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INFLUENCE OF COMPLEMENTARY FOOD ON GROWTH AND IRON STATUS OF INFANTS AGED 6-12 MONTHS IN KILOSA DISTRICT TANZANIA

INVLOED VAN COMPLEMENTAIRE VOEDING OP DE GROEI EN IJZERSTATUS VAN KINDEREN VAN 6-12 MAAND IN KILOSA DISTRICT TANZANIA

door

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Dedicated to my beloved Father Mr. Simon Mamiro and Mother Mrs. Theathildis Mamiro who were eager and keen to see the end of what they had prayed for their son to achieve.

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Summary

Childhood malnutrition remains a common and major problem in Tanzania. Protein energy malnutrition (PEM) and micronutrient deficiencies are the major problems that occur during the transitional phase from breast milk to solid complementary foods (CFs) in infants. According to WHO (1999), PEM among under-fives in Tanzania stands at 31% while iron deficiency anemia affects 32% of the infants. Others include iodine deficiency disorders (25%) and Vitamin A deficiency (6%). Most studies have associated inadequate intake and poor utilization of nutrients at the complementing age as the immediate causes of these problems.

In the first chapter various literature sources were consulted. General situation with regard to malnutrition among children including some pertinent causative factors are discussed. A brief discussion on breastfeeding, CFs and micronutrient availability from CFs is presented along with the importance of phytic acid in micronutrient availability. Various techniques that have been used to increase energy density of CFs such as germination and fermentation are described. Quality and safety aspects of CFs with regard to contamination with bacteria, cyanides and mycotoxins have been reviewed. These are important because they might be the potential sources of various diseases affecting childrens' health. Production of low cost CFs that can be afforded by the majority of children, who are faced with nutritional problems, especially in the rural areas of developing countries is discussed. Finally, success and failure stories of intervention programs that were implemented to solve nutritional problems among communities in developing countries from early senventies to the recent years community trials are presented.

In chapter 2, the *in vitro* solubility of iron and zinc in finger millet and kidney beans was evaluated. This study was important since one of our objectives was to improve the micronutrient status, especially that of iron and zinc, among infants in rural Tanzania. Determination of *in vitro* solubility was done by the HCl-Pepsin (HCl-P) and Pepsin-Pancreatin (P-P) methods after soaking, germination, autoclaving, drying and milling of finger millet and kidney beans into flour. These methods mimic the gastro-intestinal processes that take place during food digestion in humans. Germination significantly increased the solubility of iron and zinc.

Phytic acid, which is a common constituent of cereal grains, some vegetables and fruits, has mineral binding capacity and thereby reduces the bioavailability of minerals and trace elements such as iron and zinc. The processing of infants' cereal based CFs such as soaking and germination, activates a multitude of enzymes including phytases, which in turn hydrolyses the phytic acid. This activation will result in improving iron and zinc bioavailability since degraded products of phytate have a lower affinity for minerals and trace elements. During overall processing, phytic acid was reduced by 54% and 28% in finger millet and kidney beans, respectively. Iron solubility from germinated finger millet and kidney beans as determined by the pepsin pancreatin method increased more than four times with the addition of vitamin C or mango. Addition of Vitamin C or mango could be used to enhance mineral solubilities and therefore assist in alleviating micronutrient deficiencies among infants complemented with these foods.

Chapter 3 addresses the issue of pathogen outgrowth and toxin production as a result of soaking and germination of finger millet and kidney beans. Pathogenic bacteria

could be introduced to the germinating and germinated seeds in a number of pathways, including via the seeds, the water used during germination, unsanitary production practices and mishandling by the consumer. Prior to germination, seeds harbour significant levels of micro-organisms. The microflora of the seeds increases during germination, and the presence and growth of pathogenic bacteria can be a cause for concern. The study examined survival and growth of *Staphylococcus aureus* and *Bacillus cereus* during germination and solar drying of finger millet and kidney beans. Conditions during solar drying of the germinated seeds were found unsuitable for excessive growth of pathogenic bacteria because for finger millet, the water activity fell from 1 to 0.48 after 4 hr of drying while in the case of kidney beans, 7 hr were necessary to reach a water activity of 0.85. This difference in drying behaviour can be attributed to the smaller size of the millet grains. Furthermore, contamination of the grains with *B. cereus* and *S. aureus* prior to or during germination led to multiplication of both species in kidney beans and of only *B. cereus* in finger millet. Excessive growth of these pathogens in germinating legumes and cereals can lead to the production of heat-resistant toxins, resulting in unsafe germinated grains. Therefore, hazard analysis and critical control points (HACCP) procedures should be followed in all food-processing units where germination of finger millet or kidney beans is performed.

Chapter 4 discusses the contamination of mycotoxins and specifically fumonisins in food. Moulds may invade agricultural products such as finger millet, kidney beans and peanuts during their growth stage and storage. Climatic conditions and poor storage conditions frequently encountered in developing countries including Tanzania, favour the occurrence of mycotoxins. Fumonisins are important because

they have been associated with a number of diseases including cancer in humans. Maize, which is a staple for the majority of the Tanzanian rural population, is extremely vulnerable to *Fusarium*, which produces fumonisins. Determining the natural occurrence of fumonisins in human foods is important to establish the health risks associated with these toxins. Since cereal grains will continue to be the major basic diets of infants and adults in developing countries, improvement of the quality of these foods and their products should be given priority. With the growing awareness of the toxicity of fumonisins, it is of utmost importance that studies are conducted to give an indication of the extent of the problem.

Finger millet, kidney beans and peanuts were collected from various farmers, transporting lorries and market retailers in Tanzania after harvest in July 2001 and six months after harvest in January 2002. The objective was to evaluate the presence and exposure of fumonisin associated with consuming these ingredients, which are used in the formulation of complementary food (CF) for infants in Tanzania. The grains investigated were found to have fumonisin concentrations ranging from 5 $\mu g\ kg^{-1}$ to 440 $\mu g\ kg^{-1}$, which is below the suggested cut-off point of $1 \times 10^3\ \mu g\ kg^{-1}$. Peanut samples collected after harvest were found to have comparatively high fumonisin levels (mean 105 $\mu g\ kg^{-1}$), followed by kidney beans (mean 43 $\mu g\ kg^{-1}$) and finger millet (mean 5 $\mu g\ kg^{-1}$). Results also showed that 90% of samples of finger millet, kidney beans and peanuts had fumonisin contamination levels lower than 18, 77 and 194 $\mu g\ kg^{-1}$ respectively, while 90% of the children were exposed to maximum fumonisin intakes of 0.65, 0.45, 0.15 and 0.06 $\mu g\ kg^{-1}$ body weight day^{-1} from consuming all CFs, peanuts, kidney beans and finger millet, respectively. The exposure associated with the intake of fumonisin was estimated at 1%, which implied

that 99% of the infants were below the suggested tolerable total dietary intake (tTDI) of 2μg kg^{-1} body weight day^{-1}. The importance of this study is based on the fact that these ingredients are aimed at formulating a complementary food for infants where the potential presence of fumonisins and the tender age of the infants have to be compromised.

Chapter 5 evaluated the background information gathered in the proposed study area as a preliminary preparation of the intervention study. A progressive recruitment of 309 infants aged six months was undertaken in March 2001 to March 2002 with the objective of determining the prevalence of nutritional deficiencies. Hemoglobin concentration (Hb), Zinc Protoporphyrin (ZP) concentration and anthropometrical parameters were measured. A previous 24 hr dietary recall survey conducted in the same community investigated the eating habits of 378, 0-24 months old children. Anemia and iron deficiency was highly prevalent among infants in the district. Mean hemoglobin was 9.3 ± 1.9 g dL^{-1} whereby 79% of the infants were anemic (< 11 g dL^{-1}). It was equally found that the mean zinc protoporphyrin was 10.0 ± 6.2 μg g^{-1} Hb whereby 76% of the infants were iron deficient (> 5 μg g^{-1} Hb). About 35% of the infants were stunted. Low birth weight was associated with stunting and anemia, but surprisingly it was not associated with iron deficiency. Having malaria was associated with stunting, anemia and iron deficiency. Equally low maternal body mass index was associated with stunting but not with iron deficiency and anemia. The 24 hr recall revealed that few infants had an occasional mixture of ingredients in their complementary foods especially during harvest season. However, for the majority of the infants, mainly thin porridge prepared from maize flour was supplied as complementary food in most households. The results showed that nutritional

deficiencies were present in the study area, and that an intervention was necessary to improve the situation.

Chapter 6 describes the intervention, which was done with the objective of testing the effect of a locally made and processed CF on growth and iron status of 6-12 months old infants. A CF was developed at Ghent University based on locally produced crops in Tanzania. Finger millet, kidney beans, peanuts and mango were processed separately and then combined on the basis of their amino acid scores and energy content into a CF for children at complementing age. A mixture containing 65.2, 19.1, 8.0 and 7.7% of the processed finger millet, kidney beans, peanuts and mango gave a protein with a high amino acid chemical score (0.84) and provided an energy density of 1731kJ/100g DM. A gruel with 35% solids (w/v) was fluid enough for consumption by a child of complementing age. Energy intake from the CF alone covered the daily recommended amounts. The study was a double blind randomised placebo-controlled trial in which the main investigator and the mother were blinded with regard to the type of food given to the infants. Infants were continuously recruited by phases and progressively allocated to receive processed and non-processed CF when they reached the age of six months and followed up to the age of 12 months. An acceptability trial for the CF was done involving 50 mothers with their infants before intervention. All the panelists reported that they would like to give the CF to their children as well learn how to prepare the formulation. This was probably an indication that the CF will rapidly be adopted.

Mean hemoglobin for infants taking processed CF and placebo at 6 months of age was 9.2 g dL^{-1} and 9.4 g dL^{-1} and at 12 months of age was 9.7 g dL^{-1} and 9.7 g dL^{-1},

respectively. Equally, mean zinc protoporphyrin at 6 months of age was 9.9 μg g^{-1} Hb and 9.9 μg g^{-1} Hb and at 12 months of age was 5.9 μg g^{-1} Hb and 6.2 μg g^{-1} Hb, respectively. The differences of hemoglobin and zinc protoporphyrin concentrations between the two groups were not significantly different. The anthropometric measurements were also not significantly different between the two intervention and placebo groups at 12 months of age. The mean weight for length z-scores (WLZ) for infants taking processed CF and placebo declined from 0.72 and 0.45 at 6 months of age to -0.27 and -0.07 at 12 months of age, respectively. Likewise, mean length for age z-scores (LAZ) for infants taking processed CF and placebo declined from -1.54 and -1.53 at 6 months of age to -2.08 and -2.04 at 12 months of age, respectively. Malaria had an effect on both hemoglobin and zinc protoporphyrin during the intervention period. Results indicated that processing did not have the effect of improving growth, hemoglobin and iron status of infants. It is concluded that processing does not necessarily matter as long as sufficient CF is provided and nutritional guidance is appropriate. The gain in hemoglobin and ZP observed in both groups by either feeding processed or non-processed food suggests that the amount of soluble micronutrients does not necessarily reflect bioavailability and utilization by the body.

Samenvatting

Ondervoeding van het jonge kind blijft een veel voorkomend en belangrijk probleem in Tanzania. Eiwit-energie ondervoeding (PEM) en tekorten aan micronutriënten zijn de belangrijkste problemen die voorkomen tijdens de overgangsfase van borstvoeding naar vaste bijvoeding (CFs) bij zuigelingen. Volgens de WHO (1999), treft men bij 31 % van de kinderen onder de 5 jaar PEM aan terwijl er bij 32 % anemie tengevolge van ijzertekort wordt vastgesteld. Andere studies gaan over aandoeningen tengevolge van jodium tekort (25%) en vitamine A tekort (6%). De meeste studies hebben de onmiddellijke oorzaken van deze problemen in verband gebracht met een ontoereikende energieopname en een onvoldoende gebruik van nutriënten op de leeftijd waar bijvoeding wordt verstrekt.

In het eerste hoofdstuk werden verschillende literatuurbronnen geraadpleegd. Dit hoofdstuk behandelt de algemene omstandigheden met betrekking tot ondervoeding bij jonge kinderen en geeft tevens een aantal pertinente oorzakelijke factoren aan. Er wordt een korte discussie gevoerd rond borstvoeding en CFs. Hierna volgt een uiteenzetting over de beschikbaarheid van micronutriënten uit CFs, evenals het belang van fytaat hierbij. Er worden verschillende technieken besproken die werden gebruikt om de energie densiteit van CFs te verhogen, zoals kiemen en fermentatie. De kwaliteit en de veiligheid van de geproduceerde CFs met betrekking tot contaminatie met pathogenen, cyaniden en mycotoxines werd geëvalueerd, omdat dit mogelijke bronnen kunnen zijn van verschillende ziektes die de gezondheid van het kind bedreigen. Er wordt een discussie gevoerd omtrent de productie van goedkope CF's die de meeste kinderen met nutritionele problemen, vooral in de rurale gebieden in ontwikkelingslanden, zich kunnen aanschaffen. Tot slot wordt er een historiek

gegeven vanaf het begin van de jaren zeventig tot en met de huidige interventiestudies, over het succes en het mislukken van interventie programma's om aan nutritionele problemen in ontwikkelingslanden een oplossing te bieden.

In hoofdstuk 2 wordt de *in vitro* oplosbaarheid van ijzer en zink in gierst en bonen geëvalueerd. Deze studie was belangrijk omdat het verbeteren van de micronutriënten status, vooral deze van ijzer en zink, bij kinderen in landelijke gebieden in Tanzania één van onze doelstellingen was. De *in vitro* oplosbaarheid werd bepaald door middel van de HCl-Pepsine (HCl-P) en de Pepsine-Pancreatine (P-P) methoden na weken, kiemen, sterilisatie, drogen en malen van gierst en bonen tot bloem. Deze methoden zijn een simulatie van de gastro-intestinale processen die plaatsvinden in het menselijk lichaam tijdens het verteren van voedsel. Kiemen verhoogt in aanzienlijke mate de oplosbaarheid van ijzer en zink. Fytaten, die vooral voorkomen in granen en peulvruchten, hebben een minerale bindingscapaciteit en reduceren hierdoor de bio-beschikbaarheid van sporenelementen zoals ijzer en zink. De processen toegepast bij op graangewassen gebaseerde bijvoeding voor kinderen, zoals weken en kiemen, activeren verschillende enzymen, waaronder fytasen, die op hun beurt fytaat hydrolyseren. Deze activatie zal resulteren in een verbetering van de ijzer en zink bio-beschikbaarheid aangezien de degradatieproducten van fytaat een lagere affiniteit vertonen voor mineralen en sporenelementen. Tijdens het ganse proces werden fytaten in respectievelijk gierst en bonen met 54 % en 28 % gereduceerd. De oplosbaarheid van ijzer afkomstig van gekiemde gierst en bonen, zoals bepaald door de pepsine-pancreatine methode, lag viermaal hoger indien vitamine C of mango werd toegevoegd. Het toevoegen van vitamine C of mango zou kunnen gebruikt worden om

de minerale oplosbaarheid te verhogen, en zou op die manier tekorten aan micronutriënten kunnen verhelpen bij kinderen die deze bijvoeding krijgen.

Hoofdstuk 3 behandelt het essentiële probleem van pathogenen en toxine productie ten gevolge van het weken en het kiemen van gierst en bonen. Besmetting met pathogene bacteriën of toxines tijdens het kiemingsproces of van de gekiemde zaden kan gebeuren via een aantal kanalen, zoals de zaden, het water dat gebruikt wordt tijdens het kiemen, onhygiënische productiepraktijken en een verkeerde behandeling door de gebruiker. Nog voor het kiemen bevatten de zaden reeds belangrijke hoeveelheden micro-organismen. Deze microflora op de zaden neemt verder toe tijdens het kiemen en de aanwezigheid en de groei van pathogene bacteriën kan een reden zijn tot bezorgdheid. Deze studie onderzocht daarom het voorkomen en verder uitgroeien van twee pathogenen, nl. *Staphylococcus aureus* en *Bacillus cereus*, tijdens het kiemen en drogen in de zon van gierst en bonen. De omstandigheden tijdens het drogen in de zon van de gekiemde zaden bleek ongeschikt voor een overmatige groei van beide pathogene bacteriën. Wat betreft gierst daalde de water activiteit van 1 naar 0.48 na 4 uur drogen en in het geval van bonen waren er 7 uur nodig om een water activiteit van 0.85 te bekomen. Dit verschil kan in verband gebracht worden met de kleinere afmetingen van de gierstkorrels. Verder stelt men vast dat de besmetting van de zaden met *B. cereus* en *S. aureus* vóór of tijdens het kiemen leidt tot een vermenigvuldiging van beide soorten bij bonen maar enkel van *B. cereus* bij gierst. Overmatige groei van deze pathogenen tijdens het kiemen van bonen en granen kan leiden tot de productie van hitte bestendige toxines. Daarom is een HACCP (hazard analysis and critical control points) noodzakelijk in alle productie-eenheden waar het kiemen van gierst of bonen plaatsvindt.

Hoofdstuk 4 behandelt de besmetting met mycotoxines en voornamelijk fumonisines van ingrediënten gebruikt bij de productie van CFs. Schimmels kunnen landbouwgrondstoffen zoals gierst en bonen zowel vóór als na de oogst aantasten. De klimatologische omstandigheden en de gebrekkige bewaringsvoorwaarden die frequent voorkomen in ontwikkelingslanden, ook in Tanzania, bevorderen het ontstaan van mycotoxines. Fumonisines zijn belangrijk omwille van hun associatie met een aantal ziekten waaronder kanker. Vooral maïs dat voor de meerderheid van de Tanzanianen het hoofdvoedsel betekent, is bijzonder gevoelig voor *Fusarium*, dat fumonisines produceert. Het bepalen van de natuurlijke aanwezigheid van fumonisines in voedsel voor de mens is belangrijk om de gezondheidsrisico's vast te leggen die verbonden zijn met deze toxines. Aangezien graan het belangrijkste basisvoedsel zal blijven voor volwassenen en kinderen in ontwikkelingslanden zou de verhoging van de kwaliteit van dit voedsel en haar afgeleide producten prioriteit moeten krijgen. Samen met het groeiend besef van de toxiciteit van fumonisines, is het belangrijk dat er studies worden verricht die een indicatie geven over de omvang van het probleem. Staalname van gierst, bonen en aardnoten werd uitgevoerd bij verschillende boeren, bij de vrachtwagens die het transport verzekeren en bij de markthandelaars in Tanzania, en dit op twee verschillende perioden, nl. na de oogst in juli 2001 en zes maand later in januari 2002. De bedoeling was het evalueren van de aanwezigheid en de blootstelling van fumonisine geassocieerd met de opname van ingrediënten die gebruikt worden voor het aanmaken van bijvoeding (CF) voor kinderen in Tanzania. Het gehalte fumonisine in de onderzochte stalen onmiddellijk na de oogst bleek te variëren van 5 $\mu g\ kg^{-1}$ tot 440 $\mu g\ kg^{-1}$, hetgeen lager is dan de aanbevolen limietwaarde van $1 \times 10^3\ \mu g\ kg^{-1}$. Monsters van aardnoten genomen zes

maanden na de oogst bleken vergelijkbaar hoge fumonisine waarden te vertonen (gemiddeld 105 µg kg^{-1}), gevolgd door bonen (gemiddeld 43 µg kg^{-1}) en gierst (gemiddeld 5 µg kg^{-1}). De resultaten toonden ook aan dat 90% van de monsters van gierst, bonen en aardnoten een besmetting met fumonisine vertoonden lager dan 18. 77 respectievelijk 194 µg kg^{-1} terwijl 90% van de kinderen blootgesteld werden aan een maximale fumonisine inname van 0.65, 0.45, 0.15 en 0.06 µg kg^{-1} lichaamsgewicht day^{-1} afkomstig uit respectievelijk alle CFs, aardnoten, bonen en gierst. De blootstelling geassocieerd met de inname van fumonisine werd geschat op 1 % hetgeen impliceerde dat 99% van de kinderen onder de aanbevolen toelaatbare totale inname (tTDI) bleef van 2 2µg kg^{-1} lichaamsgewicht dag^{-1}. Het belang van deze studie volgt uit het feit dat deze ingrediënten bedoeld zijn om bijvoeding aan te maken voor kinderen waarbij een oplossing dient te worden gevonden voor de potentiële aanwezigheid van fumonisines en de gevoeligheid van de kinderen op deze leeftijd.

Hoofdstuk 5 evalueert de achtergrondinformatie verkregen in het voorgestelde onderzoeksgebied als een kennismaking en voorbereiding van de interventiestudie. Vanaf maart 2001 tot maart 2002 werden er in totaal 309 kinderen van 6 maanden oud opgevolgd, met als doel het bepalen van de prevalentie van nutritionele tekorten. De hemoglobine concentratie (Hb), de zink protoporphyrine (ZP) concentratie en de anthropometrische parameters werden gemeten. Voorafgaandelijk werden in dezelfde gemeenschap de eetgewoonten van 378 kinderen tussen de 1 en 24 maand oud bestudeerd, en dit via een 24 uur dietary recall. Er werd een hoge prevalentie van anemie en ijzertekort bij de kinderen in het district vastgesteld. De gemiddelde hemoglobine waarde bedroeg 9.3 ± 1.9 g dL^{-1} waarbij 79% van de kinderen anemisch

waren (<11 g dL^{-1}). Men vond tevens dat de gemiddelde zink protoporphyrine waarde 10.0 ± 6.2 µg g^{-1} Hb hemoglobine bedroeg, hetgeen betekent dat 76% van de kinderen een ijzertekort hebben (> 5 µg g^{-1} Hb). Ongeveer 35 % van de kinderen had een te kleine gestalte (lengte voor leeftijd, stunting). Een laag geboortegewicht werd geassocieerd met stunting en anemie, maar verrassend genoeg werd dit niet geassocieerd met ijzertekort. Malaria werd eveneens geassocieerd met stunting, anemie en ijzertekort. Evenzo werd een lage body mass index van de moeder geassocieerd met stunting maar niet met ijzertekort en anemie De 24 hr recall leerde dat maar enkele kinderen slechts nu en dan een mengsel van ingrediënten in hun bijvoeding kregen, voornamelijk tijdens het oogstseizoen. In de meeste gezinnen wordt aan de kinderen als bijvoeding hoofdzakelijk dunne pap gegeven op basis van maïsbloem. De resultaten toonden aan dat er nutritionele tekorten aanwezig waren in het studiegebied en dat een interventie noodzakelijk was om de situatie te verbeteren.

Het laatste hoofdstuk beschrijft de interventie die werd uitgevoerd om het effect uit te testen van een ter plaatse gemaakte en ontwikkelde bijvoeding (CF) op de groei en de ijzerstatus van kinderen tussen 6 en 12 maanden oud. Er werd een bijvoeding op punt gesteld aan de universiteit van Gent gebaseerd op lokaal geproduceerde gewassen in Tanzania. Gierst, bonen, aardnoten en mango werden afzonderlijk behandeld en daarna gecombineerd op basis van de hoeveelheid aminozuren en energie inhoud om te komen tot een bijvoeding bestemd voor kinderen van 6 tot 12 maand oud. Een mengsel dat 65.2, 19.1, 8.0 en 7.7% van respectievelijk behandelde gierst, bonen, aardnoten en mango bevatte gaf een eiwit met een hoog gehalte aan essentiële aminozuren (chemische index 0.84) en leverde een energie densiteit van 1731kJ/100g DM. Een pap met 35% vaste stof (w/v) was vloeibaar genoeg om door een kind, op de

leeftijd van bijvoeding, te worden gegeten. De energieopname uit alleen deze CF dekte reeds de dagelijks aanbevolen hoeveelheid voor CFs. De studie was een dubbel blind gerandomiseerde en placebo-gecontroleerde interventie waarbij de onderzoeker en de moeders niet op de hoogte waren van het type voeding dat aan de kinderen werd gegeven. Er werden continu kinderen toegevoegd aan de studie, waarbij zij vanaf de leeftijd van 6 maand stapsgewijze werden toegewezen om behandelde of niet behandelde bijvoeding te krijgen. Daarna werden zij opgevolgd tot de leeftijd van 12 maanden. Voorafgaand aan de interventie werd de aanvaardbaarheid van de bijvoeding getoetst waarbij 50 moeders met hun kinderen betrokken waren. Al de panelleden gaven te kennen dat zij bijvoeding aan hun kinderen zouden willen geven en dat zij ook wilden leren hoe deze aan te maken. Dit was een aanwijzing dat deze bijvoeding vrij snel aanvaard kon worden.

Het gemiddeld hemoglobine gehalte in het bloed bij kinderen die behandelde bijvoeding kregen en een placebo op de leeftijd van 6 maand bedroeg respectievelijk 9.2 g dL^{-1} en 9.4 g dL^{-1} en op de leeftijd van 12 maanden 9.7 g dL^{-1} en 9.7 g dL^{-1}. De gemiddelde zink protoporphyrine waarde op de leeftijd van 6 maand bedroeg respectievelijk 9.9 µg g^{-1} Hb en 9.9 µg g^{-1} Hb en op de leeftijd van 12 maanden 5.9 µg g^{-1} Hb en 6.2 µg g^{-1} Hb. De verschillen in hemoglobine en zink protoporphyrine concentraties tussen de 2 groepen waren niet significant verschillend. De anthropometrische waarden waren eveneens niet significant verschillend tussen de interventie en de placebo groep op de leeftijd van 12 maanden. De gemiddelde gewicht op lengte z-scores (WLZ) bij kinderen die behandelde bijvoeding kregen of een placebo, daalden respectievelijk van 0.72 en 0.45 op de leeftijd van 6 maanden tot -0.27 en -0.07 op de leeftijd van 12 maanden. Evenzo daalde de gemiddelde lengte

voor leeftijd z-scores (LAZ) bij kinderen die behandelde bijvoeding kregen of een placebo van respectievelijk -1.54 en -1.53 op de leeftijd van 6 maanden tot -2.08 en -2.04 op de leeftijd van 12 maanden. Malaria had zowel een invloed op de hemoglobine als op de zink protoporphyrine waarde tijdens de interventie periode. De resultaten toonden aan dat bewerking en verwerking van de complementaire voeding geen positieve invloed had op de groei, de hemoglobine en de ijzerstatus van de kinderen. Hieruit wordt de conclusie getrokken dat bewerking en verwerking niet noodzakelijk van belang is zolang als er voldoende CF wordt toegediend en er een aangepaste nutritionele begeleiding is. De toename van hemoglobine en ZP die werd waargenomen in beide groepen, bij zowel diegenen die behandeld voedsel kregen als niet behandeld, wijst erop dat de hoeveelheid oplosbare micronutriënten niet noodzakelijk de bio-beschikbaarheid en het nuttig gebruik door het lichaam weergeeft.

CHAPTER 1

Literature review

1 Literature review

1.1 The problem of malnutrition in children

1.1.1 Introduction

Malnutrition is a major public health problem in Asia and Africa and is one of the main causes of morbidity and mortality among infants and children (Bhandari *et al.*, 2001). In Tanzania protein energy malnutrition (PEM) is relatively low during the first six months of life, but increases rapidly, peaking between 12 to 24 months of age. The ultimate manifestation of this problem is the high infant and young child mortality rates, which stands at 104 and 165 per thousand live births, respectively (UNICEF, 2003). About 10% of the child deaths are attributable to severe PEM (Kingamkono, 1999). Among under-five children in Tanzania, PEM, which comes in various forms such as kwashiorkor, marasmus or marasmic-kwashiorkor combined with other diseases, such as malaria, diarrhea and respiratory tract infections, account for 50% of all infant and child deaths (World Bank, 2000). Anemia is also widespread and increasing among children in Tanzania especially in the rural areas (Stoltzfus *et al.*, 1998). Iron deficiency affects 32% of the children (World Bank, 2000). According to the Bureau of Statistics (1997), in 1992, a total of 43% of the children under five years of age were stunted and 6% were wasted. In 1996 the situation of malnutrition was very similar with 43% and 7% of the children under five years of age stunted and wasted, respectively. Why children's growth and development is so impaired is a very complex multi-causal problem with age specific differences in causality.

Developing countries need better complementary foods in order to combat malnutrition, which is a prevalent cause of morbidity and mortality in infants and

children. Part of the solution on global efforts to reduce malnutrition and mortality could succeed, if focus is primarily directed to infants and children (Dewey, 2001). Interventions to prevent and significantly reduce the prevalence of malnutrition among children in developing countries have traditionally focused on children who are under 5 years of age. There is however, a growing consensus that the greatest nutritional threat to children occurs in the period from about 6 to 24 months of age (WHO, 1999). Children between the age of 6-24 months represent a small proportion of beneficiaries of supplementary feeding programs. Early childhood feeding practices and patterns are important determinants of the nutritional status of the children, which in turn influences their health status. Protein energy malnutrition in young children ranks as one of the most prevalent forms of malnutrition. Although the causes behind malnutrition are diverse and complex, inadequate dietary intake, particularly at complementing-age, is a major contributing factor and deserves critical attention (den Besten *et al.*, 1998). Dewey (2001) has observed that in disadvantaged populations, growth faltering, accompanied by macro and micronutrient deficiency and high rates of infections, is most evident in the first year of life. She further reports that after two years of age it is very difficult to reduce the faltering that had occurred earlier, and suggests identification of effective interventions in promoting optimal growth in early life.

The timing of introduction of complementary foods besides breast milk has important implications for the child. Early complementation especially under unhygienic conditions can result in infections and thus lower immunity to diseases. UNICEF (1998b) estimates that as much as 50% of all children in tropical countries pass through a period of serious malnutrition when growing up. The period of

complementay feeding is particularly critical because the consequences are high morbidity as well as permanently impaired physical and intellectual development.

1.1.2 Diseases affecting children

1.1.2.1 Malaria

Worldwide, malaria causes 100 million morbid episodes and one million deaths annually (Van den Hombergh *et al.*, 1996). Sub Saharan Africa suffers most of the burden of mortality and morbidity from malaria. Severe anemia and malaria are also a significant burden to health facilities in Sub Saharan Africa accounting for much of the hospitalization (Menendez *et al.*, 1997). The disease takes the life of 5% of the children before they reach the age of five years. Malaria, a febrile disease caused by a sporozoa of the genus *Plasmodium*, is also a major public health problem in Tanzania and is often associated with anemia (Mnyika *et al.*, 2000). Anemia resulting from malaria develops as a result of mechanical destruction of red blood cells by the invading parasites and suppressing the production of new red blood cells. Thus, severe anemia is increasingly recognized as an important manifestation of severe malaria in young children (Snow *et al.*, 1994; Newton *et al.*, 1997). In some studies it has been found that iron deficiency anemia occurs in areas where transmission of malaria is endemic, most notably Sub Saharan Africa (Newton *et al.*, 1997).

According to WHO (2001), malaria as a disease does not cause iron deficiency *per se*, because much of the iron in hemoglobin released from the ruptured cells stays in the body. There have been conflicting reports among researchers whether malaria was the main cause of anemia or that it is due to dietary iron deficiency (Asobayire *et al.*, 2001). Brabin (1992) hypothesized that the severe fever caused by malaria disrupts

the normal body physiological processes, which possibly reduce absorption of iron and hence the mode through which malaria can cause nutritional anemia. However, he insists that the mechanism in subjects experiencing chronic recurrent parasitemia is unknown. Similar views were reported by INACG (1999) that iron deficiency anemia occurs in areas where malaria transmission is endemic. A study conducted by Menendez *et al.* (1997) on iron supplementation and malarial chemoprophylaxis for prevention of severe anemia and malaria in Tanzanian infants, concluded that iron supplementation of infants is important to prevent iron deficiency anemia even in malaria endemic areas.

1.1.2.2 Diarrhea

Diarrhea was estimated to be the number one killer of children under five years of age at the beginning of the 1990s and now continues to be a major cause of death among the world's children (UNICEF, 1998b). More than 3 million children are estimated to die each year as a direct cause of diarrhea (WHO, 1999). Young children 6 months to 36 months of age in particular are at high risk, with an annual number of diarrhea episodes being as high as ten per child (WHO, 1998b). In Tanzania, young children experience about five episodes of diarrhea annually, causing about 23% of paediatric admissions and 16% of deaths at the national referral hospitals (Kingamkono *et al.*, 1995). Diarrhea is oftenly caused by ingesting pathogenic micro-organisms or their toxins, which may be spread through drinking water, food, utensils, hands and vectors like flies (Afifi *et al.*, 1998). Foods prepared for infants and drinking water in developing countries are frequently contaminated by enteropathogenic bacteria. The majority of these micro-organisms are inactivated during proper cooking, but some bacteria spores and enterotoxins are heat stable (Motarjemi *et al.*, 1993).

Recontamination of the food by the germinating spores on storage may allow bacteria to proliferate very rapidly to dangerous levels. Most of the diarrhea related deaths are due to loss of large quantities of water (dehydration) and electrolytes (sodium, potassium and bicarbonate) from the body in liquid stool. Many of these deaths can be prevented with the use of oral re-hydration therapy (ORT) (WHO, 1999). In addition to ORT, efforts to control diarrhea in developing countries over the past decade have been based on multiple interventions, which included the promotion of breastfeeding, adequate complementary feeding, safe water supply and safe faeces disposal (UNICEF, 1998b). Studies done in Peru found that caloric intake decreased by 10-20% during diarrheal episodes and in one day's time, acute diarrhea could result in a loss of 2% of a childs body weight (Brown *et al.*, 1990). Death of infants and young children with acute diarrhea is normally the ultimate result if medical attention is not sought quickly.

1.1.2.3 Anemia and iron deficiency anemia

Anemia is the most prevalent nutritional problem especially in developing countries due to disease infections and blood loss, causing clinical anemia and inadequate iron nutrition causing nutritional anemia (Stoltfus *et al.*, 1997; Jackson and Al-Mousa, 2000). The most vulnerable group include infants, preschoolers and pregnant women in developing countries and especially in rural areas (WHO, 1999). For instance, about 80% of the preschool children in Cote d'Ivoire were anemic compared to 50% of school age children and women and 20% of the men (Asobayire *et al.*, 2001). Anemia is defined as a hemoglobin concentration lower than the established cut-off by the world health organization. This figure ranges from 11 g dL^{-1} for pregnant women and for children 6 months to 5 years of age to 12 g dL^{-1} for non-pregnant

women and 13 g dL^{-1} for men (WHO, 2001). Iron deficiency anemia occurs in three sequential stages. According to WHO (2001), The first stage is depleted iron stores defined by low serum ferritin (<12 g dL^{-1}), which occurs when the body no longer has any stored iron, but the hemoglobin concentration remains above the established cut-off level. The second stage known as iron deficient erythropoesis occurs when developing red blood cells have the greatest need for iron characterized by an increase in transferrin receptor concentration and increased free protoporphyrin in red blood cells. However, hemoglobin remains above the established cut-off level. The third and most severe form of iron deficiency is iron deficiency anemia. This condition develops when the iron supply is inadequate for hemoglobin synthesis resulting in hemoglobin concentrations below the established cutoff level. To diagnose iron deficiency anemia measurements of iron deficiency as well as hemoglobin are required (Tatala *et al.*, 1998; Stoltzfus *et al.*, 1999). In developing countries, many children are at high risk of iron deficiency anemia. Likewise in many parts of Tanzania iron deficiency and anemia are extremely prevalent in childhood thus forming a major public health problem (Massawe *et al.*, 1995; Mnyika, 2000). Studies in Cote d'Ivoire estimated that iron deficiency anemia accounted about 50% of the anemia observed (Asobayire *et al.*, 2001). Other causes of iron deficiency anemia include helminths infections such as hookworm and flukes such as schistosomes, which can cause blood loss and therefore iron loss (Stoltfus *et al.*, 1998). Adult hookworms attach themselves to the gut wall, where the mature larvae and adult worms ingest both the gut wall and blood (Brooker *et al.*, 1999). Hookworms change feeding sites every 4-6 hr and during feeding secrete an anticoagulant, resulting in secondary blood loss from the damaged gut wall after the worms stop feeding (WHO, 2001). Stoltfus *et al.* (1997), in their study in Zanzibar estimated that if hookworm

infections could be eradicated, prevalence of anemia could be reduced by 25% and iron deficiency anemia by 35%. There are several health effects in children associated with anemia, which include impared cognitive development, reduced physical work capacity (Hurrell, 2002). There is also evidence that anemia may result in reduced growth and increased morbidity (Stoltfus *et al.*, 1999). Therefore given the magnitude of the problem, greater efforts are needed to develop and implement programs both to prevent and to control anemia, iron deficiency and iron deficiency anemia.

1.2 Child breastfeeding

Early initiation of breastfeeding is beneficial for the mother and the child. Breastfeeding has unique biological and emotional influences on the health of both the mother and the child. It facilitates establishment of a strong bond between the mother and the child (WHO, 1998b). Breast milk alone is an ideal nourishment for infants for the first six months of life due to the combination of providing a most adequate and nutrient balanced type of food (Table 1.1). Optimal breastfeeding practices include exclusive breastfeeding (breast milk with no other foods or liquids) for the first six months of life, followed by breast milk and complementary foods (solid or semi-solid foods) from about six months of age and continued breastfeeding for up to at least two years of age while receiving complementary foods (WHO, 1998b). From the child's perspective, the first breast milk is important because it contains colostrum, which is rich in all the nutrients, antibodies, hormones and antioxidants an infant needs to thrive (Dewey *et al.*, 1998). The milk protects infants from diarrhea and acute respiratory infections, it stimulates their immune systems and according to some studies, it also confers cognitive benefits (Manda, 1999). These protective effects are the most likely explanation for the generally better growth

performance during the first few months of life in poor communities for infants who receive nothing other than breastmilk (WHO, 1998b).

Table 1.1 Estimated nutrient content in mature human milk

Nutrient	Amount	Nutrient	Amount
Lactose (g L^{-1})	72.0±2.5	Calcium (mg L^{-1})	280±26
Protein (g L^{-1})	10.5±2.0	Copper (mg L^{-1})	0.25±0.03
Fat (g L^{-1})	39.0±4.0	Iron (mg L^{-1})	0.3±0.0.1
Vitamin A (µg RE L^{-1})	500	Zinc (mg L^{-1})	1.2±0.2
Vitamin C (mg L^{-1})	40±10	Selenium (µg L^{-1})	20±5
Vitamin D (µg L^{-1})	0.55±0.1	Chromium (µg L^{-1})	50±5
Vitamin E (mg L^{-1})	2.3±1.0	Magnesium (mg L^{-1})	0.3±0.1
Vitamin K (µg L^{-1})	2.1±0.1	Manganese (µg L^{-1})	6±2
Biotin (µg L^{-1})	4±1	Potasium (mg L^{-1})	525±35
Folate (µg L^{-1})	85±37	Sodium (mg L^{-1})	180±40
Niacin (mg L^{-1})	1.5±0.2	Phosphorus (mg L^{-1})	140±22
Panthotenic Acid (mg L^{-1})	1.8±0.2	Fluoride (µg L^{-1})	16±5
Riboflavin (mg L^{-1})	0.35±0.02	Iodine (µg L^{-1})	110±40
Thiamin (mg L^{-1})	0.21±0.03	Chloride (mg L^{-1})	420±60

Source: (WHO, 1998a)

Breast milk contributes significantly to nutrition and health of infants and children, not only after birth, but also during the entire complementary period (Arifeen *et al.*, 2001). Continued breastfeeding up to two years, accompanied by appropriate complementary feeding, maintains a good nutritional status and helps to prevent diarrhea.

WHO and UNICEF recommend that children should be breastfed exclusively for the first 4-6 months and thereafter introduce safe and nutritionally adequate foods until they are at least 24 months old (Onyango *et al.*, 1999). This is because after six months of age breastmilk alone can no longer supply the required daily energy. The energy requirements increase beyond six months, which calls for increased volumes

of breastmilk. However, the amount of breastmilk produced by a mother especially in the developing countries is far lower than the required. Moreover, even if the milk was adequate, the gastric capacity of the infant wouldn't allow such intake. Table 1.2 indicates the energy intake from breastmilk by children who are 6 months to 23 months of age from mothers in developing countries. Depending on the mother's breast milk output, low, average or high, the rest of the daily energy required should be supplied by complementary food.

Table 1.2 Energy intake from breast milk by children in developing countries, by age group

Age group	Breast milk intake[1] (kJ)		
Months	Low (- 2 sd)	Average	High (+ 2 sd)
6-8	908	1728	2548
9-11	656	1586	2515
12-23	377	1448	2519

Source (WHO, 1998b). [1]The categories low, average and high correspond to energy intake from breast milk being low (Mean – 2sd), Average (Mean) and high (Mean +2sd).

Several studies in Thailand, Peru, and Honduras have documented that early initiation of complementary foods replaces breast milk and does not increase caloric intake (Brown *et al.*, 1990). None of these studies reported any benefits for the child's growth as a result of early complementary feeding. Because breast milk is generally higher in nutritional value (Table 1.1) than the complementary foods and liquids, replacing it can negatively affect macro and micronutrient intake of young infants.

The traditional societies in Tanzania have clearly set up mechanisms to protect breastfeeding. However, the changing lifestyle and the breakdown in the traditional family structures and the related lack of adequate information often reinforces harmful customs and predisposes women to modern child care practices such as provision of milk formulas (Shirima, 1996). The practice has been observed

specifically in elite and employed women urban dwellers. Studies done in developing countries (Nyagawa, 1993) show that there was a decline in duration of breastfeeding due to a number of factors. These included urbanization, relative high income, changes in lifestyles and employment of women outside their homes (Shirima, 1996). For instance in urban areas there were noticeable differences between the low and high-income mothers in the duration of breastfeeding. According to Kingamkono (1999), 11% of the low-income mothers in Tanzania had stopped breastfeeding when the infant was below one year of age compared to 70% of the high-income mothers. Equally, the bottle feeding rate among breastfed infants less than 12 months, which was about 1% in 1992 increased significantly to 9% in 1996 (Bureau of Statistics, 1997). In rural areas, prolonged coexistence of breastfeeding and complementary feeding is typical. Unless in a very rapid succession of pregnancies, there is no tendency towards abrupt complementing. Mothers continue to breastfeed until the child desists by itself, milk dries up or the mother becomes pregnant. Considering the potential advantages of breastmilk, breastfeeding should be strongly promoted as governments are obliged under Article 24 of the Convention of Rights of the Child, to ensure that all sectors of the society know the benefits of breastfeeding (UNICEF, 1998b).

1.3 Complementary foods

1.3.1 Definition

Foods or non-milk fluids that are provided in parallel with breast milk are referred to as complementary foods. The period during which other foods are provided along with breast milk is considered the period of complementary feeding. When complementary foods are specifically designed to meet particular nutritional or

physiological needs of the young child, they are referred to as transitional foods. In case complementary foods given to the young children are the same as those consumed by the rest of the family members these are refered to as family foods (WHO, 1998a). In scientific literature, definitions of weaning have varied and the term has been misused to indicate the introduction of complementary foods (Hofvander, 1981). However, there is presently a consensus to define it as a complete cessation of breastfeeding. Terms such as weaning process and weaning foods should be avoided because they imply that their purpose is the cessation of breastfeeding (WHO, 1999). Complementary feeding and complementary foods should be used instead, because they convey the notion that the foods are not intended to displace or replace breast milk but to complement it. Table 1.3 provides the recommended energy intake by children who are 6 months to 23 months of age from complementary food at average, high and low level of breast milk intake.

Table 1.3 Energy needed from complementary foods by children in developing countries to meet the daily requirements by the level of breastmilk intake

Age group			Level of breastmilk intake[1]		
Months	Daily energy requirements (kJ)	Estimates of average breastmilk intake (kJ)	High breastmilk intake + 2sd (kJ)	Average breastmilk intake (kJ)	Low breastmilk intake –2 sd (kJ)
			Energy needed from complementary foods		
6-8	2858	1728	314	1130	1946
9-11	3469	1586	962	1883	2824
12-23	4586	1448	2050	3138	4184

Source: WHO (1998a). [1]The categories low, average and high correspond to energy intake from breast milk being low (Mean – 2sd), Average (Mean) and high (Mean +2sd)

- The traditional complementary foods in Tanzania are based on starchy staples, usually cereals such as maize, sorghum, rice and finger millet and non-cereals such as cassava, sweet potato, yams, bananas and plantains (Mosha *et al.*, 2000). Nutritional

problems associated with the use of these starchy staples in complementary foods have been widely reported (Mosha, 1984; Seenappa, 1987). Generally, complementary foods in Tanzania were found to be very poor in providing nutrients required by infants. These foods are often deficient in fats, iron and vitamins (especially Vitamin A). In addition, they can be contaminated from unclean water, unwashed hands and utensils, and lack of storage facilities. According to Gibson *et al.* (1998) meeting micronutrient needs from CFs appears to be the greatest challenge. Based on requirements in Table 1.4, adequate amounts can be attained by processing, fortification, and supplementation of the staples or use of animal products.

Table 1 4 Recommended daily allowances for infants

Nutrient	Breastmilk and complementary foods		Estimated nutrients needs from complementary foods	
	6-8 months	9-12 months	6-8 months	9-12 months
Protein (g)	9 1	9.6	2.0	3.1
Fat (g)[1]	26-39	31-47	25	41
Vitamin A (RE)	350	350	13	42
Calcium (mg)	525	525	336	353
Iron (mg)[2]	7-11-21	7-11-21	7-11-21	7-11-21
Zinc(mg)	5.0	5 0	3 8	4.3

Source WHO (1998a) [1]The FAO-WHO report indicates that dietary fat should range from 30–45% of total dietary energy for children below 2 years of age, although lower fat intakes may be compatible with good health and nutrition if requirements for essential fatty acids are met [2]Depending on whether iron has a high medium or low bioavailability.

Nutrition projects such as the Joint Support Nutrition Program (JSNP) and Child Survival Development Program (CSDP) were introduced to address some of the above-mentioned problems in Tanzania (Mosha, 1984). These projects identified improved complementary foods formulation as a means of reducing malnutrition levels using local food resources available in the country. However, their main focus was on reducing dietary bulk and enriching plain porridges with nutrients for complementing children.

The dietary bulk problem was addressed by introducing "power flour" in order to achieve incorporation of more solids per unit volume and thus higher energy densities (Mosha and Svanberg, 1990). CF quality was supposedly improved by mixing other local foods, which were rich in some nutrients such as ripe bananas, cereals and legumes in order to achieve a nutrient compensatory effect. However, the blending ratios used did not consider optimal concentration and availability of specific nutrients. This resulted in inadequate intake of nutrients by the complementing children. For instance, Desikachar (1982) observed that limited quantities of malt did not exploit fully the nutritional benefits of malting in the complementary food formulation. He therefore suggested that there was a need to develop and establish affordable blends/formulations with energy and other nutrient contents that meet the recommended daily intake of nutrients. These blends/formulations have to be made based on existing household food resources.

1.3.2 Micronutrient availability

1.3.2.1 Introduction

During the past decade, efforts were essentially directed towards protein and energy content of complementary foods, and the improvement of energy and protein density to meet the children's needs. However, micronutrients did not receive as much attention until the 1980s to 1990s when the international community became aware of the importance of micronutrient deficiency as a public health problem all over the world, and in particular in low income countries (WHO, 1999). For instance, among the 48 countries of the African Region that WHO gathered their data, 40 countries had iron and iodine deficiency problems and 44 had vitamin A deficiency problems (WHO, 1999). The most vulnerable groups in these countries were infants and

pregnant women, which explains the importance of examining adequate contribution of complementary foods with regard to micronutrient requirements. Thus, in recent years there has been increasing recognition of the consequences of micronutrient deficiencies in children ranging from altered immunity, increased risk of infectious diseases, reduced growth to death (Black, 1998). Following this, governments and international agencies have given priority to the elimination of micronutrient deficiencies in developing countries (UNICEF, 1998b). Sources of micronutrients are mainly obtained from the traditional complementary foods provided to the children. The total amount of micronutrients in a food does not reflect the amount that is bioavailable. Bioavailability refers to the proportion of micronutrient in food that is absorbed and utilized for normal body functions (Watzke, 1998). The quantification of bioavailability has been done by complex processes comprising isotopic elemental digestion. However, various techniques have been established by nutritionists to determine bioavailability. For instance, *in vitro* studies range from measurements of solubility, extractability, and fractional dialysability to studies of the nurient uptake in experimental animals (Miller *et al.*, 1981; Sripriya *et al.*, 1997). The nutrients are incubated in intestinal preparations to simulate the processes that take place in the gastrointestinal tract (Blenford, 1995). The results obtained from the above methods are used as proxy to estimate bioavailability (Watzke, 1998). Several antinutritional factors have been implicated as the main cause of reduced micronutrient availability such as phytic acid and tannins although phytic acid has a more pronounced effect (Zhou and Erdman, 1995; Zdunczyk *et al.*, 1996; Antony and Chandra, 1998).

1.3.2.2 Phytic acid and its importance in micronutrient availability

Phytic acid (myoinositol hexaphosphate) is a naturally occurring compound formed during the maturation of seeds and cereal grains and constitutes about 1% to 7% of the dry weight and more than 70% of the total kernel phosphorus (Zhou and Erdman, 1995). Phytic acid has been implicated as an antinutrient due to its inhibitory effect on mineral bioavailability. The most striking chemical impact has been its strong chelating ability with multivalent cations (especially *di* and *trivalent*) to form cation-phytic acid complexes (Liu *et al.*, 1997). According to Surtadi and Buckle (1988), phytic acid is significant from a nutritional point of view because of its ability to form stable chelates. Its ability to chelate multivalent metal ions result in very insoluble salts that are poorly absorbed from the gastro-intestinal tract (Pehrsson *et al.*, 1998). Numerous studies have indicated that phytic acid reduces the bioavailability of dietary magnesium, calcium, zinc and iron in monogastric animals (Lorenz, 1983; Chompreeda and Fields, 1984; Honke *et al.*, 1998). According to Hurrell (2002) phytic acid can decrease the absorption of even the most bioavailable iron compounds to very low levels.

Several strategies have been used to reduce phytates. These include food processing unit operations (milling, extrusion cooking, frying and roasting), use of exogenous enzymes (by artificial phytases) and endogenous phytases (by germination and fermentation). According to Watzke *et al.* (1998), unit operations had little impact in lowering the phytates in cereals. However, germination of cereals and legumes has been reported to decrease the levels of phytic acid since seed germination results in increased phytase activity, which can hydrolyze phytic acid to inositol and free orthophosphate (Antony and Chandra, 1998). The commercial microbial phytases are

attractive because of their low cost and effectiveness (Harland and Narula, 1999). Svanberg *et al.* (1993), have observed that commercial phytase results in higher iron solubility than germination and fermentation alone. For instance, their research on the effects of fermentation on *in vitro* iron availability and phytate hydrolysis in maize varieties showed that the increase in soluble iron was strongly related to enzymatic degradation of phytate. The reduction of inositol hexa and pentaphosphate was about 50% with added germinated flour. Reduction was greater than 90% after soaking the flour prior to fermentation and almost complete with 50 mg phytase added in 100 mL. A number of studies have observed a significant reduction or elimination of phytates in cereals and legumes by fermentation. Chrompeeda and Fields (1984) reported that a natural lactic acid fermentation of corn meal at 32°C for four days reduced phytate phosphorus concentration by 78%. Antony and Chandra (1998) observed that phytate decreased by 39% after 48 hr of fermentation. Phytate reduction has also been reported in cereal-pulse mixtures (Chavan and Kadham, 1989), and dry beans (Barampama and Simard, 1994).

1.3.2.3 Iron and zinc availability in complementary foods

Iron and zinc deficiencies have long been prevalent in many developing countries, including Tanzania, and particularly where CFs are low in animal products and high in phytates (WHO, 1998a). Adequate intake of iron and zinc have been attained in many industrialized countries due to the following reasons: (a) improved food intakes (b) transition from cereal based diets that are rich in iron inhibitors to more mixed diets that have better bioavailability and (c) increased reliance on industrially processed foods that are fortified with iron (Ramakrishnan and Yip, 2002). In contrast, in developing countries there are minimal quantities of biavailable

micronutrients such as iron and zinc in complementary foods. Benbouzid and Benoist (1999), report that it is practically impossible to supply adequate amounts of un-modified CFs to meet the iron and zinc needs in infants 6 to11 months of age without an unrealistically high intake of animal products such as liver, fish, beef and eggs. The quantities of these foods that would be needed to meet the estimated iron requirements are generally much higher than the currently observed maximum intakes prior to 12 months (WHO, 1998a). Furthermore, Hurrell (1999) reports that iron is the most difficult micronutrient to add to foods and ensure adequate absorption. The main problem is that the water-soluble iron compounds, which are the most bioavailable, often lead to an unacceptable colour and flavor in the food vehicle. Insoluble compounds such as elemental iron powders on the other hand do not cause sensory changes but may be so poorly absorbed that they have little or no nutritional benefit (Davidsson *et al.*, 2001).

Despite the small amount of iron and zinc present in most consumed cereals in Africa, studies to enhance their solubility and hence their bioavailability in the gastro intestinal system have been done in a number of cases (Hallberg, 1981, Hurrell, 2002). Ascorbic acid is the most widely used enhancer of native consumed and iron fortified foods (Henshall, 1981). It can increase the solubility of iron and zinc by several folds in the gastrointestinal system by dissolving in the gastric juice and entering the common non-haem iron pool. The ascorbate appears to act mainly in the stomach and duodenum as both a solubilizing ligand and a reducing agent. It reduces ferric iron to the ferrous state, thus preserving its solubility as the pH rises in the duodenum (Conrad and Schade, 1968).

1.4 Techniques of increasing energy density of complementary foods

A number of techniques have been employed to increase the energy and nutrient densities of CFs while lowering viscosity to enable infants and children to be able to swallow without difficulties. These techniques include addition of oil/fat to the CF, extrusion cooking and germination of the seeds (Ashworth and Draper, 1992; Kikafunda *et al.*, 1998). Of these techniques, germination has the largest ability to reduce viscosity of cereal porridges rapidly (den Bensten *et al.*, 1998).

Amylase rich flour sometimes referred to as power flour is made with germinated cereals or legume grains. The grains are first soaked to allow for germination, dried in the sun, and then ground into flour (Weaver, 1994). Tanzania and India are some of the few countries that have actually promoted power flower through Maternal and Child Health Care (MCH) services and the media (WHO, 1999). As mentioned earlier, the most available complementary foods in developing countries are often cereal based with low energy densities. Energy-rich infant formulas are either not available or too expensive for routine use in poor communities. Starchy staples prepared for complementary purposes become highly viscous when cooked due to the water-binding capacity and gelatinization properties of starch, which limit the flour concentration that can be used for a liquid feed (Walker and Pravitt, 1989; Nout, 1993). Consequently, large quantities of water are typically added to make the consistency appropriate for infants and children. As a result, the energy and nutrient concentration is reduced significantly. Due to their small gastric capacities, infants and young children cannot consume sufficient quantities to satisfy their nutritional requirements (WHO, 1998a).

In a study conducted by Darling *et al.* (1995), it was observed that starch digestion using amylase from grain germination was an effective way of improving energy intake especially for children who are recovering from episodes of acute diarrhea. Amylase rich flour (ARF) can be produced at household level from germinating cereal grains. It is very effective in reducing viscosity of thick cereal porridges by starch digestion (Kikafunda *et al.*, 1998). Amylase synthesized within the grain during germination cleaves amylopectin and amylose within starch granules to produce maltose and low molecular weight dextrins (Mosha and Svanberg, 1983). Since these products bind little water and do not gelatinize on cooking, viscosity is markedly reduced. As a consequence, a feed with a low viscosity suitable for children can contain a much greater concentration of flour (solids) for which the nutrient density is more than doubled. According to Dijkhuizen and Wurdemann (1993), technologies like fermentation and germination have received much attention and have been tried at household level. These technologies offer interesting possibilities particularly from a nutritional point of view. However, they stress that at present they are not yet sufficiently tested for practical application. Field pilot projects to test their practical applicability and economics are therefore urgently needed.

A number of critics have argued that germination technique to improve quality of CFs will be an additional workload to women and therefore the technologies will not be adopted. This is not true because this particular task can be undertaken by a group of women in a community who could specialize in producing and selling the processed CF flour. The same scenario could be applied in that similar tasks are being done by some individuals or women groups for instance in the preparation and selling of cookies, variety of foods and local brew to other members of the community. It is

incomprehensible to anticipate that new technologies will be replicated in every household to a comparable degree of accuracy and under uniformly acceptable standards of hygiene. It is quite true that women are preoccupied by other numerous household chores, farming, and income generating activities in which processing of CFs will be an additional burden. Therefore, community organized groups engaged in processing CFs will comparatively be advantageous in the sense that they are time and cost effective. In addition, the products might be cheaper than products made by individual households.

1.5 Quality and safety of complementary foods

1.5.1 Microbial safety

1.5.1.1 Introduction

Apart from adequate availability of complementary food to infants and children it is imperative that the food is microbiologically safe. This is of particular importance with regard to food intended for young children for whom food poisoning can have serious health consequences and an important cause of infant mortality. According to Motarjemi *et al.* (1993), food safety refers to a set of conditions and practices during the production, processing, storage, distribution and storage of CFs that are necessary to guard against pathogenic micro-organisms, exogenous chemical contaminants and naturally occurring toxic substances.

Many poor households especially rural ones in developing countries lack access to clean water, soap, latrines and other resources that would provide a safer and more sanitary environment (Afifi *et al.*, 1998). The rural settings in a developing country make it very difficult to avoid microbial contamination of food during feeding due to

relatively unclean utensils and improper personal hygiene (WHO, 1999). Consequently, complementary foods are often contaminated resulting in increased morbidity. For example, about 70% of diarrheal episodes among children under five years of age are estimated to be caused by contaminated food and drinking water (Motarjemi *et al.*, 1993). If food is served while still hot or immediately after cooking, the amount of spores present in serving utensils are too few to cause any significant harm. However, if the gruel is kept at ambient temperatures for some time, micro-organisms that have contaminated the utensils will multiply rapidly in leftovers and might be hazardous to consume (Lorri and Svanberg, 1995). Most people especially in rural settings cannot afford to throw away leftovers, and it is difficult to prepare food in exactly the required amount. In the study by Lartey *et al.* (1999) in which they used thermos flask to keep the remaining CF after cooking, the incidence of diarrhea among infants was significantly reduced. Every effort is therefore required to improve the hygiene quality of complementary foods. Education of food handlers, particularly mothers, in food safety principles through primary health care and infant feeding programs should be regarded as an important strategy for the prevention of diarrheal diseases (Tomkins, 1991). Other numerous studies have shown that complementary foods prepared under unhygienic conditions are frequently heavily contaminated with pathogenic agents and are a major risk factor in transmission of diseases, especially diarrheal diseases. For example, Afifi *et al.* (1998) showed that in Egypt, of the 270 complementary foods collected, 43.7% and 21.4% were contaminated with *Escherichia coli* and *Bacillus cereus*, respectively. Milk and foods prepared separately for infants were more frequently and heavily contaminated with *E. coli* than foods prepared for adults such as boiled rice. In another study in Myanmar, 775 samples of food consumed by children aged 6-29 months were examined for enteritic bacterial

pathogens. It was found that 505 samples were positive for *E. coli*, 28 for *Salmonella sp* and 6 for *Vibrio cholerae*. Similarly, from a total of 113 samples of drinking water, *E coli* and *V. cholerae* were isolated from 29 and 5 samples, respectively (Khin-Nwe, 1991). In a similar study in Kiambu, a district in Kenya, researchers investigated factors that might explain the lower rates of diarrheal diseases in children compared to other areas of Kenya. They found that in 75% of the time, food was consumed almost immediately after being prepared. Whenever food was found contaminated, it was caused by post-preparation handling for instance when mothers added cold milk or leftover food to cool the cooked food (Pertet *et al.*, 1988). Results of the above studies indicate that in most developing countries CFs and drinking water are contaminated with a number of pathogenic bacteria and thus forming a route for the pathogens to affect children.

Many of the sources of contamination described above could also be potential sources of contamination of seeds or sprouts at farm, market level or at the germinating facility. Although seeds are suspected to be the most likely source of contamination, the water used during germination could be a source of initial contamination or a vehicle for subsequent cross contamination. Microbiological analysis of cereal grains is not performed in most cases. However, the external surfaces of cereal grains are contaminated during development of the plant along with contaminants from the soil, air and animals (Sarrias *et al.*, 2002).

Some bacteria are of interest during processing steps such as germination and autoclaving of cereal/legume based complementary foods. During germination for example, the risk of food born disease compared to other fresh produce, is the

exponential growth of bacteria during germination. Micro-organisms on seeds can grow quickly under the favorable conditions of the germination process, which are water activity, temperature, pH, time and nutrients (Piernas and Guiraud, 1997). The pathogens can grow to elevated levels since there are no inherent steps in the production of sprouts that either prevent bacterial growth or eliminate them entirely. These include, *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens*. The importance of *B. cereus*, *S. aureus* with the exception of *C. perfringens* stems from the fact that they can proliferate quickly during germination and produce heat stable toxins, which can withstand even sterilization temperatures (NACMCF, 1999).

1.5.1.2 Bacillus cereus

Bacillus cereus is a gram-positive facultative aerobic spore former found in the soil and in many raw and processed foods (Roberts *et al.*, 1982). *B. cereus* food poisoning is the general description although two recognized types of illness are caused by two distinct metabolites, i.e. the diarrheal and vomiting type. The diarrheal type of illness is caused by a large molecular weight protein while the vomiting (emetic) type of illness is believed to be caused by a low molecular weight heat stable peptide. The presence of a large number of *B. cereus* in a food greater than 10^6 cfu g^{-1} is indicative of active growth and proliferation of the organism and is consistent with potential hazard to health (Beuchat, 1996). The onset of watery diarrhea, abdominal cramps, nausea and vomiting occurs 6-15 hr after consumption of contaminated food. Children are very susceptible to *B. cereus* food poisoning and they can die if quick measures are not taken. Foods, which are associated with *B. cereus*, include milk, meats, cereals, vegetables and sprouts/germinated seeds (Krammer and Gilberts, 1989). In most instances, the actual cause of poisoning by *B. cereus* is temperature abuse of

prepared foods. Small numbers might be present after cooking, which will multiply to food poisoning levels during cool down and storage of prepared foods. In 1973, an outbreak in the USA was associated with the consumption of sprouts (a mixture of soy, cress and mustard seeds packaged in a seed sprouting kit) contaminated with *B. cereus* (Harmon *et al.*, 1987). Recently, vegetable purees were the cause of a severe *B. cereus* outbreak in a French nursing home for elderly persons (Lund *et al.*, 2000). Similarly, Carlin *et al.* (2000) reported that *B.cereus* was isolated from 80% to 100% of the samples of cooked pasteurized and chilled vegetable purees stored at 10°C. These results indicate that processed foods in spite of their low level of contamination with bacterial spores and heat treatments they undergo, may significantly contribute to the final contamination of the cooked chilled foods.

1.5.1.3 Staphylococcus aureus

Staphylococcus aureus is a gram-positive bacteria capable of producing a highly heat stable protein toxin that causes food poisoning, and which leads to a condition known as staphylocnterotoxicosis. *S. aureus* is ubiquitous, exists in air, dust, sewage, water, milk, food equipment, human and animal nasal passages and throats (Beuchat, 1996). The onset of symptoms in staphylococcal food poisoning is usually rapid and in many cases acute, depending on the individuals susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxins ingested and the general health of the individual. Infants are particularly very vulnerable. An infective toxin dose of 1μg g^{-1} in the contaminated food will produce symptoms of staphylococcal intoxication. This toxin level is reached when the *S. aureus* population exceeds 10^5 cfu g^{-1}. Intoxication is caused by ingesting enterotoxins produced in the food when the food has not been kept hot enough, i.e. at temperatures equal to or above 60°C (NACMCF, 1999).

Foods, which are associated with *S. aureus* include bakery products, eggs, poultry meat, milk and milk products and sprouts/germinated seeds (Roberts *et al.*, 1982). Foods that require considerable handling during preparation and that are kept at elevated temperatures after preparation are frequently susceptible to staphylococcal poisoning.

1.5.1.4 *Clostridium perfringens*

Clostridium perfringens is an anaerobic, gram-positive spore-forming rod. The organism causes food infection rather than food poisoning. It is widely distributed in the environment and frequently occurs in the intestines of humans and many domestic and wild animals (Anderson *et al.*, 1995). Spores of these bacteria persist in the soil sediments and areas subject to human or animal fecal pollution. Poisoning by *C. perfringens* result from ingesting a large number of bacteria (10^6 - 10^7 viable cells per gram of food) whereby the ingested bacteria sporulate in the digestive system and release a heat labile enterotoxin (Juneja and Marmer, 1998). The toxins cause abdominal cramps and diarrhea which begins 8-22 hr after consumption of foods containing large numbers of *C. perfringens* capable of producing the poisoning toxin. Diagnosis of the disease is confirmed by detecting the toxin or *C. perfringens* in the faeces of the patient. Bacteriological confirmation can also be done by finding exceptionally large numbers of the causative bacteria in implicated food. In most instances the actual cause of poisoning by *C. perfringens* is temperature abuse of the prepared foods. Small numbers of the organisms are often present after cooking and multiply to food poisoning levels during cool down and storage of prepared foods. Meat and meat products are foods most frequently implicated (Juneja and Marmer, 1998).

1.5.2 Cyanides

Some researchers identified a possible danger of children being intoxicated with cyanide from germination sprouts. Cyanide in trace amounts is widely distributed in plants and occurs mainly in the form of cyanogenic glucosides (Fennema, 1985). Glucosides that have been identified in edible plants include amygdalin (bitter almonds and fruit kernels), dhurrin (sorghum and other cereals) and linamarin (pulses, linseed and cassava). Shayo *et al.* (1998) reported high levels (513.4 ppm) of cyanide in two-day germinated finger millet used to prepare local brew in Tanzania with the cyanide levels increasing linearly with germination days. However, a previous study done by Dada and Dendy (1988) found no cyanide in two-day germinated finger millet. Similarly, Salami (1994) found levels of 18 mg kg^{-1} and 13 mg kg^{-1} in two germinated finger millet varieties from semi arid regions of Nigeria after 96 hr (4 days) of germination. The same researcher report that between 48 hr and 60 hr of germination, no cyanide was detected. He concluded however that the levels of cyanide detected, fall within the acceptable limits set for humans of 10 - 20 mg cyanide/100 g sample or 0.5 – 0.8 mg kg^{-1} body weight. In order to avoid the risk of intoxication with cyanide, mothers in Congo and India were asked to remove the sprouts before milling the grains into flour (Vester, 1999). Also, since the boiling point of hydrocyanic acid is about 26°C (Shayo *et al.*, 1998), during solar/sun drying of the sprouts, milling and cooking the cyanide can volatilize out.

1.5.3 Mycotoxins in foods

1.5.3.1 Introduction

Mycotoxins are fungal metabolites that are toxic when consumed by animals including human beings (de Nijs, 1998c). These toxins can accumulate in maturing

cereals and legumes such as maize, sorghum, rye, soybeans, peanuts and other food and feed crops in the field and during transportation. They may also accumulate during storage provided favourable conditions for the growth of the toxin producing fungi are present. Major mycotoxins include aflatoxins, trichothecenes, zearalenone and deoxynivalenol that are produced by various *Penicillium*, *Apergillus* and *Fusarium* species (Broggi *et al.*, 2002). In Africa, as in most developing countries aflatoxins are the most important mycotoxins from the point of view of occurrence, toxicity and economy. The aflatoxins are produced primarily by strains of *Aspergillus flavus* and *Apergillus parasiticus* (FAO, 1993). The aflatoxins commonly isolated from foods and feeds are aflatoxin B_1, B_2, G_1 and G_2 which are potent carcinogens and mutagens that have been associated with liver cancer in humans (de Nijs, 1998a). These toxins are primarily a problem in oil nuts such as peanuts although cereals such as maize, sorghum, wheat and rice can also be contaminated (Miller, 1995).

Fusarium species are now recognized as a major agricultural problem (Siame *et al.*, 1998). The mycotoxins frequently encountered in cereals and legumes include those produced by *Fusarium* species, which comprise the fumonisins and zearalenone (Doko *et al.*, 1996). Fumonisins are an important class of emerging mycotoxins that are present in maize, which is a staple for the majority of the Tanzanian population. Among the most common producing fumonisin species are *Fusarium moniliforme* (*F.verticillioides*) and *Fusarium proliferatum*. Other *Fusarium* species that grow on agricultural commodities in the field or during storage are also known to produce fumonisins but in low concentration. (Ross *et al.*, 1992; Kpodo *et al.*, 2000). Fumonisins are of importance because these toxins are associated with food and feed products, especially corn based commodities (Sydenham *et al.*, 1990; Ross *et al.*,

1991; Sydenham *et al.*, 1991; Hendrich *et al.*, 1993; Murphy *et al.*, 1993). Among the fumonisins currently identified are the fumonisin B_1 (FB_1), FB_2, and FB_3 of the B series (Kim *et al.*, 2002; USFDA, 2000). These are most frequently detected in fungal cultures or in natural contaminated crops, and are believed to be most abundant and most toxic (de Nijs *et al.*, 1998c). Other analogues that have been identified are classified into series A, F and P based on their chemical structure (Musser *et al.*, 1997).

1.5.3.2 Occurrence of fumonisins in food

Maize, sorghum and cassava comprise the major staples of the human diet in Africa. Other crops such as wheat, rice, millet, groundnuts, cowpeas, beans and sunflower are also used but to a lesser extent. Maize, finger millet, kidney beans and peanuts are also used in the preparation of complementary food for infants in Tanzania. The Food and Agricultural Organisation (FAO) estimates that at least 25% of the world's food crops are affected by mycotoxins annually, and contamination of maize and sorghum by *Fusarium* species is of major concern world-wide (Weidenböner, 2001). The extent of contamination of raw corn with fumonisins varies with geographic location, agronomic and storage practices, and the vulnerability of the plants to fungal invasion during all phases of growth, storage and processing. The levels of contamination are also influenced by environmental factors such as temperature, humidity and rainfall during pre-harvest and harvest periods (Ono *et al.*, 2001). Other factors include the strain, nature of the substrate and the stress level of the host plant (Rice and Ross, 1994). High levels of fumonisins are associated with stresses such as heat and drought followed by periods of high humidity. Warm wet conditions prevalent in many parts of Africa are ideal for fungal proliferation (Siame *et al.*, 1998). The levels can also

increase in grains under improper storage conditions especially in places where the grains can acquire moisture. A review of surveys on natural occurrence of FB_1 in corn and corn products prepared by the WHO (1999) working group for the Environmental Health Criteria of Fumonisin B_1 showed that 59% of 5213 samples analysed globally were contaminated by FB_1. The highest incidence of contamination was detected in samples from Oceania (82% of 82 samples), followed by Africa (77% of 383 samples), North and South America (63% of 1930 samples), Europe (53% of 1918 samples) and Asia (51% of 912 samples) (Visconti *et al.*, 1999).

Due to their water solubility, fumonisins are less likely to bio-accumulate in animal tissues than lipid-soluble compounds. Fumonisins residues have either not been detected or are detected at extremely low levels in milk, eggs and edible meat (Miller, 1994). When detected, the residues are normally found in organ tissues i.e., liver and kidney (Prelusky *et al.*, 1996). In a study done by Maragos and Richard (1994), it was reported that one cow's milk sample out of 165 tested contained a low level of FB_1 (1.29 ng mL^{-1}), indicating the possibility of fumonisin transfer to the milk. Other studies based on dosing cows orally with up to 5 mg FB_1 kg^{-1} body weight or intravenously with 0.2 mg kg^{-1} failed to show such a transfer (Scott *et al.*, 1994).

1.5.3.3 Toxicological and carcinogenic effects of fumonisins

There is no direct scientific evidence that fumonisins cause adverse effects in humans. Studies currently available demonstrate inconclusive associations of fumonisins with human diseases. Investigators in South Africa have noted a correlation between high levels of *Fusarium* in maize and oesophageal cancer in human subgroups (Rheeder *et al.*, 1992). Most of the studies are limited by the lack of controled conditions and

therefore do not allow any definitive conclusions to be made about cancer causation in humans (Chu and Li, 1994). Equally, fumonisins have been shown to play a role in animal toxicosis. Research has shown that fumonisins induce equine leucoencephalomalacia in horses (Ross *et al.*, 1991; Sydenhan *et al.*, 1992), Porcine pulmonary oedema in pigs (Colvin and Harrison 1992; Osweiler *et al.*, 1992), diarrhoea and reduced body weight in broiler chicks (Brown *et al.*, 1992), and haemorrhage in the brain of rabbits (Bucci *et al.*, 1996).

1.5.3.4 Total daily intake and limits of intake of fumonisins

The available information on human health effects associated with fumonisins is not currently conclusive. Nevertheless, since fumonisins have been shown to produce a variety of significant adverse health effects in livestock and experimental animals, and because human physiology is similar to the physiology of many animals, the association between fumonisins and human diseases is possible (USFDA, 2000).

There are no official limits that have been established for fumonisin contamination in food products, but Switzerland has established a level of $1x\ 10^3\ \mu g\ kg^{-1}$ in cereal and cereal products (de Nijs *et al.*, 1998c). United States Food and Drug Administration (USFDA) also has suggested a maximum of $2 \times 10^3\ \mu g\ kg^{-1}$ levels for fumonisins in corn and corn products intended for human consumption and animal feed. These levels were based on concerns associated with hazards shown primarily by animal studies and are considered achievable with the use of good agricultural and manufacturing practises. Equally, the European Commission's Health and Consumer Protection Directorate has suggested a tolerable total dietary intake (tTDI) cut-off for fumonisin of $2\ \mu g\ kg^{-1}$ body weight day^{-1} (SCF, 2000).

1.5.3.5 Effect of processing on fumonisins

Several studies have reported on the stability of fumonisins in various food processes (FAO/WHO, 2000). The fumonisins are believed to be stable during drying and storage, therefore there is need for the exploration of different approaches to minimise the levels to which consumers are exposed to unpredictable occurrence of fumonisins in harvested crops (FAO/WHO, 2000).

1.5.3.5.1 Heat treatment

Since fumonisins are heat stable, ordinary cooking and procedures for heat processing do not substantially reduce the toxin levels. However, other processing steps may decrease toxin levels. Thermally processed corn products (canned corn, tortillas, and grits) generally have lower levels of fumonisins than corn milled products. Extrusion cooking, under various laboratory conditions, as well as roasting have been observed to reduce fumonisin levels to varying extents (Katta *et al.*, 1999). Scott and Lawrence (1994) reported 60% loss of fumonisins in dry cornmeal heated at 190°C for 60 minutes, 70 to 80% fumonisins loss in moist cornmeal heated at 190°C for 60 minutes, and complete fumonisin eradication in dry cornmeal heated at 220°C for 25 minutes.

Another study done by Castelo *et al.* (1998) also showed that there was no significant loss of fumonisins in artificially and naturally contaminated corn muffin when baked at 204°C for 20 minutes. Only a 48% decrease was noted when corn bread was baked at 232°C for 20 minutes. They further showed that roasting (dry heating) of artificially and naturally contaminated cornmeal samples at 218°C for 15 minutes resulted in almost complete eradication of fumonisins.

1.5.3.5.2 Milling

Wet milling of corn is a major process used to obtain corn-starch for further processing into human food. The study done by Munkvold and Desjardins (1997) showed that wet milling of contaminated corn produces a starch fraction with very little or no fumonisins while the steep water, gluten, fibre, and germ fractions, in decreasing order contained most of the toxin. Dry milling of corn results in the distribution of fumonisin into the bran, germ and flour. Generally, the initial step in the process involves the removal of the pericarp (referred to as the bran fraction) and the germ. The remaining components of the endosperm are separated into the following fractions based on decreasing particle size: flaking grits, regular grits, corn meal and flour (Alexander, 1987). Examination of milled fractions obtained from a commercial dry milling of fumonisin-contaminated corn, revealed that fumonisin levels were highest in the bran and germ fractions. The lowest levels were found in the fractions with larger size particles such as flaking grits.

1.5.3.5.3 Biological processes

Ethanol fermentation of fumonisin contaminated corn resulted in very little degradation of the toxins. Most of the toxins remained in the distiller's grains, thin stillage and distiller's soluble fraction. The fermentation process did not destroy fumonisins, and hence about 85% of the toxins could be recovered in the products (Bennett and Richard, 1996). Bothast *et al.* (1992) studied the fate of fumonisins B_1 during ethanol fermentation. They found that FB_1 was not degraded during the fermentation of contaminated corn. Ethanol distilled from the whole stillage did not contain any fumonisin. However, all the other products produced from this fermentation contained FB_1 toxins.

1.5.3.5.4 Chemical processes

Nixtamalization, which is the traditional treatment of corn with calcium hydroxide Ca $(OH)_2$ and heat, which is also used to produce masa (tortilla flour), has been suggested as a decontamination treatment of fumonisin contaminated corn (Sydenham *et al.*, 1991). However, this is contrary to the finding of the study done by Hendrich *et al.* (1993), which showed that hydrolysed FB_1 (HFB_1) was produced when corn was nixtamalized and appeared to be more toxic to rats than FB_1. Sydenham *et al.* (1991) suggested that the procedure to prepare these foods, which involves a step with high pH and heat (boiling and soaking corn in a solution of calcium hydroxide) would destroy FB_1. However, when the corn was fed to rats, it was found that the toxicity had not been reduced by this treatment. On the contrary, some investigators have reported that the hydrolysed products were less toxic than the untreated corn (Voss *et al.*, 1996). A modified nixtamalization procedure incorporating various combinations of hydrogen peroxide and sodium bicarbonate in addition to calcium hydroxide, has been reported to give a 100% reduction of FB_1. However, even with this treatment, the masa products exhibited about 60% of the toxicity of the untreated corn (Park *et al.*, 1996).

1.6 Production of low cost and market oriented CF

The income of rural communities in most developing countries such as Tanzania is relatively low compared to urban and semi urban communities, which leads to a low purchasing power in most households (Bureau of Statistics, 1997). Furthermore, the majority of the vulnerable groups, especially children, which governments and international organizations target to improve their nutritional status, are in the rural

areas (UNICEF, 1998b). However, in many developing countries commercial CFs are currently available. But due to expensive packaging, extensive promotion and advertising coupled with urge for good profit margins, the prices of these products are generally about 10-15 times more than the cost of common staples (WHO, 1999). This high price is normally beyond the purchasing power of the majority of the population and therefore a limited population group can afford to purchase these products. Development and provision of low cost CFs is therefore important for the majority of the rural population provided the demand for the CF is there. Central production of CF for instance produced by associations of village women, using local staples can substantially minimize the cost of production resulting into an affordable product by the majority of the rural population.

Both industrially processed and community processed CFs are a response to the demand for convenient, nutritious foods for young children. During the 1970s many nutritionists regarded protein deficiency as the major constraint to good childhood nutrition in developing countries. Therefore, industries were encouraged to invest in CFs as a means of improving protein intake. Many of these investments proved to be un-profitable, as most of the large-scale CF factories in developing countries were unable to survive the economic recession of the seventies. In her review of commercial CFs in developing countries, Orr (1977) concluded that there was little evidence that they had played a role in the reduction of malnutrition. Poor families with the highest rates of malnutrition could not afford these foods. Given these disappointing results, various international agencies that had promoted processed CFs changed their focus to the use of local commodities to improve traditional CFs (WHO, 1999).

1.7 Intervention programs to improve the nutritional status of children in developing countries

1.7.1 Introduction

Over the past 40 years programs have been designed to address various nutritional problems (WHO, 1998a). Projects that aimed at improving child nutrition often focused on access to food, access to health services, food quality, childcare and feeding practices (UNICEF, 1998b). However, these approaches have often been unsuccessful in reducing the levels of malnutrition. There are several explanations that can account for the failure of such approaches. For example, some non-governmental organizations have been promoting home gardens as an intervention to improve family nutrition in various countries. In Indonesia, for instance, a project designed specifically to increase vegetable consumption among under-five children, supplied seeds fertilizers, fences, tools and technical assistance (Peduzi, 1990). Similar projects on vegetable gardening were carried out in Malawi through outreach programs, whereby community health workers provided women groups with nutritional and horticultural education and performed cooking demonstrations. However, assessing the impact of home gardens on child nutrition was problematic. Even when data existed, evaluators were uncertain on how to isolate and establish the impact of gardens from other interventions. So reports ended up listing the number of gardens established and people trained. Although one can assume that the gardens will enhance a household's dietary consumption or income, the extent to which this benefits young children in the family needs to be determined. This may be especially true when the problem is lack of calories whereby gardens might be unable to result in increased caloric intake but may be able to improve micronutrient consumption. According to WHO (1998a), International organizations and governments of

developing countries invested considerable resources during the 1970s and 1980s to improve complementary feeding, often by attempting to increase the availability of the CFs through centralized production of low cost food mixtures. The size of these programs varied although many were conceived to be of national scope. Unfortunately, there are relatively few good evaluations of these program activities to specific nutritional outcomes.

In some other supplementary intervention programs, various attempts have been made to ensure that supplementary feeding programs benefit children under two years of age. These efforts included promotion and protection of breastfeeding and distribution of special complementary foods. In 1990, the World Food Program (WFP) issued guidelines stating that the substitution of dry milk products for breast milk should strictly not be allowed in all situations (WFP, 1992). In addition to breast-feeding, supplementary feeding programs had tried to improve the nutrition of young children by providing a pre-mixed exotic food rather than the ingredients for home mixing. More expensive commodities such as oil and sugar that were supplied by WFP for the children, tended to be consumed by the entire household rather than the targeted children. When the WFP discovered that their goal of improving nutrition to the affected children was not realized, it decided to change its policies with regard to this intervention approach. Later, the WFP introduced locally produced precooked blended foods in feeding programs in Malawi, El Salvador and Kenya. The advantages of this approach were incentives for agricultural production, employment and reduced logistics costs (Dijkhuizen and Wurdemann, 1993). The re-orientation of food assistance to local production began in the mid-1990s. Declining food reserves in donor countries, desire to reduce food aid dependency and the attractiveness of

selling agricultural products for foreign exchange prompted donors and recipients to begin replacing imported food with locally produced food. A number of projects were established in Africa, Asia and Latin America to manufacture complementary foods for local consumption. The United States Agency for International Development (USAID) even provided technical assistance and equipment for processing plants in Sri Lanka, Costa Rica, Tanzania and Guyana (Harper and Jansen, 1985). However, despite all these efforts, the impact towards improving the nutritional status of targeted children has not been realised. According to Walker (1990), the possible reasons of failure of these projects were that, often the levels of management required for such projects were unrealistically high and sometimes imported spare-parts were needed for the plants that required hard currency. The resulting complementary foods were too expensive except for the elite.

1.7.2 Intervention trials to improve nutritional status of children

Some nutrition intervention trials to improve the nutritional status of children through improving complementary foods, attempted to modify traditional foods by fortification of either indigenous or exotic foods. Several intervention programs, which aimed at solving nutritional problems such as anemia and micronutrient deficiencies, using a food approach have either been fortifying the foods or supplementing them with micronutrient compounds. For instance, some trials have been fortifying local staples with ferrous sulphate (Mendoza *et al.*, 2001), zinc sulphate (Fairweather-Trait *et al.*, 1995), vitamin A and vitamin C (Davidsson *et al.*, 2001; Zlotkin *et al.*, 2001). However, the limitations of the effectiveness of food fortification programs has been associated with factors such as cost, constant

availability, stability, timely distribution and compliance with the prescribed fortificants (Thu *et al.*, 1999).

1.7.3 Recent scientific randomized controlled trials

There are very few scientific intervention studies that have been conducted according to a prospective randomized design, whereby one group was provided with certain types of foods that were aimed at solving a nutritional problem while another group that acted as a control was allocated coventional foods utilized in the area. Provided that the two groups were similar at the baseline then the change in nutritional status as a result of the intervention could be attributed to the dietary intervention. A longitudinal study conducted by The Institute of Nutrition of Central America and Panama (INCAP) in Guatemala is one of the richest sources of information about the importance of nutrition for growth and development in developing countries (Martorell *et al.*, 1995). The longitudinal study was carried out in two villages of Eastern Guatemala from 1969 to 1977 with the objectives of assessing differences in physical growth and behavioral development of children of less than 7 years of age. One group of children (from the *atole* community) was provided with a food supplement containing a relatively high amount of protein and energy called Incaparina (cereal legume blend) while the other group (from the fresco community) were given sugar drink mixed with vitamins and some minerals. The results showed that children in the *atole* communities grew approximately 2.5 cm more in length during the first 3 years of life than those in the *fresco* communities. These differences could be attributed to the intervention. However, even with the positive impact of the *atole*, the average growth velocity of the children in these communities was still

considerably less than the international reference data (Habicht *et al.*, 1995). Furthermore, the difference in growth did not continue after the third year.

A similar four-country study by Simondon *et al.* (1996) used a precooked porridge, which was a blend of cereals, soybean, milk powder, and fortified with vitamins and minerals with the aim of investigating the effect of early supplementation on weight and linear growth of infants from 4-7 months of age. The food was offered every day for three months, consumption monitored and both groups were free to eat local foods. The four countries under the study were Senegal, New Caledonia, Congo and Bolivia where a total of 110, 90, 120 and 127 infants respectively, were randomly allocated to supplement or no supplement (control group). About 837 kJ day^{-1} was supplied to 4-month infants and 1674 kJ day^{-1} was supplied to older infants. The results showed that the mean 4-7 months length increment was 0.48 cm higher for the supplemented than for the control infants in Senegal whereas weight increments did not differ. No significant effect was found in other countries with regard to weight or length.

Another interesting randomized intervention study conducted in Honduras aimed at investigating the optimal age of introduction of complementary foods and growth of term low birthweight, breast-fed infants (Dewey *et al.*, 1999). Mothers of low birthweight infants were recruited in a hospital and assisted with exclusive breast-feeding for 4 months. At 4 months mothers were randomly assigned to either continue with exclusive breast-feeding to 6 months or to feed complementary foods twice daily from 4 to 6 months. The results showed that there was no growth advantage of complementary feeding between 4 and 6 months of age, which meant the infants could continue to be exclusively breast fed until they reached six months of age.

A more recent study was conducted in Ghana investigating the effect of improved centrally processed complementary foods on growth and micronutrient status of Ghanaian infants from 6-12 months of age (Lartey *et al.*, 1999). *Koko*, which is a fermented maize porridge used as a primary complementary food in Ghana, was implicated for the high prevalence of malnutrition. Instead Weanimix, a cereal-legume blend developed by United Nations children's fund and the Ghanaian government was promoted as an alternative. Infants from 6-12 months of age were randomly assigned to receive weanimix, weanimix plus vitamins and minerals, weanimix plus fish powder and *koko* plus fish powder. The control group included a cross sectional anthropometrical data of 464 infants collected before and after the intervention. Anthropometrical data and blood samples were collected at 6 months and 12 months of age to assess iron, zinc and vitamin A. Results showed that there were no significant differences between the intervention groups in weight or length gain, hemoglobin and hematocrit values between 6 and 12 months of age. However, all four foods improved growth relative to the control group.

According to WHO (1998a), the results obtained from various food-based trials conducted in different parts have been very variable whereby reasons for different results are not always evident. In most cases the supplements either included some high quality animal products, with or without cereal flour or provided additional micronutrients. With such a small number of studies it is not possible to determine why growth responses were detectable in some studies but not in the others. It is also important to note that despite the positive growth responses to supplementation that were observed in some studies, in no case did the children achieve the expected growth velocities for this particular age group.

Objectives of the study

1.8 Objectives of the study

In recent years, a number of commercial CF blends have been produced in Tanzania incorporating cereals and legumes available in the country. The composition of these blends is single, double or triple cereals with legumes, mixed and milled at different ratios to form a single ingredient or composite flour. The producers of the cereal mix do not take into account the nutritional composition or the scientific basis of the ratios used in their formulations. Equally, they do not address the issue of mineral deficiencies such as iron and zinc that is prevalent in many developing countries and the presence of antinutritional factors such as phytates, and tannins that hinder bioavailability of minerals in the blends. Absence of the data on their energy values, protein content and quality makes it difficult to ascertain whether the blends were prepared to meet protein, energy or micronutrient requirement of children. The true composition is therefore not revealed. Safety of the foods with regard to microbial contamination and toxins such as fumonisin and aflatoxins are also not considered in these formulations. Moreover, for all of these CFs, there is no published information on their nutritional composition or the scientific basis of the ingredients ratios used in their formulation.

These CFs are mainly produced by petty businessmen in urban centers who are profit oriented taking advantage of the existing high demand for CF promote their blends through advertisements luring people on their high quality with regard to macro and micronutrients. Nevertheless, the relatively high price of the CF blends becomes a hinderance to the majority of the low-income population groups who are basically the ones with major nutritional problems.

Equally, trials of new formulations or blends need to be performed on the target populations such as children in order to ascertain the efficacy and quality of these CFs. In most of the commercial blends these trials are not done and therefore the basis for recommending a CF/blend in combating various nutritional problems is not often conclusive.

For the purpose of addressing the above problems there came a need to develop a CF based on locally available and produced foods crops in East Africa, formulated in such a manner to have optimum protein quality and highest possible energy content. Previous work by Mbithi-Mwikya *et al.* (2002) tackled most of the technological experiments with regard to the production of this CF. Traditionally known soaking and germination technologies were utilized in processing the ingredients and coming up with a processed CF. Laboratory experiments were done on each ingredient to be incorporated in the CF with regard to nutritional contribution. Calculations were performed on various possible means of combinations to attain a CF with the highest possible energy, optimal protein quality and with minimal contents of antinutrients. In order to ascertain the efficacy of this CF it was imperative to perform an intervention study of the CF formulation in order to establish the benefits investigated in the *in vitro* experiments.

The main objective of this study was therefore to follow-up growth and iron status of infants fed on nutritional sound and safe complementary food formulation prepared from finger millet (*Eleusine corocana*), kidney beans (*Phaseolus vulgaris*), peanuts (*Arachis hypogoea*) and mango (*Mangifera indica*) which are crops locally grown in Tanzania.

Specific objectives

(i) To investigate on the *in vitro* availability of micronutrients from ingredients used for complementary food formulation.

(ii) Ascertain the microbial safety of the produced complementary food.

(iii) To determine the presence and exposure of children to fumonisin in ingredients used for complementary food formulation.

(iv) To study preliminary nutritional status, iron deficiency anemia and feeding habits of infants in the study community.

(v) To investigate on the effect of the processed CF in improving growth and micronutrient status of infants from 6 months to 12 months of age.

CHAPTER 2

In vitro solubility of iron and zinc in finger millet and kidney beans during processing[2]

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2 *In vitro* solubility of iron and zinc in finger millet and kidney beans during processing

2.1 Abstract

Finger millet (Eleusine coracana) and kidney beans (Phaseolus vulgaris) were processed by soaking, germination and autoclaving for incorporation into a complementary food for children. In vitro solubility of iron and zinc was determined by HCl-Pepsin and Pepsin-Pancreatin methods after each processing step. Germination significantly increased the solubility of these minerals. Phytic acid was reduced by 54 and 36% in finger millet and kidney beans, respectively, during the overall processing. Iron solubility in germinated millet, as determined by pepsin–pancreatin method, increased 6.8 times with addition of vitamin C. These results show that various processing methods, especially germination, increase mineral solubility. Addition of vitamin C and mango could be used to enhance mineral extractabilities, thereby helping to alleviate micronutrient deficiencies in populations subsisting on these foods.

2.2 Introduction

A number of inorganic minerals are important either in the structure or functioning of the body and must therefore be provided by the various components that make up the diet. Cereals and legumes individually or as composite, are extensively used to prepare complementary foods in developing countries (Uwaegbute, 1991; Kingamkono *et al.*, 1995; Towo and Tatala, 1998). Minerals from plant sources, particularly those from plant seeds, are less bioavailable than those from animal sources due, in part to phytic acid, tannins and fiber present in the plants (Moeljopawiro *et al.*, 1988; WHO, 1998a). These antinutritional factors chelate

dietary minerals in the gastrointestinal tract reducing their biological availability (Frolich, 1995). Processing techniques such as soaking and germination have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing (Mosha and Svanberg, 1990; Lorri and Svanberg, 1995; WHO, 1998a). Honke *et al.* (1998) found that germination, which is often used to prepare legume seeds for consumption, causes considerable degradation of inositol phosphate (IP_6), and hence increases the bioavailability of trace elements. A study by Sripriya *et al.* (1997) revealed that germination was effective compared to fermentation in increasing the extractability of trace elements like copper, zinc and manganese from raw finger millet. Food processing and preparation may therefore influence both the content and form of the antinutritional factors thus in turn modulating mineral bioavailability (Svanberg and Sandberg, 1988; Frolich, 1995).

Ascorbic acid has been shown to enhance iron bioavailability from meals. A study by Hallberg (1981) on the effect of adding small amounts of ascorbic acid to composite whole meals has shown that the effect of ascorbic acid is significant. Pyke (1986), reports that since ascorbic acid facilitates the bioavailability of iron, supplementary amounts of ascorbic acid can be useful in the treatment of iron deficiency anaemia.

Provision of iron and zinc in children's diets is important, as deficiency of these minerals has been affecting many children in developing countries. According to Thu *et al.* (1999), micronutrient deficiencies remain common in preschool children in developing countries. Iron deficiency anemia is one of the common nutritional problems affecting millions of people in both developing and developed countries (Yip, 1994; Ziegler and Fomon, 1996). According to Gibson (1994) zinc deficiency is

increasingly becoming as common as iron deficiency, affecting young children aged 6-24 months.

Germination when carried out for extended periods may result in spoilage or excessive dry matter losses resulting from the respiring seeds or fermenting micro-organisms. Mbithi-Mwikya *et al.* (2000b) observed that germination of finger millet beyond 48 hr resulted in excessive dry matter loss without corresponding nutritional gains.

The objectives of this study were three fold. The first was to evaluate how the various processing steps, when carried out consecutively and for periods practical for processing CFs, influenced the solubilities of iron and zinc in the ingredients. Second, the two methods (HCl- pepsin and pepsin-pancreatin), which simulate gastro intestinal conditions, were compared. Finally the effect of addition of vitamin C and mango puree on the *in vitro* solubility of iron and zinc were evaluated.

2.3 Materials and methods

2.3.1 Raw materials and preliminary handling

Brown speckled kidney bean seeds (*Phaseolus vulgaris* var. Rose Coco) and brown finger millet (*Eleusine coracana* L. Gaertner) were brought from Tanzania (1999 harvest). They were sorted (by removing extraneous material and damaged seeds) and washed with distilled water, spread on trays and allowed to dry at 37°C for 24 hr. A portion of clean and dry seeds were milled with an attrition mill and sieved repeatedly until all material passed through a 200 μm sieve (no bran was, thus, removed). The flour was sealed into plastic bags and stored at -18°C until analysis.

2.3.2 Processing

2.3.2.1 Soaking

Finger millet and kidney bean samples were weighed into large plastic petri dishes and soaked in de-ionized distilled water in the ratio of 1:2 w/v. Finger millet and kidney beans were soaked for 3 and 8 hr respectively, in a ventilated room at 30°C (Mbithi-Mwikya *et al.*, 2000b) A portion of the soaked seeds were dried in a ventilated room at 37°C for 48 hr and then milled into fine flour (passing through 200 µm sieve).

2.3.2.2 Germination and autoclaving

A portion of the soaked seeds were put in petri dishes and covered with perforated aluminium foil. They were kept in the dark at 30°C for 48 hr to germinate. Germination was stopped by removing the samples and drying them in a ventilated room at 37°C and then milled into fine flour to pass 200 µm sieve (Mbithi-Mwikya *et al.*, 2000b). Another portion of the germinated seeds were placed in autoclavable bags and autoclaved at 121°C for 20 min, cooled and dried them in a ventilated room at 37°C and then milled into fine flour to pass 200 µm sieve.

2.3.3 Laboratory analysis

2.3.3.1 Total minerals

Total mineral content of the samples after the various processing stages was carried out by AOAC method No 968.08 (AOAC, 1995). Total iron and zinc was determined by atomic absorption spectrophotometry (AOAC method 970.12, AOAC, 1995).

2.3.3.2 HCl- pepsin and Pepsin-pancreatin mineral solubility

Extraction of Iron and Zinc by HCl and pepsin (HCl-P) was carried out by a method described by Kumar and Chauhan (1993). For the pepsin-pancreatin (P-P) method, extraction of the minerals was carried out as described by Miller *et al.* (1981). The amounts of iron and zinc were determined by AOAC (1995) as previously explained.

2.3.3.3 Mineral solubility after addition of vitamin C and mango puree

In 2 g flour samples of raw, soaked, germinated and autoclaved finger millet and kidney beans, 0.3 milligram of vitamin C was added. 1g of mango puree containing 0.3 mg vitamin C was added to separate 2-gram samples of raw, soaked, germinated and autoclaved, finger millet and kidney beans. Pepsin-pancreatin digestion method was used for mineral extraction as described previously. The extracted iron and zinc were evaluated by AOAC (1995) as described previously.

2.3.3.4 Analysis of phytates

A method for the rapid determination of phytates developed by Haugh and Lantzsch (1983) was used. Phytates are extracted from the grain flour by HCL and the extract heated with an acidic iron (III) solution of known iron concentration. The decrease in iron determined colorimetrically with 2,2^{l} bipyridine after centrifugation in the supernatant is the measure for the phytic acid content.

2.3.3.5 Statistical analysis

All analyses were conducted in triplicate and calculations were based on 100 g dry matter. An analysis of variance of the results was done at 95% confidence interval (P

≤ 0.05) using Tukeys Honestly Significant Difference. This analysis was done using SPSS 9.0 for Windows computer software.

2.4 Results

2.4.1 Total minerals

Total iron and zinc in raw finger millet and kidney beans were 5.48, 2.05, 9.83 and 3.28 mg/100 g DM respectively. These values did not change significantly during processing (Table 2.1).

Table 2.1. Total iron, zinc and phytic acid content in finger millet and kidney beans during processing (on 100g DM)

	Iron (mg)		Zinc (mg)		Phytic acid (g)	
Process	Mean	sd	Mean	sd	Mean	sd
			Finger millet			
Raw	5.48[a]	0.22	2.05[a]	0.01	1.31[a]	0.04
Soaked	5.43[a]	0.12	2.03[a]	0.02	1.25[a]	0.01
Germinated	5.61[a]	0.34	2.06[a]	0.02	0.63[b]	0.04
Autoclaved	5.55[a]	0.14	2.04[a]	0.04	0.60[b]	0.03
			Kidney beans			
Raw	9.83[a]	0.44	3.28[a]	0.08	1.34[a]	0.01
Soaked	9.61[a]	0.42	3.27[a]	0.06	1.34[a]	0.05
Germinated	9.90[a]	0.34	3.28[a]	0.03	1.09[b]	0.02
Autoclaved	10.22[a]	0.29	3.24[a]	0.03	0.96[c]	0.06

[1]Values are means and 1 standard deviation of 3 replicates For finger millet and kidney beans, values with the same superscript in the same row or column is not significantly different (P< 0.01)

2.4.2 Iron solubility

Iron solubility in soaked finger millet was not enhanced by vitamin C and mango addition and was not affected by the method of determination (Table 2.2). The solubility, when determined by P-P method, increased slightly during germination (4.7%) and later declined significantly (6.2%) during autoclaving.

Table 2.2. *In vitro* solubility (in %) of iron and zinc in finger millet during processing as determined by the HCl-pepsin (HCl-P) and the pepsin pancreatin (P-P) method[1].

	Finger millet (HCl-P)		Finger millet (P-P)		Finger millet (P-P) + vitamin C		Finger millet (P-P) + mango	
Process	Mean	sd	Mean	sd	Mean	sd	Mean	sd
				Iron				
Raw	5.06^{a}	0.18	4.49^{a}	0.22	5.64^{a}	0.14	4.81^{a}	0.17
Soaked	6.01^{b}	0.10	5.88^{b}	0.49	6.81^{b}	0.27	6.16^{b}	0.41
Germinated	29.91^{e}	1.82	10.57^{c}	0.81	31.20^{e}	1.99	29.02^{e}	2.00
Autoclaved	42.99^{f}	0.90	4.40^{a}	0.33	33.43^{e}	0.90	29.97^{e}	0.94
				Zinc				
Raw	50.60^{d}	0.24	30.24^{a}	1.59	50.51^{d}	0.41	46.71^{g}	0.48
Soaked	54.16^{e}	0.50	33.94^{a}	1.03	54.66^{e}	0.26	50.19^{h}	0.40
Germinated	78.41^{f}	0.58	39.20^{b}	1.30	77.19^{f}	2.55	69.98^{i}	0.48
Autoclaved	76.01^{f}	1.31	47.34^{c}	0.92	75.41^{f}	2.92	69.40^{j}	1.47

[1]Values are means and 1 standard deviation of 3 replicates. For each mineral values with the same superscript in the same row or column is not significantly different ($P < 0.01$)

The HCl-P method however, showed a rapid and significant increase in iron solubility in finger millet throughout the processing steps with an overall increase of 37.9%. In kidney beans, iron solubility increased consistently during processing, with major changes occurring during germination (Table 2.3). Overall increases in this solubility were 31.5 and 13.7 % by HCl-P and P-P methods, respectively. Addition of Vitamin C and mango in finger millet significantly increased iron solubility during

germination by 25.6% and 24.2%, respectively (Table 2.2). For kidney beans these increases were 28.0% and 26.7%, respectively (Table 2.3). However in both finger millet and kidney beans, iron solubility after addition of vitamin C was not affected by autoclaving.

2.4.3 Zinc solubility

Values of zinc solubility in both finger millet and kidney beans were lower when determined by the P-P than the HCl-P method. In finger millet, zinc solubility with HCl-P method improved with germination (27.8%) but remained constant during autoclaving. With the P-P method, zinc solubility increased gradually with processing with an overall increase of 17.1% (Table 2.2). In kidney beans, zinc solubility with HCl-P method increased with processing. The largest increase was during germination (24.8%). Zinc solubility with P-P method also increased with processing by an overall 29.2% (Table 2.3). Addition of either vitamin C or mango in finger millet showed significant increase during germination by 26.7 and 23.3% respectively, and then maintaining constancy during autoclaving. In germinated kidney beans zinc extraction increased by 26.9 and 27.5%, respectively, after addition of vitamin C and mango (Table 2.3).

2.4.4 Phytates

There was a marked reduction in phytic acid content in both kidney beans and finger millet during processing (Table 2.1). For the finger millet samples, the main decreases were observed after germination where it decreased by 49.6%. Phytic acid decreased in finger millet by 54.2% overall during processing. In kidney beans, the overall decrease was 28%.

Table 2.3. *In vitro* solubility (in %) of iron and zinc in kidney beans during processing as determined by the HCl-pepsin (HCl-P) and the Pepsin pancreatin (P-P) method

	Kidney beans (HCl-P)		Kidney beans (P-P)		Kidney beans (P-P) + vitamin C		Kidney beans (P-P) + mango	
Process	Mean	sd	Mean	sd	Mean	sd	Mean	sd
				Iron				
Raw	11 06^{d}	0.31	2.20^{a}	0.20	9.31^{c}	0.39	6.28^{g}	0.54
Soaked	15.05^{d}	0.91	3.57^{b}	0.12	15.60^{e}	0.81	6 80^{g}	0.62
Germinated	38.5^{f}	1.13	17 11^{e}	0.31	37 31^{f}	1 21	32.97^{h}	0.99
Autoclaved	42.51^{i}	0.77	15.89^{d}	0.83	36.67^{f}	1.16	31.22^{h}	0.92
				Zinc				
Raw	30.56^{e}	1.22	23.00^{a}	1 37	31 61^{e}	0 79	30.65^{e}	0.75
Soaked	37.89^{i}	0.45	31.10^{b}	0.10	34 19^{f}	0.68	31 56^{b,e}	0.40
Germinated	62.71^{j}	2.78	45.56^{c}	0.80	58 54^{g}	0.61	59.04^{h,g}	1.22
Autoclaved	68.64^{j,k}	2.62	52.15^{d}	1.32	58.91^{g}	0.03	61 36^{h}	0.42

[1]Values are means and 1 standard deviation of 3 replicates For each mineral values with the same superscript in the same row or column is not significantly different (P< 0 01)

2.5 Discussion

Anthony and Chandra (1998) found 6.53 and 2.02 mg/100 g dry matter for iron and zinc in finger millet. Contents of iron and zinc in red kidney beans (Phaseolus vulgaris) were observed at 5.16, and 3.1 mg/100 g dry matter. These values are in agreement with those observed in our study (Table 2.1). Since no biosynthesis or degradation of minerals is expected during processing, loss in mineral composition could occur mainly through leaching. In our study, solutions leached during germination were collected at the bottom of the petri dishes and incorporated into the sample thereafter. Changes in mineral composition can also occur due to apparent effects caused by loss of other nutrients. The values presented in our study were corrected for dry matter loss during processing in order to compare with the original

amounts. According to Watzke (1998), since ash values determined before and after processing do not drastically change, then equally minerals are not affected during processing. Similarly, Kumar and Chauhan (1993) observed that germination did not alter the concentrations of iron and zinc in pearl millet. Likewise Kazanas and Fields (1981) did not find any significant change in total ash content of sorghum due to germination or fermentation.

Kumar and Chauhan (1993) reported that germination of pearl millet at 30°C for 48 hr enhanced the HCl solubility of iron by 100% while during the same period, zinc solubility increased by about 50%. Divalent cations may be present as mineral-phytate chelates in ungerminated grains, which may explain the lower solubility of those minerals in HCl. Hydrolytic reduction of phytic acid in finger millet during germination has been observed by Mbithi-Mwikya *et al.* (2000b), who observed that phytic acid values decreased from 0.35 g/100 g in the raw sample to 0.02 g/100 g dry matter after 96 hr germination. Chrompreeda and Fields (1984) observed a 17.2% decrease in HCl extractable iron after autoclaving soybean (121°C, 30 min).

According to Anthony and Chandra (1998), HCl solubility of minerals and trace elements under simulated gastric conditions, is an indicator of bioavailability in foods. However, the digestion of minerals is mainly in the duodenum where some of the non-heme iron and other minerals are absorbed (Hallberg, 1981; Oski, 1993). In the stomach the pH is between 1-3 due to the presence of HCl in the gastric secretions while in the duodenum the pH is alkaline in the range of 7.5-8.0 (Rao and Prabhavathi, 1978; Miller *et al.*, 1981). A pepsin-pancreatin digestion process simulating the events in the two key areas of mineral digestion in the gastrointestinal

tract is therefore a stronger indicator of the bioavailability of minerals from foods compared to HCl-pepsin digestion. This is important in the sense that minerals in the two-pH systems behave differently. In the acidic medium of the stomach minerals are soluble since they combine with HCl to form chlorides (Hallberg, 1981; Miller *et al.*, 1981). As the pH is raised further (from 1.2 to 7.5) to simulate duodenal conditions, solubility decreases due to formation of hydroxides, which precipitate out and are unavailable for absorption (Rao and Prabhavathi, 1978). It was evident from the mineral digestions at the various processing stages, that the HCl-P (acidic) method had the highest solubility compared to P-P (alkaline) probably due to mineral precipitation.

The effect of vitamin C was very significant with the addition of 0.3 mg to each sample digested from pH 1.2 to 7.5 (Tables 2.2 and 2.3). Ascorbic acid has been shown to markedly increase the bioavailability of non-heme iron and other minerals (Hallberg, 1981; Skikne, 1988). Danisova *et al.* (1994) reports that ascorbic acid, which was not present in dry lentil seeds, reached measurable values of 20 mg/100 g in germinated samples. This might also explain a higher mineral solubility during germination. In a study by Hallberg (1981), the addition of 25 or 50 mg of ascorbic acid to a Southern Asian type of meal composed of rice, cooked vegetables and curry increased the bioavailability of non-heme iron 50 to 90% respectively. In another study by Hallberg (1981), 150 g of papaya containing 66 mg of ascorbic acid when added to a 250 g Thai rice meal increased the non-heme iron bioavailability 3 to 4 times. According to Oski (1993), orange juice doubles the bioavailability of non-heme iron whereas tea decreases bioavailability by 75%. When orange juice containing 70 mg ascorbic acid was added to continental breakfast with coffee, bioavailability of

non-heme iron increased by 2.5 times. This study however has also demonstrated that vitamin C improves mineral solubility. It has been shown that vitamin C enhances the bioavailability of non-heme iron probably by preventing the precipitation of ferric iron when the pH rises in the small intestine, either by keeping iron in a reduced form and /or by forming a ligand with ferric ions. In this way an appreciable amount of iron remains soluble and thus available for absorption (Hallberg, 1981).

Likewise addition of 1 g of mango puree containing 0.3 mg vitamin C improved solubility of iron and zinc. This might also be due to the presence of vitamin C and other substances contained in mango puree. Addition of mango might also have contributed to an increase in total dietary fibre content of the sample. Since dietary fibre is negatively correlated to mineral solubility (Frolich, 1995), this might explain the observed decrease in iron and zinc solubility of samples added with mango when compared with those added with pure vitamin C.

Phytates

As indicated by our results, the amount of phytic acid had a significant effect on the observed mineral solubility. Wolters *et al.* (1993), investigating the relationship between *in vitro* availability of minerals and food composition with a mathematical model, reported phytic acid as having a strong negative effect on calculated availability of iron and zinc and to a lesser effect on magnesium availability.

Traditional household techniques such as soaking and germination and fermentation have been shown to reduce phytates (Svanberg and Sandberg, 1988). Honke *et al.*

(1998) observed a 15 and 78% reduction in inositol phosphate (IP6) after 48 and 172 hr, respectively, of germination in faba beans.

Unit food processing operations such as milling of cereals, extrusion cooking and frying had little influence in improvement in zinc and iron bioavailability (Watzke, 1998). Other strategies of improving iron solubility by elimination of phytates such as addition of commercial microbial phytase (*Aspergillus niger*) have been suggested. Microbial phytases are universally attractive because of their low cost and effectiveness (Harland and Narula, 1999). Despite their low cost, availability in rural areas of developing countries is questionable hence natural processes of germination and fermentation remains the most sustainable approach.

Svanberg *et al.* (1993) observed that commercial phytase results in higher iron solubility than germination and fermentation alone. For instance, their research on the effects of fermentation on *in vitro* estimation of Fe availability and phytate hydrolysis in maize varieties showed that, iron availability from conventional flour was 4%, and increased to 8% with germinated flour while with flour fermented with starter culture increased to 18%. However, when 10 mg and 59 mg of phytase were added in fermented flour, iron solubility increased from 29% to 40%, respectively. The addition of the microbial phytase to a meal containing wheat bran increased Fe absorption almost two fold as measured in 10 subjects using 55 Fe and 59 Fe tracers (Sandberg *et al.*, 1996).

According to Surtadi and Buckle (1985), phytic acid is significant from a nutritional point of view because of its ability to form stable chelates or phytates. The ability to

chelate multivalent metal ions can result in very insoluble salts that are poorly absorbed from the gastro-intestinal tract (Haug and Lantzsch, 1983). Several studies have indicated that phytic acid reduces the bioavailability of dietary magnesium, zinc and iron in monogastric animals (Lorenz, 1983; Chompreeda and Fields, 1984; Honke *et al.*, 1998).

2.6 Conclusions

Germination influenced mineral solubility the most, with soaking and autoclaving showing only slight changes. Addition of vitamin C or mango puree to the ingredients showed a significant increase in mineral bioavailability. Values obtained by the HCl-pepsin method for iron and zinc solubility were consistently higher than those by the pepsin-pancreatin method. Given that the HCl-P method does not incorporate the alkaline pH conditions in the duodenum and ileum, its values can be considered as an over-estimation iron and zinc solubility. In addition, this study has further demonstrated that decrease in phytates was followed by greater mineral solubility.

CHAPTER 3

Growth of *Staphylococcus aureus* and *Bacillus cereus* during germination and drying of finger millet and kidney beans[3]

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3 Growth of *Staphylococcus aureus* and *Bacillus cereus* during germination and drying of finger millet and kidney beans

3.1 Abstract

The present study examined survival and growth of Staphylococcus aureus and Bacillus cereus during germination and solar drying of finger millet and kidney beans. Conditions during solar drying of the germinated seeds were found unsuitable for excessive growth of pathogenic bacteria. However, contamination of the grains with B. cereus and S. aureus prior to or during germination leads to multiplication of both species in kidney beans and of only B. cereus in finger millet. Excessive growth of these pathogens in germinating legumes and cereals can lead to the production of heat-resistant toxins, resulting in unsafe germinated grains. Strict GMP (good manufacturing practice) procedures should be followed in all food processing units where germination of finger millet or kidney beans is performed. The contamination level for B. cereus and S. aureus in raw finger millet and kidney beans should not exceed 100 cfu g^{-1}, and products should immediately be consumed after cooking.

3.2 Introduction

At about four to six months of age, infants' nutritional requirements begin to exceed those, which are supplied by breast milk (Cohen *et al.*, 1994). The World Health Organization (WHO) recommends that from this age onwards infants should begin to receive a variety of locally available and safely prepared foods (WHO, 1998a).

In many countries of Africa, mothers use boiled porridge made from cereals, usually maize, sorghum or millet flour to complement their children (Kingamkono *et al.*,

1995). This feeding practice has contributed to the high incidence of protein energy malnutrition reported in many developing nations (Gimbi *et al.*, 1997). The quality of complementary foods is often low; they are bulky, low in energy and contain too small amounts of micronutrients given the low gastric capacity (30-40 mL kg^{-1} body weight) of infants (WHO, 1998a). It is usually after the introduction of these complementary foods that the prevalence in deficiency status for these micronutrients as well as the overall growth deficit of the children increases.

To improve the nutritional quality of complementary foods, attempts have been made to promote production of mixtures of locally available cereals, legumes and oil seeds. Different techniques, including germination (Gimbi *et al.*, 1997) and lactic acid fermentation (Nout, 1992), have been utilised to improve the digestibility of nutrients and to lower the levels of antinutrients present in cereals and legumes. Because of their high nutritive value, germinated grains have become popular among health food consumers (Piernas and Guiraud, 1998). Germinated seeds are traditional foods in some Oriental countries and in Africa, with increasing interest in many European countries (Castro-Rosas and Escartin, 2000).

Complementary foods with traditional staples like germinated finger millet and kidney beans are used in many Tanzanian villages. Despite the nutritional advantages of germination to improve the nutritional characteristics of cereals and legumes, the process is believed to favour outgrowth of pathogens and possible toxin production, rendering safety of the germinated products questionable. Various bacterial pathogens have been isolated from sprouts of different seeds such as beans, cress mungbean, mustard and soybean (Beuchat, 1996; Weissinger *et al.*, 2001). However, little

information exists regarding outgrowth of *S. aureus* and *B. cereus* during germination of finger millet and kidney beans and thus safety of formulated foods based on such ingredients. *S. aureus* and *B. cereus* are important micro-organisms in this respect since they produce heat resistant toxins, which can even survive an autoclaving process (NACMCF, 1999). The presence of such toxins in complementary foods may partly explain the problem of complementing age children suffering from protein energy malnutrition while being complemented (Gimbi *et al.*, 1997).

In this paper, the microbial safety of germinated, autoclaved, and solar dried finger millet and kidney beans, to be used as ingredients in complementary foods for children, is evaluated. By autoclaving the seeds after germination, they cannot be used as power flour due to destruction of their amylase-activity. However, they can be added directly as ingredient to the food to reduce the bulk viscosity of starchy staple porridges (Mbithi-Mwikya *et al.*, 2000a, 2001). Since all vegetative cells, spores, and heat labile toxins are removed by autoclaving, the need for control of pathogen outgrowth during germination is limited to only *S. aureus* and *B. cereus* as these are able to produce heat stable toxins. Challenge tests were performed to study the survival and growth of these two pathogens during germination of finger millet and kidney beans. The microbial safety of germinated and autoclaved finger millet and kidney beans during solar drying was evaluated on the basis of water activity and temperature profile.

3.3 Materials and methods

3.3.1 Materials

Finger millet and kidney beans were obtained from Tanzania (1999 harvest). Cultures of *B. cereus* (LMG 12334) and *S. aureus* (LMG 8224) were ordered from the Belgian Coordinated Collections of Micro-organisms (BCCM™/LMG), Ghent. Microbiological reagents and chemicals were obtained from Oxoid Limited, England and MERCK, KgaA 64271, Darmstadt, Germany, respectively.

3.3.2 Processing

Pre-cleaned seeds (washed 5 times with distilled water) were soaked in distilled water at a 1:2 seed:water ratio and then steeped at 30°C for 2 and 8 hr for finger millet and kidney beans, respectively. Steeped seeds were spread on a tray lined with wet blotting paper, covered with another layer of wet blotting paper and then with a perforated aluminium foil. The tray was then kept in a dark room maintained at 30°C. The grains were left to germinate for 48 hr.

The microbial safety of germinated and autoclaved finger millet and kidney beans during solar drying was evaluated at the Mother and Child Care Clinic in Ilonga village, Kilosa district, Morogoro, Tanzania, using tap water that was boiled and cooled to room temperature instead of distilled water for washing and soaking of the seeds. After germination, seeds were autoclaved at 834.5 kPa for 20 min and then immediately sun dried below a glass plate from 10 am till 5 pm on sunny days during the dry season. Drying was accelerated by spreading the seeds in a thin layer approximately 20 cm below the glass plate. Each hour during drying, the product

temperature and the water content of the seeds was measured. The dried grains were milled to pass a 200 µm sieve.

The relationship between water content and water activity (a_w) was determined in the laboratory in Ghent, since a_w measurements could not be performed in Ilonga or Morogoro. Drying of the germinated and autoclaved seeds in Belgium was hereby performed in an air oven at 62 ± 2 °C, and the water content and water activity measured each hour for a total drying time of 7 hr. The following relationships were obtained:

Finger millet: $a_w = 0.491 \, x \, \ln(\%\ water\ content) - 0.724 \quad (r^2 = 0.948)$

Kidney beans: $a_w = 0.144 \, x \, \ln(\%\ water\ content) + 0.413 \quad (r^2 = 0.876)$

3.3.3 Determination of pH, water content and water activity

Ten grams of sample (wet weight) were mixed with 20 mL distilled water, filtered and the pH of the filtrate measured at 25°C. Water content of the seeds was measured according to AOAC method no. 925.10. Water activity above 0.9 was measured with a cryometer (type AWK-20, NAGY Messsysteme GmbH, Gäufelden, Germany). Water activity values below 0.9 were obtained with the thermoconstanter Humidat TH-2 (Novasina, Defensor AG, Pfäffikon, Switzerland).

3.3.4 Procedure for pure culture regeneration

A loop containing *B. cereus* or *S. aureus* was transferred from a slant (stock) of Brain Heart Infusion (BHI) agar to 5 mL BHI broth and the suspension incubated at 37°C

for 24 hr. Slants were stored for maximum 1 month at +4°C. 0.1mL of the bacterial suspension was transferred to a fresh 5 mL of BHI broth using a sterile pipette and the fresh suspension was incubated at 37°C for 24 hr. One mL of the suspension was then serially diluted using Ringer's solution and 0.1mL spread-plated on *B. cereus* agar or on Baird Parker (BCA/BP containing 5 mL egg yolk-tellurite per 100 mL medium) with incubation for 48h at 37°C, before counting.

3.3.5 Inoculation for challenge tests

The inoculation was done during the soaking process. Appropriate dilutions (10 mL each of *B. cereus* suspension and 10 mL of *S. aureus* suspension) were poured into 60 mL of distilled water in a sterile beaker to make 80 mLof steeping medium. This resulted in approximately 6.5 and 7 log cfu mL^{-1} in the case of *B. cereus* and *S. aureus*, respectively. Forty grams of finger millet or kidney beans were added to make a 1:2 seed: water ratio as required for soaking and then steeped for 2 hr for finger millet and 8 h for kidney beans.

3.3.6 Determination of microbial counts

ISO standards were followed to determine total aerobic count (ISO 4833, 1991), *S. aureus* (ISO 6888, 1983), *B. cereus* (ISO 7932, 1987), yeasts and moulds (ISO 7954, 1987), and total lactic acid bacterial count (ISO 13721, 1995). Total aerobic count, *B. cereus* and *S. aureus* counts were determined on raw materials and immediately after steeping, after 24 hr germination and at the end of the germination (48 hr). The same determination was done for lactic acid bacterial count (not for inoculated grains), and yeasts (not for inoculated grains). The total mould content was only evaluated for the raw products. *B. cereus* counts were determined by plating the dilutions after

pasteurisation at 80°C for 10 min to kill vegetative cells or other species that may out-compete the pathogens on plating and subsequent incubation for growth. Bacterial toxins were not measured directly. From a practical point of view, evaluation of the pathogen counts was considered a more stringent criterion than determination of their toxin production.

3.3.7 Calculations and statistical analysis

Unless otherwise stated, all experiments were in triplicate and the means and standard deviations reported. An analysis of variance of the results was done at 95% confidence interval ($P \leq 0.05$) using a paired sample t-test. This analysis was done using Microsoft Excel 2000.

3.4 Results and discussion

3.4.1 Microbial status during germination of naturally contaminated finger millet and kidney beans

Total aerobic plate counts increased considerably during germination of finger millet and kidney beans (Table 3.1). Similar findings were obtained by Gimbi *et al.* (1997) who studied the microbial quality of germinated millet flour. Total microbial counts of finger millet and kidney beans increased by about 2 and 4 log cycles, respectively, to a maximum level of 8 log cfu g^{-1}. In a similar study, performed by our group under field conditions in Tanzania, the average total aerobic plate count, taken from 7 raw samples in Ilonga district, gave 6.60 ± 0.42 and 6.11 ± 0.44 log cfu g^{-1} for finger millet and kidney beans, respectively. After steeping and 48 hr germination, these counts increased to 8.41 ± 0.45 and 7.69 ± 0.58 cfu g^{-1}, respectively.

Table 3.1 Changes in ph and in microbial counts (mean ± sd of log cfu g^{-1}) during steeping and germination of naturally contaminated finger millet and kidney beans[1]

Parameter	Raw grains	After steeping	After 24 hr germination	After 48 hr germination
		Finger millet		
pH	$6.23^{a} \pm 0.07$	$6.38^{b} \pm 0.04$	$5.71^{c} \pm 0.11$	$5.50^{c} \pm 0.09$
Total aerobic counts	$6.97^{a} \pm 0.13$	$5.97^{b} \pm 0.20$	$8.41^{c} \pm 0.17$	$8.70^{c} \pm 0.12$
Lactic acid bacteria	$5.47^{a} \pm 0.18$	$3.70^{b} \pm 0.51$	$5.51^{a} \pm 0.25$	$5.50^{a} \pm 0.08$
Yeast	$5.10^{a} \pm 0.14$	$3.37^{b} \pm 0.17$	$5.36^{a,c} \pm 0.26$	$5.63^{c} \pm 0.32$
		Kidney beans		
pH	$6.14^{a} \pm 0.04$	$6.25^{b} \pm 0.01$	$6.12^{a} \pm 0.09$	$6.30^{a,b} \pm 0.18$
Total counts	$3.97^{a} \pm 0.29$	$4.09^{a} \pm 1.29$	$7.24^{b} \pm 0.65$	$8.17^{b} \pm 0.46$
Lactic acid bacteria	< 2	< 2	< 2	< 2
Yeast	$0.90^{a} \pm 0.14$	$2.14^{b} \pm 0.20$	$2.95^{b,c} \pm 0.72$	$3.13^{c} \pm 0.20$

[1]pH and microbial counts with the same superscripts in the same row are not significantly different ($P \leq 0.05$)

Conditions enhancing germination, which include nutrients liberated by the seeds, temperature and humidity are conducive to bacterial growth (Piernas and Guiraud, 1998). Both in the laboratory and the field study, finger millet and kidney beans were naturally contaminated with less than 100 cfu g^{-1} of *S. aureus* and *B. cereus*. These counts were still below 100 cfu g^{-1} after germination (data not shown). This indicates that the pathogens were absent or present at such low levels that they were not competitive enough with the background flora to increase during germination to levels which lead to toxin production.

Raw finger millet contained about 5.5 log cfu g^{-1} of lactic acid bacteria, which were washed out to about 3.7 log cfu g^{-1} after steeping (Table 3.1). However, during the 48 hr of germination, lactic acid bacteria increased up to the same level as the raw finger millet. A decrease in pH of finger millet from 6.38 during steeping to 5.50 after

germination was obtained, which in view of the levels of lactic acid bacteria found after germination is probably due to other acid producing micro-organisms. Although insufficient to totally prevent the growth of pathogenic micro-organisms, the reduced pH may have induced a growth retardation of pathogens (Lorri and Svanberg, 1995). The level of lactic acid bacteria in raw kidney beans and during processing was lower than 100 cfu g^{-1} and no appreciable pH decrease was observed during their germination (Table 3.1).

Raw finger millet and kidney beans contained 4 log cfu g^{-1} and 2 log cfu g^{-1} of moulds, respectively. The level of yeasts for raw grains increased significantly during 48 hr of germination (Table 3.1). Gimbi *et al.* (1997) also found a significant increase in number of yeasts when millet was germinated for up to 72 hr between 21 and 24^{0}C. This too is attributable to the favourable conditions of water activity (0.99) and temperature (30^{0}C) prevailing during germination.

According to NACMCF (1999) pathogens grow to elevated levels since there are no inherent steps in the production of sprouts that prevents growth or eliminates them entirely. However, the frequency of food borne diseases attributable to Mung beans and soy bean sprouts is generally lower than that associated with "green" sprouts (such as alfalfa and clover) since sprouts of beans are typically cooked (NACMCF, 1999). A sufficient heat treatment after germination (e.g. cooking, autoclaving) will be necessary to reduce the levels of heat-sensitive vegetative pathogens in the seeds.

3.4.2 Survival of B. cereus and S. aureus during germination of inoculated finger millet and kidney beans.

When finger millet and kidney beans are autoclaved after germination, *Staphylococcus aureus* and *Bacillus cereus* can still pose a health hazard, through production of heat-resistant toxins by elevated numbers. It was observed that *B. cereus* multiplied during germination of finger millet and kidney beans (Table 3.2). Counts increased from about 4 log cfu g^{-1} at steeping time to 6-7 log cfu g^{-1} after 48 hr of germination. Such levels of *B. cereus* have potential for toxin production, and render the obtained grains unsafe for use in human foods (Gilbert *et al.*, 1974). In a similar study by Harmon *et al.* (1987), an increase in number of *B. cereus* during germination of wheat grains was reported. Piemas and Guiraud (1998) observed that after inoculation, rice was an excellent substrate for the growth of *B. cereus* during germination. For some *B. cereus* species, heat resistant emetic toxins are detectable at

Table 3.2 Changes in *B. cereus* levels (mean ± sd of log cfu g^{-1}) during germination of significantly contaminated finger millet and kidney beans[1].

Parameter	After steeping	After 24 hr Germination	After 48 hr germination
		Finger millet	
Total counts	$5.19^{a} \pm 0.02$	$7.72^{b} \pm 0.10$	$8.56^{c} \pm 0.04$
B. cereus	$4.16^{a} \pm 0.09$	$6.77^{b} \pm 0.03$	$7.38^{c} \pm 0.07$
		Kidney beans	
Total counts	$4.69^{a} \pm 0.37$	$7.20^{b} \pm 0.17$	$8.56^{c} \pm 0.04$
B. cereus	$3.94^{a} \pm 0.08$	$4.94^{b} \pm 0.09$	$6.16^{c} \pm 0.41$

[1]Microbial counts with the same superscripts in the same row are not significantly different ($P \leq 0.05$)

bacterial levels equal to or above 6 log cfu g^{-1} (Jay, 2000). Therefore, a maximum allowable number of 5 log cfu g^{-1} under GMP conditions at the day of consumption is often applied. Taking into account the observed 2.5 log cfu g^{-1} increase during

germination, the number of *B. cereus* in the raw materials should not exceed 320 cfu g^{-1}. Provided further contamination is prevented, these levels should be sufficient to avoid growth during germination to levels that can cause illness.

S. aureus does not increase in number during germination of finger millet (Table 3.3). A level of about 5.6 log cfu g^{-1} of the pathogen used to contaminate the grains at the steeping stage decreased slightly as the grains were germinated for up to 48 hr. This suggests that finger millet is not a good substrate for growth of *S. aureus* and that the pathogen does not compete well with the natural flora of finger millet. *S. aureus* did increase by one log cycle during germination of kidney beans.

Table 3.3 Changes in *S. aureus* levels (mean ± sd of log cfu g^{-1}) during germination of significantly contaminated finger millet and kidney beans[1].

Parameter	After steeping	After 24 hr Germination	After 48 hr germination
		Finger millet	
Total count	$6.50^{a} \pm 0.04$	$8.51^{b} \pm 0.04$	$8.60^{c} \pm 0.03$
S. aureus	$5.56^{a} \pm 0.03$	$5.06^{b} \pm 0.06$	$4.99^{b} \pm 0.12$
		Kidney beans	
Total count	$5.72^{a} \pm 0.78$	$8.13^{b} \pm 0.49$	$8.75^{b} \pm 0.28$
S. aureus	$5.69^{a} \pm 0.89$	$6.47^{a} \pm 0.62$	$6.17^{a} \pm 1.23$

[1]Microbial counts with the same superscripts in the same row are not significantly different ($P \leq 0.05$)

This is undesirable because some strains of *S. aureus* are capable of producing heat resistant enterotoxins (Naguib *et al.*, 1987). *S. aureus* produces heat resistant toxins at bacterial levels above 6-7 log cfu g^{-1} (Jay, 2000). Using a maximum allowable number of 5 log cfu g^{-1} after germination, hazardous levels of toxins will not be produced by *S. aureus* during germination when the initial contamination level of raw kidney beans is lower than 4 log cfu g^{-1}, assuming further contamination is prevented.

These results illustrate that a quality criterion of < 100 cfu g^{-1} for *B. cereus* and *S. aureus* in raw finger millet and kidney beans is sufficient to prevent production of heat resistant toxins during germination.

3.4.3 Product temperature and water activity as a function of drying time

To evaluate whether outgrowth of pathogens is possible during solar drying of germinated and autoclaved kidney beans and finger millet, a field study was performed. The product temperature, which was not significantly different between finger millet and kidney beans, reached 55°C after 1hr drying and varied between 55 and 67°C up to 7 hr of drying. For finger millet, the water activity fell from 1 to 0.48 after 4 hr drying, while in the case of kidney beans 7 hr were necessary to reach a water activity of 0.85 (Table 3.4).

Table 3.4 Temperature, water activity and moisture content during drying of germinated and autoclaved finger millet and kidney beans[1].

		Finger millet		Kidney beans	
Drying time (hr)	Product temp (°C)[2]	Water activity (a_w)	Moisture content (%)	Water activity (a_w)	Moisture content (%)
0	$51.2^{a} \pm 1.0$	1	$46.81^{a} \pm 2.09$	$0.974^{a} \pm 0.003$	$49.26^{a} \pm 1.02$
1	$55.4^{b} \pm 0.8$	1	$38.36^{b} \pm 3.43$	$0.965^{b} \pm 0.002$	$46.41^{b} \pm 0.69$
2	$64.1^{c} \pm 1.0$	$0.967^{a} \pm 0.033$	$31.39^{c} \pm 2.12$	$0.950^{c} \pm 0.004$	$41.59^{c} \pm 1.11$
3	$65.7^{d} \pm 0.8$	$0.654^{b} \pm 0.056$	$16.64^{d} \pm 1.97$	$0.930^{d} \pm 0.008$	$36.21^{d} \pm 1.85$
4	$66.7^{e} \pm 0.5$	$0.485^{c} \pm 0.061$	$11.82^{e} \pm 1.48$	$0.920^{d} \pm 0.013$	$33.85^{d} \pm 2.79$
5	$67.1^{e} \pm 0.8$	$0.378^{d} \pm 0.058$	$9.50^{f} \pm 1.23$	$0.890^{e} \pm 0.015$	$27.69^{e} \pm 2.99$
6	$66.4^{e} \pm 0.6$	$0.345^{d} \pm 0.063$	$8.89^{f} \pm 1.23$	$0.868^{e,f} \pm 0.021$	$23.84^{e,f} \pm 3.24$
7	$62.7^{c} \pm 1.9$	$0.306^{d} \pm 0.085$	$8.26^{f} \pm 1.50$	$0.843^{f} \pm 0.015$	$19.95^{f} \pm 2.15$

[1] Values are mean ± standard deviation for seven separate drying experiments. Values with the same superscripts in the same column are not significantly different ($P \leq 0.05$). [2] Product temperature during drying of finger millet and kidney beans was not significantly different ($P \leq 0.05$)

This difference in drying behaviour can also be seen in the drying curves (Figure 3.1), and can be related to the smaller size of the millet grains, or to the difference in moisture content and nutrient composition, which may influence the water binding capacity during drying (Pabis *et al.*, 1998).

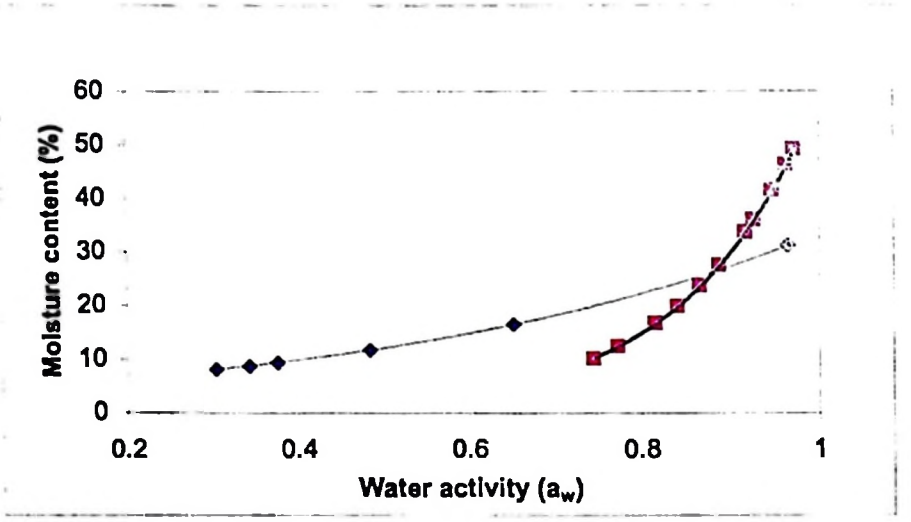

Figure 3.1. Drying curve (moisture content in function of water activity) for germinated and autoclaved finger millet (··· ◆ ···) and kidney beans (— ■ —).

Contamination of the grains during drying, especially with microbial spores, cannot be prevented. However, when the dry products are cooked after suspension in water and immediately consumed, only *B. cereus* and *S. aureus* (heat-resistant toxins) have to be taken into account for evaluation of the drying step. *Clostridium perfringens* and *Clostridium botulinum* are assumed not to grow in this aerobic environment. In view of the growth limits for *B. cereus* ($a_w > 0.92$, temp < 55°C) and *S. aureus* ($a_w > 0.85$-0.88, temp < 48°C) (ICMSF, 1996), no outgrowth of these pathogens during the solar drying process is to be expected, even in the absence of a competing microflora in the autoclaved grains. The cooked porridges can therefore be considered as microbiologically safe. However, when the product is stored after cooking, spores can germinate and pathogen outgrowth may still cause a microbial health hazard.

3.5 Conclusions

This study demonstrated that significant contamination of *S. aureus* and *B. cereus* in finger millet and kidney beans prior to or during germination, can result in the outgrowth of *S. aureus* and *B. cereus* for kidney beans and *B. cereus* for finger millet. This may lead to the presence of toxins in the germinated grains and hence in the final products, even if the grains are heat treated after germination. It is therefore recommended that proper safety control (HACCP) mechanisms including a procedure for observation of personal hygiene, selection of safe raw materials and water have to be followed in all food processing units where a germination stage for finger millet and kidney beans is involved. For units operating under GMP conditions, the contamination level for *S. aureus* and *B. cereus* in raw finger millet and kidney beans must be < 100 cfu g^{-1} prior to germination. Germinated, autoclaved and solar dried seeds should be consumed immediately after cooking, without further storage.

CHAPTER 4

Presence and exposure assessment of fumonisin in finger millet, kidney beans and peanuts, which are ingredients used for complementary food in Tanzania[4]

[4] Peter Mamiro, Bruno De Meulenaer, John Van Camp, Katleen Baert, Frank Devlieghere, Wessy Meghji, Anne Opsomer and Patrick Kolsteren. Journal of Food Additives and Contaminants: Submitted

4 Presence and exposure assessment of fumonisin in finger millet, kidney beans and peanuts, which are ingredients used for complementary food in Tanzania

4.1 Abstract

Finger millet, kidney beans and peanuts were collected from various farmers, transporting lorries and market retailers in Tanzania after harvest (AH) in July 2001 and six months after harvest (SMAH) in January 2002. The objective was to evaluate the presence and exposure of fumonisins associated with the consumption of these ingredients, which are used in the formulation of complementary food (CF) for children in Tanzania. Fumonisins were extracted from the samples by 70% methanol and quantified by enzyme-linked immunosorbent assay (ELISA) using the RIDASCREEN®FAST Fumonisin kit. Children's exposure assessment on fumonisins intake was estimated, based on the finger millet, kidney beans and peanuts consumption data of various CFs obtained from twenty-four-hour dietary recalls. All the grains were found to have fumonisin concentrations ranging from 5 μg kg^{-1} to 440 μg kg^{-1}. Peanut samples collected AH were found to have comparatively high fumonisin levels (mean 105 μg kg^{-1}), followed by kidney beans (mean 43 μg kg^{-1}) and finger millet (mean 5 μg kg^{-1}). Samples collected SMAH had fumonisin levels ranging from 5-36 μg kg^{-1}. The fumonisin levels found in these grains are below the suggested fumonisin limit of 1000 μg kg^{-1}. Ninety nine percent of the children were below the suggested tolerable total dietary intake (tTDI) of 2μg kg^{-1} body weight day^{-1}.

4.2 Introduction

Fumonisins are toxic metabolic by-products, produced by *Fusarium* molds such as *Fusarium moniliforme* and *Fusarium proliferatum* that grow on agricultural commodities in the field or during storage (Scott, 1993; Castella *et al.*, 1999; Ryu *et al.*, 1999). These mycotoxins have been found as contaminants, mainly in corn,

worldwide (Bakan *et al.*, 2002; Kim *et al.*, 2002). More than ten types of fumonisins have been isolated and characterized. Of these, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3) are the major fumonisins produced in nature (Kim *et al.*, 2002). The most prevalent of these mycotoxins in contaminated corn is FB_1, which is believed to be the most toxic (Thiel *et al.*, 1992; Bacon and Nelson, 1994; Musser and Plattner, 1997; de Nijs *et al.*, 1998c).

The fumonisins produced by *Fusarium* species are carcinogenic to rodents and possibly to humans too. The fumonisins exhibit cancer-promoting activity and experimental studies have revealed that the toxins are able to induce liver and kidney damage in several species and cancer in rats and mice (Lebepe-Mazur *et al.*, 1995). The International Agency for Research on Cancer (IARC) has evaluated these toxins derived from *F. moniliforme* (*F. verticillioides)* as group 2B carcinogens, that are possibly carcinogenic to humans (IARC, 1993). Other studies further suggested that fumonisins might be partly responsible for oesophageal cancer in humans (de Nijs *et al.*, 1998a). Maize intended for human consumption that contained high levels of fumonisins was associated with a high incidence of oesophageal cancer in Northern Italy (Doko and Visconti, 1994), USA (Castelo *et al.*, 1998), in Transkei-South Africa (Rheeder *et al.*, 1992) and in the Linxhian region of China (Sydenham *et al.*, 1991, Chu and Li, 1994). *Fusarium* contamination of cereals was also found in areas where children and adults had an osteoarthropathy known as Kashin-Beck disease, together with an important degree of selenium deficiency (Kolsteren, 1992). Selenium is an important mineral in enzymatic pathways that protects against free radical damage (Halliwell, 1994). Selenium deficiency alone could not explain the geographic occurrence of Kashin-Beck disease and a combined etiology was put forward. In a

more recent paper, Zin *et al.* (1998), demonstrated the capacity of Fumonisin B_1 to generate free radicals *in vitro*, which could serve as a pathophysiological explanation for the effects of fumonisins. Free radical damage due to a rupture of homeostasis between the protective mechanisms and free radical generating stresses have been put forward as the explanatory mechanism for oedematous malnutrition or Kwashiorkor (Golden and Ramdath, 1987). Studies in children with kwashiorkor also demonstrated high levels of aflatoxin in blood and urine (Coulter *et al.*, 1986). If fumonisin is a free radical generator, children exposed to high fumonisin intakes could be under stress with higher needs for minerals and anti-oxidative vitamins.

Fumonisins have mainly been analyzed in corn, rice, rye and wheat (Lebepe-Mazur *et al.*, 1995; Accerbi *et al.*, 1999; Kim *et al.*, 2002). Data from Kansas (Rumbeiha *et al.*, 1997) and the U.K. (Patel *et al.*, 1997) demonstrated that certain corn products contain relatively high levels of fumonisins: corn meal up to 349 mg kg^{-1}, and corn flour up to 167.7 mg kg^{-1}. Among African countries where maize is a basic dietary staple such as in Zambia, the prevalence of *F. moniliforme* in maize was widespread (Marasas *et al.* 1988). Fumonisins B_1 and B_2 isolated in maize from this country had levels up to 17.9 mg kg^{-1} and 3 mg kg^{-1}, respectively (Alberts *et al.*, 1990, Thiel *et al.*, 1991). Similarly, Doko *et al.* (1996) detected fumonisins in all maize samples from Botswana, South Africa, Mozambique and Zambia. However, the extent of contamination of the samples of maize from Malawi and Tanzania was less than 100%. These data suggest widespread occurrence of fumonisins in Eastern and Southern Africa. The high incidence of fumonisins at such high concentration levels in cereal and cereal-based products may have serious implications on the health of

intended consumers especially children who are complemented with maize porridges available in their respective areas.

Only few studies have investigated finger millet, beans and peanuts although these are frequently used as ingredients for complementary food in African countries. Munimbazi and Bullerman (1996) found no *Fusarium* growth in finger millet and low levels of fumonisin in beans and peanuts. Siame *et al.* (1998), detected no fumonisin in peanuts and beans. The objective of this study was therefore four-fold: (i) to investigate the presence of fumonisins in finger millet, kidney beans and peanut grains, which are frequently used to produce complementary food (CF) for children in Tanzania, (ii) to study the effect of storage on fumonisin levels, (iii) to evaluate the presence of fumonisin in the same ingredients at major marketing channels before reaching the final consumer, and (iv) to determine the exposure of children to fumonisins who are fed on these ingredients.

4.3 Materials and methods

A total of 90 samples of finger millet, kidney beans and peanuts from individual peasant farmers, crop haulage lorries and individual market retailers, were collected in Tanzania during year 2001 and 2002 as further explained below.

4.3.1 Samples from individual farmers collected after harvest (AH)

Samples of finger millet (harvest of July 2001) were collected from 5 randomly selected peasant farmers out of 30 farmers cultivating the crop in Mpwapwa district, Dodoma region (450 km West of Dar-es-salaam), while kidney beans and peanuts were collected from 5 randomly selected farmers out of 28 peasant farmers cultivating

the crops in Kilosa district, Morogoro region (300 km West of Dar-es-salaam) in Tanzania. The random selection of farmers was done by random numbers using the SPSS 9.0 for Windows computer software. Sampling was done in such a way to get a uniform batch of each sample. From each peasant, 2.5 kg x 10 samples were drawn from the same lot but at different locations of the pile. A total of 25 kg lot was mixed thoroughly as recommended in FAO guidelines (FAO, 1993) sampling out 2.5 kg and finally 250 g.

4.3.2 Samples from lorries collected AH

Samples of finger millet, kidney beans and peanuts were collected from the first 5 out of 10 lorries/trucks hauling crops from various regions of Tanzania and transporting them to the main Tandale market in Dar-es salaam. The market was randomly selected among five major markets in Dar-es-salaam. The investigator followed the lorries at the main market parking lot before unloading any consignment. Permission to do sampling was sought from the lorry drivers before the lot was sold to retailers. Sampling was done by random selection of 10 sacks of approximately 100 kg each, where 1 kg sample was drawn from each sack. A total of 10 kg was mixed thoroughly as recommended in FAO guidelines (FAO, 1993) and one representative sample of 250 g of each grain was taken.

4.3.3 Samples from market retailers collected AH

Samples were collected from 5 randomly selected retailers out of 30 retailers at the main market Dar-es salaam who were selling finger millet, kidney beans or peanuts. Random numbers for selecting individual retailers were used as discussed above. From each retailer, 1 kg sample was drawn from a pile for three consecutive days. A

total of 3 kg of each sample from each retailer was mixed thoroughly as recommended in FAO guidelines (FAO, 1993) and finally sampling out 250 g.

4.3.4 Samples collected six months after harvest (SMAH)

The same procedure was repeated in January 2002, that is six months after collecting the first samples, in order to evaluate the effect of storage on fumonisin levels. Samples of grains were obtained from the same farmers in Mpwapwa and Kilosa districts. Methods of storage of the grains were recorded for each farmer. It was impossible to collect samples from the same lorries, as it was difficult to trail the lorries that were sampled before. Therefore other lorries were sampled as it was done previously. However, samples were collected from the same retailers at Tandale market. A total of 45 samples weighing 250 g were obtained.

4.3.5 Methods of storage by farmers and retailers

Farmers used normal sisal sacks to store dried and threshed finger millet. The sacks were threaded and stacked one on top of the other beside the walls and raised a little from the floor using wooden crates to avoid moisture to come in contact with the grains. For kidney beans large traditional earthen pots were used. These were made insect proof by putting a layer of ash on top of the pot and covering tight with a fitting lid. Peanuts were kept un-dehusked in sisal sacks. The grains were sun dried and stored when they reached less than 13% moisture content. Storage temperatures ranged from 20°C during the night to 30°C during the day while relative humidity ranged from 30% to 35%, respectively. For the case of lorries and market retailers, normal sisal sacks were used to keep and store finger millet, kidney beans and peanut grains.

4.3.6 Packaging and transport of the sub samples for fumonisin analysis

All the above grain samples (250g each) were packed in plastic bags, sealed and stored at −18° C before transporting them by air to Ghent University-Belgium, Laboratory of Food Technology and Nutrition, for fumonisin analysis. After arrival at the Laboratory in Belgium all samples were stored in a freezer maintained at -18° C before analysis.

4.3.7 Fumonisin extraction and detection

Quantitative fumonisin analysis was performed by ELISA, cereals and feed, RIDASCREEN®FAST Fumonisin kit (Art. No. R5602) R-Biopharm GmbH, Darmstadt, Germany. Determination of fumonisin levels was done according to the manufacturer's instructions. The kit provides fumonisin standard (FB_1) ready for use, therefore the fumonisin concentrations of the samples could directly be read from the standard curve. A portion of each sample of grain was milled into fine flour with an attrition mill. A 5 g sample of each was weighed in plastic tubes and 25 mL of 70% methanol (70mL methanol: 30 mL distilled water), was added and the sample was blended for 2 x 3 minutes by a vortex shaker with a rest of 1 minute in between the shakes. The extract was filtered by Whatman filter No. 1 and 100 µL of the filtrate diluted into 1.3 mL of distilled water. Absorbance was read at 450 nm against a blank by a Multiscan Plus MKII ELISA 450M. The readings were taken within 30 minutes. Concentrations of fumonisin in the samples were read from the standard curve obtained from the standards run concurrently with the samples. All samples were screened in the presence of five standard solutions that is, 0.0 mg kg^{-1} of solid sample, 0.222 mg kg^{-1}, 0.666 mg kg^{-1}, 2 mg kg^{-1} and 6 mg kg^{-1}. The detection limit of the RIDASCREEN®FAST fumonisin test was 0.222 mg kg^{-1} of the solid sample. Samples

with fumonisin levels lower than 0.222 mg kg^{-1} were concentrated 40-fold by evaporating 4 mL of the filtrate with nitrogen gas till dry and re-solubilization in 100 µL of 70% methanol. Samples were further diluted into 1.3 mL of distilled water and analyzed to detect the fumonisin concentration as described above.

4.3.8 Sample concentration and recovery

A sample of kidney beans, peanuts and finger millet with no detectable fumonisin concentration was spiked with fumonisin standard solution to a final concentration of 1 mg kg^{-1}, 0.5 mg kg^{-1} and 0.2 mg kg^{-1}. For all solutions the recovery was 100%. The stability in nitrogen gas was evaluated by adding a known concentration of fumonisin solution to the same samples as above. A 10-fold volume of the extract was taken and evaporated in nitrogen gas, re-diluted and then analyzed to detect the fumonisin concentration. Results showed that fumonisins were stable in nitrogen gas since more than 90% of fumonisins were recovered.

4.3.9 Food consumption data from the children

A twenty-four hour dietary recall was conducted on 378 randomly selected children of age between 1 and 24 months in the same community, and using random numbers between August and October 2000. The list of children was obtained from the village MCH clinic. The objective of doing the dietary survey was to establish the variety of CFs given to children in the rural Tanzanian community in function of age. The dietary recall interviews were conducted at the homestead by a nutritionist accompanied by a village health worker. The mother was requested to show the type and amounts of foods, which she had given or cooked for her child in the last 24 hr. The amount of food given to the child by the mother was weighed using a digital

weighing scale (Tefal scales, UK) with an accuracy of 0.5 g or measured by a measuring cylinder (Pyrex, UK) with an accuracy of 0.5 mL in case of volumes.

4.3.10 Consumption data on individual ingredients and assumptions made

From the 24 hr dietary recall, all solid foods taken by the children excluding breast milk refer to all CFs. This is normally a mixture of maize, finger millet, kidney beans and peanuts. Children who consumed CF prepared from finger millet, kidney beans and peanuts were identified and the data were then used in the calculation for fumonisin exposure assessment in individual ingredients. We also assumed that if all CFs were a theoretical mixture of finger millet, peanuts and kidney beans in the ratio of 80%, 15% and 5% respectively, what would be the exposure of the children to fumonisin. This ratio is commonly promoted by Mother and Child Health Care centers in Tanzania for preparation of CF for children and hence adapted by most households especially in the rural areas. Last assumption made was if all CF was maize what would be the exposure of the children to fumonisin. Data on maize contamination by fumonisin was obtained from various literature sources (de Nijs *et al.*, 1998a; Thiel *et al.*, 1991).

4.3.11 Weight of the children

Weight of children was measured by a Salter scale (Model 235 6S - England) with a capacity of measuring up to 25 kg. The scale was adjusted to read zero and calibrated by a known 5 kg standard weight before starting the measurements. The weight was recorded to the nearest 100 g as soon as the pointer on the scale had stabilized.

4.3.12 Children fumonisin exposure analysis

Data on the children's consumption of finger millet, kidney beans and peanuts were obtained from twenty-four-hour dietary recalls conducted in the study community as described above. However, the samples for fumonisin analysis were collected from various rural and urban centers as described previously, but their history was not known. The objective was to obtain samples of the three ingredients being promoted for preparing CF for children from the various levels along the marketing chain before reaching the final consumer. The @RISK program version 4.5, Palisade Corporation, London, UK (2002) was used to estimate the exposure of children to fumonisin, which uses the technique of Monte Carlo simulation for exposure assessment. Input values of food consumption and fumonisin contaminations were fitted to the distributions. To choose the distribution with the best fit the chi-square, Anderson-darling and Komolgorov-Smirnov fitting algorithms were used. These distributions were used to calculate the probability distribution function of fumonisin exposure to the population of children. The 'auto' command was used to allow the program to select the number of iterations automatically. The total amount of fumonisin ingested by the children was a function of the amount of finger millet, kidney beans and peanuts consumed and the levels of fumonisin in the ingredients. All fumonisin contaminations obtained AH and SMAH were combined. Given a consumption of x kg of finger millet, kidney beans or peanuts per kg body weight per day, containing y µg fumonisin/kg of the respective ingredient, equaled a child randomly consuming an ingredient containing y µg fumonisin/kg of ingredient * x g of ingredient kg^{-1} body weight day^{-1}. The exposure of children to fumonisin was calculated for each of the ingredients separately as well as for all CF as per our assumptions made in the above section.

4.3.13 Statistical analysis

All analyses were conducted in triplicate. The analysis was done using Microsoft Excel 2000. The @Risk program version 4.5, Palisade Corporation, London, UK (2002), was used to estimate the probability of exposure to fumonisins.

4.4 Results and discussion

Until now no official standards have been set as the cut-off values for fumonisin contaminations in food products. However, Switzerland has suggested a level of 1 mg kg^{-1} while United States Food and Drug Administration (USFDA) has suggested a level of 2 mg kg^{-1} in corn products for the sum of FB_1, FB_2 and FB_3 intended for human consumption (de Nijs *et al.*, 1998a; USFDA, 2000). The European Commission Health and Consumer Protection Directorate has suggested a tolerable total dietary intake (tTDI) cut-off for fumonisin intake of 2 µg kg^{-1} body weight day^{-1} (SCF, 2000).

A total of ninety samples of finger millet, kidney beans and peanuts collected AH and SMAH were screened for the presence of fumonisins. The samples collected SMAH in January 2002, were independent to the ones collected AH in September 2001 since they were coming from different sources. Fumonisins were detected in 65 out of the total ninety samples collected. All the samples were found to have fumonisin levels below the suggested limit of 1 mg kg^{-1} set by Switzerland and of 2 mg kg^{-1} by USFDA respectively, with regard to the total sum of FB_1+FB_2+FB_3.

The AH samples of finger millet from farmers, lorries and retailers showed mean fumonisin levels of 5.0 µg kg^{-1} (Table 4.1). With regard to finger millet, fumonisin

levels did not differ significantly between samples collected from farmers and those collected from lorries and retailers AH. Similarly, samples of finger millet collected SMAH from the same farmers and lorries showed a little increase in mean fumonisin levels to 7.5 μg kg^{-1} and 16.8 μg kg^{-1} respectively, but overall fumonisin levels were low (Table 4.1). These results are in agreement with those obtained by Munimbazi and Bullerman (1996) who found that samples of finger millet collected from Burundi were relatively free from internal mold contamination and no *Fusarium species* were isolated from the millet.

Table 4.1 Fumonisin levels in finger millet, kidney beans and peanuts samples that were collected immediately after harvest (AH), and six months after harvest (SMAH).

Source	Samples	After Harvest (45)		Six months after harvest (45)		P-value
		Mean (μg kg^{-1})	Range (μg kg^{-1})	Mean (μg kg^{-1})	Range (μg kg^{-1})	
FARMERS	Finger millet	5.0	-	7.5	5.0 - 16.0	0.004
	Kidney beans	51.1	5.0 - 161.0	5.0	5.0 - 8.0	0.048
	Peanuts	131.8	15.0 - 252.0	5.0	5.0 - 6.0	0.0005
LORRIES	Finger millet	5.0	-	16.8	5.0 - 36.0	0.002
	Kidney beans	61.6	5.0 - 177.0	5.0	-	0.038
	Peanuts	117.9	5.0 – 440.0	7.9	5.0 - 17.0	0.059
RETAILERS	Finger millet	5.0	-	5.0	-	-
	Kidney beans	17.1	5.0 – 89.0	5.0	-	0.157
	Peanuts	64.6	5.0 – 164.0	5.8	5.0 - 10.0	0.028

Kidney beans samples collected AH from farmers, lorries and retailers showed low mean fumonisin levels of 51.1 μg kg^{-1}, 61.6 μg kg^{-1} and 17.1 μg kg^{-1}, respectively. Similarly, all samples of kidney beans collected SMAH from farmers, lorries and retailers showed very low mean fumonisin levels of 5.0 μg kg^{-1}, respectively. Siame *et al.* (1998), found similar results in that they detected no fumonisin in beans collected from storage depots and retailer outlets in Botswana. Fumonisin levels in

samples collected from farmers and lorries just AH differed significantly with the same samples collected SMAH (Table 4.1).

Peanut samples from farmers, lorries and retailers were found to have relatively high fumonisin levels for the samples collected AH with a mean of 131.8 μg kg^{-1}, 117.9 μg kg^{-1} and 64.6 μg kg^{-1}, respectively. These levels were still lower than suggested levels of 1mg kg^{-1}. Surprisingly, very low fumonisin levels in peanuts collected SMAH from farmers and lorries was found. However, these observations suggest that frequent quality checks of each raw material has to be carried out before incorporating any ingredient required for the preparation of CF for children. Similarly, Siame *et al.* (1998) also found no fumonisin in peanuts collected from storage depots and retailer outlets in Botswana. In their research on fumonisins in Zimbabwe, Gamanya and Sibanda (2001) found that there was no *Fusarium* contamination and no fumonisin detectable in sunflower and soybeans. These may be a good alternative to peanuts, which are also attacked by *Asperigillus flavus* producing aflatoxins (Munimbazi and Bullerman, 1996).

The differences in fumonisin levels AH and SMAH might have arisen from the sampling procedures AH and SMAH and probably additional processing done before storage. Although samples of finger millet, kidney beans and peanuts were collected from the same farmers AH and SMAH, the samples were not from the same piles because the farmers continued to harvest, consume, store and sell their produce. Therefore, the samples obtained SMAH were relatively different from the former ones with regard to *Fusarium* growth and distribution during maturity of the grains. A substantial number of farmers also have several small plots of farms located in

different areas and therefore there is a possibility that the samples of grains were coming from different farms. This means that different plots could have different levels of mold contamination. Similarly the samples obtained from lorries SMAH. might have been completely different from the former samples obtained immediately AH. This was because lorries hauled crops from different geographical locations in the country. Ono *et al.* (2001) also observed that the extent of contamination of raw corn with fumonisins varied with geographic location, agronomic practices, storage practices, and the vulnerability of the plants to fungal invasion during all phases of growth, storage and processing. The levels of contamination were also influenced by environmental factors such as temperature, humidity and rainfall during pre-harvest and harvest periods (Bacon and Nelson, 1994). Other factors include the strain, nature of the substrate and the stress level of the host plant (Rice and Ross, 1994). Similarly with regard to retailers, their daily survival comes from selling these produce to individual consumers as they are brought in by lorries or other means to the market from different rural localities to urban centers. For these reasons the samples obtained SMAH were presumably completely different from those obtained immediately after harvest.

Another explanation of low levels of fumonisins observed SMAH could be the result of processing done by farmers, businessmen and retailers before storage of grains, especially finger millet. Grinding of finger millet is normally done to remove the outer thin layer covering the hard testa before storing. Since moulds grow on the outer layers, by removing the layer the fumonisins produced by the moulds are also removed concurrently. Grinding the finger millet result in comparatively clean grains, which fetch more price per kilogram.

Fumonisins could also increase in grains due to improper storage conditions. Temperature and moisture conditions during storage and insect infestation are critical factors affecting fungal infection and toxin synthesis (Ryu *et al.*, 1999; Miller, 1999). The low levels of fumonisins found in samples obtained SMAH which contain more stored material compared to samples obtained AH, suggests that the storage methods and conditions employed by farmers in storing their grains were appropriate to prevent fumonisin contamination. In a study by Cahagnier *et al.* (1995) it was found that in maize a 10% reduction in water activity from 1 to 0.9 resulted in a 20-fold drop in fungal growth, and fumonisin production was reduced 300-fold. They further found out that, at a threshold water activity of 0.85-0.86, *F. moniliforme* exhibited virtually no measurable metabolic activity and hence no fumonisin production. Since the farmers were sun drying and storing their produce when dried to less than 13% moisture content and the sacks containing the produce being staked above the floor on wooden crates, it was likely that *Fusarium* was not able to grow and produce fumonisin during storage. It is also probable that these ingredients are not susceptible to *Fusarium* compared to maize. Munimbazi and Bullerman (1996) isolated no *Fusarium* moulds from finger millet and finger millet meal, and very few moulds were isolated from beans and peanuts obtained from urban markets in Burundi.

With regard to fumonisins exposure assessment, the results show that the fumonisin exposure to children consuming CF prepared from finger millet, kidney beans and peanuts was relatively low. The amount of food the children consumed is presented on the cumulative consumption distribution curves in Figure 4.1 for all complementary foods and individual ingredients (Finger millet, kidney beans and peanuts) in g kg^{-1} body weight day^{-1}. For instance, it can be seen from Figure 4.1 that,

90% of the children consumed less than 23, 10, 6 and 2 g kg^{-1} body weight day^{-1} of all CFs, finger millet, peanuts and kidney beans, respectively.

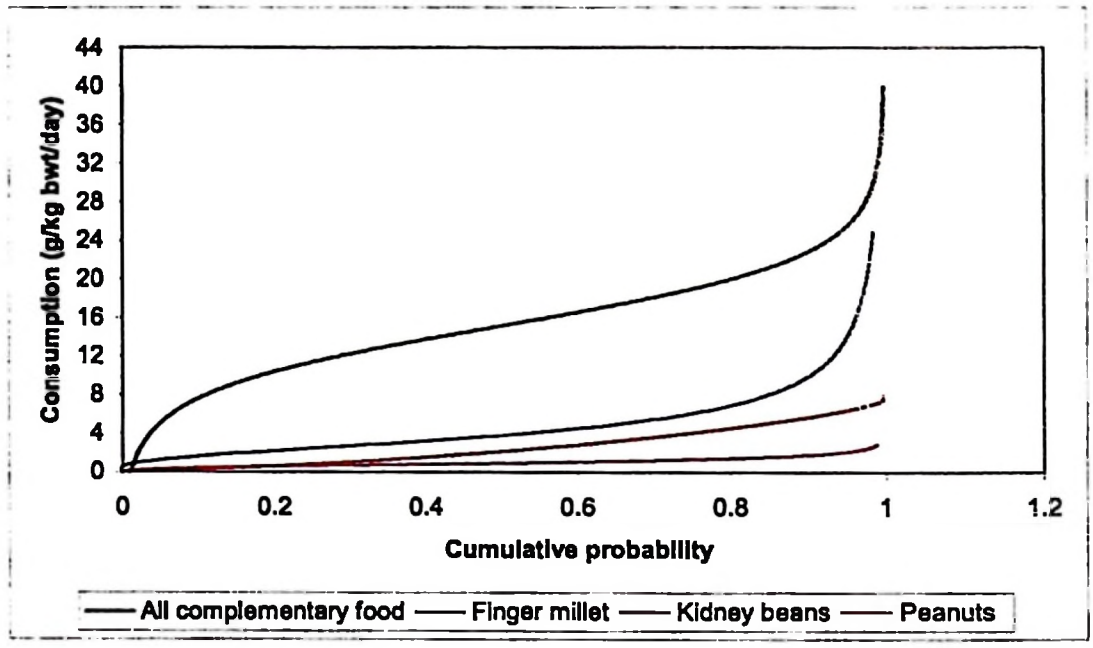

Figure 4.1 Cumulative distributions of all complementary foods, finger millet, kidney beans and peanuts consumption

With the @Risk programme the best-fit distribution function for the consumption and fumonisin contamination data was chosen. The best fit for consumption distribution functions were *logistic*, *loglogistic*, *extvalue* and *betageneral* for all CFs, finger millet, kidney beans and peanuts consumed, respectively.

Similarly, the cumulative distribution function of fumonisin contamination for the same ingredients and for the pooled data AH and SMAH, is presented in Figure 4.2a for finger millet (*beta general*), kidney beans (*Gamma*) and peanuts (*beta general*). The figure represents cumulative probability of fumonisin contamination in the samples. For instance, it can be seen from Figure 4.2a that, 90% of samples of finger

millet, kidney beans and peanuts had fumonisin contamination levels lower than 18, 77 and 194 μg kg^{-1}, respectively.

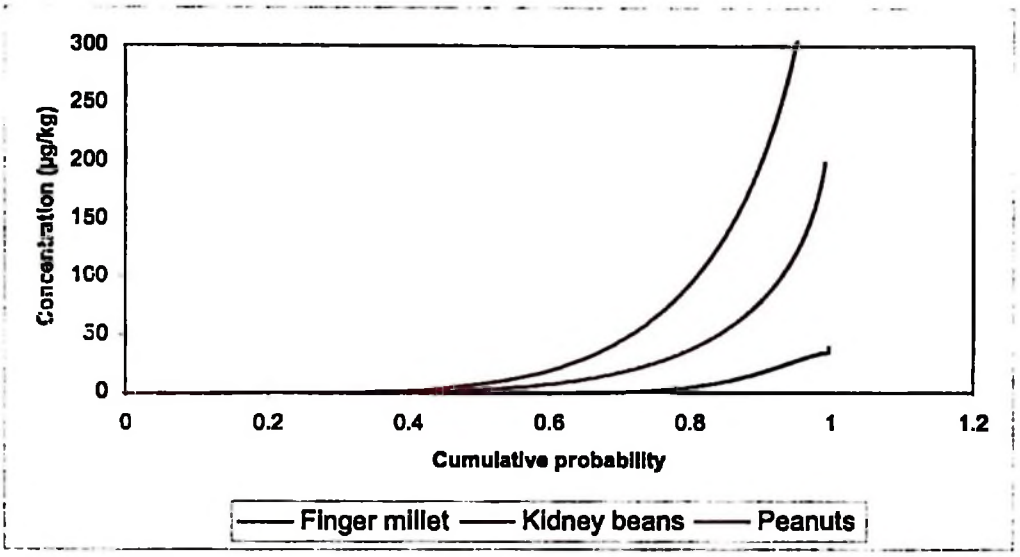

Figure 4.2a Cumulative distribution of fumonisin contamination in finger millet, kidney beans and peanuts

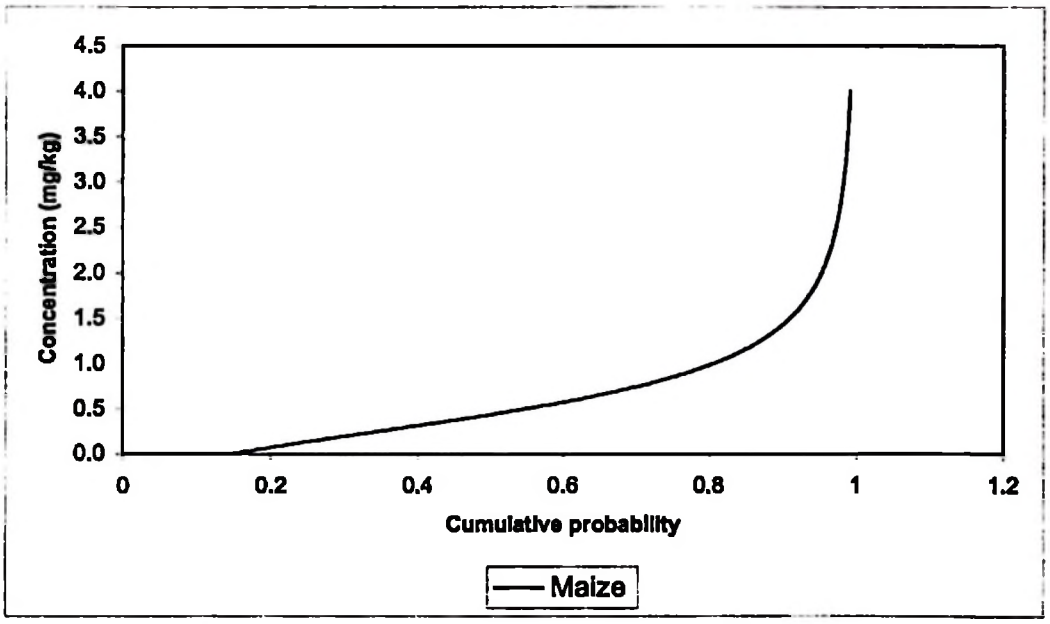

Figure 4.2b cumulative distribution of fumonisin contamination in maize

The @Risk programme then combined the distribution of the consumption with the distribution of contamination to produce a cumulative probability function for exposure to fumonisin intake in μg fumonisin kg^{-1} body weight day^{-1} from consuming all CFs, finger millet, kidney beans and peanuts (Figure 4.3a). For all the four components taken individually, the exposure to fumonisins was evidently low (Figure 4.3a). For instance, it can be seen from Figure 4.3a that, 90% of the children were exposed to maximum fumonisin intakes of 0.65, 0.45, 0.15 and 0.06 μg kg^{-1} body weight day^{-1} from consuming all CFs, peanuts, kidney beans and finger millet, respectively. In general, Figure 4.3a shows that almost 99% of the population were exposed to less than the suggested tolerable total dietary intake (tTDI) of 2 μg kg^{-1} body weight day^{-1}, which means the risk to have toxic effects from fumonisin upon consuming the CFs from a combination of these ingredients or individually was low. However, in case it is assumed that the entire CF is composed of mixture of finger millet, peanuts and kidney beans in the ratio of 80%, 15% and 5% respectively, and taking fumonisin contamination data from the AH alone, then about 91% of the children would be exposed to lower than the suggested tTDI of 2 μg kg^{-1} body weight day^{-1}. However, with the same assumptions, more than 99% of the children will be exposed to levels lower than 3 μg kg^{-1} body weight day^{-1}.

In Africa, maize is a preferred ingredient to prepare CF's for young children (Kikafunda, 1998). However, maize is reputed for high fumonisin levels (de Nijs *et al.*, 1998b; Bakan *et al.*, 2002; Kim *et al.*, 2002).

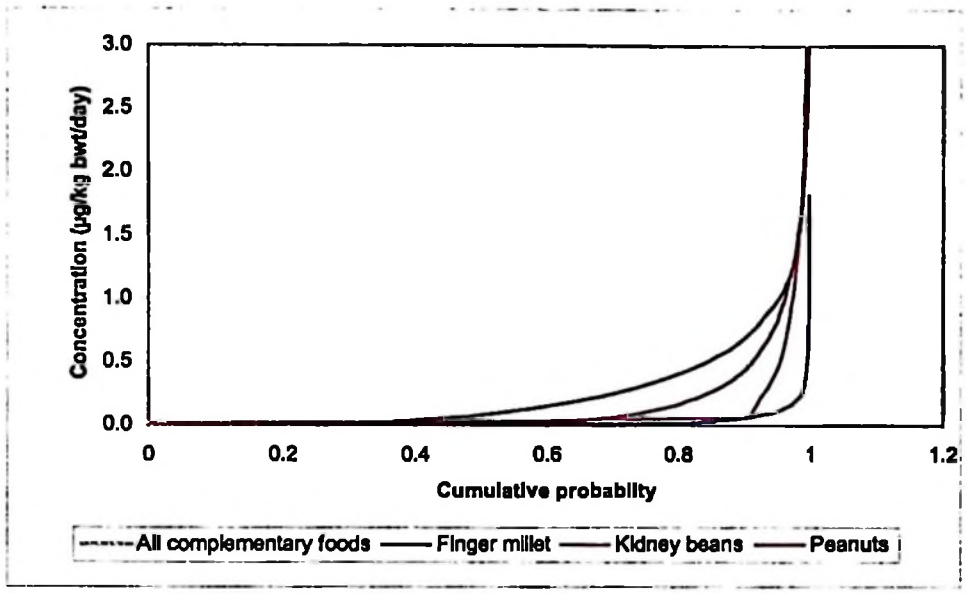

Figure 4.3a Cumulative distribution of fumonisin intake from all complementary foods, finger millet kidney beans and peanuts

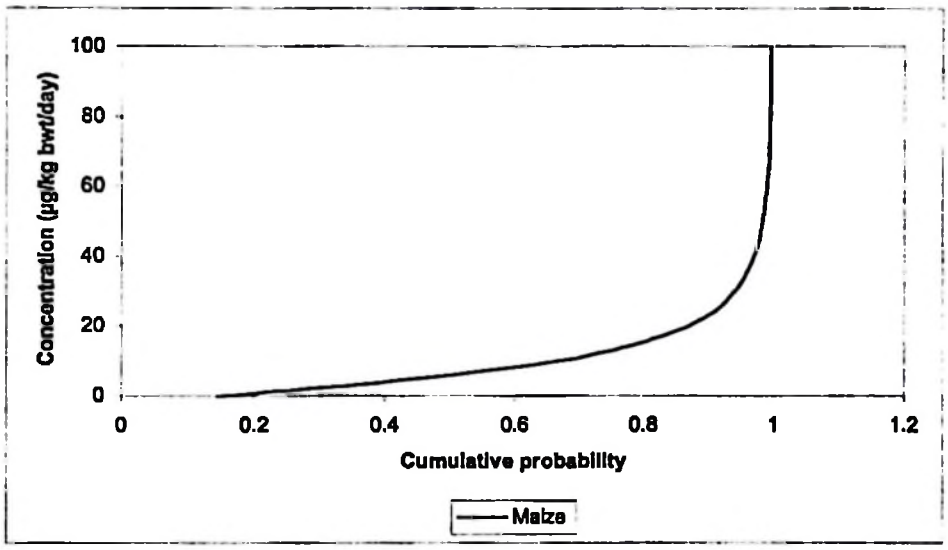

Figure 4.3b Cumulative distribution of fumonisin intake from maize

Data on fumonisin contamination in maize from various literature sources (Thiel *et al.*, 1991; de Nijs *et al.*, 1998a) and taken from 72 maize samples from European and African countries, show that the levels of contamination were extremely high ranging from 25-3350 µg kg^{-1} as presented in Figure 4.2b. Human exposure of fumonisin B_1 in The Netherlands from maize intake was assessed by de Nijs *et al.* (1998c). They found that the levels of fumonisins in maize were normally distributed and that 37%

and 97% for the group considered at risk, i.e. people with gluten intolerance were estimated to be exposed to 100 μg and 1μg of fumonisin B_1 kg^{-1} body weight day^{-1}, respectively. Otherwise, with regard to the total population in The Netherlands, the percentages for the exposure to fumonisins were 1% and 49%, respectively. Using the contamination data from Figure 4.2b, the exposure assessment of Tanzanian children was calculated. If it was assumed theoretically that the entire CF comes from maize, and that this was the only food consumed other than breast milk, more than 70% of the children could be exposed to higher levels than 2 μg kg^{-1} body weight day^{-1}, and 10% would even receive levels higher than 20 μg kg^{-1} body weight day^{-1} (Figure 4.3b). As a consequence, frequent quality checkups for fumonisin in these ingredients before processing them into CF for children is necessary. This is deemed important, taking into account the potential toxicity of fumonisins and tender age of children intended to be fed on the products of these grains.

4.5 Conclusions

The low fumonisin concentrations found in finger millet kidney beans and peanuts suggest that the exposure to fumonisin among children consuming these ingredients is relatively low, and that these ingredients are less susceptible to *Fusarium* attack as it has been found in corn. Since the majority of children in Tanzania are given maize porridge as a CF, an alternative crop such as finger millet could be promoted for CF for children since the levels of fumonisins is very low.

CHAPTER 5

Wasting, stunting and iron deficiency anemia among six months old infants in Kilosa district in Rural Tanzania: prevalence and contributing factors[5].

[5] Peter Mamiro, Patrick Kolsteren, John Van Camp, Dominique Roberfroid, Simon Tatala and Anne Opsomer. To be submitted.

5 Wasting, stunting and iron deficiency anemia among six months old infants in Kilosa district in Rural Tanzania: prevalence and contributing factors

5.1 Abstract

Infants in Tanzania are particularly vulnerable to undernutrition during the period of transition from breast milk as the only source of nourishment to solid foods. The objectives of this study were to determine the extent of wasting, stunting and iron deficiency anemia (IDA) among 6-month old infants in Kilosa district, rural Tanzania, so as to plan for a possible intervention with regard to complementary food. The study was done in two stages: first, a 24 hr dietary assessment was conducted in August-October 2000 in the community to assess the type of complementary foods given and the eating habits in function of age of 378 children, aged 0-24 months. Secondly, a progressive recruitment of 309 infants aged six months was undertaken in March 2001-March 2002 to measure weight, length, Hemoglobin concentration (Hb), Zinc Protoporphyrin (ZP) concentration and malaria parasitemia. Birth weight, the potential contributing factor to undernutrition and IDA was copied from the infants' clinic card. Mothers' weight and height were measured at the time of interview, using a bathroom scale and a stadiometer, respectively. Socio-economic variables were collected by interview.

The 24 hr dietary assessment revealed infants taking mainly thin porridge prepared from maize flour. Carbohydrates contributed the highest (69%) with respect to total energy from CF followed by fats (18.6%) and then proteins (12.1%). Food consumption data from the 24 hr dietary recall in the area showed that the CFs alone covered 21%, 55%, 61% and 60% of the total daily energy requirements for children

aged 3-5, 6-8, 9-11 and 12-23 months, respectively. The CF covered 100% of the recommended protein intake. However, it only covered 15%, 20% and 27% of the recommended iron intake for children aged 6-8, 9-11 and 12-23 months, respectively.

Anemia and iron deficiency were highly prevalent among infants in the district. Mean Hb was 9.3 ± 1.9 g dL^{-1}, 68% of the infants were moderately anemic (7-<11 g dL^{-1}) and about 11% were severely anemic with Hb below 7 g dL^{-1}, while 21% of the infants were non-anemic Hb (≥ 11 g dL^{-1}). Equally, mean ZP was 10.0 ± 6.2μg g^{-1} Hb, and 76% of the infants were iron deficient (> 5 μg g^{-1} Hb). More than 34% of the infants were stunted. The prevalence of wasting among infants was low in that only 1.3% were wasted. Low birth weight and the mothers low BMI were strong predictors of stunting. Equally, low birth weight and iron deficiency were strong predictors of anemia. Prevalence of malaria parasitemia was high affecting 50% of the infants. Having malaria was the only independent predictor associated with stunting, anemia and iron deficiency.

There is an urgent need to improve traditional CFs in terms of energy density and bioavailability of macro and micronutrients. However, the long-term solution would be to improve maternal nutrition in order to have positive effect on pregnancy outcomes since part of the harm is already done intra-uterine. A life cycle approach for the improvement of the nutritional status of mothers and their infants might be more appropriate and cost effective, thereby increasing the expected return on investment.

5.2 Introduction

Malnutrition and infectious diseases are the most widespread problems affecting young children in developing countries, and Tanzania is not an exception (UNICEF, 1998a). It is well known that protein-energy malnutrition (PEM) and micronutrient deficiencies start in utero, followed by the transitional phase from breast milk to solid complementary foods (CFs) and continue throughout the first 24-36 months (WHO, 1999).

In 1992, 43% of children under five years of age were stunted and 6% were wasted while in 1996 the level of malnutrition was very similar with 43% and 7% stunted and wasted, respectively (Bureau of Statistics, 1997). Anemia is widespread among children in Tanzania estimated to affect about 75% of the population (Tatala *et al.*, 1998). Breast milk may be a sole and sufficient source of nutrition during the first six months of life (WHO, 1998b) but there comes a time towards the middle of the first year, when breast milk provided by the best nourished mother is insufficient to support the growing infant (Weaver, 1994).

Rapid growth of infants during the first year of life and specifically after the first six months postpartum requires an adequate supply of nutrients in order to cope with the rapid build up of body muscles and other tissues (Domellof *et al.*, 2002), particularly when pregnant mothers were also undernourished. Therefore, this critical transition period is associated with a dramatic increase of malnutrition among infants. The objective of the study was therefore two-fold: the first was to investigate the feeding practices with regard to complementary foods at age 0-24 months and the second was

to investigate the nutritional status of infants at the age of six months with particular reference to iron and anemia and its determinants.

The present study follows a previous pilot study conducted in Kilosa district to determine the magnitude of nutritional deficiencies in the district on 338 infants aged 4-12 months accompanied by their mothers (unpublished data). The study revealed that the z-scores of weight for age, weight for length and length for age indicated that 21% of the infants were underweight and 2.1% wasted while 37.6% were stunted. Mean birth weight was 3000 ± 530 g. Low birth weight (LBW) was observed in 18.0% of the children (weight below 2500 g). Mean hemoglobin (Hb) concentration was 8.4 ± 1.73 g dL^{-1} whereby majority (76.4%) of the infants had Hb concentration ranging between 7 and <11 g dL^{-1}. More than 20% of the infants were severely anemic with Hb concentration below 7 g dL^{-1}. Only 3.6% of the infants had Hb concentration above 11 g dL^{-1}, the minimum WHO recommended cut-off point for normal Hb concentration for infants. There was a significant drop of Hb between 4 and 7 months old infants and stabilizing beyond 7 months although below the WHO cut off value of 11 g dL^{-1}. Comparatively, infants who were 4-6 months old had the highest Hb concentration (mean 9.64 g dL^{-1}) while infants 7-9 months old had the lowest Hb concentration, (mean 7.8 g dL^{-1}) rising a little to mean Hb concentration of 8.2 g dL^{-1} in infants 10-12 months old. The significant fall of hemoglobin between 4^{th} and 7^{th} month old infants was related to the introduction of nutritionally inadequate CFs at around 6 months of age. However, due to small number of six months old infants in this community a cross sectional study was thought to provide a firmer evidence base by a subsequent study, involving a larger group of six months old infants in this and the next chapter.

5.3 Materials and methods

5.3.1 Study area and subjects

The study was conducted in Kilosa rural district, Morogoro region, in Tanzania, located 300 km west of Dar-es-Salaam (see map in appendix). Kilosa is a district with a population of approximately 350.000 inhabitants. There are two rainy seasons: the long rains, which start in late March and last till early June and the short rains, which last from October to November. Most villagers are subsistence farmers growing maize, rice, cassava, beans, peanuts, finger millet and green vegetables and increasing numbers are petty traders. Most households keep domestic animals such as chicken, ducks and few goats. Food shortage months include February through March (lean season) while adequate food periods include July through September (harvest season). Houses are typically made of thatched roofs and mud walls. The survey was conducted in 4 villages namely, Ilonga, Mvumi, Msowero and Mambegwa, located approximately 10 km from each other. Kilosa was chosen for this study as it is among the districts in Morogoro region, which has high prevalence of infant malnutrition and iron deficiency anemia (Kilosa Hospital Annual Report, 2000).

A total of 378 children, aged 1-24 months were covered for the dietary assessment and 309 infants were surveyed for the assessment of nutritional status and related factors. They comprised 49.5% males and 50.5% females.

5.3.2 Study protocol

The study was performed in two parts. The first part comprised a cross-sectional assessment of complementary feeding patterns among children aged 1 to 24 months and was conducted in August-October 2000. About 40% of this age group (n = 378

infants) were selected using random numbers. Sample size was not calculated but 40% was considered representative of the age group selected. The list of children was obtained from the village mother and child health care (MCH) clinic. To have a homogenous infant population to study determinants of nutritional status and iron status before complementary foods were normally introduced, in the second part infants, six months of age were the subjects. A transversal design would have yielded too small numbers in the study community. Therefore infants were enrolled consecutively in the period March 2001 to March 2002 when they reached the age of six months whereby inclusion criteria and nature of recruitment were as presented in Chapter 6 under exclusion, inclusion and ethical considerations section. Sample size was calculated as presented in Chapter 6 under sampling and sample size section.

5.3.3 Dietary assessment

For the dietary assessment, home visits were made and a twenty-four hour semi-quantitative dietary recall was used. For some mothers, who were not found at home at the time of the first visit, a new appointment was fixed leaving a message to request the mother to be peresent at home. During the dietary recall interviews the mother was requested to show the type and amounts of foods, which the infant had consumed in the last 24 hr. The amount of food consumed by the infant was weighed using a digital weighing scale (Tefal scales UK or measured by a measuring cylinder, Pyrex-UK). No attempts were made to estimate the amount of spilled food. To calculate total energy and nutrient intake from CF, the average amount in the range of reported CF intake of children in the age range in developing countries was considered (WHO, 1998a). These amounts of CFs were then converted to macro and micronutrient

values, using the FAO food composition tables (FAO, 1984) and computed by Microsoft Excel Office 2000 software.

5.3.4 Anthropometric indicators

Most of the infants were born in hospitals or maternity centers, where birth weight was measured soon after birth by health care personnel. The child's birth weight was obtained from his or her clinic card where the prevalence of LBW could be determined.

Recumbent lengths of the infants were measured using an infant measuring board, which had a fixed head rest, and a movable foot piece placed on a flat surface (Perspective Enterprises, Portage, MI). The length was measured with the subject lying in a supine position by two persons one holding the head in a lateral position and the other keeping the knees down, while moving the foot piece. Length was recorded to the nearest 0.1 cm.

Weight of infants was measured by a Salter scale (Model 235 6S - England) with a capacity of measuring up to 25 kg. The scale was adjusted to read zero before starting the measurements. The Salter scale was hung on one rafter at the MCH centre. The infants were slipped into a weighing sling one at a time and hung on the scale. The weight was recorded to the nearest 100 g as soon as the pointer on the scale had stabilized. Maternal weight and height were measured with a bathroom scale and stadiometer respectively, which was later used to calculate body mass index (BMI).

5.3.5 Measurement of hemoglobin concentration and zinc protoporphyrin

Hemoglobin concentration was measured from a finger prick blood sample by the Hemocue B-Hemoglobin System (Hemocue AB Ängelholm, Sweden) using disposable sterile lancets. The hemocue device for measuring hemoglobin concentration has been evaluated by Von Schenck *et al.*, (1986) and used in various studies (Stoltzfus *et al.*, 1999; Van de Broek and Letsky, 2000; Pehrsson *et al.*, 2001).

A drop of blood on a cover glass was also used in the determination of zinc protoporphyrin (ZP) measured with a portable hematofluorometer (Aviv Biomedical Inc, Lakewood, NJ). The instrument was standardized daily by using control solutions (low, medium and high ZP controls) provided by Aviv Biomedical Inc. To determine the prevalence of iron deficiency, elevated ZP was used as an indicator of circulating free erythrocyte protoporphyrin. Values > 5μg ZP g^{-1} Hb indicated iron deficiency erythropoiesis. The device and method has been used in various studies (Himes *et al.*, 1997; Jackson and Al-Mousa, 2000; Asobayire *et al.*, 2001). According to Cook *et al.* (1992), techniques that are simple and inexpensive that require finger prick rather than a venous blood are preferred. Therefore haemoglobin and zinc protoporphyrin has been suggested as one of the combinations, which can be used in field conditions (Cook *et al.*, 1992).

5.3.6 Determination of malaria parasitemia

A finger prick blood sample by disposable sterile lancets was used to prepare thick blood smears on a glass slide and within 10 hr of blood sampling, quantitative determination of malarial parasites was done. The blood smears were dried and stained with Giemsa buffered staining solution and left to dry under room conditions

for 30 minutes (WHO, 1990). The slides were then washed and allowed to dry and examined for the presence of malaria parasites under a light microscope (Wild-Heerbrug, Switzerland) with a X100 oil immersion lens. Malaria parasite counts were made per 200 white blood cells.

5.3.7 Ethical considerations

Verbal consent was sought from the mothers for their willingness to participate in the study and their infants to be tested for various parameters. Infants found infected with malaria and those found with other diseases such as severe anemia and diarrhoea were referred at the respective health centres and treated free of charge. The ethics committee of the Tanzania Food and Nutrition Centre and University of Gent reviewed the protocol and gave approval of the study.

5.3.8 Socioeconomic aspects and reasons for dichotomisation of some variables

Structured questionnaires were used to obtain social-economic information of the mother and the household, such as maternal education, maternal age, marital status, parity and family income. Maternal education was dichotomised because 50% of the mothers had reached up to primary school level, while 27% knew how to read and write and the remaining 23% had no education. This was the same as 50% of the mothers had no education and the other 50% had reached primary school level. Likewise maternal age, marital status and maternal BMI were dichotomised to check on the prevalence of teenage mothers, single mothers and malnourished mothers, respectively. With regard to BMI, for rural settings in most developing countries, obesity does not prominently feature as a major problem because of poor diets and infections among women populations (Schroeder and Martorell, 1999). Therefore the

decision to dichotomise the BMI into either nourished or malnourished mothers was made. The cut-off for chronic energy deficiency is BMI < 18.5 (Bailey and Ferro-Luzzi, 1995; Kusin *et al.*, 1994).

Income was also dichotomized since the majority of the women (79%) were getting less than Tanzanian Shillings (TShs) 10,000 (equivalent US$ 10) per month and therefore the cut-off for income was set at either less than or equivalent to US$ 10.

5.3.9 Statistical analysis

Data were entered by using EPI-INFO (version 6.04d; Centres for Disease Control and Prevention), and analyses done with Stata 5.0 package *(Stata version 5.0; STATA, College Station, Texas)*. Z-scores of weight for length and length for age were computed using EPINUT according to National Centre for Health Statistics standards published January 2001.

Three main outcomes (dependent variables) were considered i.e. anemia (Hb < 11 g dL^{-1}), iron deficiency (zinc protoporphyrin > 5µg ZP g^{-1} Hb) and stunting that is, length for age < -2 standard deviation of the population reference median (WHO, 1983). Wasting was not considered in the analysis because it was virtually non-existent in our sample. We were interested in determining biological or environmental factors associated with the outcomes.

The considered factors (independent variables) were the following ones. Child parameters: zinc protoporphyrin > 5µg ZP g^{-1} Hb (0 = no, 1 = yes), hb < 11 g dL^{-1} (0 = no, 1 = yes), blood smear positive for malaria (0 = no, 1 = yes), birthweight < 2500

grams (0 = no, 1 = yes), length-for-age < -2 Zscore (0 = no, 1 = yes), sex (0 = girl, 1 = boy), season at entry in the study (0 = harvest season, 1 = other season). Mother's parameters: mother's education lower than primary school (0 = no, 1 = yes), mother living alone (0 = no, 1 = yes), mother's age < 20 years (0 = no, 1 = yes), parity ≥ 3 (0 = no, 1 = yes), BMI (0 = between 18.5 and over 25, 1 = under 18.5), income lower than 10000 shillings (0 = no, 1 = yes).

The strategy of data analysis was set in two steps. First the association was studied between each of the main outcomes and the potential determinants analyzed by the Mantel-Haenszel test and secondly, a multivariate analysis was performed by applying a logistic regression. Three sets of models were defined, one for each of the main outcome dependent variable coded 0/1. For all sets, all the variables significantly ($P < 0.05$) associated with the dependent variable in the univariate analysis were included in the regression model. To determine which variable was to be kept in the final model, a stepwise backward procedure was used. Removal of variables was at ($P > 0.05$) for the likelihood ratio test. The presence of multicolinearity and other numerical problems in the regression analysis was appraised by verifying the presence of a high estimated standard error (Hosmer and Lemeshow, 1989).

5.4 Results

5.4.1 Twenty-four hour dietary recall

Food consumption data from the 24 hr dietary recall in the area showed that the CFs alone covered 21%, 55%, 61% and 60% of the total daily energy requirements for children aged 3-5, 6-8, 9-11 and 12-23 months, respectively. The highest contribution

to the daily energy from CFs alone was from carbohydrates ranging from 51% to 90% with a mean of 69%. Protein contribution was the lowest ranging from 7.9% to 15.9% with an average of 12.1%. Fats contributed on average 18.6% ranging from 1.3% to 34.3% between ages 3 and 24 months (Table 5.1).

Table 5.1 Percentage energy contribution of carbohydrates, proteins and fats from complementary foods in Kilosa district

Age in months	Carbohydrates	Range	Proteins	Range	Fats	Range
	Mean±SD		Mean±SD		Mean±SD	
3-5	73.5±10.9	53.7-89.4	11.9±1.3	9.0-14.8	14.6±10.1	1.6-33.8
6-8	69.8±13.4	51.0-89.7	12.0±1.7	8.9-14.7	18.3±11.9	1.4-34.3
9-11	69.9±12.4	53.7-90.8	11.9±1.7	7.9-14.6	18.3±11.0	18.3-11.0
12-24	68.2±11.4	51.0-90.8	12.2±1.6	7.9-15.9	19.6±10.2	1.3-34.3

Food intake analysis showed that most complementary foods were cereal based porridges with a global iron content insufficient to meet the daily requirements of children as per WHO's recommendations (WHO, 1998a). Figure 5.1 shows that before six months, there are no recommended values because it is assumed that infants get adequate supplies from breast milk. Recommendations for iron intake only start from 6 months onwards as this is the period, which CFs are introduced. Not a single infant's total iron intake could cover the iron needs with a 5% bioavailability level taking into consideration that most of the CFs are cereal based and high in phytates (Figure 5.1). The main foods given to these children included plain maize porridge, finger millet, rice and peanuts composite flour porridge, beans and sardines. However, very few families fed their children with beans and sardines.

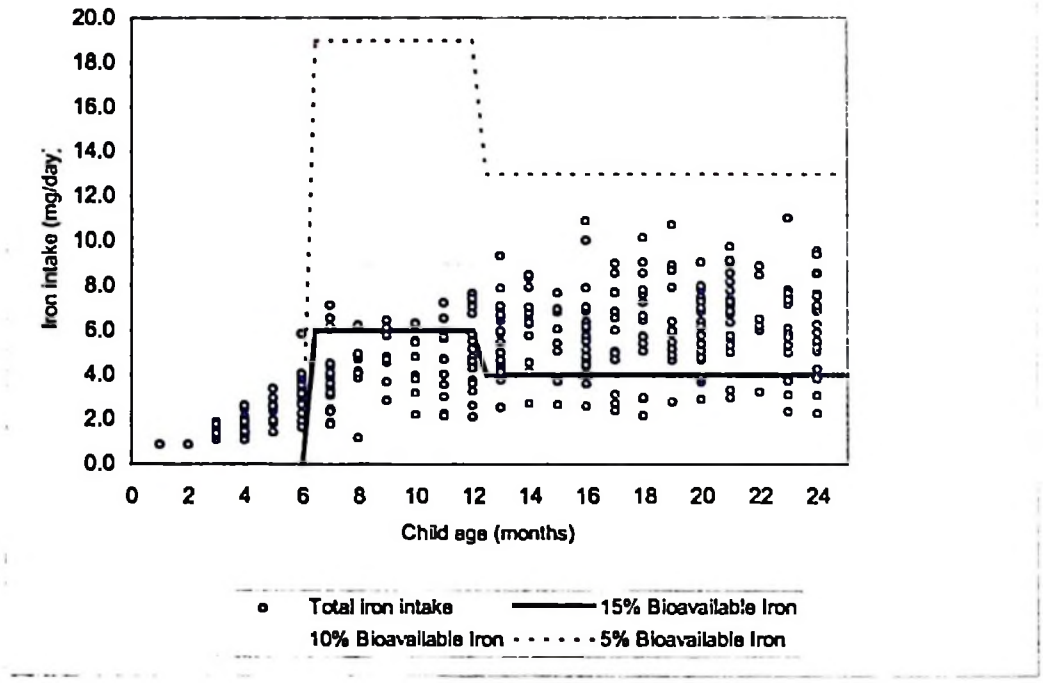

Figure 5.1 Daily iron intake among children in Kilosa District

5.4.2 Baseline characteristics

5.4.2.1 Infant characteristics

Average birth weight was 2900 g and the prevalence of LBW was 11%. Sex distribution was not significantly different within the group of infants. Prevalence of stunting among infants of six months of age was relatively high. At six months the infants were already stunted with a mean z-score of -1.53 ± 1.1 with prevalence as high as 35% (Table 5.2). Wasting was not very pronounced with only one percent of the infants being wasted. Prevalence of anemia and iron deficiency was 79% and 76%, respectively. On average the breast-feeding frequency was about 17 times per 24 hr.

5.4.2.2 Maternal and household variables

Average maternal BMI was 21.9 higher than the cut-off value (<18.5) for malnourished women (Table 5.2). Prevalence of mothers who never finished primary school was 50% while others knew how to read and write (27%) and illiterates (23%). Prevalence of single mothers was 22% while others were married or cohabiting with men. Average age of the mothers was 26 years. Maternal live children and parity were on average 2.7 and 3.4, respectively (Table 5.2). Household size was an average of 6 persons.

Table 5 2 Characteristics of infants, aged 6 months, their mothers and households

Variables	Mean ± SD (309)	Category	Cut-off	%
Sex (male / female, infants)	153/156	Infants with low birth weight	< 2 5 kg	11
Birth weight (kg)[1]	2.9 ± 0 6	Infants with low hemoglobin	< 11.0 g dL^{-1}	79
Hemoglobin concentration (g dL^{-1})	9.3 ± 1 9	Infants with iron deficiency	> 5 0 µg g^{-1} Hb	76
Zinc protoporphyrin (µg g^{-1} Hb)	10.0 ± 6 2	Stunted infants	< -2 0 z-scores	35
Length/Age z-scores (LAZ)	-1 53 ± 1 13	Wasted infants	< -2 0 z-scores	1
Weight/Length z-scores (WLZ)	0 59 ± 1 20	Infants with malaria	≥ 1 0 parasites	48
Malaria (parasites/200 white blood cells)	139 ± 377	Mothers with primary education		50
Mid upper arm circumference (cm)	13 7 ± 1.2	Mothers who can read and write		27
Breastfeeding frequency per 24 hr	16.6 ± 5 5	Mothers with no education		23
Household Size (persons)	5 6 ± 2.1	Married mothers		47
Maternal Body Mass Index (kg m^{-2})	21 9 ± 2.4	Mothers cohabiting with men		31
Maternal live children (persons)	2.7 ± 1.8	Single mothers		22
Mother age (years)	25.6 ± 6 9	Mothers with low BMI		5.5
Maternal parity (persons)	3 4 ± 2.2			

[1]Based on 280 infants, 29 infants were home deliveries

5.4.3 Stunting

Six factors were significantly associated with stunting in the crude analysis, that is, having malaria, being born with a low birthweight (LBW), being iron deficient,

having a single mother, coming from a low income family and having a mother with low BMI (Table 5.3). Among these factors, only malaria, LBW, low income and low BMI of the mother were kept in the final logistic model. LBW (adjusted odds ratio 5.1, 95% CI 2.3 - 11.2) and low BMI (adjusted odds ratio 5.2, 95% CI 1.2 - 22.3) were the two strongest independent predictors of stunting in our sample.

Table 5.3 Odds ratio for the likelihood of the association between stunting and other variables

Variable	Stunted[1]			Stunted[1]		
	Crude odds ratio[2]	p-value	95% CI	Adjusted odds ratio[2]	p-value	95% CI
Having malaria	1.9	0.01	1.1-3.0	1.9	0.02	1.1-3.2
Low birth weight	4.1	0.01	1.8-8.7	5.1	0.01	2.3-11.2
Iron deficient	2.1	0.02	1.1-3.8	-	-	
Sex	1.3	0.23	0.8-2.1	-	-	
Low hemoglobin	1.7	0.08	0.9-3.1	-	-	
Mother low education	1.5	0.11	0.9-2.3	-	-	
Mother age	1.2	0.48	0.6-2.1	-	-	
Mother living alone	1.8	0.03	1.0-3.0	-	-	
Low Income	2.0	0.02	1.0-3.8	2.0	0.04	1.0-4.2
Parity	0.7	0.10	0.4-1.0	-	-	
Low BMI	4.6	0.01	1.1-18.5	5.2	0.03	1.2-22.3
Lean season	0.7	0.16	0.4-1.1	-	-	

[1]Low LAZ (< -2 z-scores), [2]Crude odds ratio is derived from Mantel-Haenszel test and adjusted odds ratio is derived from logistic regression

5.4.4 Anemia and iron deficiency

The results showed that the prevalence of iron deficiency anemia was quite high among the infants as revealed by the ZP values (Table 5.2). The mean ZP was 10.0 ± 6.2 μg g^{-1} Hb. Seventy six percent of the infants were iron deficient (> 5 μg g^{-1} Hb). Mean Hb was 9.3 ± 1.9 g dL^{-1} with sixty eight percent of the infants moderately anemic (7 - < 11 g dL^{-1}) and about 11% severely anemic with Hb below 7 g dL^{-1}.

Five factors were significantly associated with anemia in the crude analysis, that is, having malaria, being born with a LBW, being iron deficient, having a mother with low education and being from a low income family (Table 5.4). All the five factors were kept in the final logistic model. The two strongest independent predictors of anemia in our study sample were LBW (adjusted odds ratio 5.8, 95% CI 1.2 - 26.1) and iron deficiency (adjusted odds ratio 2.9, 95% CI 1.5 - 5.5). Equally, four factors were significantly associated with iron deficiency in the crude analysis, that is, having malaria, being stunted, being from a family with low income and being in a lean season. Only malaria, stunting and lean season were kept in the final logistic model. Malaria (adjusted odds ratio 6.5, 95% CI 3.3 - 12.7), stunting (adjusted odds ratio 2.5, 95% CI 1.2 - 5.0) and lean season (adjusted odds ratio 3.2, 95% CI 1.7 - 6.1) were the three strongest predictors of iron deficiency in our study sample (Table 5.5).

Table 5.4 Odds ratio for the likelihood of the association between anemia and other variables

Variable	Anemia[1]			Anemia[1]		
	Crude odds ratio[2]	p-value	95% CI	Adjusted odds ratio[2]	p-value	95% CI
Having malaria	2.8	<0.01	1.5-5.0	1.9	0.05	0.9-3.6
Low birth weight	5.8	0.01	1.3-25.5	5.8	0.02	1.2-26.1
Iron deficient	4.1	<0.01	2.2-7.5	2.9	0.01	1.5-5.5
Sex	0.9	0.71	0.5-1.5	-	-	
LAZ	1.7	0.08	0.9-3.1	-	-	
Mother low education	1.9	0.02	1.0-3.2	1.9	0.04	1.0-3.4
Mother age	0.9	0.62	0.4-1.6	-	-	
Mother living alone	1.0	0.95	0.5-1.9	-	-	
Low Income	2.3	0.01	1.2-4.3	1.8	0.01	0.9-3.5
Parity	0.9	0.92	0.5-1.6	-	-	
Low BMI	0.9	0.09	-	-	-	
Lean season	1.3	0.33	0.7-2.2	-	-	

[1]Low hemoglobin (< 11g/dL) = Anemia. [2]Crude odds ratio is derived from Mantel-Haenszel test and adjusted odds ratio are derived from logistic regression

Table 5.5 Odds ratio for the likelihood of the association between iron deficiency and other variables

Variable	Iron deficient[1]			Iron deficient[1]		
	Crude odds ratio	p-value	95% CI	Adjusted odds ratio[2]	p-value	95 % CI
Having malaria	6 2	<0.01	3 1-12.2	6.5	< 0.01	3.3-12.7
Low birth weight	2 4	0 12	0 8-5 8	-	-	
Sex	1.1	0 66	0 6-1.8	-	-	
LAZ	2.1	0 01	1.1-3.8	2 5	0.01	1.2-5.0
Mother low education	1 2	0.44	0.7-2 0	-	-	
Mother age	0 8	0 38	0.4-1.4	-	-	
Mother living alone	1.4	0 34	0.7-2.6	-	-	
Low Income	1.9	0.03	1.0-3.4	-	-	
Parity	0 9	0.59	0.5-1.4	-	-	
Low BMI	0.1	0.07	-	-	-	
Lean season	3.0	<0 01	1 6-5 3	3 2	< 0.01	1.7-6.1

[1]Low zinc protoporphyrin (> 5 μg g^{-1} Hb) = Iron deficient. [2]Crude odds ratio is derived from Mantel-Haenszel test and adjusted odds ratio is derived from logistic regression.

It was observed that the odds for developing anemia and iron deficiency were 2 and 6 times higher when having malaria parasitemia than infants with normal Hb and iron status, respectively (Tables 5.4 & 5.5). In this study, prevalence of malaria was 48% with the same distribution in both sexes. The average number of parasites per infant was 139±377 per 200 white blood cells ranging from zero parasites to 2760 parasites/200 white blood cells. Diarrhoea and upper respiratory tract infections were other major diseases affecting infants in the study area.

5.5 Discussion

The total energy intake from CFs without breast milk was covering about 69% of the total daily energy requirements for children from 6 - 23 months of age. This shows that the contribution of the CFs alone did not cover the daily requirements for CFs adequately, which means that there is a need for improving the energy density of CFs. The high contribution of carbohydrates in the CFs of children as depicted in Table 5.1

was a characteristic of CF given to children in most of Tanzania (UNICEF, 1998b). This is mainly the result of complementing breast milk with gruels made up of locally produced staples, such as maize, cassava, sorghum, finger millet and sweet potatoes (den Bensten *et al.*, 1998). These starch based CFs are characterized by a high water content and low energy and nutrient density. To achieve an intake that was sufficient to meet energy and nutrient requirements, a child is supposed to ingest relatively large volumes of such foods (WHO, 1998a). The dietary bulk characteristics of CF could be among important factors contributing to the etiology of protein energy malnutrition in children.

Dietary fat supply from complementary foods remained low contributing an average of 18.6% of energy from CFs. At 3 months and 24 months of age fats were contributing about 14.6% and 19.6% of the total energy intake from CF, respectively. The fat intake from CF was mostly contributed by peanuts in the habitual CF mix. Similar findings were reported by Prentice and Paul (2000) in Gambia where they found the percentage of total energy from fat was initially >50%, but declined to 30% by 17 months of age. Dietary fat was also provided mainly by peanuts. According to WHO (1998a) and Prentice and Paul (2000), dietary fats provide young children with essential fatty acids and allow adequate absorption of fat-soluble vitamins. Current guidelines suggest that dietary fat should range from 30% to 45% of the total dietary energy for children less than 2 years of age (Michaelsen and Jørgensen, 1995). By removing the amount supplied by breast milk, complementary foods that were currently consumed by children in the study area did not provide adequate dietary fat.

Protein from CFs contributed about 12% of energy from CF. Between 6 - 8 months, 9 - 11 months and 12 - 24 months of age the average protein intake was 12.0 g day^{-1}, 11.9 g day^{-1} and 12.2 g day^{-1}, respectively, which was adequate as per WHO recommendations. According to WHO (1998a) the recommendations for protein intake were 9.1 g day^{-1} (6 - 8 months), 9.6 (9 - 11 months) and 10.9 g day^{-1} (12 - 23 months). Our findings are different from those found in Bangladesh where protein consumption was only 8.2 g day^{-1} or 75% of the requirements of children 9-18 months old. It was observed that the low protein consumption was due to inadequate energy intake (Brown and Zeitlin, 1991). Dewey *et al.* (1996) expressed similar sentiments that protein intake will be adequate if energy intake from CFs was sufficient.

Infants were already iron deficient at 6 months of age as revealed by ZP values probably because of early introduction of CFs which replaces adequate intake from breastmilk. This study revealed that the majority of the mothers start to complement their infants with solid foods by three months of age on average. The low dietary iron correlated well with ZP values obtained in which 76% were iron deficient. According to WHO (1998a), meeting micronutrient needs from complementary foods appears to be the greatest challenge. It has been reported that it was practically impossible to supply enough iron from unmodified CFs to meet the calculated needs of infants 6 - 11 months of age without high intakes of animal products such as liver, fish, beef and eggs (WHO, 1999). However, affordability of animal products in rural Tanzania by the majority of households is questionable. One possibility could be to modify currently consumed grains by germination to reduce the antinutritional factors such as phytates and tannins that interfere with the bioavailabilty of micronutrients (Lorri and Svanberg, 1993; Mamiro *et al.*, 2001).

The high prevalence of stunting observed in these rural Tanzanian infants at six months of age was similar to the data obtained from the Demographic and Health Surveys (DHS) conducted in Tanzania. Findings from 1996 DHS showed that in 22 administrative regions of Tanzania, the proportion of stunted children increased rapidly from 1 - 22 months of age (Bureau of Statistics, 1997; USAID, 1998). At 3 months and 12 months of age, the prevalence of stunted children were between 10% and 40%, respectively. Our study showed that already 34.5% of the infants at 6 months of age in Kilosa district were stunted and that stunting was a very early occurrence, before the effects of complemenatry feeding are felt on child growth. This is also true as the analysis showed that LBW was significantly associated with stunting. Similar observations were reported by Kardjati *et al.* (1994), that a continuation of intrauterine growth retardation into postnatal period seems more likely although dietary inadequacy due to frequent illness cannot be excluded. A study conducted in Madura Indonesia, also showed that growth started to falter very early almost from birth onwards (Kolsteren *et al.*, 1997a; Kolsteren *et al.*, 1997b).

Continuation of growth faltering during the second half of infancy is often seen in African populations (Hautvast *et al.*, 2000) and Asian populations (Kolsteren *et al.*, 1997a; Shrimpton *et al.*, 2001). Wasting was less prevalent than stunting as also observed in other studies (Hautvast *et al.*, 2000). Stunting has frequently been associated with maternal factors. Three factors that have most impact are mother's poor nutritional status before conception, short stature and poor nutrition during pregnancy (Kusin and Kardjati, 1994; Krammer and Victoria, 2001). Inadequate weight gain during pregnancy is particularly important since it accounts for a large proportion of fetal growth retardation (Dyck and Tan 1995). In our results mother's

BMI was the strongest predictor of stunting in children. According to Sarlio-Lähteenkorva and Lahelma (2001) mothers' low nutritional status reflects low food availability or accessibility facing households. The household food insecurity phenomenon will also be translated to other members of the household including children who are most vulnerable. Low income, which dictates the purchasing power of households, was also a predictor of stunting. Income level has a great influence on the food security system of the household and thereby reflecting in the nutritional status of household members especially women and children who are most vulnerable. In a review and analysis of literature on the relation between income generation programs and nutrition improvement, Shirima (1996) found that increases in household income were most likely to be associated with nutritional improvements and vice versa.

Diseases were found to have significant bearing on infants' nutrition in Kilosa community. Diseases most frequently encountered were malaria and diarrhoea. Malaria was observed to be a predictor of all three main outcomes in our study, i.e. stunting, anemia and iron deficiency. The study area is a malaria endemic area where re-infection is very common. According to Menendez *et al.* (1997), continuous re-infection causes disturbance in physiological growth processes, which end up in growth faltering among infants.

Our study further observed a positive association of anemia at 6 months of age with LBW. Similar results were observed in the Honduran children whereby LBW was strongly related to low hemoglobin, hematocrit and ferritin values (Dewey, 2001). Because of the well known relation between birth weight and iron reserves it is

commonly recommended that infants with birth weights less than 2500 g receive iron supplements in the form of medicinal drops beginning at 2 - 3 months of age. However, it was rather surprising that LBW was not associated with iron deficiency. This is quite contrary to findings from other studies where they found strong associations of iron deficiency and LBW. In the developing world, low birth weight stems primarily from the mother's poor health, nutrition and age (Dreyfuss *et al.*, 2001), and carries a range of serious health risks for children. Teenage pregnancies increase the risk that an infant will be born with LBW. For example, in a study in Mali and Burkina Faso, LeGrand and Mbacke (1993) found out that teenage mothers were twice as much likely to give birth to LBW infants as older mothers. In our study prevalence of teenage mothers was 22% while LBW was 11%.

Mother's low educaton was also a predictor of anemia. Maternal education is related to knowledge of good childcare practices (USAID, 1998), such as appropriate meals to be given to the infants, diseases and treatment required. However, in Kilosa rural setting, primary level education was the highest education level reached by most mothers. This is considered to be a very low level of education for a mother to synthesize and articulate issues comprehensively in comparison with uneducated mothers. Low income earned by the family was also a predictor of anemia in infants. As explained above the same effects that income might have on stunting are the same for anemia.

Our findings confirm that iron deficiency anemia was a major problem among infants in rural Tanzania. Similar findings were reported by Davidsson *et al.* (2001), where they observed that iron deficiency manifesting as nutritional anemia was a major

public health problem during early life, particularly in developing countries. Full term infants are normally endowed with adequate iron stores at birth, which is also reflected in high hemoglobin concentrations during the first few months of life (Lartey *et al.*, 2000). Hemoglobin and iron stores decline as body iron is mobilized in support of the rapid growth during this period. By 6 months of age, typical normal birth weight infants exhaust most of their body iron stores present at birth and depend on complementary foods for most of their iron intake. The most critical period at which iron deficiency anemia develops is between 6 and 12 months of age because iron requirements are the highest during this period (Yip and Ramakrishnan, 2002).

Dietary deficiency in iron might be among the major reasons for iron deficiency at this age because for the majority of infants and young children in rural Tanzania, CFs are cereal based and low in iron content and bioavailability. The condition of iron deficiency is aggravated in persons who have low caloric intake and high-energy requirements like infants. This was reflected in our results in that the lean season was a strong independent predictor of iron deficiency in infants at six moths of age. Tanner and Lukmanji (1987) reported that during the post harvest period infants were given more superior foods and became healthier than in the lean season where porridge made of cassava was the main dish. However, it was surprising in our study that the lean season was not associated with anemia in infants.

The study by Menendez *et al.* (1997), on iron supplementation and malarial chemoprophylaxis for prevention of severe anemia and malaria in Tanzanian infants concluded that, iron supplementation of infants was important to prevent iron deficiency anemia even in malaria endemic areas. This observation is consistent with

our findings that in areas with high malaria prevalence iron deficiency exist. Menendez *et al.* (1997), further report that malaria may contribute to iron deficiency and thus anemia, by reducing iron absorption during acute episodes. The results suggest that malaria could be one of the causes of anemia but not the major contributing factor at this age.

5.6 Conclusions

In summary, this study has identified several potential factors responsible for malnutrition and iron deficiency anemia among infants in rural Tanzanian communities. Poor nutrition and diseases have been seen as the main factors responsible for health deterioration in infants at complementary stage. If nutrition could be improved at this age there might be beneficial effects on growth and health of infants in the short run. Therefore, there is an urgent need to improve traditional CFs in terms of energy density and bioavailability of macro and micronutrients. However, the long-term solution would be to improve maternal nutrition in order to have positive effect to pregnancy outcomes since part of the harm is already done intra-uterine. A life cycle approach for the improvement of the nutritional status of mothers and their infants might be more appropriate and cost effective, thereby increasing the expected return on investment.

CHAPTER 6

A randomized controlled trial of processed complementary food on growth and iron status of Tanzanian infants from 6-12 months of age in Kilosa district in rural Tanzania[6]

[6] Peter Mamiro, Patrick Kolsteren, John Van Camp, Dominique Roberfroid, Simon Tatala and Anne Opsomer. To be submitted.

6 A randomized controlled trial of processed complementary food on growth and iron status of Tanzanian infants from 6-12 months of age

6.1 Abstract

A baseline survey in Kilosa district revealed that 76% and 20% of 4-12 months old infants had moderate and severe anemia, respectively. A previous food intake study in the same population found iron intake to be low. A processed complementary food (CF) using locally produced crops, was developed, which increased iron solubility from 4.8% to 19%, as measured by in vitro techniques. The CF contained germinated finger millet, kidney beans, roasted-peanuts and mango puree. The porridge prepared with the processed CF had a high-energy content 1731 kJ/100 g and provided 1194 kJ day^{-1} and 1956 kJ day^{-1} for infants aged 6-8 months and 9-11 months, respectively. The research aimed to test the effect of the locally made and processed CF on growth and iron status of 6-12 months old infants. The study was a double-blind-randomized, placebo-controlled trial, conducted from March 2001 to March 2002 involving 309 infants. The infants were randomized individually to receive processed CF or the placebo, a blend of the same composition but not processed, from the age of 6 months until 12 months. Parameters followed were weight for length z-scores (WLZ) and length for age z-scores (LAZ), hemoglobin (Hb), zinc protoporphyrin (ZP) and malaria-parasitemia. Measurements were made at 6 months and 12 months of age. Mean WLZ for infants taking processed CF and placebo at 6 months of age was (0.72 and 0.45) versus at 12 months of age was (-0.27 and -0.07), respectively. Likewise, mean LAZ at 6 months of age was (-1.54 and -1.53) versus at 12 months of age was (-2.08 and -2.04), respectively. Mean Hb for infants taking processed CF and placebo at 6 months was (9.2 g dL^{-1} and 9.4 g dL^{-1}) versus at 12 months of age was (9.7 g dL^{-1} and 9.7 g dL^{-1}) and ZP at 6 months of age was (9.9 μgZP g^{-1} Hb and 9.9

μgZP g^{-1} Hb) versus at 12 months of age was (5.9 μgZP g^{-1} Hb and 6.2 μgZP g^{-1} Hb), respectively. Malaria affected both Hb and ZP during the follow-up. None of the outcome variables in the two groups were significantly different at age 12 months. The absence of the effect of processed relative to the non-processed CF in improving growth, hemoglobin and iron status of infants was most likely due to the feeding frequency. Mothers in the non-processed group fed their infant on average 5-6 times per day, compared to 1-2 time in the processed CF group. We conclude that processing does not necessarily matter, as long as sufficient CF was provided and nutrition guidance was appropriate. Likewise, the positive gain in terms of hemoglobin and iron status in both groups suggests that the amount of soluble micronutrients does not necessarily reflect bioavailability and utilization by the body.

6.2 Introduction

The growth and chance of survival at birth is to a large extent determined by the intra-uterine period (Martorell *et al.*, 1998). In the first 4-6 months exclusive breastfeeding further contributes to these indicators of health. This is due to the combination of breast milk, providing adequate amounts of macro- and micronutrients and the low prevalence of infectious diseases directly through protecting factors present in breast milk and indirectly though elimination of the ingestion of contaminated foods and fluids.

Young infants are most vulnerable at the time complementary foods are introduced. Traditional complementary foods are often bulky, have a low energy density and contain too small amounts of micronutrients, in particular iron and zinc (Bennett *et al.*, 1999; Lartey *et al.*, 1999). Moreover, complementary foods are largely cereal

based and contain considerable amounts of anti-nutrients, which affect micronutrient bioavailability considerably. A food complement can further decrease the total amount of breast milk intake and thus does not necessarily increase the total energy intake (WHO, 1998a).

Infectious diseases, notably respiratory tract infections and diarhoea, are the other important causes of growth faltering and mortality of children after the age of six months. These repeated infections will aggravate the downward spiral of malnutrition, which in turn increase the risk of infection (Tomkins and Watson, 1989). An infection period however, does not necessarily need to have a lasting effect. Once the child is better and his/her appetite has returned he/she will be able to regain the weight loss, provided that sufficient foods of adequate quality are consumed to allow catch-up growth (Kolsteren *et al.*, 1997b). Low child feeding frequency further contributes to under-nutrition of children. Surveys carried out in Tanzania (UNICEF, 1998a) show that most children are fed only two or three times a day.

Iron deficiency anemia (IDA) is an important nutritional problem in Tanzania. It is estimated that 45% of the children under the age of five years are suffering from nutritional anemia (UNICEF, 1999). It is more prevalent in infants and pregnant women and is usually the result of low bioavailability of dietary iron (Fox *et al.*, 1998). Although the effects of iron deficiency anemia are reversible, including impaired intellectual development, in the local sub-optimal conditions this will rarely be the case. Kolsteren *et al.* (1999) also indicated that despite the fact that diagnosis

of anemia is fairly simple and treatment cheap, the prevalence of anemia remains high.

The correction of dietary iron deficiency has been done in most cases by food fortification and/or iron supplementation (Stoltzfus *et al.*, 1998). Local staples have been fortified with ferrous sulphate (Mendoza *et al.*, 2001), zinc sulphate (Fairweather-Trait *et al.*, 1995), vitamin A and vitamin C (Davidsson *et al.*, 2001; Zlotkin *et al.*, 2001). Effectiveness of large-scale fortification programs has been reduced due to factors such as cost, constant availability, timely distribution of fortificants and compliance with the prescribed fortificant (Thu *et al.*, 1999). Similar experiences have been observed in many places with iron supplementation (Schultink and Gross, 1996).

The above mentioned limitations of fortification and supplementation underline the importance of preventing growth faltering and micronutrient deficiencies, such as iron deficiency anemia through a food-based approach (Fox *et al.*, 1998). Food modification approaches employing natural processes such as germination to combat micronutrient deficiencies and improvement of infants growth (WHO 1998a), deserve more attention since they are likely to be more sustainable in the long term.

Based on the knowledge of the traditional complementary foods (CFs) in Tanzania collected by the authors earlier, a processed CF prepared from locally produced crops was formulated. Through germination and other processing measures (see below) the CF had high energy density, increased protein digestibility, low anti-nutritional factors with a normal viscosity for a semi-liquid gruel (Mbithi-Mwikya *et al.*, 2002).

The present study therefore aimed to compare growth and iron status of Tanzanian infants, from 6 to 12 months, provided with processed and non-processed CF. Our main hypothesis was that a processed CF with higher energy density and lower concentration in anti-nutrients would improve growth of infants and decrease iron deficiency.

6.3 Materials and methods

6.3.1 Study area and subjects

The study was conducted from March 2001 to March 2002 in Kilosa rural District in Morogoro region of Tanzania. Morogoro is located about 300 km west of Dar-es-Salaam (see map in appendix). Kilosa is a district with a population of ≅ 350,000 inhabitants. There are two rainy seasons: the long rains, which start from late March to early June and short rains, which begin from October to November. Most villagers are subsistence farmers growing maize, rice, finger millet, cassava, beans, peanuts and green vegetables and increasing numbers are petty traders. Most households keep domestic animals such as chickens, ducks and few goats. Food shortage months include February through March (lean season) while the adequate food period includes July through September (harvest season). Houses are typically made of thatched roofs and mud walls. Complementary food given to infants in Kilosa is mainly porridge prepared from cereal grains. Kilosa was chosen for this study as it is among the districts in Morogoro region, which has high prevalence of iron deficiency anemia (Kilosa Hospital Annual Report, 2000).

6.3.2 Sampling and sample size

A total of 364 infants, aged 6 months were enlisted from the Mother and Child Health (MCH) register book. A pilot study conducted in the area prior to the trial revealed that the mean Hb concentration among children aged between 4-12 months was 8.4±1.7 g dL^{-1}. The sample size for the trial was computed to detect difference of ~ 0.8 g dL^{-1} (equivalent to ½ standard deviation) between the two groups with a significance level of 0.5% and power of 95%. The calculated minimum sample size for the experimental and placebo groups was 117 infants. Allowing for a 10 % dropout, the total sample size was set at 260 infants or 130 infants per group.

6.3.3 Design of the study

The study was a double blind randomised placebo-controlled trial in which the main investigator and the mother were blinded with regard to the type of food given to the infants. Infants were continuously recruited by phases and progressively allocated to receive processed and non-processed CF when they reached the age of six months. Allocation to the treatment or control group was determined using a bloc randomisation technique. The code was broken to the main investigator at the end of the study when the last infant was measured. The two types of CF were distributed until the infant reached the age of 12 months. Mothers were free to give their infants any other food of their choice apart from this supplement. Measurements were taken twice at the age of 6 and 12 months. Verbal consent was sought from mothers for their infants to participate in the trial. The ethics committee of the Tanzania Food and Nutrition Centre and University of Gent reviewed the protocol and gave approval of the trial.

6.3.4 Composition, preparation and distribution of the food

The processed CF consisted of 65.2% finger millet, 19.1% kidney beans, 8% peanuts and 7.7% mango puree. Finger millet and kidney beans were washed and soaked in pre-boiled water for 2 and 7 hr respectively, and germinated for 48 hr at 30°C. Later the lot was autoclaved and then solar dried for about 6 hr. The reasons for autoclaving are given in chapter three. Peanuts were roasted in an oven at 150°C for 20 minutes. Mangoes were washed, peeled, sliced and puree extracted. Later the puree was dried in the solar drier for 12 hr. The ingredients were mixed together by weight and milled to composite flour. The non-processed CF was a blend of the same composition but not processed. These were milled and mixed in the indicated ratios above and then packed. Packing and labeling was done by the director at the production site. All the packages and labeling order was the same for the two groups and the two preparations were virtually indistinguishable.

Before the intervention an acceptability trial for the CF was done involving 50 mothers with their infants. Various quality control measures were undertaken to ensure safe CF. Procurement of the ingredients was from individual cultivating farmers and later transporting the consignments to the production site in Ilonga village. The objective of buying from individual farmers was to make sure the ingredients were coming from a known reliable source and was from a recent harvest.

Quality control was observed from purchase of raw materials to processing and distribution. An Ultra-Violet (UV) lamp (Type B-4 watt. Kurtz-und langwellig) for qualitative and quick checking of aflatoxin contamination in the grains was used. At

various CF production stages samples were checked for contamination with fumonisins (RIDASCREEN® FAST Fumonisinkit), aflatoxins (RIDASCREEN® FAST Aflatoxin kit) and cyanides (AOAC, 1995). Fumonisins and aflatoxins concentrations were less than recommended limits of 1 ppm and 2 ppb respectively, while cyanides were not detected. Outgrowth of pathogens including *Bacillus cereus, Staphylococcus aureus* and *Clostridium perfringens* was investigated for each batch of CF following the HACCP plan (HACCP, 1998) and found to be less than 100 cfu g^{-1} (Kimanya *et al.*, 2003).

The CF was placed in a polyethylene bag and seamed by a seaming machine. The polyethylene bag was then transferred to a khaki bag, which was closed by cello tape. All packages were given a label with appropriate preparation (cooking) instructions for the mother. Every two weeks, 1 and 1.6 kg of CF were allocated for infants 6-8 months and 9-11 months old, respectively. On a daily basis this means that each child was supplied with 69 g and 113 g dry matter of CF, which gave an energy supply of 1194 kJ day^{-1} and 1956 kJ day^{-1} for infants aged 6-8 months and 9-11 months, respectively. The CF food amounts were determined in such way to provide at least on average 275 kcal day^{-1} for children 6-8 months, 450 kcal day^{-1} for children 9-11 months according to the WHO guidelines (WHO, 1999) so as to close the deficit in energy and protein. The formulation of the CF is described in Mbithi-Mwikya *et al.* (2002). On the basis of the CF ingredients' amino acid profiles and scores and their energy content, the solver function (linear programming) in excel 2000 for windows, was used to maximize energy in the blend at the greatest possible amino acid chemical score. Every fortnight the Women and Childs' Center's vehicle was used to

distribute the required amounts for the period to respective villages, where they were stored in a cupboard and securely closed to prevent spoilage.

The mothers came to collect their share every two weeks. The MCH nurses supervising the mothers had a list of the infants and recorded every food collection. The MCH nurses always demonstrated to the mothers how to prepare the CF (although the packets had a self-explanatory label) and reminded the mothers how much in local measures (table spoons) of the CF powder to use each day. The nurses also adviced the mothers to add 1-2 teaspoonfuls of oil to each portion of the CF. So, at least about 1151 kJ (275 kcal) and 1883 kJ (450 kcal) per day from CF were aimed at. In case of absence, the nurse made certain that a message was sent to the responsible mother to collect her consignment on the same day. Nutrition officers from the center followed up the mothers in their homestead to make sure the CF is prepared in the correct way at least twice per week. Surprise visits especially in the mornings were done, which enabled them to observe actual feeding and frequently asking about the consumption in the past days and problems encountered if any.

6.3.5 Anthropometric indicators

Weight and length were recorded at enrolment (age 6 months) and at the end of the trial (age 12 months). Recumbent lengths of the infants were measured using an infant measuring board, which had a fixed head rest and a movable foot piece (Perspective Enterprises, Portage, MI). Length was measured with the infant in supine position according to standard procedures and recorded to the nearest 0.1 cm. Weight of infants was measured by a Salter scale (Model 235 6S - England) with a capacity of measuring up to 25 kg. The scale was adjusted to read zero before starting the

measurements. The weight was recorded to the nearest 100g as soon as the pointer on the scale had stabilized. Recorded length and weight were expressed as Z-scores of the reference (NCHS) values. The infant's birth weight was obtained from his or her clinic card.

6.3.6 Measurement of hemoglobin concentration and zinc protoporphyrin

Hemoglobin concentration was measured from a finger prick blood sample by the Hemocue B-Hemoglobin System (Hemocue AB Ängelholm, Sweden). The hemocue device for measuring hemoglobin concentration has been evaluated by Von Schenck *et al.* (1986) and used in various studies (Stoltzfus *et al.*, 1999; Van de Brock and Letsky, 2000; Pehrsson *et al.*, 2001). A drop of blood was also used in the determination of ZP measured with a portable hematofluorometer (Aviv Biomedical Inc, Lakewood, NJ). The instrument was standardized daily by using control solutions (low, medium and high ZP controls) provided by Aviv Biomedical Inc. To determine the prevalence of iron deficiency, elevated ZP was used as an indicator of circulating free erythrocyte protoporphyrin. Values > 5μg ZP g^{-1} Hb indicated iron deficiency erythropoiesis. The device and method has been used in various studies (Himes *et al.*, 1997; Jackson and Al-Mousa, 2000; Asobayire *et al.*, 2001).

6.3.7 Determination of malaria parasitemia

A finger prick blood sample by disposable sterile lancets was used to prepare thick blood smears on a glass slide and within 10 h of blood sampling quantitative determination of malarial parasites was done. The blood smears were stained with Giemsa buffered staining solution and left to dry under room conditions for 30 minutes (WHO, 1990). The slides were then washed and allowed to dry and examined

for the presence of malaria parasites under a light microscope (Wild-Heerbrug, Switzerland) with a X100 oil immersion lens. Malaria parasite counts were made per 200 white blood cells. Infants found infected with malaria during subsequent follow-up and those who fell sick at any time during the trial were treated free of charge at the respective health centres.

6.3.8 Assessment of dietary intake

A twenty-four hour dietary recall was conducted on 137 randomly selected sub-samples of infants aged between 6 and 12 months. We aimed to carry out the dietary recall on about 50% of the total number of infants who came for measurements with equal number of infants from each group. However, for some mothers who were not found at home at the time of the visit, a new appointment was fixed. Random numbers were used to select infants from each group. The dietary recall interviews were conducted at the homestead by a nutritionist accompanied by a village health worker. The mother was requested to show the type and amounts of foods, which the infant had consumed in the last 24 hr. The amount of food consumed by the infant was weighed using a digital weighing scale (Tefal scales UK) with an accuracy of 0.5 g or measured by a measuring cylinder (Pyrex-UK) with an accuracy of 0.5 mL in case of volumes. Consideration was not taken for the amount of food, which was spilled. These amounts of CF were then converted to macro and micronutrients values obtained from food composition tables (FAO, 1984) and computed by Microsoft Excel Office 2000 software.

6.3.9 Nutrient composition of samples from the field

Samples weighing 250 g of CF from each production were sampled for determination of protein, fats and iron. Proteins were determined by Kjedahl Method AOAC method 920.87 (AOAC, 1995) a nitrogen conversion factor of 5.83 for millet and 5.3 for beans and peanuts was used. Fat content was determined by Weibull method (Egan *et al.*, 1981). Total iron was determined by atomic absorption spectrophotometry AOAC method 970.12 (AOAC, 1995). Solubility of iron was determined by the method of Svanberg and Sandberg (1993). Phytate content in the CF was measured by Haugh and Lantzsch (1983).

6.3.10 Morbidity and socioeconomic information

Structured questionnaires were used to obtain household social-economic information and occurrence of common diseases affecting the study infants. The mothers were asked whether their infants had fever, diarrhea, dysentery and cough a week before each measurement, that is, at 6 months and at 12 months of age.

6.3.11 Exclusion, inclusion criteria and ethical considerations

Infants were excluded from the trial if they had received blood transfusions or presented with serious health conditions as assessed by a medical doctor at the time of enrolment. These excluded infants were referred to the local health facilities for appropriate treatment. Mothers gave their verbal consent in participating in the trial. All the children were investigated for malaria at six months of age and treated accordingly. At the end of the trial, the children were re-evaluated and treated for most likely etiology of anemia by the supervising medical doctor.

6.3.12 Statistical analysis

Data were entered by using EPI-INFO (version 6.04d; Centres for Disease Control and Prevention, World Health Organization, 1996), and analysis was done by using Stata 5.0 package *(Stata version 5.0; STATA, College Station, Texas).* Z-scores, weight-for-length and length-for-age were computed using EPINUT according to National Centre for Health Statistics standards published January 2001.

Descriptive statistics were done on each variable to identify outliers and assess the normal distribution of continuous variables. Comparability of food intake between groups by twenty-four hour dietary recall was also assessed including mean number of breast-feeding and percentage of children finishing the porridge.

Outliers were defined from the box plot as values more extreme than 3-interquatile range of the box. In presence of outliers, a new variable was created excluding these values. However, in case of doubt, the dataset was cross-checked with original data in the rosters. All tests were done first with the original variable, and then redone with the new variable to assess influence of such outliers. Normal distribution of continuous variables was appraised by a Kolmogorov Smirnoff test. In case of severe departure from normality, the variables were log-transformed[1].

The strategy of data analysis was set in 2 steps. First, a difference at 12 months of age between the 2 intervention groups was assessed for each primary outcome. These primary outcomes were mean zinc protoporphyrin and mean hemoglobin. Differences in anthropometry indicators i.e. mean weight-for-length Z-score and length-for-age Z-

score at 12 months of age were also looked at. A standard t test was used for continuous variables, and a Chi-square test for categorical ones. Likewise, the general trend in main outcomes between the beginning and the end of the trial were assessed by applying a paired t-test or a McNemar test for categorical variables.

Secondly, a logistic regression analysis was applied. We used an EVW[2] model (Kleinbaum, 1994). Continuous variables were transformed in categorical ones as indicated below. The dependent variable in all models were:-

(i) High zinc protoporphyrin at 12 months of age (ZP > 5, coded 0/1) for the first set of models.

(ii) Anemia (Hb < 11g dL^{-1}, coded 0/1) for the second set of models.

(iii) Length-for-age Z-score (LAZ < -2 SD, coded 0/1) for the third set of models.

The exposure variable was the type of CF received. The following covariates were inserted in the initial model because they were meaningful confounding factors or effect modifiers (biological or environmental):

(i) Child parameters[3]: zinc protoporphyrin > 5 at baseline (0 = no, 1 = yes), Hb < 11 g dL^{-1} at baseline (0 = no, 1 = yes), blood smear positive for malaria (0 = no, 1 = yes), birthweight < 2500 grams (0 = no, 1 = yes), length-for-age < -2 Z-score (0 = no, 1 = yes), sex (0 =

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[1] Find zero-skewness log transformation where the new variable=ln (variable exp-k), choosing k and the sign of exp so that skewness of the new variable is zero

[2] The E denote the exposure variable (independent variable) ; the V's denote covariates that do not involve E ; the W's denote covariates that go into the model as product terms with E.

[3] Morbidity during the last week was not introduced in the model because at least 1 disease episode was reported for all the children ; weight-for-length indicator was not introduced in the model because 99% of the children were in the normal range (mean +/- 2 standard deviations).

girl, 1 = boy), season at entry in the study (0 = harvest season, 1=other season).

(ii) Mother's parameters: mother's education lower than primary school (0 = no, 1 = yes), mother living alone (0 = no, 1 = yes), mother's age < 20 years (0 = no, 1 = yes), parity ≥ 3 (0 = no, 1 = yes), BMI (0 = between 18.5 and 25, 1 = under 18.5, 2 = over 25), income lower than 10000 shillings (0 = no, 1 = yes).

All covariates were also considered as potential effect modifiers and introduced in the initial model as product terms involving E (type of food). The presence of multicolinearity and other numerical problems in regression analyses was appraised by verifying the presence of high estimated standard errors for the regression estimates (Hosmer and Lemeshow, 1989). Then a hierarchical backwards elimination procedure was applied to eliminate non-significant variables.

Removal of variables was at $P > 0.05$ for the likelihood ratio test. First effect modifications were tested using a likelihood ratio test for the entire collection of interactions term (Kleinbaum, 1994). Then variables were removed one by one according to the likelihood ratio test. The model including the remaining co-variatess was considered the gold standard. Then confounding was assessed by monitoring changes in the effect measure (OR) for subsets of covariates. The subset of covariates included in the final model was the one allowing the best gain in precision.

6.4 Results

6.4.1 Subjects and compliance

For operational reasons, infants were short-listed from the MCH register book according to date of birth, and randomized to receive a processed or a non-processed CF after contacting their mothers for acceptance in participating in the trial when their infants reached six months of age. However, this approach resulted in some loss of infants at the beginning of the trial. The 55 infants who never came back for measurements despite acceptance of the mother, had different reasons, the major being admission to the district hospital to receive blood transfusion.

The other reasons included absence due to out-migration or travels to other parts of the country, husbands restricting their wives from participating in the trial, death due to malaria, severe anemia and diarrhea. To make up for the unexpected extra losses, more infants were enrolled than the calculated required sample size of 260 infants. The drop out rate did not differ significantly between the two groups. Out of 364 eligible infants enrolled in the trial, 133 infants in the intervention group and 125 infants in the placebo group completed the trial (Figure 6.1).

To check on compliance, nutrition officers from the center followed up the mothers in their homestead to make sure the CF was prepared in the correct way. Compliance was regarded good if the mother collected the CF, correctly prepared and consumed according to instructions, by making surprise home visits once or twice weekly and conducting the 24 hr dietary recalls. However, we were not able to monitor full compliance in the strict sense.

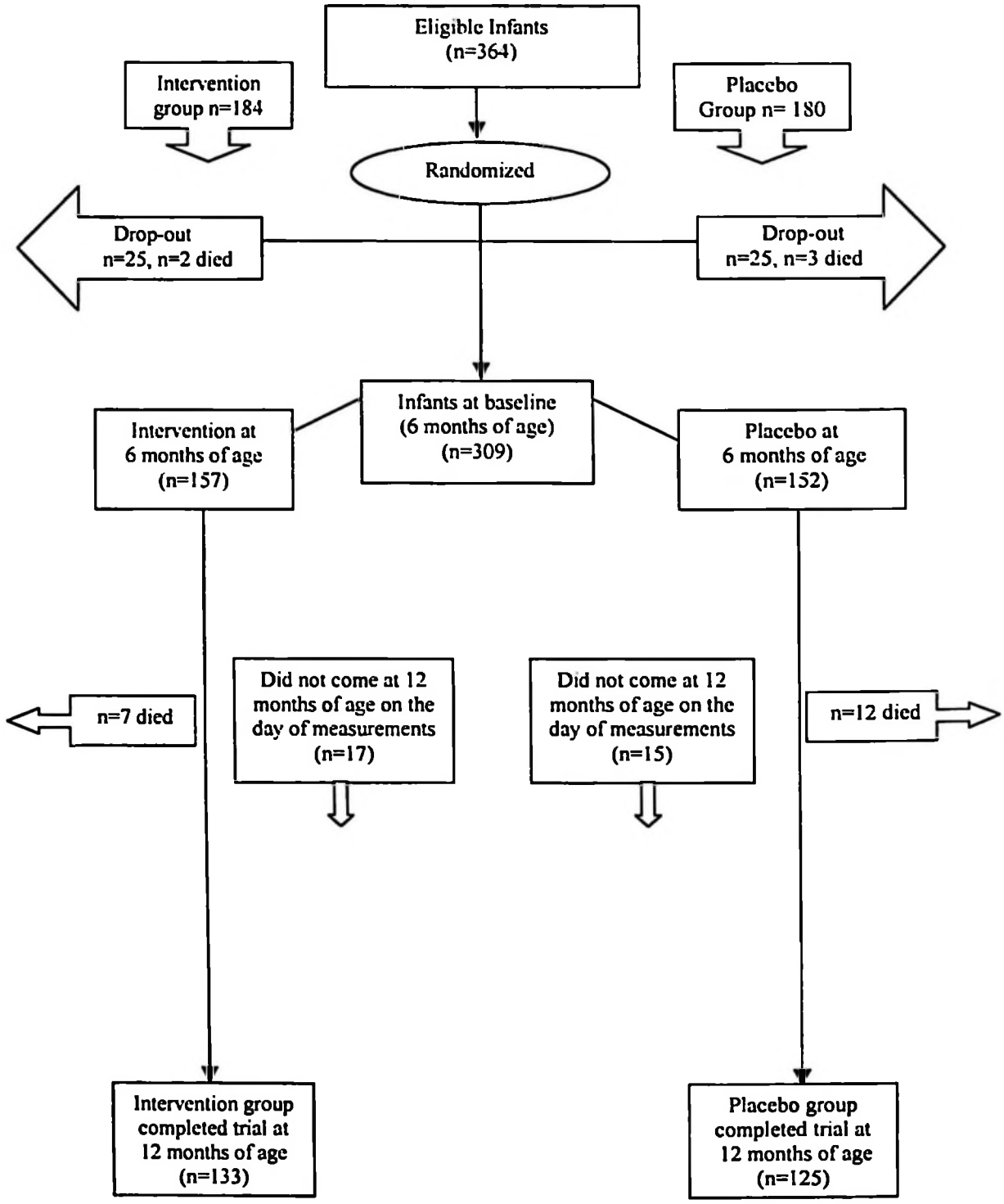

Figure 6.1 The randomized controlled trial profile

6.4.2 Baseline characteristics

The characteristics of infants, mothers' and household variables in both groups were similar at enrolment of the infants, aged 6 months (Table 6.1).

6.4.2.1 Infant characteristics

Birth weight of infants was on average 3000 g, while weight and length did not significantly differ in the two groups. Anthropometrical index of wasting differed slightly while stunting was the similar in both groups. Sex ratio was also 1:1. About 50% of the infants had malaria at baseline while 50% were malaria free for both groups. Morbidity was high for both groups with prevalence of over 80%.

6.4.2.2 Maternal characteristics

Mothers body mass index was on average 21 kg m^{-2}. Maternal live children and parity were on average 2.7 and 3.3, respectively. Maternal age was not significantly different in that the average age was 25 years. Mother educational status did not differ significantly between the two groups. Similarly widowed, divorced, single and those who lived with a man without formal marriage did not differ significantly.

6.4.2.3 Household characteristics

Income category was based on annual per capita income of Tanzania, which at the time of the study was equivalent US$ 240*. However, the average monthly income of households in the rural areas was equivalent to 2% of the per capita income (Bureau of Statistics, 1997). Thus, income levels between farmers in this study were not significantly different since more than 40% of the households were on the ≤ 5 US$ category (Table 6.1). This implies that the farmers were more or less receiving the

same income and therefore there were minimal differences in their purchasing power. Household size was an average of 6 persons.

Table 6.1 Baseline characteristics

Continuous variables		Processed Food (157)	Placebo (152)	P-value
		Mean[1]	Mean[1]	
Birth weight (kg)[2]		2.9 ± 0.5	3.1 ± 0.5	0.06
Weight (kg)		6.7± 1.0	6.9± 1.0	0.93
Length (cm)		62.7± 3.1	62.8± 3.1	0.06
Hemoglobin concentration (g dL^{-1})		9.2 ± 1.9	9.4 ± 2.1	0.27
Zinc protoporphyrin (μgZP g^{-1} Hb)		9.9 ± 6.1	9.9 ± 5.8	0.97
WLZ		0.45 ± 1.24	0.72 ± 1.14	0.04
LAZ		-1.53 ± 1.16	-1.54 ± 1.11	0.96
Household Size (persons)		5.8 ± 2.3	5.4 ± 2.1	0.11
Maternal Body Mass Index (kg m^{-2})		21.9 ± 2.5	21.9 ± 2.7	0.96
Maternal live children (persons)		2.7 ± 1.9	2.7 ± 1.8	0.86
Mother age (years)		25.6 ± 7.0	25.3 ± 6.6	0.63
Maternal parity (persons)		3.4 ± 2.3	3.3 ± 2.1	0.63
Sex ratio of infants (male: female)		1.01:1	0.95:1	
Categorical variables	**Category**	**Percent**	**Percent**	
Morbidity	Yes	83.4	82.2	0.77
Malariaprevalence	Malaria positive	48.4	50.7	0.92
Low birth weight prevalence	Low	16.4	8.6	0.04
Mother education	No education	26.1	19.7	0.11
	Can read & write	21.7	32.2	
	Primary school	52.2	47.4	
	Secondary School	0	0.7	
Mother status	Married	49.0	44.7	0.36
	Cohabit with a man	26.1	34.2	
	Divorced	1.3	0.7	
	Widowed	1.3	0	
	Single	22.3	20.4	
Monthly income (US$ equivalent)	≤ 5	46.5	42.8	0.43
	5.1-10	30.6	38.2	
	10.1-15	8.9	9.9	
	15.1-20	13.4	9.2	
	> 20	0.6	0	

[1]Mean ± SD. No significant differences between the groups, [2]Based on 280 infants, 29 infants were home deliveries

* 1US$ = 1000/- Tanzanian Shillings

6.4.3 Nutritional characteristics of the complementary food

The nutrient contents of the processed and non-processed CF as determined by laboratory analyses are shown in Table 6.2. The random samples from each month's CF production unit and traditional CF had an energy, fat, protein and iron content, which were not significantly different. However, the porridge prepared with the processed CF had a higher energy density, significantly higher iron solubility and a lower concentration of phytate when compared to the porridge prepared with the non-processed CF. Energy density values should be viewed in light of its density in the ready to eat form of a CF because of the differences in viscosity leading to different bulk densities. There was a small but significant reduction in total iron content in the processed CF compared to the non-processed CF probably due to leaching in the course of processing the CF (Watzke, 1998). This disadvantage was counter-balanced by the higher amount of soluble iron in the processed CF compared with non-processed CF (18.83% versus 4.76%), respectively.

Table 6.2 Composition of the field complementary food[1]

Parameter	Complementary Food		Mean difference	P-value
	Processed	Non-processed		
Energy (kJ/100 g DM)	1731± 11	1731± 18	-0.84	0.89
Protein (g/100 g DM)	12.87± 0 57	12.64± 0.56	0.23	0.33
Fat (g/100 g DM)	4.64± 0.52	5.08± 0.74	-0.44	0.11
Ash (g/100 g DM)	2.38± 0 08	2.88± 0.4	-0.50	0.001
Total iron (mg/100 g DM)	4.74± 0.41	5.89± 0.87	-0.93	0.0002
Energy density of porridge (kJ/mL)	6.1	1.7	-	-
Soluble iron (%)	18.83± 0.72	4.76± 0.80	14.07	0.0001
Solids% (w/v) in pap at optimum viscosity[2]	35	10	-	-
Phytates (% DM)	0.66± 0.02	1.15± 0.03	0.34	0.04

[1]Mean±SD of 12 production batches, [2]Discussed in Mbithi-Mwikya *et al.* (2002)

Food consumption data from the 24 hr dietary recall in the area showed no significant difference in daily energy intake, proteins, fats, and iron intake from the processed and non-processed CF between the two groups of children (Table 6.3). The energy intake from the project CF was 1752 kJ and 1679 kJ in the processed and non-processed CF groups. This was increased to 1922 and 1943 kJ in both groups, respectively due to the addition of oil. Overall the project complementary food contributed more than 50% of the total daily energy intake. The energy intake from CF exceeded the WHO recommendations. Other foods consisted of plain maize flour porridge, stiff porridge, mixture of maize, rice, peanuts and finger millet flour and milk. Other foods and breast milk contributed substantially in total daily energy, fats and protein intake. The estimated total energy intake in comparison to the recommended daily energy requirements was on average 96% and 107% for infants 6-8 months and 106% and 103% for infants 9-11 months respectively (Table 6.3).

Table 6.3 Twenty-four hour dietary recall of six to twelve months old infants[1] receiving processed and non-processed (placebo) complementary food

Average daily intake	Infants receiving processed food (n=71)	Infants receiving placebo (n=66)	Mean difference	P-value
Total energy intake (kJ day^{-1})	3427± 915	3426±784	1	0 99
6-8 mo infants % recommended energy intake	95 7± 26.0	105.9± 22.7	-9.3	0.20
9-12 mo infants % recommended energy intake	106.7± 24.0	103.4± 22.5	3.3	0.49
Energy from project CF + oil (kJ day^{-1})	1922± 793	1943±691	-21	0.47
Energy from other CFs (kJ day^{-1})	657± 333	636± 345	21	0.49
Breast milk (kJ day^{-1})[2]	847	847	0	-
Total proteins (g)	18.3± 6 3	17 9 ± 5 5	0.4	0.68
Total fats (g)	29.9± 7.2	31 3± 6.4	-1.4	0.24
Total iron intake (mg)	6.8 ± 2.7	6.5±2.4	0.3	0.55
Frequency of meals day^{-1}	1-2	5-6		

[1]Mean±SD, [2] Based on minimum milk production by women in developing countries (WHO, 1998a)

6.4.4 Iron dosage for the infants in the entire six months of intervention

The recommended iron intake from foods with low bioavailability is 21 mg iron (Fe) per day (WHO, 1998a). Thus:-

21 mg per day x 5% bioavailability x 30 days x 6 months = 189 mg Fe. The amount taken by infants taking processed and non-processed complementary food was a mean of 4.74 mg iron and 5.89 mg iron per 100 g of processed and non-processed CF. Assuming that the *in vitro* calculated amount of soluble iron is equivalent to its bioavailability: -

(i) Processed CF: 4.74 mg/100g CF x 104 g CF/day x 19% (soluble Fe) x 30 days x 6 months = 169.1 mg Fe. Therefore, 169.1 mg Fe /189 mg Fe x 100% = 89.5% of the recommended intake.

(ii) Non-processed CF: 5.89 mg/100g CF x 100 g CF/day x 5% (soluble Fe) x 30 days x 6 months = 53.0 mg Fe. Therefore, 53.0 mg Fe /189 mg Fe x 100% = 28% of the recommended intake.

Thus for the entire six months, the theoretical calculated amount of iron the infants could have received from processed CF and non-processed CF was 169 mg Fe and 53 mg Fe, respectively representing 89.5% and 28% of the recommended amount. However, although a difference *in vitro* bioavailability exists between the processed and unprocessed CF, it is emphasized that this is an assumed estimate and not the true bioavailability.

6.4.5 Effect of complementary food on growth

There were no significant differences in measurements at entry into the study (6 months old infants) between the two groups except for prevalence of wasting and low birth weight, which might have occurred by chance (Table 6.1). Similarly, there were

no significant differences ($p > 0.05$) between the groups at 12 months of age (Table 6.4). The differences in weight gain was not significant 1.4±0.6 kg versus 1.3±0.7 kg at 12 months of age, respectively. Likewise, mean WLZ and LAZ parameters of the two groups of infants did not differ significantly. Infants fed with processed CF the mean length of infants increased from 62.7±3.1 cm at 6 months of age to 69.4±2.9 cm at 12 months of age. Infants fed with non-processed CF the mean length of infants increased from and from 62.8±3.1 cm at 6 months of age to 69.4±3.0 cm at 12 months of age. This was a change of 6.7±2.0 and 6.9±1.9 cm for the processed and non-processed CF groups, respectively. There was a significant overall decline in LAZ between 6 months and 12 months of age for both groups (Table 6.5). Mean LAZ was -1.60, below the NCHS reference median at the age of 6 months and –2.06 at the age of 12 months ($p < 0.05$). A similar trend was observed in WLZ. There was a significant decline between 6 months and 12 months of age ($p < 0.05$). Mean WLZ was 0.57, above the NCHS reference median at 6 months of age and –0.17 at 12 months of age (Table 6.5).

Table 6.4 Comparison of Hemoglobin (Hb), Zinc protoporphyrin (ZP), WLZ and LAZ of infants at 12 months of age by t-test[1]

	Mean at 12 months of age			
Variable	Processed food (133)	Placebo (125)	Mean Difference	P value
Hb (g dL^{-1})	9.7± 0.2	9.7± 0.2	+0.01	0.96
ZP (µg g^{-1} Hb)[2]	5.8± 3.5	6.2± 3.1	-0.39	0.34
WLZ[2]	-0.27± 0.97	-0.07± 0.98	-0.19	0.12
LAZ	-2.08± 1.02	-2.04± 1.07	-0.04	0.78
Change Hb (g dL^{-1})	0.48±0.18	0.15±0.2	-0.33	0.19
Change of ZP (µg g^{-1} Hb)[2]	-4.40±0.5	-3.63±0.5	0.77	0.39
Change in WLZ	-0.67±0.1	-0.81±0.1	-0.14	0.31
Change in LAZ[2]	-0.50±0.1	-0.42±0.1	0.08	0.40

[1]Mean ± SD, [2]t-test applied on zero-skewness log-transformed variables

Table 6.5 Comparison of Hb, ZP and anthropometry of infants at six and twelve months of age[1] for the whole group by paired t-test and McNemar test

Variable	At 6 months of age (309)	At 12 months of age (258)	Mean Difference	P - value
Hemoglobin (g dL^{-1})	9.3± 2.0	9.7± 1.9	+0.32	0.01
Anemic (<11 g dL^{-1})	78.6 %	76.3%	-2.3%	0.39
Zinc protoporphyrin (µg g^{-1} Hb)[2]	10.1± 6.3	6.0± 3.3	-4.02	0.001
High ZP (>5 µg g^{-1} Hb)	76.0%	55.8%	-20.2%	0.001
Weight	6.8± 1.0	8.1± 1.1	+1.37	0.001
Length	62.8± 3.1	69.4± 3.0	+6.81	0.001
WLZ	0.57± 0.75	-0.17± 0.98	-0.74	0.001
Wasted (<-2SD)	1.3%	1.3%	0	-
LAZ	-1.60± 1.15	-2.06± 1.05	-0.06	0.001
Stunted (<-2SD)	34.6%	50.3%	+15.7%	0.001

[1]Mean ± SD, [2]t-test applied on zero-skewness log-transformed variable

6.4.6 Effect of complementary food on hemoglobin and zinc protoporphyrin

Clinical measurements of Hb and ZP were not significantly different between groups at 12 months of age (Table 6.4). Conversely, both groups gained significantly from 6 months to 12 months of age with regard to Hb and ZP. The overall mean for Hb at the age of 6 months was 9.3 g dL^{-1} increasing significantly to 9.7 g dL^{-1} at the age of 12 months (Table 6.5). This showed however that the majority of the infants were still anemic based on WHO standards as depicted by the percentage of anemic infants in Table 6.5. Likewise, ZP declined significantly from a mean of 10.1 µg g^{-1} Hb at the age of 6 months to a 6.0 µg g^{-1} Hb at the age of 12 months as shown by the percentage reduction in infants with iron deficiency indicating infants improvement in iron status (Table 6.5).

Table 6.6 shows the results of the logistic regression analysis. No significant interaction between covariates and the type of CF was detected (likelihood ratio test

for set 1, p-value 0.20, likelihood ratio test for set 2, p-value 0.11, likelihood ratio test for set 3, p-value 0.11). After removal of non significant variables the models included high ZP at baseline, anemia at baseline, stunting at 12 month of age and low income in set 1 (dependent variable: anemia); high ZP at baseline and season at entry in the study in set 2 (dependent variable: high ZP); mother's age and LBW in set 3 (dependent variable: low length-for-age z-score). Eventually, for all 3 sets of models, all covariates could be removed from the models without notably affecting the size of the odds ratios for type of CF (no confounding effect). The final models included only the dependent variable and the variable type of CF as independent variable. The crude odds ratios showed that the processed CF had no effect on anemia, ZP deficiency or on stunting. Outliers represented less than 2% of the dataset, consequently excluding them from the analysis where appropriate did not modify the results.

Table 6.6 Odds ratio (OR) for CF derived from logistic regression analysis

Dependent variable	Crude OR	95% Confidence interval	P-value	Adjusted OR	95% Confidence interval	p-value
Anemia	1.04	0.58 - 1.84	0.90	0.93[1]	0.50 - 1.71	0.81
High ZP	0.92	0.56 - 1.51	0.75	0.85[2]	0.53 - 1.59	0.54
Low LAZ	1.20	0.74 - 1.96	0.45	1.02[3]	0.60 - 1.72	0.94

[1] Adjusted for high ZP at baseline, anemia at baseline, low LAZ at month 12 and low income
[2] Adjusted for high ZP at baseline and season at entry in the study
[3] Adjusted for age of mother and LBW

6.5 Discussion

The results show that the processing did not have the effect of improving growth, hemoglobin and iron status of infants. Similar results were found by Lartey *et al.* (1999), in Ghana, who evaluated the effects of feeding weanimix and three other locally formulated, centrally processed CF's on nutritional status of breastfed infants (6-12 months of age). They found no significant differences between intervention

groups in weight or length gain, hemoglobin and hematocrit values. Equally, insignificant results were observed in a study by Thu *et al.* (1999), investigating the efficacy of weekly and daily micronutrient supplementation in reducing anemia prevalence and growth status in 6-24 months old children in Vietnam. Though they were supplementing the children both daily and weekly with elemental iron, zinc and vitamin A, the Hb, zinc and retinol concentrations were the same. Their growth status was also not significantly different between the children who were being supplemented with micronutrients and those who received placebo.

Stevens and Nelson (1995) investigating the effect of feeding 6 months old infants a milk formula with no iron against one with 1.2 mgFe/100mL for 12 months, found no differences with respect to mean hemoglobin, ferritin concentrations and growth between the two groups of infants. Furthermore, a study by Simondon *et al.* (1996), investigating the effect of supplementation with high energy density CF fortified with minerals and vitamins, on weight and linear growth of 4-7 months old infants in 4 developing countries, found no significant difference in linear growth.

However, there are other studies that observed increased physical growth by supplementation. In a study by Chinamma and Golpaldas (1993), two groups of children aged 6-24 months were fed a complementary food of either high-energy low viscosity or high-energy high viscosity for 6 months. The former group increased significantly in weight and length over the latter. The difference of this study with our study and other studies is that the children were restricted to one experimental meal per day although they were allowed to eat *ad libitum* in that single meal. In a sense therefore one group was getting less experimental food than other per day.

Another longitudinal study conducted by Mora *et al.* (1981) in Columbia investigating the impact of supplementary feeding and home education on physical growth of disadvantaged children observed significant results. Families with high risk of having malnourished children were randomly assigned to a supplemented or unsupplemented study group. Pregnant women in the supplemented group received food rations weekly beginning with the third trimester of pregnancy and continuing up to 36 months after birth of the infants. By 3 months the infants born to supplemented mothers were significantly heavier by 197 g, while at 36 months there was a cumulative difference between the groups of 476 g in weight and 2.2 cm in length. The difference of this study with our study is that, it was a purposive sample of poor families with malnourished children. Furthermore, supplementation was done for the whole family members (supplemented group) with an objective of monitoring and following-up the pregnancy outcome. However the results of the study do suggest that there is an advantage of nourishing the mother for better pregnancy outcome.

The advantage of nourishing the mother for the benefit of the baby was also reported by Kusin and Kardjati (1994). In their study high or low energy supplements were given to the mother in the last trimester of pregnancy whereby a significant positive effect was observed. The children born to high energy supplemented mothers were significantly heavier till 24 months and taller up to 60 months than the low enery supplemented children.

Bhandari *et al.* (2001), insist that better understanding the etiology of infant growth faltering with regard to illnesses and breastfeeding rates instead of assuming that merely increasing CF intake might bring the desired effect.

In our study there was a trend towards wasting (0.57 to – 0.17 z-scores) and stunting (-1.6 to –2.06 z-scores). However, with an estimated food intake that normally covers needs, we did not expect to observe a reversal in the trends of stunting. At present it is not clear why this was the case as it was different for the other studies such as Mora *et al.* (1981) and the Institute of Nutrition of Central America and Panama (INCAP) (Habicht *et al.*, 1995). Stunting is a very complex multi-causal problem, and it is probable that other nutrients were limiting. Similar trends were observed by Martorell *et al.* (1998), whereby 25% of infants at baseline (4 months old) were already stunted, presumably due to intrauterine growth retardation (IUGR). They concluded that infants who experience IUGR, usually never completely catch-up in size to their normal birth weight peers even when raised under optimal conditions.

Despite an overall significant rise in hemoglobin between ages 6 and 12 months for all infants, the rise was quite marginal. Mean hemoglobin showed a large proportion of infants still being anemic at the end of the study even when low birthweight groups were eliminated from the analysis.

The effect of malaria seemed to have had an impact to the two supplemented infant groups as it supposedly affected the utilization of nutrients. In a study done in Tanzania by Msuya *et al.* (1991), it was observed that after effective malarial parasite clearance with sulphadoxine (fansidar), almost all children became re-infected within

two to four weeks after disappearance of the protective effect of the drug. Thus failure to restore the hemoglobin concentrations to normal concentrations could be explained by the continuous malaria re-infection among the infants despite the treatment given when they fell sick. This was evident in our study that the majority of infants treated against malaria were re-infected despite of being thoroughly treated and thus malaria intensity in infants remained high throughout the study period with no significant differences between the two study groups.

Furthermore with regard to morbidity especially diarrhoea, which is attributed to poor basic hygiene, sanitation and poor methods of preparation and preservation of food (Afifi *et al.*, 1998), the high disease incidence could also increase energy needs considerably (Lartey *et al.*, 2000; Dewey, 2001). Thus, even when energy intake was according to recommendations, it might not have covered the needs of our study children who had frequent disease episodes and a high malaria attack rate in the two groups.

In spite of the fact that there was no significant difference in iron status between infants complemented on processed and non-processed CF, there was an overall significant gain in hemoglobin and iron status (i.e. lowered zinc protoporphyrin) in both groups which means that the processed CF did not have a better bio-availability of iron. There are several schools of thought regarding this aspect. The results seem to suggest that there was no need of processing the ingredients for CF preparation, instead provision of adequate CF in terms of quality and quantity and as long as nutrition guidance was appropriate is enough. Therefore, part of the reason could be the provision of adequate food free of charge by the project, which improved the

overall food availability and both groups benefiting on the CF despite the processing effect. Similarly another reason could be that the mothers were very keen on measurement outcome and therefore fed their infants adequately because there was a tendency for a mother to be mocked by others in case her infant scored low measurements.

Adequate feeding was also evident in the 24 hr recalls whereby infants were provided with the recommended amount of food per day as directed by project personnel. Age specific energy intake was adequate compared to the recommendations whereby 6-8 months and 9-12 months old infants total energy intake was on average 100%, though the quantity might have been overestimated since we did not consider the spilled food. Similar remarks were given by Dewey (2001) who observed that safe and adequate complementary feeding for breastfed infants is a recognised critical factor in preventing malnutrition. The complementary food that was provided did increase food intake overall if the consumption figures from the main study are compared with the information obtained from the baseline study. In the previous baseline study, habitual CF provided to children as revealed from the 24 hr dietary recall did not meet the daily needs of the children. Thus, provision of the project's CF that was adequate in terms of quantity and quality, met the recommendations and therefore the theoretical advantage of a higher energy dense food was neutralized.

Another school of thought may be based on, results by Domellof *et al.* (2002), who concluded that regulation of iron absorption undergoes developmental changes between 6 and 9 months of age. These changes enhance the infant's ability to adapt to a low-iron diet and provide mechanism by which some infants may avoid developing

iron deficiency despite low iron intakes in the second 6 months of life. This means that the infants improved their physiological efficiency with regard to absorption of iron and other minerals from this main plant source.

Furthermore it could be that the duration of supplementation was not sufficient enough for the CF to have an effect. Compared to fortified foods, which needs less time to show their effects (Mendoza *et al.*, 2001), probably the processed CF required a longer duration than the six months we had intervened.

Likewise, reduction of phytates in the processed CF by 34% as shown by laboratory tests, improved iron solubility to 19% in which case, for the entire 6 months of intervention, the infants taking processed CF theoretically absorbed about 89.5% of the recommended amount of iron from plant food sources. It is surprising however that, even infants who received the non-processed CF improved in terms of iron status although they theoretically absorbed only 28% of the recommended amount. This might probably suggest that the amount of soluble iron was not necessarily correlated with bioavailability (Domellof *et al.*, 2002). The laboratory *in vitro* bioavailability tests were appreciated and used but still they might not be reflective of the actual bioavailability in the real sense. However, Hurrell *et al.* (1992) suggested that in order to have significant effect, phytates have to be reduced by more than 95%.

Another explanation could be that the infants covered their iron needs but that was not adequate enough to show any significant increase with regard to covering their needs and allowing for recovery from iron deficiency (Domellof *et al.*, 2001). Thus despite the deficiencies observed in both groups and regardless of the type of the

supplement offered no significant differences on change in iron status indicators could be noted.

6.1 Conclusions

The fact that the effect of processed relative to the non-processed CF in improving growth, hemoglobin and iron status of infants was not observed, we conclude that processing did not necessarily matter, as long as nutrition guidance was appropriate. Adequate CF was provided to each child and therefore the theoretical advantage of a higher energy dense food was therefore neutralized. Moreover, the positive gain in terms of hemoglobin and iron status in both groups suggests that the amount of soluble micronutrients does not necessarily reflect bioavailability and utilization by the body. But even so, energy intake from complementary food covered recommended amounts. We are still at a loss to explain the decrease in z-scores for the growth parameters. One recommendation can however, be made at this stage namely that an intensive malaria control in infancy should be part of the feeding program in highly endemic areas.

CHAPTER 7

General discussion and perspectives

7 General discussion and perspectives

7.1 Introduction

This thesis concentrated only on food based approaches to combat nutritional deficiencies. Since the inception of this CF project the main aim was to come up with long-term strategies, which might be sustainable in fighting nutritional problems in the rural areas of developing countries.

Childhood malnutrition remains a common problem in much of the developing world. Numerous studies demonstrate that inappropriate complementary feeding practices including premature or late introduction of foods other than breast milk, inadequate amount of food, nutritionally inadequate or unsafe foods and early cessation of breastfeeding are important determinants of malnutrition among young children. However contemporary research on factors that influence children's dietary intake and growth performance has been providing new insights into the etiology of childhood malnutrition. These findings suggest that novel programmatic approaches may be more successful in improving child feeding and reducing the current high rates of malnutrition.

7.2 Micronutrient bioavailability

Micronutrients are required in relatively small quantities although they participate in major physiological functions of the body (Frolich, 1995). However, meeting micronutrient needs from complementary foods especially of plant origin appears to be the greatest challenge (Towo and Tatala, 1998). It has been shown that it is practically impossible to supply enough micronutrients especially iron and zinc from

unmodified complementary food to meet calculated needs of infants 6-11 months of age without unrealistically high intakes of animal products (WHO, 1998a).

The bulk of children's diet in most developing countries is composed of cereals and legumes, which are the major source of micronutrients in their diet (Kingamkono *et al.*, 1995). But as reported by several researchers, cereal and legumes also contain antinutritional factors such as phytates, tannins and dietary fibre which have been shown to decrease the bioavailability of micronutrients (Frolish, 1995). Food processing such as germination, fermentation and food preparation has been shown to positively influence both the content and form of these antinutritional factors thus in turn, modulating micronutrients bioavailability (Honke *et al.*, 1998). Implementation of attainable technologies such as selection of processing and cooking conditions has therefore, the potential to reduce the risk of micronutrient deficiencies in vulnerable groups.

Conventional laboratory *in vitro* methods simulating the events taking place during digestion in the human stomach and small intestines, have shown that processing prior to ingesting the food, has an advantage in improving micronutrient availability (Sripriya *et al.*, 1997). However, it is not very clear if the *in vitro* simulation will equally respond to the complexity of the events happenning in the gastro-intestinal sytem.

7.3 Use of Caco-2 cell line to measure iron bioavailability

In the former work, iron bioavailability in the complementary foods has been evaluated in the laboratory by using an *in vitro* assay based on solubilising the iron

simulating gastro-intestinal digestion (Mamiro *et al.*, 2001). Although the solubility of the iron in the processed food was significantly increased in this assay, there was no significant difference found in iron status of the 12 months old infants who received the processed and non-processed complementary foods, respectively. At this moment, it is unclear whether the food as such had no effect, or whether the failure to see a difference was caused by other factors, which could not be controlled during the field trial, e.g. malaria and diarrhoea. Therefore, in the future there is a need to use a Caco-2 cell assay to measure iron bio-availability in both the processed and unprocessed complementary food samples so that one knows whether processing had improved bioavailability or not. Several references indicate that this assay is more reliable compared to iron solubility used as a proxy for iron bioavailability in laboratory settings (Glahn *et al.*, 1998).

The Caco-2 cell line is a useful model for studies of intestinal human iron uptake in that the food undergoes a simulated peptic digestion followed by intestinal digestion (Glahn *et al.*, 1996). This model measures iron solubility in addition to providing a measure of uptake via a living component called Caco-2 monolayer. Iron availability from foods is monitored by ferritin formation by Caco-2 cells as an indicator of iron uptake. This methodology circumvents the need for using radioactive iron and thus eliminates the costs and controversies associated with food radiolabeling. The Caco-2 monolayer is an advancement over the use of *in vitro* digestion alone, which measures only iron solubility and therefore is not a complete measure of iron bioavailability.

7.4 Microbial safety of complementary foods

Safety is an important parameter in food processing and production especially when the food is intended for children. Sources of contamination of the complementary foods can be numerous. These can include the raw ingredients, the water used for processing, the personnel involved and even cross contamination from one of the sources. A centrally located food production facility is relatively easier to monitor safety by following the Hazard Analysis and Critical Control Principles (HACCP) than individual household food processing and production.

HACCP is a management sysem in which food safety is addressed through the analysis and control of biological, chemical and physical hazards from raw material production, procurement and handling to manufacturing, distribution, and consumption of the finished product (HACCP, 1998). HCCP is designed for use in all segments of food industry from growing, harvesting to food preparation and consumption. The principles are general logical procedures, which can be applied at all levels.

The poor hygienic conditions of any rural setting in a developing country make it very difficult to avoid microbial contamination of food during feeding by dirty utensils and improper personal hygiene. If food is served immediately after cooking, however there is no reason for serious concern, as the amount of micro-organisms present at this stage are too few to cause harm. On the other hand if the porridge is kept at ambient temperatures for some time, micro-organisms that have contaminated utensils will multiply rapidly in leftovers, and might then be hazardous to consume (Kingamkono *et al.*, 1995). Most people cannot always afford to throw away

leftovers, and it is difficult to prepare foods in exactly the required amounts. This is truly a dilemma for the majority of people living in the rural areas of most developing countries such as Tanzania.

The important aspect lacking in most of the rural settings in addressing food safety is nutrition education, hygiene and environmental sanitaton. These aspects need to be continuously reinforced if behavioural change is to be sustained. A pilot study in Bangladesh showed that nutrition education, using social marketing techniques could affect maternal hygiene behaviour. The study included a rapid assessment survey in home diagnosis, focus groups and household trials. Food hygiene messages were written into poems that used popular proverbs and folk songs. Before intervention, all the 25 community women who participated in the study fed their babies food that had been prepared the previous day. After the promotion of the hygiene messages, 75% accepted the advice to refrain from serving leftover food that could be contaminated (Ahmed *et al.*, 1992). This strategy could also be emulated in other developing countries like Tanzania.

7.5 Mycotoxins and complementary foods

There is increasing worldwide awareness of the serious consequences, which undesirable levels of mycotoxins may have on food supplies and on human health. Mycotoxins have been high on the agenda in the world's scientific forum (WHO, 1999). Setting internationally agreed upon tolerance levels for mycotoxins in foods has been encountering difficulties because of divergent views which exist between cereal and legume grain importing and exporting countries on the levels to adopt (FAO, 1993, WHO, 1999). Thus, until now only suggested tolerance limits set up by

some governments and organizations are being used, although they are not based on scientific experiments. In order to rationally enforce tolerance levels, reliable analytical procedures are needed. The lack of tolerance limits might lead to children and adults being exposed to high levels of toxins without themselves being aware. Contamination of cereals and legumes with mycotoxins in developing countries has been a major problem and has a significant economic and nutritional impact (Kim *et al.*, 2002).

In sub-Saharan countries including Tanzania maize is widely consumed. Porridge made from maize flour is a popular complementary food for infants and children in many households (Kikafunda, 1998). Peanuts have also been promoted to prepare composite flour of maize and other cereals for complementing children (Kingamkono, 1999). However, maize and peanuts have been implicated as having relatively high levels of mycotoxins (Munibazi and Bullerman, 1996, Siame *et al.*, 1998). High levels of contamination of maize and peanuts with mycotoxins have been associated with a serious health risk, which might lead to acute toxicity. For instance, mycotoxins have been implicated to produce free radicals, which have been associated with growth retardation in children (Zin *et al.*, 1998). Furthermore, growth among exclusively breast-fed infants has been observed to deviate from the reference growth curve as early as two months (Garza and De Onis, 1999). This might possibly be an effect of mycotoxins since Kolsteren (1992) observed that children and adults with osteoarthropathy known as kashin beck disease were from areas whereby cereals were highly contaminated with *Fusarium* moulds. Other potential risks of mycotoxins associated with human health are cancer and liver damage (Siame *et al.*, 1998). In our study in Kilosa, despite provision of adequate food meeting daily energy and protein

requirements infants continued to be stunted by 12 months of age. Perhaps mycotoxins might be playing a role because 95% of household's main meal in Kilosa constitutes of maize flour through a popular meal called stiff porridge. The mycotoxins might be finding their way to infants through breast milk and later through complementary and other foods.

Therefore, in future research it is important to evaluate better mycotoxin levels in breast milk and in CFs used in rural areas in developing countries. This is because raw materials for CFs for children originate from various places with different climate regimes, production and storage methods. The results of such experiments will enable researchers to come up with some suggestions of better storage conditions and frequent quality checks of the cereal and legume grains for preparing CFs employing hazard analysis and critical control point principles (HACCP). These are general principles of logical procedures allowing risk of contamination to be identified and controlled and can be applied at all levels from small to medium-scale food industries.

7.6 Complementary food fortification versus food based approach

Food based approach to fight nutritional problems is still the best choice in most of the developing countries compared to fortification and supplementation. This is because of the mere fact that majority of the people in developing countries are residing in the rural areas, where they consume most of what they produce.

7.6.1 Dietary modification, supplementation and fortification

Dietary modification and diversification involve changes in Complementary Food (CF) selection patterns and traditional household methods for preparing and

processing indigenous CF. These strategies are more culturally acceptable, economically feasible and sustainable than CF supplementation and fortification. Apart from improving energy and protein intake through reduction of bulkiness and density, the CF modification approach can be used to alleviate several micronutrient deficiencies simultaneously without the risk of antagonistic interactions although it might take a longer time to show results (Gibson *et al.*, 1998).

CF fortification on the other hand involves adding a specific micronutrient to processed staple foods, such as iron in maize, sorghum, millet or wheat flour (Mannar and Gallego, 2002) while supplementation refers to provision of supplementary feeding, usually a processed complemenary food or the actual form of micronutrient e.g. ferrous sulphate/fumarate syrup or tablets (Thu *et al.*, 1999). Several studies have shown that micronutrient supplements and fortified CF are effective in preventing and treating deficiencies (Hurrell, 2002). For example the study by Zlotkin *et al.* (2001) treating 6-18 months anemic children in rural Ghana using microencapsulated ferrous fumarate, had a 58% success in treating anemia in 2 months, though compliance was often poor due to unpleasant side effects of the supplement. In a study by Moffatt *et al.* (1994) on prevention of iron deficiency in infants using iron fortified formula, a double blind randomised controlled trial involving 230 healthy bottle-fed infants, one group was allocated 12.8 mg iron per litre while another group was provided with regular formula with 1.1 mg iron per litre. After three months the group receiving iron fortified formula was significantly different from the group receiving regular formula in the sense that the former group was better off with regard to all measures of iron status.

Food fortification does pose a number of limits. According to Nantel and Tontisirin, (2002), fortification is potentially very costly for developing countries. First, fortification usually involves a manufacturing step, which is an expensive process. Secondly, there is a need for quality control when a fortificant is added. This introduces a level of technical sophistication that requires training, investment and recurrent costs. Excess intake of micronutrients can also lead to toxicity, which needs a close surveillance on average intake and side effects. In addition, adding micronutrients to a food item can induce stability problems. It is, for instance, well known that a number of metals can oxidise unsaturated lipids (Davidsson *et al.*, 2000). The usefulness of a food fortification approach is also limited by the population served. In urban localities most food is purchased and thus the population is dependent on the offer. There is little by-pass. In rural areas, most households consume what they have produced. Fortification will miss many of the vulnerable groups, either because they have no access to the fortified food or because they rely on their own production for food intake as is the case in rural areas.

7.6.2 Effect of the processed complementary food

The lack of the CF showing an improvement in children growth, hemoglobin and iron status suggest that the conventional laboratory performed test does not always translate to reality that the same benefits will be reflected in the complex human systems. Alternatively, we might have erred by trying to solve the children nutritional problems at the wrong entry point. This is because from the baseline survey, it was seen that nutritional problems had already started way back in the first six months of life. Other problems like LBW are even associated with foetal development in the mother during pregnancy (Kusin and Kardjati, 1994). Thus it is imperative that in

order to have longterm solution to nutritional problems facing children the feasible strategy is investing on maternal nutrition.

Numerous intervention studies have been conducted with the goal of preventing growth faltering and to identify the specific nutrient deficiencies and other mechanisms that may be responsible for this phenomenon (Waterlow and Schürch, 1994). The results of such studies have often been conflicting due to factors such as differences in amount, duration, and type of supplement among trials, the baseline nutritional status of children and the probable simultaneous occurrence of multiple nutrient deficiencies. In addition many interventions occurred late in, or even after the period of growth faltering, so although they could have improved catch up growth, they could not have prevented growth failure (WHO, 1998a).

7.7 Diseases and infections

Malaria has been rated as number one killer disease in Tanzania (Premji, 1995, Gonzalez *et al.*, 2000). Kilosa district was not an exception and it was very evident in this study because the prevalence of malaria was 50% in infants, which we also assume that the disease was one of the confounding factors. Comparatively, it was the leading district in malaria caused deaths in Morogoro region in the year 2002 with most of the deaths occurring in children below five years and pregnant women. As a preventive measure, the government should supply impregnated mosquito nets to malaria endemic areas free of charge. Currently, the mosquito nets are sold at an equivalent of US$ 5 in MCH clinics. However, most of the rural population cannot afford that price. If the Government can supply anti-malarial drugs, which are

relatively more expensive and free of charge in the rural places, there is no reason whatsoever for failing to supply the nets free as a long-term prevention measure.

Diarrhea has been sighted as a disease second to malaria in afflicting children's health. Each infant in this study had an episode of diarrhea from birth to six months, which explains its high prevalence. Diarrhea has a great impact on nutrient utilization by the body. In most cases this is due to unhygienic environments in the rural localities, unclean water and utensils used to serve CFs. Almost 70% of the diarrheal episodes among children under five years of age in Kilosa district are estimated to be caused by contaminated drinking water, which acts as a vehicle in cross contamination (Kilosa Hospital Annual Report, 2000). This problem requires more emphasis on awareness and nutritional education such as use of boiled water, clean utensils and better disposal of waste and general environmental sanitation. In places such as Kilosa where villagers still use river water, the government should as a matter of urgency be in the forefront to direct some resources in improving safe water supplies in the form of tap or borehole water.

8 Summary of conclusions and recommendations

(i) The conventional laboratory *in vitro* bioavailability methods does not necessarily reflect the actual bioavailability of micronutrients from complementary foods. Therefore reliable methods should be sought. A suggestion is made on the recent developed Caco2 monolayer cell, which is relatively inexpensive compared to the food radiolabeling techniques.

(ii) Food safety should be a matter of priority during processing, production, storage conditions, distribution and consumption of complementary food for children. Therefore ingredients intended for complementary food preparation should be microbiologically free from of potential pathological bacteria. The hazard analysis critical control point principles should be adhered at all stages of handling of the complementary food. Moreover, maize flour, which is used to prepare CFs for the majority of children in Tanzania, has been found to be susceptible to mould attack, which produces a variety of mycotoxins. Ingredients low in mycotoxin content should be promoted for the preparation of complementary food for infants, as the hazards caused by various mycotoxins are immense considering the tender age of infants.

(iii) Prevalence of stunting is still high among children in rural localities in Tanzania. Results of this study confirm that it was related to maternal nutritional status, as indicated by their low BMI. Studies have shown that maternal nutrition during pregnancy influences the growth of offspring beyond the intraruterine period. Long-term investiment, which is deemed

cost effective, should be directed to nutrition of adolescent and or pregnant mothers so as to have healthy pregnancy outcome.

(iv) After birth, the main causes of growth faltering are the availability of appropriate complementary food, morbidity and childcare. This study shows that improving the type of complementary food is not sufficient for a beneficial effect. It needs to be complemented by behavioural change through proper guidance aimed at frequent feeding to provide the quantity and quality of complementary foods for adequate growth.

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10 Map of Tanzania showing Kilosa district

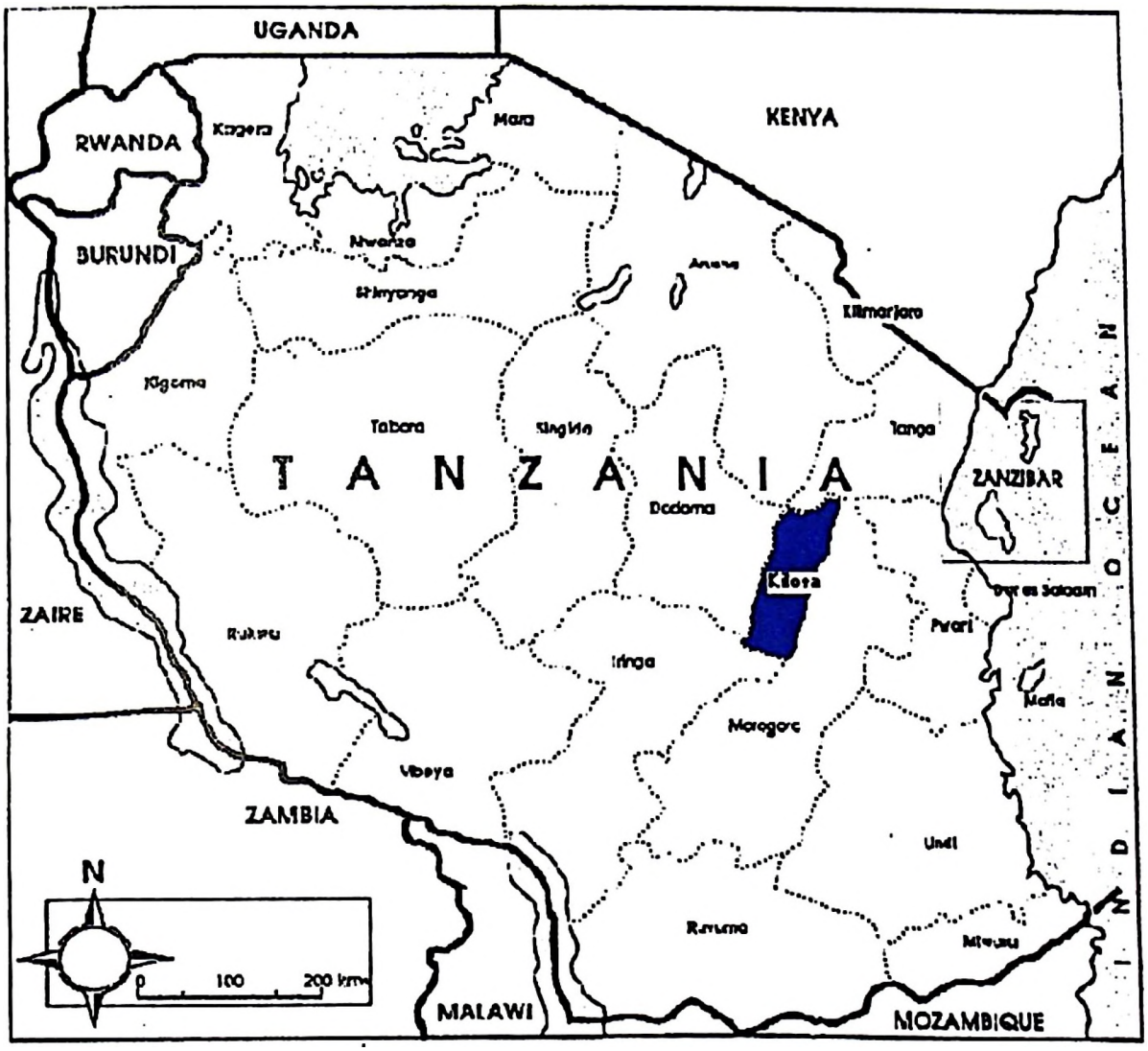

Figure 9.1 Kilosa district where the randomized controlled trial was undertaken

11 Curriculum vitae

Personal history

Full name	Peter Ruwaichi Simon Mamiro
Date of birth	23.08.1958
Place of birth	Marangu Moshi Kilimanjaro
Nationality	Tanzanian.
Marital status	Married
Number of children	Three

Academic qualifications

B.Sc. (Agriculture) Food Science Option- Sokoine University of Agriculture – 1986.

Postgraduate Diploma (Management of Natural Resources and Sustainable Agriculture) - Agricultural University of Norway - 1990.

M.Sc. (Management of Natural Resources and Sustainable Agriculture)- Agricultural University of Norway - 1991.

Membership of academic institutions:

Food and Nutrition Association of Tanzania (FONATA).
Agricultural Economists Society of Tanzania (AGREST).
Convocation member Sokoine University of Agriculture.

Research reports

Mamiro, P.S. (1986) Studies on some characteristics and oxidative behaviour of sunflower and cottonseed oil produced by Morogoro Oil Processing Company (MOPROCO). A Bachelor of Science Degree - Dissertation -1986.

Mtebe, K., Mamiro, P.S., Nyang'ali, E., Njebele, C. (1989) Household food security in child survival and development programme area. A survey report (CSDP) Morogoro Region - 1989. Presented for UNICEF.

Mamiro, P.S. (1991) Household food security in rural Tanzania: adequacy stability and accessibility - A case study of Moshi and Kilosa districts. M.Sc. Thesis.

Mwaseba, D., Mamiro, P.S. (1997) The contribution of Nile perch in Lake Victoria to the Welfare of Women Fish Traders in Tanzania.The case of Magu district. A Report Submitted to the Organization for Social Sciences Research in Eastern and Southern Africa (OSSREA).

Papers

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Temu, A.E., Mamiro, P.S. (1995) Structural adjustment programme: preliminary situation and outlook of coffee based small-scale farmers in Kilimanjaro. *Agricultural Economic Analysis and Rural Development*, 5 (1), 14-19.

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Congress proceedings

Mdoe N.S.Y., Nyagori, H., Isinika, A.C., Mamiro, P.S. (2000) Evaluation of small-scale food processing enterprises in Dar es salaam and Morogoro. Paper presented during the University wide research workshop held on 5th –7th April, 2000.

Mamiro, P., Van Camp, J., Roberfroid, D., Kolsteren, P., Huyghebaert, A. (2001) Prevalence of malnutrition and anaemia among infants aged 4-12 months in Kilosa district-Rural Tanzania. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, 66 (4), pp.69-73.

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Van Camp, J., Mamiro, P., Demeulanear, B., Devlieghere F., Kolsteren, P. (2003) The balance between safety and improvement of nutritional value for complementary foods in African countries. Food Africa Conference, Yaounde Cameroon, 4th – 9th May 2003.

Mamiro, P., Van Camp, J., Kolsteren P., Roberfroid, D. (2003) An intervention trial to improve growth and iron status among infants in Kilosa District-rural Tanzania. Food Africa Conference, Yaounde Cameroon, 4th – 9th May 2003.

Posters

Van Camp, J., Mamiro, P.S., Mbithi-Mwikya, S., Huyghebaert, A. (2001). Bioavailability of micronutrients in relation to public health. 30 mei – 1 Juni 2001. Interlaken, Zwitserland. Poster: "Formulation and evaluation of a millet based complementary food".

Van camp, J., Mamiro. P., Roberfroid, D., Kolsteren, P., Huyghebaert, A. (2001). Promoting growth and development of under fives. International colloquium organized by the Prince Leopold Institute of Tropical Medicine. 28-30 November 2001. Antwerp. Poster: "Nutritional habits of young children in Kilosa district - Tanzania".

Mamiro, P., Van camp, J., Kolsteren, P., Roberfroid, D., Huyghebaert, A. (2001). Nutritional problems of infants in Kilosa district, Tanzania and appropriate interventions. PhD symposium, Faculty of Agricultural and Applied Biological Sciences, Ghent University, October, 10th, 2001.

Mamiro, P., Kolsteren, P., Van Camp, J., Roberfroid, D., Tatala, S., Opsomer, A. (2003). Improvement of iron deficiency anemia through a processed complementary food: a randomized trial in rural Tanzania. Presented at the International Anemia consultative Group Symposium- Marakech-Morocco, On the 6th February 2003.

Mamiro, P., Kolsteren, P., Van Camp, J., Roberfroid, D., Tatala, S., Opsomer, A. (2003). Prevalence of malnutrition and anemia among infants aged 4-12 months in Kilosa district-Tanzania. Presented at the International Anemia consultative Group Symposium- Marakech-Morocco, On the 6th February 2003.

Mamiro, P., Demeulanear, B., Van Camp, J., Kolsteren, P., Baert, K., Opsomer, A., Huyghebaert, A. (2003). Exposure of fumonisins in finger millet, kidney beans and peanuts, which are ingredients used for complementary food in Tanzania. Presented at the International Networking conference for the Food and Feed Industry in The Netherlands on 17th-18 February 2003.

Teaching compendia

The Open University of Tanzania:

Mamiro, P.S., Mamiro, D.P. (1996) Biology for home economics course for Bachelor of Science general. A teaching manual prepared for The Open University of Tanzania.

Mamiro, P.S., Mamiro, D.P. (1996) Plant metabolism for Bachelor of Science. A teaching manual prepared for The Open University of Tanzania.

Mamiro, P.S., Mamiro, D.P. (1997) Plant taxonomy for Bachelor of Science. A teaching manual prepared for The Open University of Tanzania.

Consultancies

UNICEF:

Household food security survey for villages under child survival and development programme in Morogoro Region - 1989.

UNICEF:

Household food security situation in selected districts of Tanzania: knowledge attitude and practices of community leadership. April 1998.

NORAGRIC:

Rapid organizational appraisal of the national environment management council - 1993.

Areas of research interest

Human Nutrition.

Food security at household level and post-harvest technologies.

Course attended

Promotion of rural development in the tropics and subtropics - Feldafing - Germany, May to June, 1993.

Promotion of rural development in the tropics - Kumasi - Ghana, February, 1994.

Community based land use planning in rural areas - Masvingo, Zimbabwe, October to November1996