a result of fermentation which do not lead to the complete break-down of starch and its loss as a nutrient are preferred. Such changes would include the partial hydrolysis of the starch.

The reduction in the dietary fibre content of the gruels as a result of replacing some of the finger millet gruel with skim milk in the composite gruels means that infants, who do not require high levels of fibre in their diets, will benefit from consuming the composite gruels compared to a situation where they consume gruels with cereal only where the fibre content is higher.

The protein content of the composite gruels was significantly improved by replacing some of the finger millet gruel with skim milk. The improvement in the protein content of cereal-based foods with supplementation using milk or legumes has been observed by other workers. Since cereals tend to be low in protein, feeding cereal-based diets on their own to infants would lead to inadequate protein intake. As a result of their higher content of protein, the composite gruels contributed more to the protein requirements of both adults and infants compared to the gruels that had cereal only.

The quality of the protein as measured by the lysine content was higher in the finger millet-skim milk composite gruel compared to the gruels with finger millet only. The improvement in the lysine content with the addition of skim milk was such that the pattern for requirements for infants was met in the 50% finger millet and skim milk composite gruel. It has been suggested that the improvement probably results from the proteolytic activity of bacterial starter cultures which leads to an increase in the free amino acid content of the gruels. This is important in the development of weaning foods for infants who have a high requirement for a high quality protein for growth, the maintenance of tissue integrity and health.

On the whole, the results that were obtained in this study show that it is possible to produce a fermented finger millet and skim milk composite gruel using bacterial starter cultures such as YC380 and V2 which are normally used in the manufacture of yoghurt. A pH of 4.5 or lower can be achieved if incubation is carried out at 37°C or 45°C even when the level of skim milk is low. From a nutritional point of view and in agreement with work published by other workers, the composite gruel is superior when compared with the gruel that is prepared using cereal only.

It is, however, important to appreciate that optimal nutritional value, when a composite gruel such as the one prepared in this study, is used for weaning, as a supplementary food for pre-school children and children of school-going age or as a high energy snack/beverage for adults, optimal nutritional benefits are observed only when the composite is part of a total balanced diet. Ultimately, balanced nutrition comes from a wide choice of foods belonging to all the major food groups i. e. dairy, meat and poultry, cereals and legumes and fruits and vegetables.

In terms of future research on the finger millet-skim milk composite, it might be important to look at the effect of adding different levels of skim milk to a constant quantity of finger millet gruel as one possible way of further improving the energy density. The possibility of tannins (which have been found to be present in red varieties of finger millet) influencing protein digestibility of the composite gruels may also need to be investigated. This would provide information on how much of the protein present is available for utilisation as a nutrient.

CHAPTER 7

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