

## 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

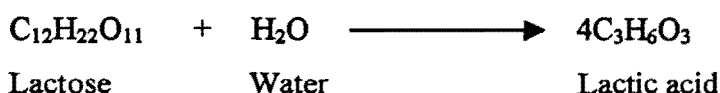
concentration of a population of lactic acid bacteria (Daeschel, Andersson & Fleming, 1987) and not the formation of pure cultures. Some of these bacteria may be thermophilic while others may be mesophilic and this might explain why starter culture JC developed for fermentation under mesophilic conditions 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, CO<sub>2</sub> 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  $\beta$ -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 (McCrary, 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  $\alpha$ -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  $\alpha$ -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  $\beta$ -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.  $\beta$ -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  $\alpha$ -keto acid group leading to the formation of a new amino acid (from the  $\alpha$ -keto group) and the regeneration of  $\alpha$ -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  $\epsilon$ -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.