Protein, iron and zinc content and bioaccessibility of a ready-to-eat sorghum and cowpea meal developed for 2- to 5-year old African children

By

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Declaration

I hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other university or institution of higher learning.

Nokuthula Vilakati

Dedication

You were friends, lovers, family and confidants. Mdayiseni na Lomdundu, eMaphephetse, boNdukuzabafo, "Umndeni". This holds a piece of each and every one of you, thank you for all your support. With all my Heavenly Fathers' blessings and His undying love, He made it possible; I dedicate this to you my Lord.

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Abstract

Protein, iron and zinc content and bioaccessibility of a ready-to-eat sorghum and cowpea meal developed for 2- to 5-year old African children

By

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Protein, iron and zinc deficiencies are among the most prevalent nutritional deficiencies among children under the age of five years living in developing countries. Nutritional deficiencies are caused by insufficient dietary intake due to food insecurity. Sorghum and cowpea are important staple plant foods indigenous to Africa. They are inexpensive sources of protein, iron and zinc. Hence, they are suitable vehicles that could be used for improving nutrition in young children. Their use is, however limited by the long cooking time required for their preparation and the restricted types of food products available for young children.

A ready-to-eat (RTE) composite meal suitable for young children aged two to five years was formulated using extrusion cooked decorticated sorghum (ES) and micronised (infrared treatment) dehulled cowpea (MC) to make an ESMC RTE meal. The ESMC RTE meal was supplemented with a cooked cowpea leaf relish. The study investigated the effects of the high temperature short time (HTST) (extrusion cooking and micronisation) heat treatments, compositing and adding a cooked cowpea leaf relish on the protein, iron and zinc contents; trypsin inhibitor activity (TIA), total phenolic content (TPC), tannins and phytate contents. The study also compared the effects of HTST heat treatment, compositing and adding the relish on protein quality, in vitro protein digestibility (IVPD) and in vitro iron and zinc bioaccessibilities against raw sorghum and cowpea, the raw composite and a commercial fortified corn (maize): soy RTE porridge. Assessments for protein quality were: calculated Protein Digestibility Corrected Amino Acid Score (PDCAAS), pepsin and multienzyme IVPD assays for IVPD and dialysability assay for in vitro iron and zinc bioaccessibilities. A mineral solubility assay was used to estimate iron and zinc bioaccessibility.

Micronisation inactivated TIA and instantised the cowpea flour resulting in excellent hydration properties. A daily serving of the recommended portion size of the ESMC RTE

meal with cooked cowpea leaf relish would meet approximately 40% of the protein and lysine requirements for children aged two to five years. Further, the calculated PDCAAS would be similar to the commercial fortified RTE porridge. This was despite the negative effect on protein digestibility of the high tannin content in cooked cowpea leaf relish.

A recommended serving of ESMC RTE meal with cooked cowpea leaf relish could contribute ≈85 and 18% towards children's iron and zinc recommended dietary allowance, compared to the commercial fortified RTE porridge (84 and 125%, respectively). However, the higher iron and zinc bioaccessibilities of the ESMC RTE meal with cooked cowpea leaf relish compared to the commercial fortified RTE porridge (11.8 vs. 5% and 18.9 vs. 2.7%) resulted in higher and similar levels of bioaccessible iron (2.24 vs. 0.86 mg/100 g, db) and zinc (0.35 vs. 0.32 mg/100 g), respectively. Thus, the ESMC RTE meal with cooked cowpea leaf relish compared to the commercial fortified RTE porridge would provide more available iron and similar levels of zinc towards the basal requirements of children.

This ESMC RTE meal with cooked cowpea leaf relish can provide protein, iron and zinc nutrition for young children, increase diversity and food security in Africa.

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List of Abbreviations and Definitions

AR	- Absolute requirements
ARF	- Amylase rich flour
Bioaccessibility	- The estimated potential of a food matrix to release a substance upon digestion.
Bioavailability	- The amount of a substance that is released from a food matrix upon digestion that is capable of being absorbed and available for use or stored.
CE	- Catechin equivalents
CP	- Cowpea
CPL	- Cowpea leaves
CSB	- Corn soy blend
DB	- Dry basis
DFS	- Defatted soy flour
EAR	- Estimated Average Requirements
ESMC	- Extruded sorghum-micronised cowpea
FAO	- Food and Agriculture Organization
HTST	- High temperature short time
ICP-OES	- Iron coupled plasma optical emission spectrophotometry
IDA	- Iron deficiency anaemia
IVPD	- In vitro protein digestibility
LMW	- Low molecular weight
LNS	- Lipid based nutrient supplement

MCP1	- Micronised cowpea 1
MCP 2	- Micronised cowpea 2
MRP	- Maillard reaction products
PD	- Protein digestibility
PDCAAS	- Protein digestibility corrected amino acid score
PEM	- Protein energy malnutrition
RDA	- Recommended dietary allowance
RPM	- Revolutions per minute
RTE	- Ready-to-eat
RUTF	- Ready-to-use therapeutic food
SAM	- Severe acute malnutrition
TIA	- Trypsin inhibitor activity
TIU	- Trypsin inhibitor unit
TPC	- Total phenolic content
UN	- United Nations
Undernutrition	- The outcome of insufficient food intake, inadequate care and infectious diseases ad can include underweight, stunting, wasting and micronutrient deficiencies
UNICEF	- United Nations Children's Fund
USA	- United States of America
USAID	- United States Agency for International Development
USDA	- United States Department of Agriculture
VAD	- Vitamin A deficiency

WAI	- Water absorption index
WFP	- World Food Programme
WSB	- Wheat soy blend
WSI	- Water solubility index
WHO	- World Health Organization

1 Introduction

Children under the age of five years living in developing countries are nutritionally among the most vulnerable. Malnutrition is estimated to have been responsible for approximately 6.3 million deaths in 2013 and 45% of the deaths of children under five years in 2011 (World Hunger Education Services, 2015). In sub-Saharan Africa, even though improvements have been reported in the poverty levels, the region still remains with the highest prevalence of undernutrition (UN, 2015).

In 1999 in South Africa, a National Food Consumption Survey (NFCS) for children aged one to nine years found that almost 9% of the children were underweight and 21% were stunted (Labadarios, Steyn, Maunder, MacIntyre, Gericke, Swart, Huskisson, Dannhauser, Vorster, Nesamvuni & Nel, 2000). In 2008, the National Food Consumption Survey Fortification Baseline (NFCS-FB-I) in South Africa, found that 67% of the children were estimated to have poor vitamin A status, 14% had poor iron status and 45% had poor zinc status (Labadarios, Swart, Maunder, Kruger, Gericke, Kuzwayo, Ntsie, Steyn, Schloss, Dhansay, Jooste, Dannhauser, Nel, Molefe & Kotze, 2008). In South Africa, children living in the most rural parts of provinces such as the North-West, Free State, Northern Cape, Eastern Cape and Limpopo provinces were identified as the most vulnerable nutritionally (Shisana, Labadarios, Rehle, Simbayi, Zuma, Dhansay, Reddy, Parker, Hoosain, Naidoo, Hongoro, Mchiza, Steyn, Dwane, Makoae, Maluleke, Ramlagan, Zungu, Evans, Jacobs, Faber & SANHANES-1 Team, 2013; Labadarios et al., 2008).

Inadequate food intake, poor maternal and child care and chronic infectious diseases lead to underweight, stunting, wasting and micronutrient deficiencies among children (UNICEF, 2009). Nutritional deficiencies in developing countries are often established during childhood where the common cause is the familiar practice of feeding young children nutritionally inadequate foods (MacIntyre, De Villiers & Baloyi, 2005). Nutritional deficiencies in children are intensified by chronic infections and diseases (WFP, 2005), which in turn increase the need for micronutrient-rich foods to manage the arising conditions (Müller & Krawinkel, 2005).

Prevention of malnutrition in resource poor communities is often a challenge as foods rich in nutrients that are suitable for young child feeding are not readily available (Lutter, Daelmans, de Onis, Kothari, Ruel, Arimond, Deitchler, Dewey, Blössner & Borghi, 2011). Prevention is even more difficult for rural communities as undernutrition is more prevalent in rural than in urban areas. A lack of good quality foods and inadequate intakes of micronutrients such as iron, zinc, iodine and vitamin A are common occurrences in developing countries (Black, Allen, Bhutta, Caulfield, de Onis, Ezzati, Mathers & Rivera, 2008).

Ideally, the food given to young children should give the relevant nutrients in the right quantities required for growth and development (Dewey & Brown, 2003). In South Africa, as in many other developing countries, malnutrition in food insecure areas is caused by poor food accessibility which leads to the inadequate dietary intakes among children (Labadarios et al., 2000). According to a 2008 study in South Africa, 52% households nationally were food insecure and experiencing hunger with only 20% being food secure (Labadarios et al., 2008). The children's diets were found to contain less than 50% of the recommended dietary allowance (RDA) for energy, calcium, iron, zinc, selenium, vitamin A, vitamin D, vitamin C, vitamin E, riboflavin and niacin (Labadarios et al., 2000).

Commercial fortified ready-to-eat (RTE) foods intended for young children are mostly nutritionally adequate in terms of quality and the quantities per serving as they are fortified by adding minerals and vitamins (Porter & Shafritz, 1999). However, the cost of such products may be generally out of reach for people in developing countries, more especially those in rural communities. Lack of access to commercially available infant and young children foods leads to situations where children are fed locally available monotonous diets made from the local staples especially cereals (Egli, Davidsson, Zeder, Walczyk & Hurrell, 2004).

The main staple foods used in developing countries are composed of cereals, roots and tubers (Gibson, Ferguson & Lehrfeld, 1998). Locally produced cereals, roots and tubers offer a valuable source of nutrition for rural people. To supplement the cereals, roots and tubers, legumes are often used to add a protein component and improve the nutritional value of the meal (Egli et al, 2004; Gibson, Perlas & Hotz, 2006). These plant foods also often contain antinutrient components which affect the absorption and bioavailability of some nutrients (Gemede & Ratta, 2014). Consumption of nutritionally inadequate plant foods that contain antinutrients is widespread in Africa (Stephenson, Amthor, Mallowa, Nungo, Maziya-Dixon, Gichuki, Mbanaso & Manary, 2010).

However, with adequate processing, indigenous foods can contribute significantly to a nutritionally balanced diet by supplying essential vitamins and minerals (FAO, 1988). They not only provide nutrition but also contribute to food security, create jobs, and help with income generation (Bille & Shemkai, 2006). In Africa, such indigenous foods include sorghum and cowpea.

Sorghum (*Sorghum bicolor* [L.] *Moench*) is an important staple cereal crop indigenous to Africa (FAO/ICRISAT, 1996). Sorghum is genetically suited to arid and semi-arid conditions of Africa where other crops cannot grow. Sorghum serves as a major source of nutrition for food insecure communities in Africa. It is, however, a poor source of protein, especially the essential amino acid lysine (Serna-Saldivar & Rooney, 1995). This shortfall is often overcome by supplementing sorghum with legumes such as cowpeas which are a good lysine source.

Cowpeas (*Vigna unguiculata*) are pulse type legume similar to common beans also indigenous to Africa (Taiwo, 1998). Like sorghum, cowpeas are also well adapted to growing in semi-arid regions. Several parts of the cowpea that serve as a good sources of protein and minerals can be consumed. Young cowpea leaves are used as a relish and the grain can be consumed providing a good source of micro- and macronutrients (Mamiro, Mbwaga, Mamiro, Mwanri & Kinabo, 2011).

Together with nationally and globally endorsed interventions to address malnutrition such as supplementation, fortification and dietary diversification, there is a need for greater collaboration and strategies to improve infant and child nutrition especially in developing countries. This study therefore serves as an investigation into how non-traditional technologies can be used to eliminate antinutrients, minimally process and deliver nutritionally adequate foods made from locally available staples such as sorghum and cowpeas. The aim is to formulate a product using high throughput equipment that will benefit the people that are food insecure where these grains are consumed.

1.1 Structure of the thesis

This PhD thesis is a presentation of independent original work that seeks to make a meaningful contribution to scientific knowledge. The thesis is presented in scientific paper type format which consists of two articles. Each article deals with an objective that was investigated as a sub-study and then presented in the form of a journal publication. The thesis is presented in the technical style, dialect and referencing method according to the style stipulated by the Department of Food Science, University of Pretoria. The work in sub-Chapter 4.1 has been published in LWT-Food Science and Technology and the work in sub-Chapter 4.2 is has been submitted to a journal for peer review.

The first three chapters of the thesis comprise an introductory chapter which gives background to the problem and why the study was done (Chapter 1). Chapter 2 is the literature review that gives an account of what has been published and is currently available in literature that are related to the proposed topic. The literature review chapter helps to identify the gaps in knowledge. Chapter 3 comprises the hypotheses and objectives which are an explanation of what the research problem is and what the possible outcome of the research or expected outcome is using supporting evidence from literature to explain the logic.

Chapter 4 has the two research sub-Chapters. The first sub-Chapter (4.1) investigated the influence of high temperature- short time (HTST) processing, compositing and adding a cooked cowpea leaf relish on the protein nutritive value of the formulated ready-to-eat meal. The second sub-Chapter (4.2) investigated the effects of HTST processing, compositing and the addition of the cooked cowpea leaf relish on the iron and zinc nutritive value of a ready-to-eat porridge aimed at preschool aged children. Chapter 5 discusses and summarises the importance of the findings of this study and a conclusion and recommendations for further studies are given in Chapter 6. Chapter 7 comprises a list of all the references that were used.

2 Literature review

Malnutrition contributes to high levels of mortality and morbidity among young children in developing countries. The leading causes are the poor quality and quantity of the diets given to children. Cereals and legumes such as sorghum and cowpea are good sources of nutrition and they are used as the main dietary components in developing countries. Exploration of the nutritional contribution of these important foods has been carried out through multi-sectorial food-based strategies addressing malnutrition. Even with these efforts, malnutrition is still a major problem facing many developing countries. This literature review will therefore focus on the causes of malnutrition and nutritional deficiencies that affect children in developing countries and interventions that are used to address the nutritional deficiencies. Additional topics that will be covered include; infant and young child feeding practices, importance of indigenous foods, antinutrient factors in sorghum and cowpea and processing methods of cereals and legumes. This will help to identify where gaps still exist in trying to address the problem of nutrition in developing countries, especially in sub-Saharan Africa.

2.1 Malnutrition

Malnutrition is the lack of proper nutrition (Blössner & de Onis, 2005). It includes both over and undernutrition. Malnutrition, however, tends to be primarily used synonymously with undernutrition (Saunders & Smith, 2010). Undernutrition comes about as a result of inadequate food intake and is the most common form of malnutrition in developing countries (Saunders & Smith, 2010). For example, seven percent of the children born in Africa are at risk of dying before the age of five years, which is almost seven times higher than in Europe (1.2%) (World Hunger Education Services, 2015). In Africa, undernutrition is responsible for more than half of the deaths in children under five year of age (Black, Allen, Bhutta, Caulfield, de Onis, Ezzati, Mathers & Rivera, 2008). In 2014, it was estimated globally that about 161 million children under the age of five years were stunted and almost 99 million were underweight, while 51 million were wasted (UNICEF/WHO/World Bank, 2014).

The UNICEF conceptual framework of malnutrition (Figure 2.1) (UNICEF, 1990) gives a visual presentation of the different factors that lead to malnutrition. The causes of malnutrition are classified into basic, underlying and immediate factors. They can either be environmental, household, individual or political factors that affect the overall health

(Bizouerne, 2005). The following section will briefly review each cause of malnutrition according to the UNICEF conceptual framework.

2.2 Causes of malnutrition

The conceptual framework helps to broaden the understanding of each of the causes of malnutrition. Consideration is given of possible factors from a macro level up to a micro level. At the macro level, are those factors that indirectly contribute to malnutrition, which include the basic and underlying causes (Schuftan, 2009). At the micro level are the factors which directly relate to nutritional problems. These are the immediate causes of malnutrition.

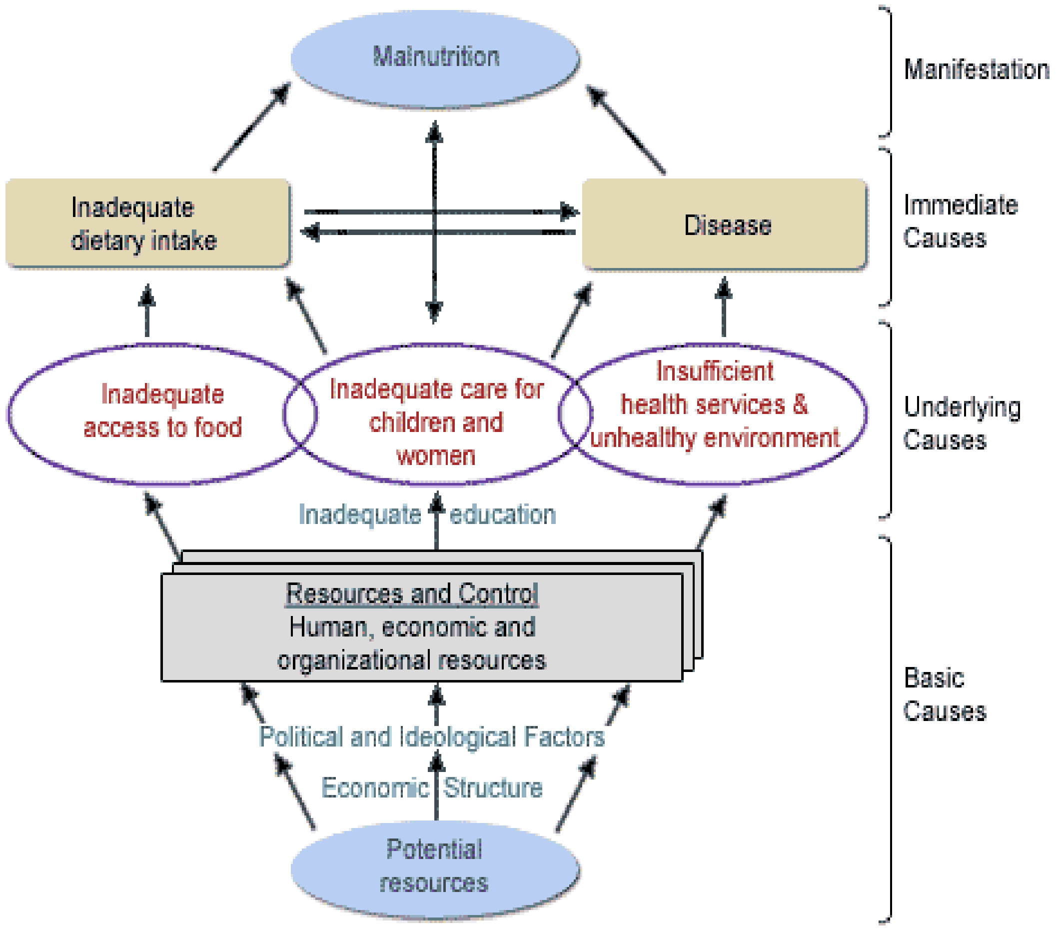

Figure 2.1: The UNICEF conceptual framework of malnutrition (UNICEF, 1990).

2.2.1 Immediate causes of malnutrition

The immediate causes of malnutrition are those factors that can lead directly to malnutrition and death. These factors include inadequate dietary intake and disease (Black et al., 2008). If inadequate dietary intake (nutrient supply) and disease exist together uncontrolled, they lead to the risk of developing diseases such as wasting and stunting and diabetes later in life (Bizouerne, 2005; UNICEF, 2012).

The connection between diseases and malnutrition has been described as the vicious cycle of malnutrition and infections (Tomkins & Watson, 1989; Bhaskaram, 2002). The interaction between malnutrition and infection leads to poor disease resistance, the development of illness, strains the body's immunity and reduces the body's capacity to fight off the illness. The reduction in immunity increases an individual's susceptibility to other diseases (Katona & Katona-Apte, 2008).

2.2.2 Underlying causes of malnutrition

Underlying causes of malnutrition include those characteristics of a household that increase an individual's susceptibility to malnutrition. They include factors that prevent individuals from accessing proper nutrition in the household (Reinhardt & Fanzo, 2014). The underlying causes of malnutrition are: inadequate access to food, inadequate child and maternal care, poor access to healthcare, unsanitary environment and poor water supply (UNICEF, 2005).

Underlying causes of malnutrition mostly affect women and children as they are the most vulnerable groups (Benson, 2005; Heady, 2013). The excessive work load placed on women reduces their ability to produce enough food and take care of their families. Women headed households are therefore more likely to be food insecure than male headed ones (Ndobo & Sekhampu, 2013).

In Africa, food insecurity has been found to be more pronounced in rural than in urban households (Bogale & Shimelis, 2009). The education level, gender and marital status of the head of a household affect food security of a rural household (Ndobo & Sekhampu, 2013). Additional contributing factors to food insecurity especially for farming communities include the income from farm production, distance to markets, dependency ratio, location and overall household income (Kaloi, Tayebwa & Bashaasha, 2005).

In South Africa, food insecurity remains a big challenge especially in female-headed households living in small-farm households in rural areas (HSRC, 2012). Using the Household Food Insecurity (Access) Scale (HFIAS) to assess the level of food insecurity in the Limpopo province, food insecurity was highest in households with a low monthly household income (<R2098.47), low income per capita (<R332.68), a large household

(number of people) and those with a young head of the household (De Cock, D'Haese, Vink, van Rooyen, Staelens, Schönfeldt & D'Haese, 2013). Lack of transportation and the high transportation costs (when available) are some of the problems experienced by rural people (van der Zijpp, Wilke & Carsan, 2010).

A study in Ghana examining the effects of giving attention by caregivers and mothers to the children's needs (Nti & Lartey, 2008) found that giving attention to children's needs resulted in well-nourished children compared to those who received little or no attention. In Botswana, older children were found to be more likely to have poor nutritional status than infants (Gobotswang, 1998). The reason for the inequality was that less time was spent by the mothers or caregivers feeding and taking care of their older children than the younger children.

2.2.3 Basic causes of malnutrition

The basic causes of malnutrition are affected by the structures at a national or societal level that are involved with the organisation or governing a population (Smith, Ramakrishnan, Ndiaye, Haddad & Martorell, 2003). Basic causes are not closely connected to the individuals that they affect because they are indirectly connected to malnutrition (Schuftan, 2009). The basic causes affect all individuals that are part of a country or community.

The basic causes of malnutrition can be found at the political, economic, cultural, religious and institutional structures which govern society (UNICEF, 2005). The basic causes give an indication of whether a country has the capacity to take care of the health of the most vulnerable groups in the country who are children, the aged and women (UNICEF, 2003a). Countries with better governance and political stability try to bridge the inequality gap by making sure that basic resources such as food are available to the poorer sections of the population (Pridmore & Hill, 2009). Developed countries have had more success with making sure that basic resources are available to the poor than developing countries. The way in which a country or community provides and distributes its most basic resources impacts on the nutritional status of the population (Benson, 2005). This is observed when the nutritional status of children is compared between developing and developed countries.

Following this discussion on the causes of malnutrition the common types of malnutrition among children in developing countries will be examined.

2.3 Forms of malnutrition that affect children in developing countries and children's nutritional requirements

Malnutrition in children is manifested in the forms of stunting, wasting, undernutrition and micronutrient deficiencies (Food and Agriculture Organization of the United Nations & Food and Nutrition Division, 1997; WFP, 2015). Nutritional deficiencies commonly found in children younger than five years in developing countries are protein-energy malnutrition (PEM), iodine deficiency, vitamin A deficiency (VAD) and iron-deficiency anaemia (IDA) (Black et al., 2008).

An analysis of risks to health in 2002 by the Global Burden of Diseases ranked iron, zinc and vitamin A deficiencies ninth, eleventh and thirteenth, respectively among 25 major causes of morbidity and mortality in different populations and geographic regions (Ezzati, Lopez, Rodgers, Vander Hoorn & Murray, 2002). The analysis also found that in the developing regions, the leading causes of death among children and mothers included underweight (15%), iron deficiency (3%), vitamin A deficiency (3%), and zinc deficiency (3%). Micronutrient malnutrition is caused by either not consuming enough micronutrients, frequent micronutrient losses and high/increased bodily demands for micronutrients (FAO/ILSI, 1997). Micronutrient deficiencies are characterised by a long-lasting lack of vitamins and minerals, which may not be immediately identified (Kennedy, Nantel & Shetty, 2003). The existence of unidentified micronutrient deficiencies is termed the "hidden hunger".

Because their deficiencies are among the most prevalent in developing countries affecting a high proportion of children in Africa and Southeast Asia (Black, Victora, Walker, Bhutta, Christian, de Onis, Ezzati, Grantham-McGregor, Katz, Martorell & Uauy, 2013), iron, zinc, vitamin A, iodine and protein and energy will be reviewed. These nutrient deficiencies are also a concern in South Africa (Labadarios et al., 2008).

2.3.1 Iron requirements and deficiency

Infants are born with adequate iron stores and continue to receive an adequate supply of iron from the mother's breast milk (WHO/FAO, 2004). Supplementation is therefore not required for exclusively breastfed infants during the first six months. From six months onwards, iron requirements are considerably increased due to the rapid growth, and there is a need to supplement iron with the diet (WHO/UNICEF/UNU, 2001).

Unlike adults, the iron requirements for male and female children are the same up to the age of 10 years (WHO, 2001b). For children aged six to 12 months, with an average body weight of nine kg, the recommended intakes at different levels of bioavailability (15, 12, 10 and 5% bioavailability) are 6.2, 7.7, 9.3 and 18.6 mg/day (Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004). For children aged one to three years (13 kg) and four to six years (19 kg) the recommended intakes at the different bioavailability levels are 3.9, 4.8, 5.8 and 11.6 mg/day; 4.2, 5.3, 6.3 and 12.6 mg/day respectively.

Iron deficiency is defined as the lack of mobilisable iron stores (WHO/UNICEF/UNU, 2001). Iron deficiency is a state in which the body has insufficient iron to produce haemoglobin and to supply the body with oxygen. Severe iron deficiency leads to anaemia (Cançado, 2012; Sanou & Ngnie-Teta, 2012). Among the many causes of anaemia, iron deficiency is the leading cause (de Benoist, Cogswell, Egli & McLean, 2008). Other causes of anaemia include heavy blood loss from menstruation (not applicable to children), maternal-foetal bridge of iron deficiency, parasite infections such as hookworms, ascaris, and schistosomiasis (Miller, 2013), and acute and chronic infections that lower haemoglobin concentrations (malaria, cancer, tuberculosis, and HIV) (de Benoist et al., 2008). Deficiencies of vitamins A and B12, folate, riboflavin and copper can increase the risk of anaemia (Tulchinsky, 2010).

Between 1993 and 2005, the global prevalence of anaemia was highest among preschool-children (aged zero to five years) at 47% (293 million) (McLean, Cogswell, Egli, Wojdyla & de Benoist, 2009). Anaemia prevalence among children was high in five sub regions of the world which were, South East Asia II (Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Maldives, Myanmar, Nepal) (66%), Eastern Mediterranean (63%), Africa (60%), Southeast Asia I (Indonesia, Sri Lanka, Thailand) (49%) and Latin America (46%) (Stoltzfus, 2003).

In 2013, the South African National Health and Nutrition Examination Survey (SANHANES-1) reported the prevalence of iron deficiency and anaemia in children under the age of five years as 10.7% and 1.9%, respectively (Shisana et al., 2013). In a smaller study in Vhembe district, Limpopo province, South Africa, a high prevalence of iron and zinc deficiency in rural preschool children, aged three to five years was found (Motadi, Mbhenyane, Mbhatsani, Mabapa & Mamabolo, 2015). The Vhembe study found a somewhat higher prevalence of iron deficiency (28%), which was attributed to poor socioeconomic situations, poor nutritional qualities of diets and insufficient food intake.

2.3.2 Zinc requirements and deficiency

Zinc is a trace mineral involved in all major human biochemical pathways (Hotz & Brown, 2004). Similar to iron requirements, the need for zinc for the growing body increases when breastfeeding ceases. Breast milk provides enough zinc for the first six months of life (Krebs, 2000). The recommended nutrient intake (RNI) for dietary zinc (mg/day) required to meet the normative storage requirements at different zinc bioavailability for children aged one to six years is 2.4-2.9 mg/day at high bioavailability, 4.1-4.8 mg/day at medium and 8.3-9.6 mg/day at low bioavailability (WHO/FAO, 2004).

High intakes of cereal and legume foods with high phytate contents inhibit the absorption of zinc (Brown, Wuehler & Peerson, 2001). Low zinc intake, however, is not common in diets containing sufficient animal foods. Zinc deficiency is a condition that exists when the levels of zinc intake fall below the body's requirement (Deshpande, Joshi & Giri, 2013). Zinc deficiency in children impairs immunity and increases the incidence of infections, growth failure and impaired neurobehavioral development (Brown et al., 2001). Zinc deficiency is also associated with the risk of morbidity and mortality related to diarrhoea, pneumonia and malaria in children (Caulfield & Black, 2004).

Globally, zinc deficiency is estimated at 17.3% (Wessells & Brown, 2012). Regions with the highest risk are South Asia (29.6%), closely followed by sub-Saharan Africa (25.6%). The 1999 National Food Consumption Survey in South Africa revealed inadequate zinc intake in 52–69% of all children aged one to nine years that were included in the study sample (Labadarios et al., 2000). Zinc intake was even lower in the diet of children living in rural areas. A similar observation was made in rural KwaZulu-Natal, in two to five year old children (Faber, Jogessar & Benadé, 2001). A recent study revealed a high prevalence of zinc deficiencies in preschool children aged three to five years in Vhembe district, Limpopo province (42.6%), mainly affecting girls (Motadi et al., 2015).

2.3.3 Vitamin A requirements and deficiency (VAD)

Vitamin A is a fat soluble vitamin whose physiological functions include maintaining good vision, cell differentiation, gene regulation and immune system functions (Gallagher, 2008). The recommended vitamin A intakes for children aged two to three and four to five years are 400 and 450 μg RE/day respectively (Joint FAO/WHO Expert Consultation on human vitamin and mineral requirements, 2004). Dietary sources include preformed sources of vitamin A which are found in animal foods such as liver and pro-vitamin A carotenoids in coloured fruits, vegetables and red palm oil. Vitamin A deficiency is diagnosed when the concentration of serum retinol falls below 0.70 mmol/l (Rice, West Jr. & Black, 2004).

In 2004, it was reported that 40% of all children under the age of five years from developing countries had a vitamin A deficiency (UNICEF, 2004). An earlier report found that more than 70% vitamin A deficient children were in the regions of South Asia and sub-Saharan Africa (Mason, Lotfi, Dalmiya, Sethuraman & Deitchle, 2001). In 2013, the prevalence of vitamin A deficiency in South Africa was estimated at 43.6%, which had improved from the prevalence reported in the year 2005 of 63.6% (Shisana et al., 2013).

2.3.4 Iodine requirements and deficiency

Iodine is an important part of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) (Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, 2004). The recommended iodine intake for children aged zero to 59 months is 90 µg/day (WHO/UNICEF & ICCIDD, 1996). Dietary sources of iodine include fish, seafood, kelp, drinking water and vegetables grown in iodine sufficient soil and iodated table salt (Vitti, Ross & Mulder, 2014).

Iodine deficiency is diagnosed when serum iodine levels fall below recommended levels (WHO, 2001a) resulting in the failure of the thyroid gland to synthesize enough thyroid hormone. Severe iodine deficiency leads to development of goitre and cretinism (Delange, 1994; 1996) and is the leading cause of brain damage, psychomotor retardation, intellectual impairment and mental retardation in children (Eastman & Zimmermann, 2009).

In 2002, the Eastern Mediterranean region had the highest prevalence of iodine deficiency (74%), followed by Africa (50%) (Ramakrishnan, 2002). In 2006, two million people worldwide were estimated to have inadequate iodine nutrition, with the global prevalence of iodine deficiency estimated at 35% and 43% in Africa (Allen, de Benoist, Dary & Hurrell, 2006). In 2008, it was reported that an estimated 32% school-age children had insufficient iodine intake (de Benoist, McLean, Andersson & Rogers, 2008). Of the children estimated to be iodine deficient, 73 million were living in South-East Asia and 58 million were living in Africa.

Mandatory iodisation was introduced in South Africa in December 1995 and has had positive results. The mandatory iodisation programme in South Africa helped to improve iodine status in areas where mild iodine deficiency had previously been identified (Jooste & Joubert, 2004). Mandatory salt iodisation with regular monitoring of the iodine content of retail salt in South Africa has been successful in ensuring that salt consumers meet the recommended level of iodine through consuming iodized table salt (Jooste, 2004).

2.3.5 Protein and energy requirements and Protein Energy Malnutrition (PEM) in children

Energy and protein in childhood are required for height and weight development, maturation of bodily functions and maintenance thereof (Joint FAO/WHO/UNU Expert Consultation, 2004). Adequate energy is required from childhood in order to meet the rapid energy expenditure and the requirements for growth and development.

In order to meet requirements of growing children, at infancy; from six to eight months the required average intake is 836 kJ/day, at nine to eleven months, the requirements are some 1254 kJ/day and for children aged 12–24 months, 2299 kJ/day (Dewey & Brown, 2003). It is recommended that a child's diet must at least provide an average energy amount of 4.18 kJ/g/food in order to meet the energy requirements when served in the recommended quantities for the age group. It is also recommended that 20-30% of the total energy intake must be provided by healthy fats (mono and polyunsaturated), 10-15% must come from protein and 50-60% from carbohydrates (Institute of Medicine, 2005; Thompson, Manore & Vaughan, 2005).

The relative protein requirement (by body weight) is higher for infants and younger children and decreases as they grow older. Safe protein intake for children aged two to five years ranges between 0.85 to 0.97 g protein/kg body weight per day (WHO/FAO/UNU Expert Consultation, 2007). The minimum protein intake must be adequate enough to balance the losses of nitrogen and maintain energy balance at minimum levels of physical activity while supporting tissue growth (Joint FAO/WHO/UNU Expert Consultation, 1985).

Amino acids are important as they are required for the synthesis of protein and for regulating key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity (Wu, 2009). Availability of all the essential amino acids in the diet is of critical importance. Lysine, however is often deficient in cereal based diets which are commonly consumed in developing countries (Fürst & Stehle, 2004). Lysine is an essential (indispensable) amino acid and is often the first-limiting amino acid. The lysine requirement by children aged one to two years is 45 mg/g protein/ day and requirement for children aged three to 10 years is 35 mg/g protein/ day (WHO/FAO/UNU Expert Consultation, 2007).

Inadequate energy and protein intake can lead to an energy imbalance, which in severe cases can lead to the development of PEM (Reilly, 2002). PEM is characterised by a decrease in body fat and muscle mass resulting in conditions of low weight for height, low height for age and low weight for age among children younger than five years of age (Tulchinsky & Varavikova, 2014). Anthropometric indices in the forms of stunting, wasting and underweight are therefore used to describe the different types of and to diagnose PEM in young children (Rice, Sacco, Hyder & Black, 2000). PEM is associated with increased morbidity and mortality and is among the top ten causes of years of life lost in developing countries such as Rwanda, Somalia, South Sudan, Tanzania, Zambia and Uganda (Global Burden of Disease, 2013).

2.3.5.1 Classification of PEM

Anthropometric indices are used to classify the nutritional status of children under the age of five years using the children's weight and height in comparison to the reference population. Anthropometric measures are interpreted using the z-score, percentile or percent median (WHO, 1995). The z-score (SD score) indicates how many standard deviations is the individual being measured far from the mean or median of reference population. The z-score is calculated as:

$$\frac{(\text{observed value}) - (\text{median value of the reference population})}{\text{standard deviation value of reference population}}$$

Using the statistically defined cut off values, stunting, wasting and underweight can be diagnosed when a child's height or weight falls below two standard deviations (<-2 SD) of the median weight for height or height for age or a combination of both (weight for age) (de Onis, 2000). Severe stunting or wasting can be diagnosed when a child's height or weight fall below three standard deviations (<-3) below the median (WHO, 2009).

- *Wasting* (Weight for height (WHZ)): Wasting is an indication of severe undernutrition that lasts for short periods of time (Wang & Chen, 2012). Wasting is a sign of fat mass loss to levels below the reference value (WHO Working Group, 1986). Moderate wasting is defined using a z-score <-2 and severe wasting <-3 (WHO, 1999). Using the WHO standards, wasting numbers among infants under six months of age in developing countries are some two and a half million for moderately wasted and three million for severely wasted (Kerac, Blencowe, Grijalva-Eternod, McGrath, Shoham, Cole & Seal, 2011). Estimates of wasting prevalence among children in South Africa are six percent (UNICEF, 2013). Amongst grade one learners in the North-West province of South Africa, wasting prevalence was almost seven percent due to poor household's food security, unavailability of school feeding schemes and socioeconomic status (Kruger, Pienaar, Coetzee & Kruger, 2014).

- *Stunting* (Height for age (HAZ)): Stunting is an indicator of low height for age (Pelletier, 1994; UNICEF, 2013). Stunting is a result of prolonged periods of inadequate nutrition during early life resulting in the slowing down or suppression of skeletal growth (WHO Working Group, 1986). Moderate stunting is defined by a z-score of <-2 while severe stunting is defined a z-score of <-3 (WHO, 1999). The estimated prevalence of stunting in preschool children in the years 1990 and 2005 globally was 34% and 24%, respectively, while in Africa it was 37% and 35% (de Onis, Blössner, Borghi, Morris & Frongillo, 2004). Recent South African country statistics reported stunting at 33% in children under five years (UNICEF, 2013). The South African National Health and Nutrition Examination Survey (SANHANES-1) reported that nationally, young children aged zero to three years had the highest prevalence of stunting of up to 26.9%. Among the boys, those living in rural informal areas were more stunted (23.2%) while girls living in urban informal areas were the most affected by stunting (20.9%) (Shisana et al. 2013). In the rural north eastern parts of South Africa, stunting was found to be the most frequent form of undernutrition in children aged one to four years (Kimani-Murage, Kahn, Pettifor, Tollman, Dunger, Gómez-Olivé & Norris, 2010).
- *Underweight* (Weight for age (WAZ)): Underweight includes elements of both stunting and wasting (WHO, 1995). Underweight can begin early in a child's life

and worsens with age (WFP, 2005). The worldwide prevalence of underweight in children under the age of five years in 2013 was estimated at 99 million (UNICEF/WHO/World Bank, 2014). Asia has twice the number of underweight children (66%) to those found in sub-Saharan Africa (33%) which has the second highest numbers. In South Africa, statistics for moderate underweight in 2013 were estimated at nine percent (UNICEF, 2013). The percentage of underweight amongst grade one learners in the North-West province of South Africa was found to be four percent (Kruger et al., 2014).

2.4 Feeding practices and food intakes of young children

Infant and young child feeding (IYCF) in the first 1,000-days of life is important in order to achieve the proper developmental health and the future wellbeing of the growing child (World Health Organization Department of Child and Adolescent Health and Development, 2008). The first 1,000-days of life are initiated at the beginning of the pregnancy and last until the child's second birthday. IYCF focuses on continued exclusive breastfeeding for the first six months followed by the introduction of appropriate soft, semi-solid and solid foods with continued breastfeeding and the introduction of balanced family meals (UNICEF, 2013).

Recommendations for feeding children under five years that have been extensively documented are very age specific and only include exclusive breastfeeding for the first six months and complementary feeding with continued breastfeeding from zero to 23 months (Ruel, Brown & Caulfield, 2003). The limited age range for the child feeding guidelines is a challenge when deciding on what guidelines to use for feeding children over the age of two years. The challenge is because of the age specificity of guidelines which do not include children from 24-59 months, even though the age range is still within the critical ages of rapid development (under five years).

The newly proposed paediatric food-based dietary guidelines (FBDG) for South Africa (Naidoo, 2013; Bourne, Pilime, Sambo & Behr, 2013) which support the earlier published guidelines for infants and young children (Bourne, Marais & Love, 2007) have helpful recommendations to support proper child growth. The guidelines have been specifically designed to accommodate children's growth and developmental needs taking into account the cultural diversity of South Africans (Love, 2001). According to the guidelines, children aged six to 12 months should be introduced to solids with continued breastfeeding. By the age of

12 months, all the solids must have been introduced to ensure children partakes in family meals and enjoys a variety of meals.

Even though there are no specific recommendations for feeding children from the age of 24 months, there are benefits of a good start in life if the guidelines for exclusive breastfeeding and complementary feeding from zero to 23 months are observed (UNICEF, 2003b). Burgess & Danga (2008), however, acknowledged that as undernutrition can be a common problem in children older than 23 months, the risk might be slighty lower compared to younger children as older children have developed immunity to fight off infections. Continued adequate good nutrition, therefore remains the overall aim for a healthy body at any age.

Cereals, roots and tubers remain the main sources of nutrition in developing countries (FAO, 2005), and are widely supplemented with legumes to provide the protein and mineral components (FAO, 1992). The low nutrient content of traditionally prepared children's foods in developing countries is often due to the extreme watering down of the cereal gruel (Gibson, Ferguson & Lehrfeld, 1998). Even in more recent years, children in developing countries continue to receive nutritionally inadequate diets that are given in small quantities resulting in insufficient nutrition (Lutter & Rivera, 2003; Kimmons, Dewey, Haque, Chakraborty, Osendarp & Brown, 2005).

Traditionally, in Kenya, cassava, sorghum and finger millet are ground into flour and used to prepare gruel for children aged three to four months (K'Okul, 1991). In Ethiopia, a cereal gruel together with cow's milk has been used as a weaning food (Bekele & Berhane, 1998). However it did not sufficiently meet the children's nutrient requirement.

Introduction of foods from the age of six months is done using one food type at a time (Van der Merwe, Kluyts, Bowley & Marais, 2007). Even though the FBDG have been made available in South Africa, one study found that in some cases solids were being introduced as early as five weeks, six weeks or two months old (MacIntyre, De Villiers & Baloyi, 2005; Van der Merwe, 2012; Kruger & Gericke, 2003). The first solid food introduced was an overcooked, over-diluted maize soft porridge. By seven or nine months the children were already introduced to a mixed family diet. The South African National Food Consumption Survey for children aged one to nine years reported that the most commonly consumed food item by the children was maize porridge (Labadarios et al., 2000).

In South Africa, the diet of children aged between two to six years in rural areas has been found to be insufficient in energy, calcium, iron, zinc, niacin, riboflavin, thiamine, vitamins A, B6, C and D content (Dannhauser, Bester, Joubert, Badenhorst, Slabber, Badenhorst, Du Toit, Barnard, Botha & Nogabe, 2000; Labadarios et al., 2000). More recently, the SANHANES-1 study also found that the diets of children in similar age groups had insufficient vitamin A, resulting in 44% vitamin A deficiency among children (Shisana et al., 2013). Similarly in Ethiopia, complementary foods were reported to be deficient in protein, carbohydrates, fat, minerals and vitamins (Temesgen, 2013). Similar to the Ethiopian diet, children in Samfya District in Zambia had insufficient energy, protein, calcium, iron and vitamin A in their diet (Hautvast, van der Heijden, Luneta, van Staveren, Tolboom & van Gastel, 1999).

2.5 Ready-to-use therapeutic foods (RUTF)

RUTF are effective and safe foods used at home to rehabilitate severely malnourished children (Manary & Manary, 2005). They provide the energy density that is sufficient for correcting a nutritional deficiency. Home-based therapeutic treatments are more successful than the standard inpatient therapy as they are more cost effective than the commercial ones (Ashworth & Khanum, 1997; Collins, 2001). A locally produced energy-dense RUTF yielded positive results when caregivers were taught how to make and administer high calorie cereal milk in India (Singh, Kang, Ramachandran, Sarkar, Peter & Bose, 2010). In Malawi, RUTF were found to achieve positive results in 95% of HIV-negative malnourished children and malnourished children (Manary, Ndkeha, Ashorn, Maleta & Briend, 2004; Ciliberto, Sandige, Ndekha, Ashorn, Briend, Ciliberto & Manary, 2005). In Niger, RUTF was successfully used with a minimum number of defaulters when compared to children receiving treatment at therapeutic feeding centres where a high number of defaulters was recorded (Gaboulaud, Dan-Bouzoua, Brasher, Fedida, Gergonne & Brown, 2007).

2.6 Commercial foods for young children

In developing countries the first foods given to young children include local staples, mainly cereals (Onofiok & Nnanyelugo, 1998). In some countries, for example such as Ghana, some of these traditional foods have been commercialised to achieve a wider reach to include people who would not normally consume the traditional products (Nagai, Staatz, Bernsten, Sakyi-Dawson & Annor, 2009).

Even though commercial cereals for children are widely available in developing countries, their use is limited by their high cost (Lutter, 2003; Owino, Amadi, Sinkala, Filteau & Tomkins, 2008). Hence, food insecure households are the least likely to use commercial foods, choosing instead traditionally prepared foods (Owino et al., 2008). Creating fortified children's foods that are locally produced at a low-cost can help to increase their use and provide nutrition for young children (Nestel, Briend, de Benoist, Decker, Ferguson, Fontaine, Micardi & Nalubola, 2003).

While use of fortified commercial children's foods may be an ideal option for improving young children's nutrition, some researchers have advised against using commercial foods over home prepared foods. Children given commercial foods as part of the first foods are less likely to accept home prepared food when it is later introduced in their diet (Cameron, Taylor & Heath, 2013).

2.7 Nutrition intervention strategies to combat micronutrient deficiencies

Various types of interventions are used to combat malnutrition. Interventions aimed to increase the supply of micronutrients include increasing production and consumption of micronutrient rich foods; food fortification; supplementation and global public health interventions (FAO/ILSI, 1997). Agriculture interventions, for example home gardening (Berti & FitzGerald, 2004) and supplementation (Jukes, 2005), have been successfully used together with nutrition education to improve nutrition. All the various intervention strategies complement each other and no single strategy is able to meet optimal nutrition on its own. This section therefore provides an overview of the commonly used approaches for reducing malnutrition.

2.7.1 Micronutrient fortification

Food fortification is an intervention that involves adding nutrients to commonly eaten foods at levels higher than those found in the original food (FAO/ILSI, 1997). Food fortification can also be used to restore nutrients lost during food processing (Codex Alimentarius Commission, 1991). Fortification of staple foods has been used successfully in many countries to enhance the iron, folate, iodine, and zinc intakes (Thompson, 2007).

Food fortification reaches a much wider range of the population than other nutrition intervention strategies without imposing any significant changes in the consumption patterns of the target group (Allen et al., 2006). Also, because fortification uses the local staple foods consumed by the target population, it is considered a cost-effective intervention (Horton, Alderman & Rivera, 2008).

In South Africa, mandatory addition of essential nutrients is regulated by the government (Department of Health) for maize meal and wheat flour (The Department of Health South Africa and UNICEF South Africa, 2007). The staple foods that are used as fortification vehicles were identified in the 1999 National Food Consumption Survey for children (Labadarios et al., 2000). The fortification mix includes vitamin A palmitate, thiamine mononitrate, riboflavin, nicotinamide, pyridoxine hydrochloride (Vitamin B6), folic acid, electrolytic iron and zinc oxide (The Department of Health South Africa and UNICEF South Africa, 2007).

Challenges, however, are experienced with food fortification in rural populations (Salgueiro, Zubillaga, Lysionek, Caro, Weill & Boccio, 2002). This is because the fortification food vehicle may not always be accessible to rural areas or it may not be a readily used food even though it may be available commercially. Poor infrastructure also poses a challenge in developing successful fortification programmes. Additional challenges with fortification programmes include poor stability of the micronutrients in the fortification mix for example light sensitivity of unstable micronutrients such as vitamin A, riboflavin and folic acid over time. It is therefore important that fortification programmes be monitored closely upon implementation with all the target food manufacturers, suppliers and importers (UNICEF South Africa, Department of Health and GAIN, 2008).

2.7.2 Micronutrient supplementation

Supplementation is defined as products in the forms of food, tablet or syrup containing either a vitamin, mineral or a herb that is given to an affected population to provide immediate relief from a nutritional deficiency (Thompson, 2012). It is a direct approach of delivering micronutrients of concern to an affected population (Harrison, 2010). It is the strategy which is often used to address nutritional deficiencies immediately (Allen et al., 2006). Supplementation provides nutrients in a highly absorbable form (Harrison, 2010).

Supplementation has an advantage as it reduces risks of overconsumption of the supplemented nutrient by segments of the population that are not affected by the deficiency of concern (Harrison, 2010). This is done by supplying the supplement directly to the portions of the population most affected. For example, in South Africa where anaemia is identified in children, supplementation from the ages of six to 12 months has been given consisting of 12.5 mg iron and 50 µg folic acid supplementation daily (Stoltzfus & Dreyfuss, 1998).

To address the widespread vitamin A deficiency affecting young children in developing countries vitamin A supplementation is recommended for children between six and 72 months of age (WHO, 1998). In South Africa, vitamin A supplementation was initiated after VAD was identified by the SAVACG survey (DOH, 2012). Vitamin A supplementation is given to children aged between six to 60 months twice a year. Such a supplementation programme has to be culturally acceptable and practical as a short term solution.

Successful implementation of supplementation programmes requires a closely monitored and effective distribution system where clients are cooperative (Allen et al., 2006). Monitoring systems to ensure the successful running and adherence are also required (Du Plessis, Najaar, Koornhof, Labadarios, Petersen, Hendricks & Kidd, 2008). Even with monitoring systems in place, lack of compliance from affected individuals remains a challenge. In a South African daily multi micronutrient supplementation study, the dropout rate was up to 27% over the duration of the intervention due to low compliance (Smuts, Dhansay, Faber, van Stuijvenberg, Swanevelder, Gross & Benadé, 2005). Community resettlements, lack of cooperation, lack of motivation, lack of supplies, lack of support and side effects especially in women taking iron supplements are among the reasons given for non-compliance in such programmes (Galloway & McGuire, 1994).

2.7.3 Food-based dietary modification and diversification strategies

Food-based strategies are economically feasible and culturally acceptable strategies that are appropriate for rural communities in that they can be sustained (Gibson, 1994). They can successfully be used to enhance availability of nutrients of locally available foods with no external resources necessary. Food-based dietary strategies also require less resource investments than single-nutrient based interventions such as supplementation and fortification. Further, they prevent multi-micronutrient deficiencies and pose fewer risks of interactions with other nutrients (Thompson & Amoroso, 2011).

A focus of the food-based intervention strategies is to increase dietary diversity (Ruel & Levin, 2000). Dietary modification and diversification can be achieved through combining activities in agricultural food production by plant breeding, biofortification of plant foods, using traditional processing, preservation, storage and preparation methods and using food combinations that complement each other to increase nutrient density (Tontisirin, Nantel & Bhattacharjee, 2002; Thompson & Amoroso, 2011). Other types of food-based interventions that have been successful include small livestock production such as chickens, goats, rabbits and guinea pigs and their by-products (eggs, milk) (Gibson, Yeudall, Drost, Mtitimuni & Cullinan, 2003).

Home processing methods such as soaking, sprouting (malting) or fermenting raw grains can be useful to eliminate or reduce antinutrients in grains (Yeudall, Gibson, Cullinan & Mtimuni, 2005; Gibson, 2007). Such processing eliminates or reduces the effects of antinutrients such as phytic acid and polyphenols in cereal-based meals. Enriching cereal-based porridges with legumes and adding ascorbic acid and provitamin A carotenoid-rich foods are also used to increase mineral absorption as a food modification strategy (Gibson, Perlas & Hotz, 2006).

To improve food nutrient density, in Bangladesh and Peru children have been given an energy-dense diet liquefied by adding amylase-rich flour (ARF) so that it was easy for the children to consume the meal without compromising the nutrient density (Bennett, Morales, González, Peerson, López de Romaña & Brown, 1999; Hossain, Wahed & Ahmed, 2005). Similarly, in the North-West province of South Africa, alpha-amylase has been added to an infant food to reduce its dietary bulk but still maintain the energy and nutrient density (den Besten, Glatthaar & Ijsselmuiden, 1998). In Jamaica, children received a gruel made from germinated sorghum flour (Stephenson, Gardner, Walker & Ashworth, 1994). The germinated sorghum flour provided a high energy and micronutrient dense gruel compared to the traditionally prepared thin gruel that was low in micronutrients and energy. This type of strategy facilitates consumption of a nutrient quality that would normally be impossible for such children (Gardner, Walker, Gavin & Ashworth, 2002). Energy-dense liquefied diets prepared using ARF increase the dietary intake of both malnourished (Rahman, Mitra, Mahalanabis, Wahed, Khatun & Majid, 1997) and healthy children (Islam, Peerson, Ahmed, Dewey & Brown, 2006). Alternatively, addition of sugar and margarine can be used to increase the energy density of porridges fed to malnourished and healthy children (Ashworth, 2002).

Vitamin A intake in rural villages can be improved by home-gardening programmes where butternut squash, carrots, orange-fleshed sweet potatoes, and spinach are planted, plus an education component to highlight the relation between vitamin A and health and integration with community-based growth-monitoring systems (Faber, Phungula, Venter, Dhansay & Benadé, 2002). The study reported an increase in serum retinol concentrations in the experimental village while there was no improvement seen in the control village.

The effectiveness of food-based strategies are strongly supported by the use of locally available indigenous plant foods. Sorghum and cowpea are such indigenous foods that can be used to improve protein, iron and zinc nutrition which will be explored by this research.

2.8 Importance of indigenous foods in rural diets

Indigenous foods are an important part of the diet of many rural African communities. Rural communities have maintained these indigenous foods as a source of subsistence and cultural heritage for years (Kinyuru, Konyole, Kenji, Onyango, Owino, Owuor, Estambale, Friis & Roos, 2012). They offer food security and nutrition especially in drought prone regions where other crops fail to thrive (Jacks, 1994). Due to the ability of indigenous crops to survive extreme agronomic conditions, they can be produced with minimal inputs (Orech, Aagaard-Hansen & Friis, 2007). Sorghum has been shown to display stay green traits, producing higher yield in extreme agronomic conditions such as during drought periods, compared to crops without the trait (Borrell, Mullet, George-Jaeggli, van Oosterom, Hammer, Klein & Jordan, 2014; Borrell, Hammer & Henzell, 2000). Further, some indigenous crops have income generation potential which could be beneficial in rural communities for maintaining food security. Indigenous foods are ideal because they add to the diversity and micronutrient supply in resource poor communities (FAO, 1995a).

According to the FAO (1988), indigenous foods can either be wild or cultivated in a community where they are used to provide food security and nutrition. In Africa, some of the important indigenous plant foods are sorghum, finger millet, teff, sweet potato, cowpeas, pigeon pea, bambara nuts and locust beans (FAO, 1988).

The importance of cereals, legumes, fruits and vegetables in addressing micronutrient deficiencies and chronic diseases cannot be stressed enough. The wavering food supply coupled with an increase in demand for food can be addressed by increasing the promotion and use of cereals, legumes, fruits and vegetables that are indigenous to the food deficient areas (Mastny, 2004). For example in South Africa, traditional leafy vegetables play an important role in the nutrition of vulnerable groups (Vorster, Jansen van Rensburg, Van Zijl & Venter, 2007). The leafy vegetables serve as an important food source that can be used fresh or dried in order to be consumed during times of food shortages when other food are scarce (Vorster & Jansen Van Rensburg, 2005).

Further, indigenous foods can increase the food supply, provide variety and improve the nutrient content of rural people's diet (Venter, Jansen van Rensburg, Vorster, van den Heever & van Zijl, 2007). In Sekhukhuneland, an arid area in Limpopo province South Africa, sorghum is the main crop and is therefore important in the area in terms of both production and consumption (Bichard, Dury, Schönfeldt, Moroka, Motau & Bricas, 2005; Department of Agriculture, Forestry and Fisheries, 2010). In Sekhukhuneland, sorghum is produced mainly for subsistence and not commercial purposes (NAMC, 2007). Sorghum and cowpea are used in the present research because they have the ability to offer a good source of nutrition in resource poor communities and they are reviewed below.

2.8.1 Sorghum

Sorghum is an important staple in the semi-arid tropic areas of Asia and Africa (FAO, 1995b). It is one of the oldest and most important cereals grown in Africa (FAO/ICRISAT, 1996). It provides nutrition for millions of people living in the arid and semi-arid regions. As indicated, in parts of South Africa such as the Limpopo province, sorghum is one of the most consumed cereals mainly as breakfast porridge (Bichard et al, 2005). Sorghum has additional health benefits such as being a good source of phytochemicals with anti-inflammatory properties (Awika & Rooney, 2004; Burdette, Garner, Mayer, Hargrove, Hartle & Greenspan, 2010).

However, as with most cereals, sorghum has a relatively low level of the essential amino acid lysine (Cordain, 1999). Complementing legume and cereal proteins helps improve the protein quality of plant-based protein foods to meet the required level and quality of essential amino acids and protein (Young & Pellett, 1994). To increase the protein and lysine contents of sorghum, sorghum and cowpea composite foods are a common combination that has been found to have a protein content comparable to commercially available products (Pelembe, Erasmus & Taylor, 2002). Products that are made from sorghum commonly consumed in Africa include breads, porridges, flatbreads, couscous, gruels, steam-cooked products, alcoholic and non-alcoholic beverages (Kulamarva, Sosle & Raghavan, 2009). Various preparation methods are also used in order to enhance sorghum's nutritional value.

2.8.2 Cowpea

Cowpeas, like sorghum, are cultivated in many parts of Africa (Ba, Pasquet & Gepts, 2004), including South Africa (Van Rensburg, Van Aver eke, Slabbert, Faber, Van Jaarsveld, Van Heerden, Wenhold & Oelofse, 2007). Cowpeas can also survive in temperate climates where other crops fail to survive. In South Africa, cowpeas are grown and consumed in KwaZulu-Natal, Mpumalanga and Limpopo provinces (Van Rensburg et al., 2007). Importantly, many parts of the cowpea plant can be consumed, including the young pods, seeds and leaves (fresh or dried).

Compared to cowpea with an average of 26.1 g/100 g (Rivas-Vega et al., 2006), soy contains 49.2 g/100 g (USDA, 2009) protein. Even though soy may contain a good quality protein compared to cowpea and is used as a good animal protein substitute (USSEC, 2006), it is not indigenous to Africa. Soy beans are indigenous to Manchuria, China, hence most of the soy bean in South Africa comes from imports (Department of Agriculture, Forestry and Fisheries, 2010). Of the amount produced in South Africa, 20% goes towards human consumption, 60% towards animal feed and only 20% towards oil and oil cake.

Cowpeas on the other hand are an important source of protein especially in the least developed regions of Africa and Asia. This is related to the fact that they are cheaper and more easily available than animal protein (Adebooye & Singh, 2007). Cowpeas are low in the essential sulphur-containing amino acid methionine (1.5 to 2.3 g/16 g N) but high in lysine (6.7 to 8.1 g/16 g N) (Kochhar & Walker, 1988). The high lysine content of cowpea proteins makes it a good complement to be used with cereals that have low lysine and have higher methionine content. Cowpeas have been used with sorghum to create various products with improved protein quality (Badi, Pedersen, Monowar & Eggum, 1990; Pelembe et al., 2002). Further, cowpeas are also a good source of antioxidants (Zia-Ul-Haq, Ahmad, Amarowicz & De Feo, 2013).

As mentioned, a major challenge with plant based foods is the presence of antinutrients which inhibit digestion, absorption and bioavailability. Antinutrients in sorghum and cowpea will be discussed briefly in the next section.

2.9 Antinutrients in sorghum and cowpea

Antinutritional factors are important metabolites produced by grains which often serve as a natural defence against attacks from insects, animals and other pathogens (Serna-Saldivar & Rooney, 1995). However, they can be harmful to human health as they affect the digestion and bioavailability of nutrients when consumed (Kataria, Chauhan & Punia, 1989).

2.9.1 Protease inhibitors

Trypsin and chymotrypsin inhibitors affect protease action in the digestive tract (Gemede & Ratta, 2014). Protease inhibitors in legumes block protease enzyme activity resulting in a reduction in the protein nutritional quality (Kochhar & Walker, 1988). Legumes contain varying levels of trypsin inhibitor activity (TIA), which in some cowpea varieties can be as much as 32 TIU/mg (Rivas-Vega, Goytortúa-Bores, Ezquerra-Brauer, Salazar-García, Cruz-Suárez, Nolasco & Civera-Cerecedo, 2006). Levels up to 58 TIU/mg have been reported in some grain legumes (Mulimani & Vadiraj, 1994). Trypsin inhibitors have also been identified in some sorghum varieties (Mulimani & Vadiraj, 1993).

Protease inhibitors can be decreased by applying different heating methods such as autoclaving at 121°C and one bar pressure for five and 10 minutes and in an oven (100 °C) for 10, 20, 30, 40 and 50 minutes (Mulimani & Vadiraj, 1993). A gradual decline in trypsin and chymotrypsin inhibitory activities has also been found when sorghum was sprouted over six days.

2.9.2 Phytic acid

Phytic acid (myo inositol hexaphosphate) reduces the nutritional value of foods by binding essential minerals, such as calcium, iron, and zinc. The phytic acid content varies between raw and processed cowpea grains and flours. Phytic acid levels in legumes such as cowpea can be reduced through leaching into water, especially during soaking or cooking (Lopez, Leenhardt, Coudray & Remesy, 2002). Phytate can be reduced by other processing methods such as fermentation which activates the endogenous phytase (Marfo, Simpson, Idowu & Oke, 1990). Sorghum has similar phytic acid content as cereals such as maize and finger millet (approximately 10 mg/g) (GarcõÂa-Estepa, Guerra-HernaÂndez & GarcõÂa-Villanova, 1999).

2.9.3 Polyphenols

Both sorghum and cowpeas contain varying levels of polyphenols which can affect the availability of minerals such as iron and zinc (Towo, Matuschek & Svanberg, 2006). The phenol content (phenolic acids, flavonoids and condensed tannins) where present in sorghum varieties can range very widely, between 170-10 260 mg/100g (Bravo, 1998). In sorghum, the polyphenols are concentrated in the pericarp (Dykes & Rooney, 2007). Sorghum varieties can be tannin (proanthocyanidins/anthocyanidins) or non-tannin containing where the tannin containing varieties have the highest level of polyphenols. Likewise, cowpeas contain varying levels of polyphenols ranging between 34-1710 mg/100 g (Bravo, 1998).

2.9.4 Oligosaccharides

Cowpeas also contain oligosaccharides in varying levels (Egounlety & Aworh, 2003). These include raffinose, stachyose and verbascose α-galactosides (Sreerama, Sashikala, Pratape & Singh, 2012). Oligosaccharides cannot be digested easily by humans hence they cause flatulence, abdominal cramps and diarrhoea due to their bacterial fermentation in the lower gut. These oligosaccharides can be reduced by processing technologies such as fermentation, soaking, dehulling, washing and cooking (Egounlety & Aworh, 2003).

2.9.5 Lectins (Haemagglutinins)

Cowpeas contain lectins which are proteins that bind reversibly to a specific mono- or oligosaccharide (Lis & Sharon, 1998). Lectins can cause nausea, bloating, vomiting and diarrhoea when consumed (Vasconcelos & Oliveira, 2004). Even though lectins are protein in nature, they are resistant to proteolytic enzyme action (Carbonaro, Cappelloni, Nicoli, Lucarini & Carnovale, 1997).

Other antinutrients found in grains include oxalates (oxalic acid which is a strong organic acid in both plants and animals responsible for the formation of kidney stones) (Çalişkan, 2000; Ogbonna, Abuajah, Ide & Udofia, 2012). Effective removal of the antinutrients can be achieved through the use of different processing methods which are discussed in the following section and summarized in Table 2.1.

2.10 Traditional processing methods

Cereals and legumes are processed using various methods. As stated, some of the processing methods can be used to effectively reduce antinutrients levels (Sathe & Salunkhe, 1981). These include methods such as decortication (dehulling), soaking, cooking, malting, sprouting and fermentation (Marfo et al., 1990; Khattab & Arntfield, 2009; Mugendi, Njagi, Kuria, Mwasaru, Mureithi & Apostolides, 2010; Krishnan, Dharmaraj & Malleshi, 2012). Often a combination of these methods is more effective in achieving the desired removal of antinutrients compared to the use of a single method (Akande & Fabiyi, 2010).

In as much as some of the processing methods are deemed effective in eliminating antinutrients and enhancing the nutritional value of cereals and legumes, they are not all equally effective. The commonly used processing methods of germination, fermentation, soaking, dehulling and conventional cooking (hydrothermal processing) will be reviewed together with some less commonly used processing methods.

Table 2.1.1: Summary of the effects of different processing technologies on some nutrients and antinutrient factors in cowpea and sorghum grains

PROCESSING METHOD	EFFECTS ON ANTINUTRIENTS	EFFECTS ON NUTRIENTS	REFERENCE SOURCES
Soaking	• ↓ tannin and non-tannin polyphenols • ↓ phytate • ↓ trypsin inhibitor activity	• ↑ essential amino acids • ↑ in vitro protein digestibility • ↑ niacin • ↓ protein • ↓ mineral • ↓ thiamine	Barampama & Simard, 1994; Wang, Lewis, Brennan & Westby, 1997; Alonso, Orue & Marzo, 1998; Alonso, Aguirre &Marzo, 2000; Adb El-Hardy & Habiba, 2003; Khattab, Arntfield & Nyachoti, 2009; Khattab & Arntfield, 2009.
Boiling/cooking	• ↓ tannin and non-tannin polyphenols • ↓ phytate • ↓ trypsin inhibitor activity	• ↑ essential amino acids • ↑ in vitro protein digestibility • ↑ in vitro starch digestibility	Tuan & Phillips, 1991; Barampama & Simard, 1994; Vijayakumari, Siddhuraju, Pugalenthi & Janardhanan, 1998; Nergiz & Gökgöz, 2007; Khattab et al., 2009; Khattab & Arntfield, 2009;.
Microwave cooking	• ↓ tannin and non-tannin polyphenols • ↓ phytate • ↓ trypsin inhibitor activity • ↓ lectins	• ↑ essential amino acids • ↑ in vitro protein digestibility	Hernández-Infante, Sousa, Montalvo & Tena, 1998; Khattab et al., 2009; Khattab & Arntfield, 2009.
Hydrothermal processing	• ↓ tannin and non-tannin polyphenols • ↓ phytate • ↓ trypsin inhibitor activity	• ↑ essential amino acids • ↑ in vitro protein digestibility	Vijayakumari, Siddhuraju & Janardhanan, 1996; Siddhuraju & Becker, 2001; Rehman & Shah, 2005; Khattab et al., 2009; Khattab & Arntfield, 2009.

Table 2.1.1 continued

PROCESSING METHOD	EFFECTS ON ANTINUTRIENTS	EFFECTS ON NUTRIENTS	REFERENCE SOURCES
Fermentation	• ↓ tannin and non-tannin polyphenols • ↓ phytate • ↓ trypsin inhibitor activity	• ↑ in vitro protein digestibility • ↑ protein • ↑ energy • ↑ niacin and thiamine • ↓ carbohydrate content • ↓ minerals (calcium, iron, magnesium, zinc and potassium)	Akinyele & Akinlosotu, 1991; Barampama & Simard, 1994; Alonso et al., 1998; Egounlety & Aworh, 2003; Giami, 2005; Khattab et al., 2009; Khattab & Arntfield, 2009.
Germination	• ↓ tannin and non-tannin polyphenols • ↓ phytate		Alonso et al., 1998; Bvochora, Reedb, Reada & Zvauya, 1999; Gurumoorthi & Uma, 2011
Dehulling	• ↓ tannin and non-tannin polyphenols • ↑ trypsin inhibitor activity • ↓ L-Dopa	• ↑ energy • ↑ protein • ↑ fat • ↓ minerals (calcium, magnesium, iron, potassium, phosphorus) • ↓ protein (sorghum)	Egounlety & Aworh, 2003; Mugendi et al., 2010.

2.10.1 Soaking

Soaking can reduce the phytic acid content of legumes such as faba beans, chickpeas, peas and kidney beans (Adb El-Hady & Habiba, 2003). Soaking for 12, 24, 48 and 72 hrs. was found to progressively and substantially reduce phytic acid, condensed tannin and polyphenol contents of faba and kidney beans (Alonso et al., 2000). Soaking causes a reduction of the phytic acid content by leaching phytic acid out of the grain into the soaking medium (Alonso et al., 1998; Alonso et al., 2000). Soaking also leaches water soluble phenols into the soaking medium (Adb El-Hady & Habiba, 2003). Soaking is, however, also responsible for the unintended loss of minerals into the soaking medium (Akinyele & Akinlosotu, 1991).

2.10.2 Hydrothermal processing

Hydrothermal processing is the basic method of cooking which involves blanching, sterilisation, pasteurisation, steaming and cooking (Grajek & Olejnik, 2010). Moist heating (hydrothermal processing) has been identified as one of the most effective treatments for eliminating trypsin inhibitors (Liener, 1975). Due to the aqueous soluble nature of phenolic compounds, they are also affected by cooking in moist heat (Vijayakumari, Pugalenthi & Vadivel, 2007). Soaking and cooking lablab beans in water resulted in a 61% reduction in tannin content (Vijayakumari, Siddhuraju & Janardhanan, 1995). Similar, wet cooking reduced total phenolics in indigenous Indian pulses (Vijayakumari et al., 1996; Siddhuraju & Becker, 2001). Reduction in the tannin and phytate contents using autoclaving ranged between 33–45% and 28–51%, respectively was achieved for red kidney beans, black gram, chick pea, lentils, white kidney beans and red kidney beans (Rehman & Shah, 2005).

2.10.2.1 Boiling

Boiling is a commonly used cooking method for grains. Boiling has been reported to reduce the trypsin inhibitor activity in soybean, cowpea and ground beans by 82%, 86% and 76%, respectively (Egounlety & Aworh, 2003). Boiling was able to remove 66% trypsin inhibitor activity in raw lablab beans (Osman, 2007). Boiling legumes can cause a significant reduction in water soluble and heat sensitive antinutrients such as tannins, phytic acid, trypsin inhibitors and oligosaccharides (Khattab & Arntfield, 2009; Abd-El Hady & Habiba, 2003; Alonso et al., 1998; Vijayakumari et al., 1998). During boiling, the water soluble antinutrients are leached into the water medium and heat sensitive antinutrients such as protease inhibitors are denatured by the high temperatures (Vidal-Valverde, Frias, Estrella, Gorospe, Ruiz & Bacon, 1994). Since traditional household cooking of legumes generally involves soaking the grains in either hot or cold water prior to boiling (Filipiak-Florkiewicz, Florkiewicz, Cieślik, Wałkowska, Walczycka, Leszczyńska & Kapusta-Duch, 2011), the combination of soaking and boiling was found to be very effective in reducing the level of antinutrients in dry beans (Nergiz & Gökgöz, 2007).

2.10.2.2 Microwave cooking

Microwave cooking is an effective way of reducing the levels of antinutrients in legumes. Microwave cooking velvet beans resulted in an 88%, 70%, 82%, 91% and 96% reduction in total phenolics, tannins, phytic acid, trypsin inhibitors and chymotrypsin inhibitor activity, respectively (Gurumoorthi, Janardhanan & Kalavathy, 2013). In another study, microwave heating reduced lectins and trypsin inhibitors without affecting the protein quality of the legumes (Hernández-Infante et al., 1998). With broad beans, it resulted in reduced trypsin inhibitors and increased protein digestibility (Pysz, Polaszczyk, Leszczyńska & Piątkowska, 2012). Microwave cooking was found to effectively reduce trypsin inhibitors, tannins, phytate and oligosaccharides in Egyptian cowpea (*Vigna sinensis* L.), pea (*Pisum sativum* L.), white kidney bean (*Phaseolus vulgaris* L.), Canadian cowpea (*V. sinensis* L.), pea (*P. sativum* L.) and red kidney bean (*P. vulgaris* L.) (Khattab & Arntfield, 2009).

2.10.3 Fermentation (lactic acid bacteria fermentation)

Fermentation effectively reduces the content of phytic acid in grains (Alonso et al., 1998). Fermentation of soybean, cowpea and peanuts for 30 and 36 hours reduced phytic acid by 30.7% in soybean, 32.6% in cowpea and 29.1% in ground bean (Egounlety & Aworh, 2003). Fermentation activates endogenous enzymes responsible for hydrolysis of certain antinutrients (Sandberg & Andlid, 2002). Fermenting soya beans, for example initiates the activity of the enzyme phytase which hydrolyses phytates and reduces its content (Buckle, 1985). Fermentation of sorghum can also significantly increase protein digestibility and reduce the tannin content (Raihanatu, Modu, Falmata, Shettima & Heman, 2011). The reduction in tannin content was found to be even higher when fermentation was combined with sprouting of the fermented grain. Fermentation can also reduce other polyphenols in grains, which is brought about by the action of polyphenol oxidase present in the grain (Svanberg, Lorri & Sandberg, 1993).

2.10.4 Malting/ sprouting

Malting which is also referred to as fermentation is the sprouting of cereals and legumes. The duration of the malting or sprouting is an important factor for the effective reduction of antinutrients in legumes (Kayembe & Jansen van Rensburg, 2013). For example, a longer duration increased reduction in phytic acid, condensed tannins and polyphenols in faba and kidney beans (Alonso et al., 1998; Gurumoorthi & Uma, 2011). Malting or sprouting activates phytase which is responsible for the hydrolysis and reduction of the phytate content. Malting or sprouting of velvet beans for 24 hours resulted in a 50% reduction in total phenolics and 41% tannins reduction (Gurumoorthi & Uma, 2011). When velvet beans were germinated for 120 hours a reduction of up to 76% total phenolics and 71% tannin was achieved. Reduction in polyphenols by malting or sprouting is attributed to activation of the polyphenol oxidase enzyme (Gurumoorthi & Uma, 2011). Steeping (48 hours) and germinating (56 hours) was found to reduce tannins (mainly proanthocyanidins) by up to 33% in sorghum (Bvochora et al., 1999).

2.10.5 Dehulling

Dehulling can reduce the level of antinutrients concentrated in the seed coat or bran of grains. While reduction is expected for antinutrients concentrated in the seed coat, it can, however, result in an increase in concentration of those antinutrients located in the cotyledon of legumes such as phytic acid (Alonso et al., 1998).

For example, dehulling removed the tannins in the outer seed coat in coloured mucuna beans (Mugendi et al., 2010), while phytic acid content increased during the dehulling of dry beans (Deshpande, Sathe, Salunkhe & Cornforth, 1982; Anton, Ross, Beta, Fulcher & Arntfield, 2008). Another study found that dehulling caused an increase in trypsin inhibitors, chymotrypsin inhibitor activity and α-amylase inhibitors in dry beans (Deshpande et al., 1982). Using combined processing can be more effective as was observed with the combined effect of soaking and dehulling which significantly reduced phytic acid in Great Northern beans (Sathe & Salunkhe, 1981). However, in sorghum because of pericarp and aleurone layer removal, unintended mineral losses have been reported (Raihanatu et al., 2011).

Because traditional food processing methods are sometimes not very effective in achieving the desired reduction of antinutrients, other newer technologies can be used. These technologies can also be used for income generation in rural areas. Two such technologies, extrusion cooking and micronisation, selected for the purpose of the present study, will be discussed in the following section.

2.11 Modern processing technologies

Modern methods of food processing can be used to create employment opportunities and increase food security (Fellows, 2004). Promoting local food production that is low-cost and uses non-traditional yet efficient food processing and preparation technologies can be a source of income generation for small scale farmers in particular (Habwe, Walingo & Onyango, 2008). Several modern food-processing and preparation methods such as extrusion cooking have successfully been used to enhance the bioavailability of micronutrients and to eliminate antinutrients in plant-based diets (Alonso et al., 2000). Due to the scope of the current study however, only extrusion cooking and micronisation will be reviewed.

2.11.1 Micronisation

Micronisation is a high temperature short time (HTST) treatment which uses infrared electromagnetic energy (an invisible light with longer wavelengths than visible light) that can directly transfer heat from a source to the object to heat food (Krishnamurthy, Khurana, Soojin, Irudayaraj & Demirci, 2008; Sharma, 2009). Micronisation is used extensively in the food industry to dry (dehydrate) low moisture foods (Fasina, Tyler, Pickard, Zheng & Wang, 2001; Skjoldebrand, 2001), thaw, pasteurise (Sakai & Hanzawa, 1994) and to reduce the cooking time of cereals and legumes (Arntfield, Scanlon, Malcolmson, Watts, Ryland & Savoie, 1997; Fasina et al., 2001).

With micronisation processing, dry grains are first soaked or tempered to raise the moisture content which allows the grain to be more sensitive to the effect of heat (Bellido, Arntfield, Cenkowski & Scanlon, 2006). Raising the moisture in the grain is also important so as to achieve the cooking effect without burning the grain. Soaking or tempering raises the moisture content, reduces the cooking time, causes starch gelatinization and denatures the protein (Arntfield et al., 1997). Soaking can also help to reduce grain toughness.

Due to micronisation's ability to increase starch gelatinization and protein denaturation, it reduces the amount of slowly digestible starch (Emami, Meda, Pickard & Tyler, 2010). Intact starch granules which may be resistant to digestion are ruptured by micronisation, causing an increase in the amount of more digestible starch. Micronisation can also be used to reduce indigestible resistant starch contained in undamaged cell as a result of incomplete grinding of cereal grains (Leszczynski, 2004). Hence, micronisation has been found to be useful in improving digestibility, retaining the nutritional value and yet at the same time destroying antinutritional factors. Further, micronisation can also cause desirable colour changes and improve functional properties of food products (Batham, Sharma, Khan & Govindara, 2013).

2.11.2 Extrusion cooking

Extrusion cooking is a high throughput method that has high potential for creating market standard food products (Riaz, Anjum & Khan, 2007). Extrusion cooking is a popular high temperature short time (HTST) energy efficient cooking method used to produce many commercial products (Guy, 2001). Extrusion cooking includes conveying, mixing, shearing, separation, heating or cooling, shaping, co-extrusion, venting volatiles and moisture, flavour generation, encapsulation and sterilisation of the food product (Figure 2.2). The principle involves the application of pressure and shearing to the food material fed into the extruder barrel exerted by heat and the rotating screws. Extrusion cooking is effective at reducing trypsin inhibitors and chymotrypsin inhibitor activity in legumes (Alonso et al., 1998). The high temperatures and high shearing action of the process disrupts and causes the denaturation of the proteinaceous inhibitors. Extrusion cooking can also reduce the levels of phytic acid, α-amylase inhibitory activity, lectins activity, condensed tannins and polyphenols in legumes (Alonso et al., 2000; Alonso et al., 1998).

Figure 2.2: Type of extruder similar to the TX 32 twin screw, co-rotating extruder (CFAM Technologies, Potchefstroom, South Africa) used in the study. Image courtesy: Prof J. R. N. Taylor.

Extrusion cooking has been used to create a variety of ready-to-eat (RTE) foods using different grains such as finger millet (Sawant, Thakor, Swami & Divate, 2013), maize (Anderson, Conway, Pfeifer & Griffin, 1969), rice (Choudhury & Gautam, 1998), wheat (Barres, Vergnes, Tayeb & Della Valle, 1990) and sorghum (Pelembe et al., 2002). Extrusion cooking has also been used to create fibrous meat analogues, breakfast cereals, pasta, pet foods and snacks (Osen, Toelstede, Wild, Eisner & Schweiggert-Weisz, 2011; Moscicki, 2011). Grain products created using extrusion cooking have an increased water absorption capacity, due to the presence of the gelatinized and ruptured starch granules (Moscicki, Mitrus, Wojtowicz, Oniszczuk & Rejak, 2013).

Nutritionally, extrusion cooked products have been found to have improved protein and starch digestibility (Alonso et al., 1998). However, it was found that high extrusion temperatures and low extrusion water contents, negatively affected the amino acids isoleucine, leucine, lysine, threonine and valine in maize flour (Paes & Maga, 2004). Importantly, high temperature and low moisture in the presence of reducing sugars causes Maillard reaction, resulting in the loss of available lysine which is the most reactive amino acid (Pelembe et al., 2002).

2.12 Conclusions

Malnutrition affects a large number of young children in developing countries, especially in sub-Saharan Africa. One of the causes has been identified as the poor nutritional quality of the diet received by the children. Sorghum and cowpea are important crops grown in Africa and can be used to address nutritional challenges in the diet of young children. One of the reasons sorghum and cowpea use is limited is the long cooking time they require. Sorghum and cowpeas are also underutilized because they contain antinutrients such as enzyme inhibitors, phytic acid and tannins which reduce the bioavailability of nutrients such as iron, zinc and protein. The nutritive value of these grains can be improved by compositing and then used as a vehicle for protein, iron and zinc nutrition where they are staples. Their long cooking time and antinutrients can be addressed by using newer processing technologies to create RTE mineral- and protein- enhanced product suitable for the nutrition of young children. The present study therefore aims to use the two indigenous African grains sorghum and cowpeas, to develop a RTE meal with improved protein, iron and zinc content that is suitable for young children.

3 Hypotheses and objectives

3.1 Hypotheses

1. Applying non-conventional cooking methods to sorghum that has been decorticated (debranned) (extrusion cooking) and cowpeas that have been dehulled (micronisation heat treatment) will reduce the antinutrient content (trypsin inhibitor activity (TIA), phytate and total phenolic content (TPC) and tannins) and enhance the protein, iron and zinc contents, in vitro protein digestibility (IVPD) and iron and zinc bioaccessibility of the flours. Extrusion cooking pea seeds effectively reduced the levels of phytic acid, α-amylase inhibitory activity, lectins activity, condensed tannins and polyphenols (Alonso et al., 2000; Alonso et al., 1998).

2. A composite made from extruded decorticated sorghum (ES) and micronised dehulled cowpea (MC) to form an ESMC ready-to-eat (RTE) meal will have improved nutritional value in terms of protein, iron and zinc contents compared to a sorghum porridge alone. Cowpeas have high mineral content and protein content compared to sorghum (USDA, 2009). Composites of cereals and legumes have increased protein amino acid quality and digestibility (Protein Digestibility Corrected Amino Acid Score (PDCAAS)) Kannan, Nielsen & Mason, 2001) compared to cereals alone.

3. Adding a cooked cowpea leaf relish to the ESMC RTE meal will significantly improve the protein, iron and zinc contents and the IVPD to levels comparable to a commercial fortified corn (maize): soy RTE porridge. Compositing sorghum with cowpea increased the protein digestibility (PDCAAS) of traditional African sorghum foods (Anyango, De Kock & Taylor, 2011). The consumption of African leafy vegetables (ALV) can contribute significantly to vitamin A and iron intakes (van Jaarsveld, Faber & van Heerden, 2012).

4. The ESMC RTE meal with a cooked cowpea leaf relish will have a high level of bioaccessible iron and zinc comparable to a commercial fortified corn (maize): soy RTE porridge. Several green leafy vegetables were found to contain high amounts of iron that

increased bioaccessible iron, in spite of the presence of antinutrients (Gupta, Lakshmi & Prakash, 2006).

5. Adding the cooked cowpea leaf relish to the composite will improve the diversity of the meal and it will make a meaningful contribution to meeting the protein, iron and zinc RDA of children aged two to five years (taking into account that there are other foods that are consumed by the children that will also contribute to the overall RDA). Food-based dietary guidelines (FBDG) for infants and children in South Africa recommend a diversified diet that includes a variety of fruits and vegetables to meet the daily micronutrient requirements for children (Bowley, Pentz-Kluytz, Bourne & Marino, 2007; Vorster, Badham & Venter, 2013).

3.2 Objectives

The research was undertaken in order to:

1. Formulate and develop a RTE composite meal using raw and processed (extruded) decorticated sorghum flour and (micronized) dehulled cowpea flour with and without a cooked cowpea leaf relish to create a RTE composite meal with improved protein, iron and zinc nutritive value.
2. Evaluate the effects of extrusion cooking on decorticated sorghum (ES), micronisation heat treatment on dehulled cowpea (MC) and compositing to form an ESMC RTE meal and adding a cooked cowpea leaf relish on the nutrient (protein, iron and zinc) and antinutrient (trypsin inhibitors, total phenolics, tannins and phytates) contents.
3. Determine the effects of extrusion cooking, micronisation, compositing and adding a cooked cowpea leaf relish on in vitro protein digestion and in vitro iron and zinc bioaccessibilities compared to a commercial fortified corn (maize): soy RTE porridge for children.
4. Determine the contribution of the ESMC RTE meal with and without cooked cowpea leaf relish to the protein, iron and zinc RDA for children aged two to five years and compare it to the contribution of the commercially fortified corn (maize): soy RTE porridge.

4 Research

4.1 Influence of micronisation (infrared treatment) on the protein and functional quality of a ready-to-eat sorghum-cowpea African porridge for young child-feeding

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Abstract

Indigenous plant foods play a major nutritional and cultural role in the diets of rural people in Africa. However, they can contain high levels of antinutrients, which may exacerbate nutritional and health problems in young children consuming nutrient deficient diets. Also, the rapid increase in urbanisation in Africa has led to the need for convenience type meals. This study investigated the potential of micronisation (infrared treatment) in combination with extrusion cooking in developing a ready-to-eat sorghum and cowpea based porridge supplemented with a cooked cowpea leaf relish for young child-feeding. Micronisation not only inactivated the trypsin inhibitors in cowpea, it also produced an instantised product with excellent hydration properties. When served as a stiff porridge with cooked cowpea leaf relish in the recommended portion sizes for children aged two to five years, one daily serving would meet 40% of the children's protein and lysine requirements. Further, the calculated Protein Digestibility Corrected Amino Acid Score was comparable to commercial fortified corn (maize): soy RTE porridge products. This is notwithstanding that the cooked cowpea leaf relish had a negative effect on protein digestibility due to their high tannin content. This nutritious ready-to-eat meal from locally available plant foods could contribute substantially to food security in both urban and rural communities in Africa.

4.1.1 Introduction

Traditionally, in many resource poor African communities, meals are prepared mainly using indigenous cereals and legumes (Young & Pellett, 1994). The use of legumes and cereals to make composite foods suitable for infant and young child feeding is well founded (FAO/WHO, 1994). Sorghum and cowpea are indigenous African cereal grains and pulse-type legume of considerable nutritional and cultural importance in Africa (Anyango et al., 2011). Indigenous green leafy vegetables also make an important contribution to rural African diets, adding high levels of vitamins and minerals (Uusiku, Oelofse, Duodu, Bester & Faber, 2010). Indigenous African green leafy vegetables are widely consumed in farming communities in Africa. Traditional dishes made from indigenous African green leafy plants have been found to be well-accepted and are consumed by children seven to 10 years (Van der Hoeven et al., 2013).

However, these African plant foods can have substantial levels of antinutrients such as enzyme inhibitors, polyphenolic compounds and anti-metals (phytates and oxalates) (Soetan & Oyewole, 2009). Consumption of foods that contain antinutrients by infants and young children can predispose them to malnutrition (Gibson, Ferguson & Lehrfeld, 1998). This in turn affects the children's immune systems leading to diseases such as pneumonia, diarrhoea, malaria and acute malnutrition (Black, Allen, Bhutta, Caulfield, De Onis, Ezzati, Mathers & Rivera, 2008).

In addition to these nutritional problems, the rapid increase in urbanisation in Africa has led to the need for convenience type meals prepared from easily available foods (De Pee & Bloem, 2009) such as sorghum and cowpea. High Temperature-Short Time (HTST) extrusion cooking has been used successfully to produce nutritious ready-to-eat (RTE) meals such as a protein-rich instant porridge (Pelembe et al., 2002; Singh, Gamlath & Wakeling, 2007). Another thermal technology, micronisation (infrared heating) (Sharma, 2009), has been found to be particularly effective at reducing the cooking time of legumes such as cowpea (Mwangwela, Waniska & Minnaar, 2006) and eliminating antinutrients in cowpea (Khattab & Arntfield, 2009). Micronisation has also been found to improve the starch pasting properties of kidney beans, green beans, black beans, lentils and pinto beans (Fasina, Tyler, Pickard, Zheng & Wang, 2001).

Thus, this study investigated the potential of micronisation in combination with extrusion cooking in developing a ready-to-eat sorghum and cowpea based porridge supplemented with cooked cowpea leaf relish for young child feeding (two to five years) and the effects of these technologies on the protein quality and functional quality of the meal. The products were compared with a commercial fortified corn (maize): soy RTE porridge flour for children (one to four years).

4.1.2 Materials and methods

4.1.2.1 Raw materials

Red non-tannin sorghum (cultivar MR Buster) which was produced by the Agricultural Research Council, Potchefstroom, North-West province, South Africa and cowpeas (cultivar Bechuana white) from Delareyville, North-West province were used. The grains were cleaned and stored at 4°C. Young cowpea leaves were handpicked at the Ukulima Research Farm, Limpopo province (Figure 4.1). Super (highly refined) white maize meal (Premier, Johannesburg), defatted soy flour (DFS) (Nedan Oil Mills, Potgietersrus) and a commercial fortified corn (maize): soy RTE porridge flour suitable for children from one year to four years (Futurelife®, Durban) were obtained.

4.1.2.2 Sorghum processing

Sorghum grains were decorticated to an extraction rate of 70–80% using an abrasive dehuller (Rural Industries Innovation Centre, Kanye, Botswana). The decorticated grains were milled using a hammer mill with a 1.5 mm opening screen size. The milled grains were extruded in a TX 32 twin-screw, co-rotating extruder (CFAM Technologies, Potchefstroom, South Africa). The feed rate was 30 kg/h and moisture content of the feed was adjusted to 20%. The screw rotation speed was 200 rpm and barrel temperature was maintained between 130°C and 159°C with a residence time of 30–90 s. The die diameter was 3 mm and the cutter speed 310 rpm.

The extrudate (Figure 4.2) was cooled at ambient temperature for 8 h before being packaged into plastic buckets with tight fitting lids.

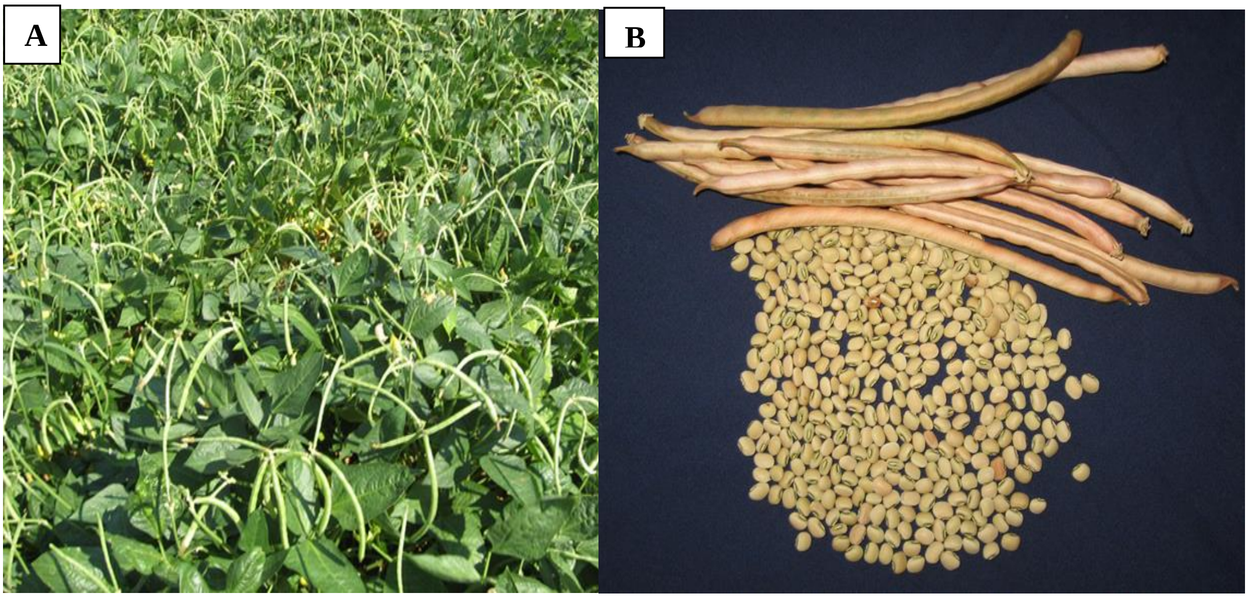

Figure 4.1: (A) Young cowpea leaves and pods growing in the field on Ukulima Research Farm, Limpopo province, South Africa and (B) mature dried cowpea pods and grains. Images courtesy: Prof J. R. N. Taylor.

Figure 4.2: Sorghum extrudate. Images courtesy: Prof J. R. N. Taylor.

4.1.2.3 Cowpea processing

Micronised cowpea flour type 1 (pre-conditioned, micronised, cooled, dehulled and then milled).

The cowpeas were pre-conditioned to 41% moisture by steeping in de-ionized water for 6 h and then allowed to equilibrate for 12 h. The grains were micronised using a table top microniser at a temperature of 153-160°C for six minutes (Technilamp, Johannesburg, South Africa) (Figure 4.3). After micronisation, the grains were cooled to ambient temperature and then manually dehulled. The dehulled grains were milled using a hammer mill fitted with a 500 µm opening screen and then packed into zip lock-type polyethylene bags and kept at 10°C.

Micronised cowpea flour type 2 (pre-conditioned, dehulled, micronised, cooled and then milled).

This was prepared by first pre-conditioning as described, after which the grains were manually dehulled. Micronisation, milling and packaging of the dehulled grains was as described.

4.1.2.4 Processing of cowpea leaves

Cowpea leaves were prepared essentially as described by Faber, Van Jaarsveld, Wenhold & Van Rensburg (2010) for boiled amaranth. The leaves were washed using running tap water and then cooked in the water remaining trapped between them. The cooked cowpea leaf relish was cooled and then frozen at -20°C. These steps were completed on the day the leaves were picked. The cooled cooked cowpea leaf relish was vacuum packed and then freeze-dried and stored at 4°C. Freeze-drying was used simply as a matter of convenience for research purposes as it is the best method of preserving the nutritional quality of fresh produce. In real practice, fresh or air-dried leaves would be used.

Figure 4.3: Table top microniser (Technilamp, Johannesburg) which was used in the study. Images courtesy: Prof J. R. N. Taylor.

4.1.2.5 Formulation of meals

Composite flours were prepared from the raw and extruded sorghum flour and the raw and micronised cowpea flours at a 70:30 (w/w) ratio. Composites of sorghum, cowpea flour and freeze dried cooked cowpea leaf relish were prepared at a 7:3:5 (w/w/w) ratios. The cooked cowpea leaf relish was included in accordance with recommendations by the Nutrition Information Centre of the University of Stellenbosch (NICUS) (2003) and Vorster, Badham & Venter (2013) that children's diets should include a high intake of fruit and vegetables in order to provide better micronutrient nutrition.

4.1.2.6 Analyses

Protein content

Protein content (N x 6.25) was determined by Dumas combustion, Method 46-30 (AACC International, 2000).

In vitro pepsin protein digestibility (IVPD)

IVPD was determined using the procedure of Hamaker, Kirleis, Mertz & Axtell (1986) as modified by Taylor & Taylor (2002) and using pepsin ≥250 units/mg solid (P7000) (Sigma–Aldrich, St. Louis, MO). The protein digestibility was expressed as a percentage.

In vitro multi-enzyme protein digestibility

In vitro multi-enzyme protein digestibility was determined according to Hsu, Vavak, Satterlee & Miller (1977). Samples were digested with trypsin, 13,000-20,000 BAEE units/mg protein (T03030, Sigma-Aldrich), bovine Chymotrypsin type II, 60 units/mg protein (C 4129, Sigma-Aldrich) and Protease XIV, 3.5 units/mg solid (P5747, Sigma-Aldrich). Protein digestibility was expressed as a percentage.

Lysine content

Lysine content was determined by the Pico-Tag method after acid hydrolysis of the protein (Bidlingmeyer, Cohen & Tarvin, 1984).

Trypsin inhibitor activity (TIA)

TIA was determined according to Method 22-40 (AACC International, 2000). The TIA data were expressed as Trypsin inhibitor units (TIU).

Total phenolic content (TPC)

TPC was determined by a Folin–Ciocalteu method (Waterman & Mole, 1994). Catechin (Sigma-Aldrich) was used as a standard. The TPC data were expressed as catechin equivalents (CE).

Tannin content

Tannin content was determined using the modified Vanillin-HCl method of Price, Van Scoyoc & Butler (1978). Extract blanks were used to correct for highly coloured samples where the colour detected was not only due to tannins. Catechin (Sigma-Aldrich) was used as a standard and the data were expressed as catechin equivalents (CE).

Water absorption (WAI) and water solubility (WSI) indexes

Flour WAI and WSI were determined according to the method of Anderson, Conway, Pfeifer & Griffin (1969). WAI gives an indication of the amount of starch in a food sample that can disperse in water after centrifuging. WSI was ascertained by using the amount of a sample that is recovered after the supernatant has been dried out.

4.1.2.7 Statistical analysis

Data were analysed using one way analysis of variance (ANOVA). Means were compared using Fisher's least significant difference test at a 95% level. All the experiments were conducted three times.

4.1.3 Results and discussion

4.1.3.1 Protein and lysine contents

As expected, the protein contents of the raw and micronised cowpea flours were some three times higher than the sorghum and raw maize flours (Table 4.1.1). The protein content of the cowpea flours (28-29%) was within the range reported for cowpea flour processed using traditional preparation methods (Akinyele, 1989). The protein content of the freeze-dried cooked cowpea leaf relish (27%) was similar to that of the cowpea flours (29%) and to that of other dried leafy vegetables such as spinach and amaranth (Singh, Kawatra & Sehgal, 2001).

The two micronised cowpea flours had a slightly higher protein content compared to raw cowpea flour. This was presumably as a result of leaching out of minerals, simple sugars and amino acids (Barampama & Simard, 1995) during pre-conditioning prior to the micronisation cooking process.

Compositing sorghum flour with cowpea flour in the ratio 70:30 resulted in a 36-38% higher protein content compared to sorghum flour alone. This increase was similar to where the same ratio for sorghum and dehulled cowpea flours were used to formulate composite traditional African food products (Anyango et al., 2011). The protein contents of the sorghum and cowpea composites were, however, considerably lower than the laboratory prepared and commercial fortified corn (maize): soy RTE porridge flours. This is due to the notably high protein content of DFS, 47% (USDA, 2009). As a result of their high dry basis protein content, inclusion of the cooked cowpea leaf relish further increased protein content of the extruded sorghum and micronised cowpea flour composite, by approximately 30%.

Lysine is well-known as being the first limiting indispensable amino acid in cereals (WHO/FAO/UNU Expert Consultation, 2007). As expected, the sorghum and maize flour samples had by far the lowest total lysine and the protein lysine contents (Table 4.1.1). Compositing sorghum with cowpea flour more than tripled the lysine content. Inclusion of the cooked cowpea leaf relish further increased the lysine content of the meal because although lysine content of the cooked cowpea leaf relish was somewhat lower than that of the cowpea flour, because the portion of the sorghum in the meal was reduced, the lysine content of the meal was increased.

Table 4.1.1: Effects of cooking sorghum by extrusion and cowpea by micronisation in combination with compositing sorghum with cowpea and the inclusion of cooked cowpea leaf relish on the protein, lysine contents and lysine scores of the flours, in comparison with the maize-soy composites

Treatment	Percentage contribution (dry basis) of sorghum and cowpea flours and cooked cowpea leaf relish in the formulations					Protein (g/100 g dry basis)[1]	Lysine (g/100 g dry basis)[1]	Lysine (g/100 g protein)[1]	Lysine score (amino acid score)[4]	
	Sorghum	Cowpea	Micronised cowpea1	Micronised cowpea2	Cooked cowpea leaf relish				2 years	3-5 years
Raw										
Decorticated sorghum flour	100	0	0	0	0	9.2[a]	0.20[7]	2.17	0.42	0.45
Dehulled cowpea flour	0	100	0	0	0	28.3[g]	1.60[7]	5.65	1.09	1.18
Super maize meal flour	0	0	0	0	0	8.6[a]	0.25[7]	2.91	0.56	0.61
Defatted soy flour	0	0	0	0	0	49.3[i]	2.65[7]	5.38	1.03	1.12
Cooked										
Extruded sorghum flour	100	0	0	0	0	9.4[ab]	0.16[a]	1.70[a]	0.33	0.35
Micronised cowpea 1 flour[2]	0	0	100	0	0	29.3[h]	1.60[7]	5.46	1.05	1.14

Table 4.1.1 continued

Micronised cowpea 2 flour[3]	0	0	0	100	0	29.4[h]	1.60[7]	5.44	1.05	1.13
Cooked cowpea leaf relish	0	0	0	0	100	27.0[f]	1.16[c]	4.30[b]	0.83	0.90
Composites										
Raw decorticated sorghum and dehulled cowpea flour[5]	70	30	0	0	0	14.9[c]	0.62[8]	4.16	0.80	0.87
Raw maize and defatted soy flour[5]	0	0	0	0	0	23.8[e$]	0.97[9]	4.08	0.78	0.85
Extruded sorghum and micronised cowpea 1 flour[5]	70	0	30	0	0	14.7[c]	0.61[b]	4.15 [b]	0.80	0.86
Extruded sorghum and micronised cowpea 2 flour[5]	70	0	0	30	0	15.0[c]	0.61[b]	4.07[b]	0.78	0.85
Extruded sorghum and micronised cowpea 1 flour+ cooked cowpea leaf relish[6]	46.7	0	20	0	33.3	18.9[d]	0.80[b]	4.23[b]	0.81	0.88
Extruded sorghum and micronised cowpea 2 flour+ cooked cowpea leaf relish[6]	46.7	0	0	20	33.3	19.1[d]	0.80[b]	4.19[b]	0.81	0.87
Commercial fortified corn (maize): soy RTE porridge[5]	0	0	0	0	0	26.9[f@]	0.97[9]	3.61	0.69	0.75

[1]Values are means (n=3 for protein and n=2 for lysine). Means followed by different superscripts in a column are significantly different ($p < 0.05$)
[2]Pre-conditioned, micronised, cooled, dehulled and then milled into flour
[3]Pre-conditioned, dehulled, micronised, cooled and then milled into flour

Table 4.1.1 continued

[4]WHO/FAO/UNU Expert Consultation (2007)

[5]7 parts sorghum/maize flour: 3 parts cowpea/soy flour (dry basis)

[6]7 parts sorghum flour: 3 parts cowpea flour: 5 parts cooked cowpea leaf relish (dry basis)

[7]Lysine values are from FAO (1981), except for extruded sorghum, cooked cowpea leaf relish, extruded sorghum and micronised cowpea flours 1 & 2 and extruded sorghum and micronised cowpea flours + cooked cowpea leaf relish 1 & 2.

[8]Calculated using the raw sorghum and cowpea values based on a 70% sorghum and 30% cowpea formulation.

[9]Calculated using the raw maize and soy values based on 70% maize and 30% soy formulation

[$] A simulation of the raw form of the corn (maize): soy blend was made in the laboratory using maize flour (70%) and DFS (30%) and no micronutrients were added. @ The values of the commercial fortified corn (maize): soy RTE porridge presented on the table are of the actual commercial product that were analysed in our laboratory and not the values given on the product's information label.

The Lysine Scores of the extruded sorghum flours of 0.33 and 0.35 for children aged two and three to five year old fell far below the WHO/FAO/UNU Expert Consultation (2007). The sorghum Lysine Score was, however, within the range reported in a study of seven Algerian sorghum varieties (Mokrane, Amoura, Belhaneche-Bensemra, Courtin, Delcour & Nadjemi, 2010). Micronised cowpea, however, met the lysine requirements of two to five year old children. The cooked cowpea leaf relish was also much higher in lysine than sorghum but had a slightly lower Lysine Score than the cowpea flour. Hence, inclusion of cooked cowpea leaf relish with the extruded sorghum and micronised cowpea composite flour did not affect the Lysine Score.

4.1.3.2 In vitro protein digestibility (IVPD) and Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The multi-enzyme and pepsin in vitro methods were used as they are simple, rapid and reproducible and have given comparable results to in vivo methods in rat models (Boisen & Eggum, 1991) and in young children (Mertz, Hassen, Cairns-Whittern, Kirleis, Tu & Axtell, 1984). There was generally good agreement in terms of ranking of the treatments between the multi-enzyme and pepsin IVPD methods (Table 4.1.2). Overall, the raw sorghum flour and the cooked cowpea leaf relish had the lowest IVPD, while the extruded sorghum and micronised cowpea composite and the commercial fortified corn (maize): soy RTE porridge flour composite had the highest. Compared to raw sorghum flour, the extruded sorghum flour had a seven and 10 percentage points higher IVPD with the pepsin and the multi-enzyme methods, respectively. The high IVPD of the extruded sorghum and micronised cowpea composite flour was presumably a result of structural changes to the sorghum protein during the extrusion cooking process. Changes include improvement in the solubility of sorghum protein kafirin due to its disruption by friction during the extrusion cooking process (Hamaker, Mertz & Axtell, 1994).

Micronising cowpea resulted in little or no change in IVPD using the multi-enzyme method, while the pepsin method gave an 11 percentage point decrease in the IVPD. This difference is probably due to reactions between amino groups and reducing sugars during micronisation, which could result in the formation of low molecular weight Maillard reaction products (Oste, Dahlqvist, Sjostrom, Noren & Miller, 1986). The Maillard reaction products formed have some antioxidant activity (Vhangani & Van Wyk, 2013), which can inhibit protein digestion (Oste & Sjodin, 1986). It is likely that the pepsin method was more sensitive to inhibition by

Maillard reaction products as only one enzyme is used, unlike the multi-enzyme method. The multi-enzyme method estimates hydrolysis in the stomach, small intestine and hind-gut of simple-stomach animals (Hsu et al., 1977). In contrast, the pepsin IVPD method, only estimates hydrolysis in the stomach (Boisen & Eggum, 1991).

Compositing cowpea flour with sorghum flour generally increased IVPD, due to the higher digestibility of the cowpea. Similar increases in the digestibility of several traditional sorghum African foods when composited with cowpea have been reported (Anyango et al., 2011).

Table 4.1.2: Effects of cooking sorghum by extrusion and cowpea by micronisation in combination with compositing sorghum with cowpea and the inclusion of cooked cowpea leaf relish on the in vitro protein digestibility (IVPD) and Protein Digestibility Corrected Amino Acid Score (PDCAAS) of the flours, in comparison with maize-soy composites

Treatment	IVPD-Multi-enzyme (%)[1]	IVPD-Pepsin (%)[1]	PDCAAS[4]			
			IVPD-Multi-enzyme (%)		IVPD-Pepsin (%)	
			2 years	3-5 years	2 years	3-5 years
Raw						
Decorticated sorghum	78.8[b] ±0.1	80.1[ab] ±6.9	0.34	0.35	0.34	0.36
Dehulled cowpea flour	83.7[c] ±0.4	98.6[g] ±0.1	0.91	0.99	1.07	1.16
Super maize meal flour	87.9[efg] ±0.3	86.5[d] ±6.6	0.49	0.54	0.48	0.53
Defatted soy flour	87.6[ef] ±0.3	98.1[g] ±2.6	0.90	0.98	1.01	1.10
Cooked						
Extruded sorghum flour	88.8[fg] ±0.6	87.0[de] ±1.0	0.29	0.31	0.29	0.30
Micronised cowpea 1 flour[2]	86.4[de] ±0.1	88.7[e] ±2.8	0.91	0.98	0.93	1.01
Micronised cowpea 2 flour[3]	84.8[cd] ±0.3	87.3[de] ±1.3	0.89	0.96	0.92	0.99

Table 4.1.2 continued

Cooked cowpea leaf relish	76.5 ^{a} ±0.6	79.3 ^{a} ±2.3	0.63	0.69	0.66	0.71
Composites						
Raw decorticated sorghum and dehulled cowpea flour[5]	83.2^{c} ±0.3	92.3 ^{f} ± 3.9	0.67	0.72	0.74	0.80
Raw maize and soy composite flour[5$]	86.7^{e} ±0.1	93.4 ^{f} ± 4.1	0.68	0.74	0.79	0.79
Extruded sorghum and micronised cowpea 1 composite flour[5]	90.5^{h} ±0	91.9 ^{f} ± 1.4	0.72	0.78	0.74	0.79
Extruded sorghum and micronised cowpea 2 composite flour[5]	89.5^{gh} ±0.6	93.0 ^{f} ± 0.1	0.70	0.76	0.73	0.79
Extruded sorghum and micronised cowpea 1 flour+ cooked cowpea leaf relish[6]	78.8^{b} ±0	81.7^{bc} ± 0.4	0.64	0.69	0.66	0.72
Extruded sorghum and micronised cowpea 2 flour+ cooked cowpea leaf relish[6]	79.0^{b} ± 1.4	82.9 ^{c} ± 0.4	0.64	0.69	0.67	0.72
Commercial fortified corn (maize): soy RTE porridge[5@]	88.5^{fg} ±.5	92.0 ^{f} ± 0.2	0.61	0.66	0.63	0.69

[1]Values are means ± standard deviation (n=3). Means followed by different superscripts in a column are significantly different ($p< 0.05$).
[2]Pre-conditioned, micronised, cooled, dehulled and then milled into flour
[3]Pre-conditioned, dehulled, micronised, cooled and then milled into flour
[4]PDCAAS = Lysine score x IVPD
[5]7 parts sorghum/maize flour: 3 parts cowpea/soy flour
[6]7 parts sorghum: 3 parts cowpea: 5 parts cooked cowpea leaf relish (dry basis)
[$] A simulation of the raw form of the corn (maize): soy blend was made in the laboratory using maize flour (70%) and DFS (30%) and no micronutrients were added. [@] The values of the commercial fortified corn (maize): soy RTE porridge presented on the table are of the actual commercial product that were analysed in our laboratory and not the values given on the product's information label.

The cooked cowpea leaf relish had the lowest IVPD. Adding the leaves to the composites reduced IVPD by 10-11 percentage points. The low IVPD of the cooked cowpea leaf relish and the sorghum-cowpea flour composite with cooked cowpea leaf relish was undoubtedly due to the high tannin content of the leaves (2.19 g CE/100 g) (Table 4.1.3). Tannins interact with the grain proteins forming complexes, which reduce enzyme action (De Bruyne, Pieters, Deelstra & Vlietinck, 1999).

PDCAAS is a standard measure of how well the protein in a food can be used by the body (WHO/FAO/UNU Expert Consultation, 2007). PDCAAS of the products ranged from 0.29 for extruded sorghum flour to 1.16 in raw cowpea flour (Table 4.1.2). Raw and extruded sorghum and raw maize flours had the lowest PDCAAS because of their low lysine contents (Table 4.1.1). Due to the addition of cowpea flours with high lysine contents, the PDCAAS of the extruded sorghum and micronised cowpea composite flours were two to three times higher than the extruded sorghum flour (Table 4.1.2). In fact, the PDCAAS for extruded sorghum and micronised cowpea composites were all >0.70, the level recommended by the WHO as a reference for children two to five years (Codex Alimentarius Commission, 1991). A similar increase in PDCAAS after compositing traditional African sorghum foods with cowpea has been reported (Anyango et al., 2011). Considerably increased PDCAAS of other cereal and legume composites have been reported for a rice and beans weaning product (Kannan, Nielsen & Mason, 2001) and a sorghum-soy composite biscuit (Serrem et al., 2011).

Adding the cooked cowpea leaf relish somewhat reduced the PDCAAS compared to the extruded sorghum and micronised cowpea composite flour alone (Table 4.1.2). Nevertheless, the PDCAAS of the composites with the cooked cowpea leaf relish (0.64-0.72) were the same as the calculated PDCAAS for the commercial fortified corn (maize): soy RTE porridge flour (0.61-0.69).

4.1.3.3 Antinutrients

The highest TIA was in the raw dehulled cowpea flour (Table 4.1.3). Micronising cowpea, however, significantly reduced the TIA by 88-90%. The micronisation procedures (micronised cowpea 1 and 2) were equally effective in reducing TIA. The percentage reduction was similar to that reported for micronisation of cowpeas, kidney beans and peas (Khattab & Arntfield, 2009). Exposure of the trypsin inhibitors to heat causes denaturation and subsequent inactivation of the inhibitors (Lajolo & Genovese, 2002). Because of micronisation of the cowpea, the level of TIA in the extruded sorghum and micronised cowpea composite flours with cooked cowpea leaf relish was reduced to the same level as the commercial fortified RTE porridge flour.

It appeared that TIA inactivation by micronisation of the cowpea resulted in only small changes in IVPD, a 0-3% increase with the multi-enzyme method and a 10-11% decrease with the pepsin method (Table 4.1.2). Roasting and micronisation treatment of cowpeas, peas and kidney beans was reported to reduce their IVPD (Khattab et al., 2009). It is likely that micronisation, a thermal treatment led to some non-enzymatic browning and cross-linking of the protein (Hsu et al., 1977; Fagbemi, Oshodi & Ipinmoroti, 2005), resulting in reduced protein digestibility, as reported for sorghum (Duodu, Taylor, Belton & Hamaker, 2003). Notwithstanding this, the level of protein digestibility of the extruded sorghum and micronised cowpea composites were comparable to (pepsin method) or higher than (multi-enzyme method) the commercial fortified RTE porridge product.

Table 4.1.3: Effects of cooking sorghum by extrusion and cowpea by micronisation in combination with compositing sorghum with cowpea and the inclusion of cooked cowpea leaf relish on the trypsin inhibitor activity, total phenolic content and the tannin content of the flours, in comparison with maize and soy composite flour

	Trypsin inhibitory units (TIU/ g)[1]	Total phenolic content (g CE/100 g)[1]	Tannin content (g CE/100 g)[1]
Raw			
Decorticated sorghum flour	ND[a4]	0.09[a] ± 0.00	0.24[abc] ± 0.1
Dehulled cowpea flour	29292[f] ± 727	0.19[c] ± 0.01	0.25[abc] ± 0.2
Super maize meal flour	3340[cd] ± 295	0.26[e] ± 0.01	ND[a]
Defatted soy flour	2146[cd] ± 746	0.43[g] ± 0.03	0.09[ab] ± 0.2
Cooked			
Extruded sorghum flour	ND[a4]	0.18[c] ± 0.01	0.18[abc] ± 0
Micronised cowpea 1 flour[2]	3421[cd] ± 72	0.19[c] ± 0.00	ND[a]
Micronised cowpea 2 flour[3]	2983[cd] ± 284	0.18[c] ± 0.01	ND[a]
Cooked cowpea leaf relish	4476[d] ± 432	3.35[k] ± 0.01	2.19[d] ± 1.7

Table 4.1.3 continued

Composites			
Raw decorticated sorghum and dehulled cowpea flour[5]	$10413^{e} \pm 442$	$0.16^{b} \pm 0.01$	$0.05^{a} \pm 0.1$
Raw maize and defatted soy flour[5$]	$1925^{bcd} \pm 74$	$0.35^{f} \pm 0.00$	ND^{a}
Extruded sorghum and micronised cowpea 1 flour[5]	$3590^{cd} \pm 70$	$0.23^{d} \pm 0.01$	$0.14^{abc} \pm 0$
Extruded sorghum and micronised cowpea 2 flour[5]	$4343^{d} \pm 72$	$0.23^{d} \pm 0.01$	$0.13^{abc} \pm 0$
Extruded sorghum and micronised cowpea 1 flour+ cooked cowpea leaf relish[6]	$768^{bc} \pm 72$	$0.83^{i} \pm 0.03$	$0.71^{c} \pm 0.5$
Extruded sorghum and micronised cowpea 2 flour+ cooked cowpea leaf relish[6]	$1378^{bcd} \pm 358$	$0.86^{j} \pm 0.01$	$0.65^{bc} \pm 0.5$
Commercial fortified corn (maize): soy RTE porridge[5@]	$1436^{bcd} \pm 145$	$0.53^{h} \pm 0.06$	ND^{a}

[1]Values are means ± standard deviation (n=3). Means followed by different superscripts in a column are significantly different ($p < 0.05$).
[2]Pre-conditioned, micronised, cooled, dehulled and then milled into flour.
[3]Pre-conditioned, dehulled, micronised, cooled and then milled into flour.
[4] ND not detected.
[5]7 parts sorghum/maize flour: 3 parts cowpea/soy flour
[6]7 parts sorghum: 3 parts cowpea: 5 parts cooked cowpea leaf relish (dry basis)
[$] A simulation of the raw form of the corn (maize): soy blend was made in the laboratory using maize flour (70%) and DFS (30%) and no micronutrients were added. [@] The values of the commercial fortified corn (maize): soy RTE porridge presented on the table are of the actual commercial product that were analysed in our laboratory and not the values given on the product's information label.

The tannin levels in the sorghum flours were low and within the acceptable recommended range for sorghum flour of < 0.3% (Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, 1996). The noticeable difference of the tannin content between the raw and micronized cowpea grains was probably due to the effects of association between protein and tannins upon heating. The association creates a complex as a result the tannins cannot be detected by the assay (Lewis & Serbia, 1984). It is also possible that improper mixing during the sampling procedure could have resulted in a systematic error resulting in a raw composite flour with far less tannin content than the individual cowpea and sorghum flours. Importantly, adding the cooked cowpea leaf relish to the extruded sorghum and micronised cowpea greatly increased the tannin content of the meals to 0.65-0.71 g/100 g db. This tannin level is more than twice the recommended Codex maximum level for sorghum flour (Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, 1996). Further, as described, the tannins in the cooked cowpea leaf relish substantially reduced the IVPD of the meals (Table 4.1.2).

4.1.3.4 Hydration properties of the flours

The instant porridge making properties of the flours were determined using the water absorption index (WAI) and water solubility index (WSI) parameters (Anderson et al., 1969). All the raw flours had low WSI and had low WAI, with exception of DFS (Table 4.1.4). The high DFS WAI was due to the high globulin type protein content of soy (Koshiyama, 1983). As previously reported, (Pelembe et al., 2002), extrusion cooking greatly increased the WAI and WSI of the sorghum flour.

Both micronisation treatments increased the WAI and WSI of cowpea flours substantially, 1.7-2.4 and 2.7 times, respectively. Micronisation causes changes in cellular structure and gelatinization of starch (Cenkowski, Ames & Muir, 2006). Gelatinization of starch by micronisation has been shown in other pulse-type legumes, navy and black beans (Bellido, Arntfield, Cenkowski & Scanlon, 2006). Although micronisation of cowpeas has been investigated in-depth as a technology to reduce cooking time (Mwangwela et al, 2006) and inactivate trypsin inhibitors (Khattab & Arntfield, 2009), this is the first report of micronisation instantising of cowpea flour. Importantly, both extruded sorghum and micronised cowpea composite flour products had the same WAI and a higher WSI than the commercial fortified RTE porridge product.

Table 4.1.4: Effects of cooking sorghum by extrusion and cowpea by micronisation in combination with compositing sorghum with cowpea and cooked cowpea leaf relish on the Water Absorption Index (WAI) and Water Solubility Index (WSI) of the flour in comparison with the maize-soy composites

Treatment	WAI (%)[1]	WSI (%)[1]
Raw		
Decorticated sorghum flour	2.47[b]	12.7[bcd]
Dehulled cowpea flour	1.94[a]	9.4[a]
Maize flour	2.85[bc]	15.6[de]
Defatted soy flour	4.23[fg]	10.8[abc]
Cooked		
Extruded sorghum flour	4.26[fg]	33.1[i]
Micronised cowpea 1 flour[2]	4.70[g]	25.7[g]
Micronised cowpea 2 flour[3]	3.22[cd]	25.6[g]
Composites[4]		
Raw decorticated sorghum and dehulled cowpea flour	2.34[ab]	9.95[ab]
Raw maize and defatted soy flour	3.17[c]	13.1[cd]
Extruded sorghum and micronised cowpea 1 flour	3.33[cde]	26.1[gh]
Extruded sorghum and micronised cowpea 2 flour	3.28[cde]	25.6[g]
Commercial fortified corn (maize): soy RTE porridge	2.83[bc]	22.2[f]

[1]Values are means (n=3). Means followed by different superscripts in a column are significantly different ($p < 0.05$).
[2]Pre-conditioned, micronised, cooled, dehulled and then milled into flour.
[3]Pre-conditioned, dehulled, micronised, cooled and then milled into flour.
[4]7 parts sorghum/maize flour: 3 parts cowpea/soy flour
[$] A simulation of the raw form of the corn (maize): soy blend was made in the laboratory using maize flour (70%) and DFS (30%) and no micronutrients were added. [@] The values of the commercial fortified corn (maize): soy RTE porridge presented on the table are of the actual commercial product that were analysed in our laboratory and not the values given on the product's information label.

Of the two micronisation treatments, treatment 1 (pre-conditioning and micronisation before dehulling) showed a significantly greater increase in WAI than treatment 2 (pre-conditioning and dehulling before micronisation). This was probably because in treatment 1 the cowpea hull retained more moisture within the grain during micronisation. A similar effect was observed during micronisation of hulled barley where the hull provided some resistance to the moisture removal leading to expansion of the kernel (Fasina, Tyler, Pickard & Zheng, 1999).

4.1.3.5 Estimated contributions of the composite porridge meals to the protein and lysine requirements of young children

When the lysine and protein contents in a 100 g serving of a composite RTE porridge meal for children aged two to three years and 125 g serving for those aged four to five years were calculated, there was a 36-50% increase in protein and a 74% increase in lysine in the extruded sorghum and micronised cowpea composite porridge meal compared to extruded sorghum porridge (Table 4.1.5). Inclusion of the cooked cowpea leaf relish with the extruded sorghum and micronised cowpea composite porridge meal resulted in further increases of 21-22% and 3-11% in protein and lysine, respectively.

Table 4.1.5: Effects of cooking sorghum by extrusion and cowpea by micronisation in combination with compositing sorghum with cowpea and cooked cowpea leaf relish on the protein and lysine contents of the flours and the calculated percentage contribution of stiff porridges made from these flours to the WHO/FAO/UNU Expert Consultation (2007) protein and lysine requirements of young children (2-5 years), in comparison with maize-soy composites

Treatment	Protein per serving of porridge (g) [Lysine per serving (mg)]		% per serving of porridge of safe level of protein intake[6]		% per serving of porridge of lysine requirement[6]			
	2-3 years (portion size 100 g)[5]	**4-5 years** (portion size 125 g)	**2-3 years** (safe level of protein intake 0.9-0.97g protein/kg body wt/ day)[6,10]	**4-5 years** (safe level of protein intake 0.85-0.86 g protein/kg body wt/day)[6,10]	**2-3 years** (lysine requirement 45 mg/kg body wt/day)[6]		**4-5 years** (lysine requirement 35 mg/kg body wt/day)[6]	
					Girls	Boys	Girls	Boys
Raw								
Decorticated sorghum flour	3.04 [66]	3.77 [82]	23.8-26.7	24.3-27	12.8-13.5	12.2-13.0	13.2-13.8	12.8-13.4
Dehulled cowpea flour								
Maize flour	2.84 [83][7]	3.53 [103][7]	22.1-24.9	22.8-25.3	16.0-16.9	15.3-16.4	16.6-17.3	16.1-16.8

Table 4.1.5 continued

Defatted soy flour								
Cooked								
Extruded sorghum flour	3.10 [53]	3.85 [65]	21.8-26.4	21.1-23.7	10.2-10.8	9.8-10.4	10.6-10.9	10.2-10.6
Micronised cowpea 1 flour[1]								
Micronised cowpea 2 flour[2]								
Cooked cowpea leaf relish								
Composites								
Raw decorticated sorghum and dehulled cowpea flour[3]	4.92 [205][8]	6.11 [254][8]	34.5-41.9	33.5-37.6	39.6-41.5	38.0-40.4	41.1-42.7	39.8-41.5
Raw maize and defatted soy flour[3$]	7.85 [320][9]	9.76 [398][9]	55.1-66.8	53.5-60.1	61.8-65.3	59.3-63.1	64.2-66.9	62.3-65.0
Extruded sorghum and micronised cowpea 1 flour[3]	4.85	6.03	34-41.3	33-37.1	38.8-41.0	37.2-39.2	40.4-42.0	39.1-40.8

Table 4.1.5 continued

	[201]	[250]						
Extruded sorghum and micronised cowpea 2 flour[3]	4.95 [201]	6.15 [250]	34.7-42.1	33.7-37.8	38.8-41.0	37.2-39.2	40.4-42.0	39.1-40.8
Extruded sorghum and micronised cowpea 1 flour+ cooked cowpea leaf relish[4]	6.24 [208]	7.75 [258]	43.8-53.1	42.5-47.7	40.2-42.4	38.5-41.0	41.7-44.3	40.4-45.7
Extruded sorghum and micronised cowpea 2 flour+ cooked cowpea leaf relish[4]	6.30 [225]	7.83 [280]	44.2-53.6	42.9-48.2	43.5-45.9	41.7-44.3	45.2-47.1	43.8-45.7
Commercial fortified corn (maize): soy RTE porridge[3@]	8.88 [320][9]	11.0 [397][9]	62.3-75.6	60.3-67.7	61.8-65.3	59.3-63.1	64.2-66.7	62.2-64.8

[1]Pre-conditioned, micronised, cooled, dehulled and then milled into flour,
[2]Pre-conditioned, dehulled, micronised, cooled and then milled into flour
[3]7 parts sorghum/maize flour: 3 parts cowpea/soy flour
[4]7 parts sorghum: 3 parts cowpea: 5 parts cooked cowpea leaf relish (dry basis)
[5]Portion size data from the Nutrition Information Centre of the University of Stellenbosch (NICUS) (2003)
[6]Child weight data is from WHO (2014)
[7]Lysine values from FAO (1981)
[8]Calculated using the raw sorghum and cowpea lysine values based on the 70% maize and 30% soy formulation
[9]Calculated using the raw maize and soy lysine values based on the 70% maize and 30% soy formulation
[10]Protein/kg body wt/ day – protein per kilogram body weight per day
[$] A simulation of the raw form of the corn (maize): soy blend was made in the laboratory using maize flour (70%) and DFS (30%) and no micronutrients were added. [@] The values of the commercial fortified corn (maize): soy RTE porridge presented on the table are of the actual commercial product that were analysed in our laboratory and not the values given on the product's information label.

However, compared to commercial fortified corn (maize): soy RTE porridge meal, extruded sorghum and micronised cowpea composite porridge meal with cooked cowpea leaf relish contribution was 30-35% lower in terms of both their protein and lysine requirements (WHO/FAO/UNU Expert Consultation, 2007). This is because of the higher protein content and quality of the soy. Notwithstanding this, if the sorghum-cowpea porridge and cowpea leaf meal was consumed by the children once a day, it would meet 40% of their protein and lysine requirements.

4.1.4 Conclusions

Micronisation cannot only be used to reduce cooking time and inactivate TIA in cowpea, it can produce instant flours for making porridges with excellent water absorption and solubility. Composite flours of micronised cowpea with HTST extrusion cooked sorghum have equivalent, if not better, hydration properties than a commercial fortified corn (maize): soy RTE porridge product. When served as a stiff porridge in the recommended portion sizes for children aged two to five years once a day, the micronised cowpea and extruded sorghum composite porridge with cooked cowpea leaf relish would meet 40% of the children's protein and lysine requirements and the calculated PDCAAS would be comparable to commercial fortified corn (maize): soy RTE porridge products. This is notwithstanding the negative effects of the cooked cowpea leaf relish on protein digestibility.

Furthermore, using sorghum and cowpeas to produce such nutritious ready-to-eat foods can contribute substantially to food security in both urban and rural communities in Africa. This helps in the development of food-processing enterprises in the rural areas that these crops are produced, which in turn create a commercial market for small-holder farmers (Mwaniki, 2006). Further, elsewhere in the world where other grains are traditional staples, for example amaranth and common beans in South America, these could be similarly processed to produce nutritious ready-to-eat foods. Also, as the product is in the form of a dry powder it has the advantage that it will remain microbiologically stable if kept dry. However, the powder would be subject to oxidation. The use of ascorbic acid as an antioxidant which is a permitted additive in cereal-based infant foods (European Food Safety Authority, 2010) is suggested.

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4.2 Effects of processing and addition of a cowpea leaf relish on the iron and zinc nutritive value of a ready-to-eat sorghum-cowpea porridge meal aimed at young children

Abstract

While dietary diversification of monotonous cereal-based diets using legumes and vegetables can alleviate the high prevalence of iron and zinc deficiencies in sub-Saharan African children, laborious cooking times limit the use particularly of legumes.
This study investigated the effects of high-temperature short-time (HTST) processing on sorghum (extrusion cooking) and cowpea (micronisation), compositing 70:30 sorghum-cowpea (ESMC) in a ready-to-eat porridge, addition of cowpea leaves on iron and zinc bioaccessibilities compared to a commercial fortified maize: soy ready-to-eat porridge.

HTST processing increased iron bioaccessibility from both grains and the zinc bioaccessibility from the sorghum. One serving of ESMC porridge with cowpea leaves could contribute ≈85 and 18% towards the iron and zinc RDA of preschool children, compared to the commercial product at ≈84 and 125%, respectively. However, the higher iron and zinc bioaccessibilities from the ESMC porridge with cowpea leaves, compared to the commercial product (11.8 vs. 5.0% and 18.9 vs 2.7%, respectively) means it would provide more bioaccessible iron (2.24 vs. 0.86 mg/100 g, db) and similar levels of zinc (0.35 vs. 0.32 mg/100 g) towards the absolute/basal requirements of preschool children.

The ESMC porridge with cowpea leaves could improve the iron and zinc nutritive value of preschool sub-Saharan African children's diets.

4.2.1 Introduction

Globally, Africa has the highest estimated prevalence of anaemia in preschool aged (0-5 years) children at 64.6% (McLean, Cogswell, Egli, Wojdyla, & De Benoist, 2009). While information on the prevalence of zinc deficiency is limited, it is believed that where iron deficiency persists, zinc deficiency is very likely to also occur (Ramakrishnan, 2002).

Many households in Africa, where iron and zinc deficiencies are prevalent, depend on monotonous cereal-based diets for micronutrients as well as energy (Oniango, Mutuku, & Malaba, 2003). These diets often contain high levels of antinutrients such as phytate and sometimes tannins, which reduce the already low bioavailability of the non-heme iron and zinc in the diet (Hunt, 2003). Legumes, often used to increase the protein nutritive value of the cereal based diets, have also been found to increase mineral nutritive value (Anigo, Ameh, Ibrahim, & Danbauchi, 2009).

Legume preparation, however, is time consuming and laborious, which consequently limits their use (dos Santos Siqueira, Vianello, Fernandes, & Bassinello, 2013). With the increase in urbanization and more women working outside the home, even in the low socioeconomic and/or rural areas (Tacoli, 2012), there is an increased need for culturally acceptable and convenient foods (Kennedy, Nantel, & Shetty, 2004).

The use of underutilised crops is increasing as their economic potential is realised (Gruère, Giuliani, & Smale, 2006). Underutilised crops are commercialised for various reasons including; providing culturally acceptable options, increasing commercial opportunities for local farmers, lowering production costs and/or increasing the nutritive value of the product.

Information on mineral bioavailability from processed indigenous foods, however, is still severely lacking. This is especially important in areas where mineral deficiencies are often prevalent, but interventions such as supplementation and fortification are impractical.

Sorghum and cowpeas are very important as traditional staple foods in sub-Saharan Africa, as shown by Oyarekua (2010). In a previous study with the aim of providing a high protein quality product, we explored the potential of using sorghum and cowpeas to develop a ready-to-eat (RTE) composite porridge as a complementary food for preschool aged children (2-5 years old) (Vilakati, MacIntyre, Oelofse, & Taylor, 2015). It was found that a single serving of the RTE porridge, with a cowpea leaf relish, could meet 40% of the children’s protein and lysine requirements.

In the current study the effect of high-temperature short-time (HTST) processing technologies on sorghum (extrusion cooking) and cowpea (micronisation), their compositing (70:30) and addition of a cooked cowpea leaf relish on the iron, zinc and phytate contents and iron and zinc bioaccessibilities was evaluated. The extruded sorghum (ES)-micronised cowpea (MC) (ESMC) without or with a cooked cowpea leaf relish porridge's iron and zinc nutritive value was also compared with a commercial fortified maize: soy RTE porridge.

4.2.2 Materials and Methods

4.2.2.1 Raw and processed materials

The sample acquisition, preparation and the formulation of the composite meals have been described in previous work (Vilakati et al., 2015). In short, red non-tannin sorghum (cultivar MR Buster) grains were decorticated, milled and extruded in a TX 32 twin-screw, co-rotating extruder (CFAM Technologies, Potchefstroom, South Africa). Cowpeas (cultivar Bechuana white) were pre-conditioned to 41% moisture, manually dehulled, micronised (Techni lamp, Johannesburg, South Africa) and then milled. Young cowpea leaves were handpicked, cleaned and boiled, as has been described for amaranth (Faber, Van Jaarsveld, Wenhold, & Van Rensburg, 2010). A commercial maize: soy RTE composite porridge (FUTURELIFE®, Durban, South Africa) formulated for children aged 1-4 years was used. The commercial product had also been fortified with multiple micronutrients, including iron, zinc and calcium to levels of 30, 15 and 800 mg/100 g, dry basis (db), respectively (information provided on the packaging).

4.2.2.2 Analyses

Phytate content

Phytate content was determined using an indirect quantitative anion exchange chromatography method described by Frubeck, Alonso, Marzo, & Santidrian (1995). Glass barrel Econo-columns, 0.7 x 15 cm (BioRad, Johannesburg, South Africa), Dowex 1; anion-exchange resin-AG 1 x 4, 4% cross-linkage, chloride from, 100-200 mesh (Sigma, Johannesburg, South Africa) were used.

In vitro iron and zinc bioaccessibilities

Iron and zinc bioaccessibilities were determined using a dialysis method described by Luten et al. (1996) and modified by Kruger et al. (2012). In short, simulated gastric digestion was performed at pH 2 using porcine pepsin (P-700) (Sigma, Johannesburg, South Africa).

Intestinal digestion was simulated at pH 7 using porcine pancreatin (P-1750), bile extract (B-8631) (Sigma, Johannesburg, South Africa) and dialysis tubing (Spectra/Por 7 (ø = 20.4 mm), molecular weight cut-off of 10kDa G.I.C. Scientific, Johannesburg, South Africa). The dialysate was decanted and acidified using concentrated nitric acid to ensure that the minerals did not absorb to the sides of the container and did not precipitate out. The iron and zinc that passed through the dialysis tubing was measured as bioaccessible. The assay is based on the theory that smaller, soluble iron and zinc compounds are better absorbed than large compounds (Fairweather-Tait et al., 2005). Results are displayed as both the amount of bioaccessible iron and zinc (mg/ 100 g) as well as the percentage (%) of bioaccessible iron and zinc relative to total respective contents.

Mineral analysis

The total iron, calcium and zinc contents of the raw and processed flours, digested samples (dialysates) and blanks were determined using inductively coupled plasma optical emission spectrometry (ICP-OES), (SPECTRO ARCO model, Spectro Analytical Instruments, Kleve, Germany) with a dual-view torch, spray chamber and cross-flow nebulizer. Multi-element standard solutions were prepared by dilution of stock solutions with deionized water (1000 mg/l Merck, Darmstadt, Germany) and a range of calibration standards to match expected concentration for Ca, Fe and Zn in samples were used (Operation conditions of the SPECTRO ARCO are given in Table 4.2.1). Prior to analysis of the raw undigested samples, acid assisted microwave digestion was performed using ultrapure nitric acid (65%, Merck, Darmstadt, Germany) and 2 mL hydrogen peroxide (30%, Merck, Darmstadt, Germany). The iron and zinc contents of the flour samples were measured in triplicates.

Vitamin C determination

Vitamin C was determined only in the cowpea leaf relish using the method described by Nielsen (2010) which is similar to AOAC International Methods of Analysis (Method 967.21) (AOAC, 1995).

4.2.2.3 Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) using STATISTICA 10 (Stat Soft, Johannesburg, South Africa). Fisher’s LSD Post-hoc test was applied to determine significant differences between specific means at a confidence level of 95% ($p<0.05$).

Table 4.2.1: ICP-OES working conditions

Parameters	Units
RF Power	1400
Coolant flow rate (L/min)	12.0
Nebulizer flow rate (L/min)	1.00
Auxiliary flow rate (L/min)	1.00
Pump speed (rpm)	30
Rinse time (s)	30
Replicate read time (s)	15
Element	**Emission line (nm)**
Ca	317.9
Fe	238.2
Zn	213.9

4.2.3 Results and Discussion

4.2.3.1 Mineral and phytate contents

Compositing the raw and HTST treated sorghum and cowpea substantially ($p \leq 0.05$) increased the iron, zinc and calcium contents of both the raw and HTST processed composites by 47, 83, 59 and 67, 180, 410%, respectively, compared to the sorghum flours (Table 4.2.2). Adding the cooked cowpea leaf relish to the extruded sorghum-micronised cowpea (ESMC) RTE porridge greatly ($p \leq 0.05$) increased the iron and calcium contents, four and five folds respectively, but did not affect the zinc content. Importantly, the iron content was increased to 19.0 mg/100 g, db, the same level as the commercial fortified maize: soy RTE porridge.

After micronisation and extrusion cooking, the cowpea and sorghum flours had significantly higher iron and zinc contents, respectively. These increases were possibly due to some iron and/or zinc contamination, occurring during the processing. The large increase in iron (76%) and zinc (67%) content after extrusion was probably due to the use of tap water, which could contain significant levels of minerals and abrasion of the ferrous extruder screw and barrel, as was also reported in a study by Mutambuka (2013).

While the phytate contents of the raw and micronised cowpea were significantly higher than those of the raw extruded sorghum, only compositing of HTST processed grains resulted in significantly increased ($p \leq 0.05$) phytate content (13%), compared to sorghum flour alone.

Table 4.2.2: The effects of high-temperature short-time (HTST) processing of sorghum and cowpea and their compositing on the iron, zinc, calcium, phytate contents (mg/100 g db) and the respective phytate: mineral molar ratios

	Iron (n=3)*	**[Phytate :Fe]**	**Zinc** (n=3)	**[Phytate : Zn]**	**Calcium** (n=3)	**[Phytate x Ca:Zn]**	**Phytate** (n=4)
Raw and processed sorghum, cowpea and their composites							
Decorticated sorghum	1.7^{a} (0.0)	[47]	0.6^{a} (0.2)	[154]	17^{b} (1)	[65]	935^{ab}(133)
Dehulled cowpea	4.4^{d} (0.1)	[23]	2.6^{d} (0.0)	[46]	48^{d} (0)	[56]	1220^{c}(64)
Sorghum: cowpea*	2.5^{b} (0.1)	[33]	1.1^{b} (0.1)	[87]	27^{c} (1)	[59]	971^{ab}(186)
Extruded sorghum (ES)	3.0^{b} (0.4)	[25]	1.0^{b} (0.0)	[89]	10^{a} (2)	[22]	901^{a}(50)
Micronised cowpea (MC)	5.0^{e} (0.3)	[21]	2.8^{d} (0.2)	[44]	51^{d} (2)	[55]	1210^{c}(106)
ESMC ready-to-eat (RTE) porridge*	**3.7^{cW} (0.3)**	**[24]**	**1.5^{cW} (0.3)**	**[68]**	**22^{bW} (2)**	**[37]**	**1022^{bW}(91)**
Cowpea relish and other composite RTE porridges							
Cowpea leaf relish	**49.6^{Z} (3.2)**	**[2]**	**2.4^{X} (0.1)**	**[54]**	**374^{Y} (26)**	**[508]**	**1319^{Y}(102)**
ESMC RTE porridge and cowpea leaves**	**19.0^{X} (1.1)**	**[6]**	**1.8^{W} (0.2)**	**[67]**	**126^{X} (9)**	**[210]**	**1101^{X}(91)**
Commercial maize: soy RTE porridge @	**18.7^{X} (1.9)**	**[4]**	**12.9^{Y} (0.9)**	**[7]**	**602^{Z} (57)**	**[113]**	**976^{W}(122)**

Values are means with 1 standard deviation in parentheses. Mineral analyses were performed on 3 individual samples (n=3); phytate analysis was performed on two individual samples in duplicate (n = 4). abc - Mean values of raw and processed sorghum and/or cowpea flours within a column with different superscripts differ significantly ($p \leq 0.05$). WXY-Mean values of different composite dishes (**in bold**) within a column with different superscripts differ significantly ($p \leq 0.05$). *Composite ratio of cereal: legume, 70:30 (db) (reconstituted to 33% solid content), **composition ratio of extruded sorghum: micronised cowpea: cooked cowpea leaves, 70:30:50 (db) (reconstituted to 33% solid content). ES -extruded sorghum, MC-Micronised cowpea and ESMC RTE – extruded sorghum-micronised cowpea ready-to-eat. @ The values of the commercial fortified corn (maize): soy RTE porridge presented on the table are of the actual commercial product that were analysed in our laboratory and not the values given on the product's information label.

4.2.3.2 Mineral bioaccessibility

4.2.3.2.1 Effect of the high temperature short time (HTST) processing and compositing of sorghum and cowpea flours on their iron and zinc bioaccessibilities

The amount of bioaccessible mineral (mg /100 g food product) together with the mineral bioaccessibility (percentage of total mineral) of a specific mineral gives a good indication of the sum of the effects of the mineral bioaccessibility inhibitors and enhancers when the total mineral contents differ substantially (Kruger, Mongwaketse, Faber, van der Hoeven, & Smuts, 2015).

Despite the higher bioaccessibility of iron from cowpea flour, compositing with sorghum flour did not increase the iron bioaccessibility ($p>0.05$) from the raw composite (sorghum 20.7% vs. composite 19.5%) or ESMC RTE porridge (extruded sorghum 32.4% vs. composite 32.3%) (Table 4.2.3). This is probably because the phytate: iron molar ratios of the composite flour at 33 and the ESMC RTE porridge at 24 (Table 4.2.2) were much higher than the critical level of 1, above which iron bioavailability is seriously impaired (Hunt, 2003).

HTST processing substantially increased the iron bioaccessibility (%) from sorghum (32.4% vs. 20.7%) and cowpea (40.4% vs. 25.2%) (Table 4.2.3). However, while the phytate: iron molar ratios were somewhat reduced (21-25) (Table 4.2.2), they were still far above the critical level of 1. The increased iron bioaccessibility (%) was probably due to the previously observed increased water absorption index (WAI) and water solubility index (WSI) of the RTE porridge (Vilakati et al., 2015). It was found that extrusion and micronisation increased the WAI and WSI by approximately 40 and 60% in sorghum and 50 and 60% in cowpea respectively, possibly resulting in more minerals solubilising from the food matrix.

Table 4.2.3: The effect of high-temperature short-time (HTST) processing of sorghum and cowpea and their compositing on the percentage iron and zinc bioaccessibilities (% of total iron) and amount of bioaccessible iron and zinc (mg bioaccessible iron/100 g) assessed by a dialysability assay.

	Iron bioaccessibility		**Zinc bioaccessibility**	
	% of total iron (n=6)	mg/100 g, db	% of total zinc (n=6)	mg/100 g, db
Raw and processed sorghum, cowpea and their composites				
Decorticated sorghum	20.7[a] (3.5)	0.31[a](0.05)	18.2[c] (5.7)	0.11[a](0.03)
Dehulled cowpea	25.2[ab] (1.8)	1.28[c](0.09)	12.8[ab] (1.7)	0.37[cd](0.05)
Sorghum: cowpea*	19.5[a] (1.1)	0.44[a](0.02)	15.6[bc] (3.9)	0.17[b](0.05)
Extruded sorghum (ES)	32.4[c] (5.9)	0.92[b](0.17)	31.3[d] (6.0)	0.30[c](0.06)
Micronised cowpea (MC)	40.4[d] (4.4)	1.95[d](0.21)	13.5[ab] (2.0)	0.36[d](0.05)
ESMC ready-to-eat (RTE) porridge*	**32.3[cY] (3.6)**	**1.05[bW](0.10)**	**11.2[X] (0.8)**	**0.20[bW](0.01)**
Cowpea relish and other composite RTE porridges				
Cowpea leaf relish	**6.2[WX] (0.6)**	**3.08[Y](0.28)**	**12.9[X] (2.0)**	**0.30[X](0.05)**
ESMC RTE porridge and cowpea leaves**	**11.8[X] (1.2)**	**2.24[X](0.34)**	**18.9[Y] (3.4)**	**0.35[X](0.07)**
Commercial maize: soy RTE porridge@	**5.0[W] (0.3)**	**0.86[W](0.05)**	**2.7[W] (0.3)**	**0.32[X](0.03)**

Values are means with 1 standard deviation in parentheses, analyses were performed on two individual samples in triplicate (n = 6). [abc]-Mean values of raw and processed sorghum and/or cowpea flours within a column with different superscripts differ significantly ($p \leq 0.05$). [WXY]-Mean values of different composite dishes (**in bold**) within a column with different superscripts differ significantly ($p \leq 0.05$). *Composite ratio of cereal: legume, 70:30 (db) (reconstituted to 33% solid content), **composition ratio of extruded sorghum: micronised cowpea: cooked cowpea leaves, 70:30:50 (db) (reconstituted to 33% solid content). ES -extruded sorghum, MC-Micronised cowpea and ESMC RTE – extruded sorghum-micronised cowpea ready-to-eat. [@] The values of the commercial fortified corn (maize): soy RTE porridge presented on the table are of the actual commercial product that were analysed in our laboratory and not the values given on the product's information label.

The iron bioaccessibilities (6.2 %) from the cowpea leaf relish and the commercial fortified maize: soy RTE porridge (5.0%) were substantially lower than those of the sorghum and cowpea grains. For the cooked cowpea relish, this was despite containing ascorbic acid, an iron bioavailability enhancer (Teucher, Olivares, & Cori, 2004) at levels of 42 mg/100 g (db) (data not tabulated) and having a much lower phytate: iron molar ratio (2 vs. 21-47). The low iron bioaccessibility in the cooked cowpea leaf relish was probably due to its high tannin content (219 mg/100 g, db) (Vilakati et al., 2015). Tannins have been found to be extremely potent iron bioavailability inhibitors (Santos-Buelga, & Scalbert, 2000). However, despite the low percentage iron bioaccessibility from the cooked cowpea leaf relish (Table 4.2.3), the amount of bioaccessible iron was high (3.08 mg/100 g, db), because of the high total iron content (49.6 mg/100 g db) (Table 4.2.2). For the commercial fortified maize: soy RTE porridge it is possible that its high calcium content (602 mg/100 g db) could have decreased the iron bioaccessibility, as founded by Roughead, Zito, & Hunt (2005).

HTST processing of sorghum, but not cowpea, significantly increased the percentage and amount of bioaccessible zinc (Table 4.2.3). Alonso, Rubio, Muzquiz, & Marzo (2001) in a rat model study, observed an increase in iron absorption after extrusion of peas and kidney beans, but also did not observe any increased zinc absorption. The increase in zinc bioaccessibility from sorghum resulting from extrusion cooking may have been because of the two and three fold decrease in the phytate: zinc and phytate x calcium: zinc ratios (Table 4.2.2), respectively. HTST processing had no effect on the phytate: zinc and phytate x calcium: zinc ratios of cowpea.

Percentage zinc bioaccessibility from the commercial fortified maize: soy RTE porridge was very low, despite the low phytate: zinc molar ratio of 7, well below the critical range of 10-14, above which the bioavailability is seriously impaired (Hunt, 2003). This was probably due to the addition of soy isolate to the product, which has been found to inhibit zinc bioavailability (Lönnerdal, 2000).

4.2.3.2.2 Comparison of iron and zinc bioaccessibilities of the different ready-to-eat (RTE) meals

The ESMC RTE porridge had substantially higher ($p \leq 0.05$) iron bioaccessibility (32.3% vs. 5.0%) compared to the commercial fortified maize: soy RTE porridge. This resulted in both porridges having similar amounts ($p > 0.05$) of bioaccessible iron (1.05 and 0.86 mg/100g db), despite the substantially higher total ($p \leq 0.05$) iron content of the commercial fortified maize:

soy RTE porridge compared to the ESMC RTE porridge (18.7 vs. 3.7 mg/100 g) (Table 4.2.2). Similarly with zinc, the ESMC RTE porridge had much ($p \leq 0.05$) higher zinc bioaccessibility (11.2%) compared to the commercial fortified maize: soy RTE porridge (2.7%). The amount of bioaccessible zinc from the ESMC RTE porridge (0.20 mg/100 g db) was, however, 38% lower than that from the commercial fortified maize: soy RTE porridge, due to the much higher zinc content of the commercial fortified maize: soy RTE composite porridge (12.9 vs. 1.5 mg/100 g db) (Table 4.2.2).

The effect of adding the cooked cowpea leaf relish to the ESMC RTE porridge on the iron and zinc bioaccessibilities varied. While the bioaccessibility of the iron decreased from 32.3 to 11.8%, the amount of bioaccessible iron doubled from 1.05 to 2.24 mg/100g, db (Table 4.2.3). Importantly, both the percentage and amount of bioaccessible iron from the ESMC RTE porridge with cooked cowpea leaves was more than double that from the commercial fortified maize: soy RTE porridge. Both the percentage and amount of bioaccessible zinc increased substantially ($p \leq 0.05$) with the addition of the cooked cowpea leaf relish. The zinc bioaccessibility (%) from the ESMC RTE porridge with cooked cowpea leaf relish was seven-fold higher than that from the commercial fortified maize: soy RTE porridge, and despite the much higher zinc content of the commercial fortified maize: soy RTE porridge, the amounts of bioaccessible zinc were similar (0.35 vs. 0.32 mg/100 g) ($p > 0.05$).

4.2.3.2.3 Estimated contributions of the porridges to iron and zinc requirements of preschool aged children

The contribution that a recommended portion (NICUS, 2003) of the ES, ESMC porridge, without and with cooked cowpea leaves, and the commercial fortified maize: soy RTE porridges could make towards the iron and zinc recommended dietary allowances (RDA) of preschool children aged 2-3 (100 g portion) and 4-5 years (125 g portion) were calculated. Each porridge (with and without cowpea leaves) were reconstituted to 33% solid content. The RDA of children aged 2-3 and 4-5 years for iron and zinc is 7 and 10 mg/day and 3 and 5 mg/day, respectively (Institute of Medicine (IOM), 2001). Compositing the extruded sorghum with the micronised cowpea increased the contribution the RTE porridge could make towards the RDAs of children aged 2-3 and 4-5 years (averaged across age groups) from 13 to 17% for iron and 10 to 15% for zinc, compared to extruded sorghum alone (Figure 4.4a). Further, inclusion of the cooked cowpea leaf relish with the ESMC RTE porridge increased the contribution to the iron RDA of preschool children to an average of 85%, the same as the commercial fortified maize: soy RTE porridge (84%). The contribution of the commercial fortified maize: soy RTE porridge to the zinc RDA was much higher (125%) compared to the ESMC RTE porridge without or with the cowpea leaf relish, which could only provide on average 15 and 18%, respectively.

When evaluating the contribution that bioaccessible minerals can make towards the absolute/basal requirements (AR), expressed as mg bioavailable iron or zinc/day (Figure 4.4b), the direction of the effect is more reliable than the magnitude (Fairweather-Tait et al., 2005). For this reason, the percentage contribution that each porridge could make towards the absolute iron and zinc requirements is expressed relative to the commercial fortified maize: soy RTE porridge's contribution (% contribution of RTE porridge / % contribution of commercial fortified maize: soy RTE porridge x 100).

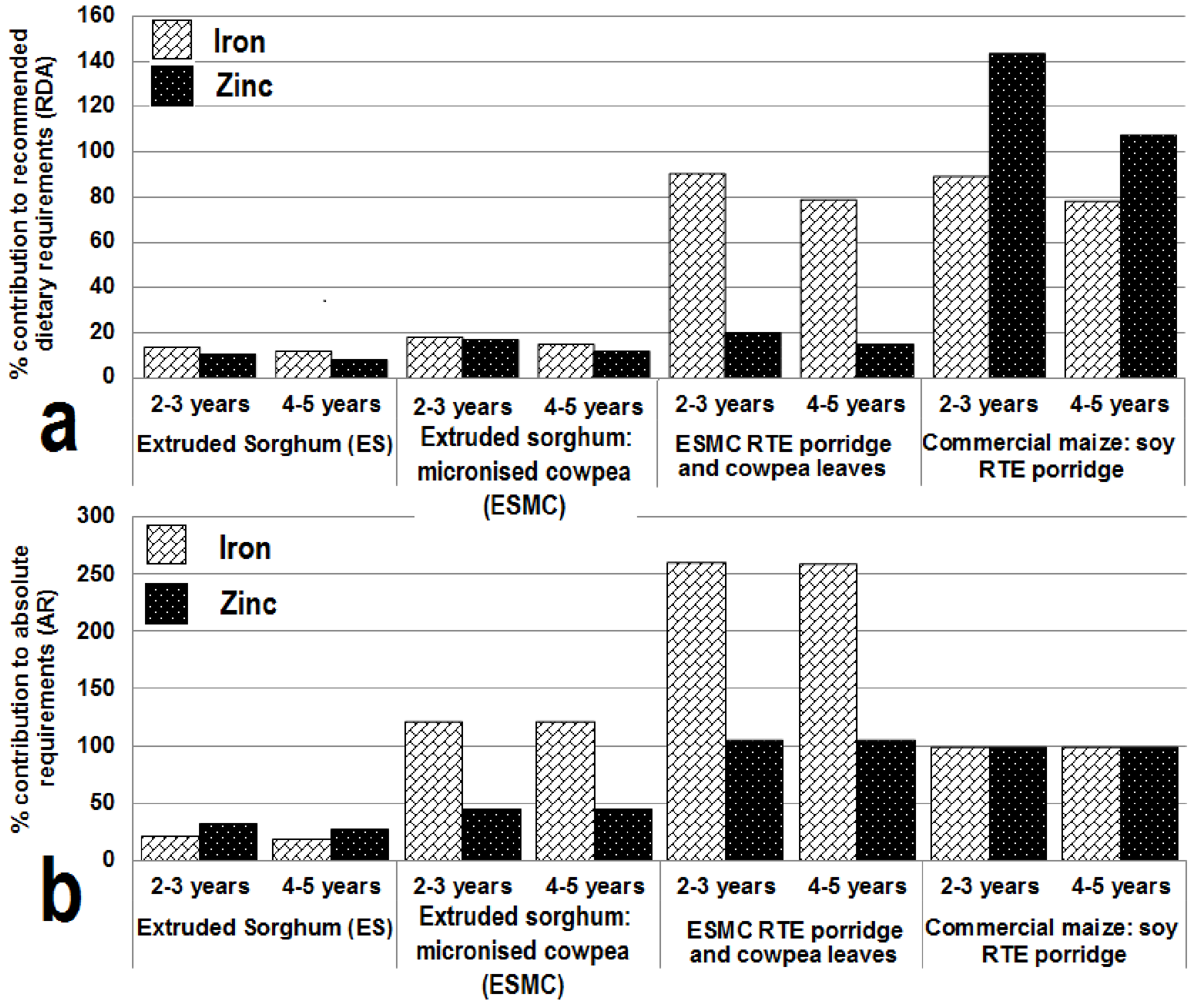

Figure 4.4: The contribution that 100 and 125 g[$] (reconstituted to 33% solid content), as consumed, each ready-to-eat (RTE) porridge can make towards (a) the recommended dietary allowance (RDA)* and (b) absolute requirements (AR)** of pre-school children aged 2-3 and 4-5 year old, respectively.

[$]Recommended portion size per serving as consumed is according to the Nutrition Information Centre of the University of Stellenbosch (NICUS) (2003). *RDA for iron and zinc: 7 and 3 mg/day for children aged 2-3 years and 10 and 5 mg/day for those aged 4-5 years, respectively (Institute of medicine (IOM), 2001). **AR is the % contribution that the amount of bioaccessible iron and zinc (Table 3) in each meal can make towards the absolute requirements for iron (World Health Organization (WHO), 2008) and zinc (Food and Agriculture Organization/World Health Organization (FAO/WHO), 2001): 0.46 and 0.92 mg/day for children aged 2-3 years, respectively and 0.50 and 1.09 mg/day for those aged 4-5 years, respectively. The percentage contributions are expressed relative to the contribution of the commercial product displayed as 100.

While the extruded sorghum could contribute approximately 20 and 30%, respectively of what the commercial fortified maize: soy RTE porridge could towards the iron and zinc AR, the ESMC RTE porridge could contribute the same as the commercial product towards the iron AR and almost 50% towards the zinc AR. Addition of the cooked cowpea leaf relish doubled the contribution that the ESMC RTE porridge could make towards the absolute iron requirements compared to the commercial fortified maize: soy RTE porridge. Importantly, despite the small contribution the ESMC RTE porridge with cooked cowpea leaf relish could make towards the zinc RDA (≈18%) compared to the commercial fortified maize: soy RTE porridge (≈125%), the contribution towards the zinc AR is similar.

4.2.4 Conclusions

Despite the lower total iron and zinc contents of the extruded sorghum-micronised cowpea RTE porridge and cooked cowpea leaf relish meal, the bioaccessibility of the intrinsic iron and zinc is much higher compared to a commercial fortified maize: soy RTE product. If the intrinsic iron and zinc contents of a sorghum: cowpea RTE porridge can be increased further, for example through high iron and zinc cultivar selection, it has the potential to have an iron and zinc nutritive value far superior to the commercial fortified maize: soy RTE product. Although the commercial fortified maize: soy RTE product is not intended for the same market segment which the ESMC RTE porridge with cooked cowpea leaf relish is intended for, the common nutritional benefit for children aged 2-5 years was motivation to use it for comparison.

The ESMC RTE porridge on its own and even more so when consumed as a meal with added cooked cowpea leaf relish, shows it could improve the iron and zinc nutritive value of diets of sub-Saharan African children. Hence, this composite RTE porridge produced from sorghum and cowpea, grains that are staples in Africa, could be a sustainable community/country specific product or commercial alternative type products for young child feeding.

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5 General discussion

5.1 Introduction

As reviewed earlier, the global malnutrition estimates for children under the age of five years in 2005 were that 32% were stunted, 20% were underweight and four percent were severely wasted (Black et al., 2013). In 2014, it was estimated that 161 million children under the age of five years were stunted, almost 99 million were underweight, while 51 million were wasted (UNICEF/WHO/World Bank, 2014).

South African children's diets have been found to have insufficient energy, calcium, iron, zinc, selenium, vitamin A, vitamin D, vitamin C, vitamin E, riboflavin and niacin to meet the children's RDA's (Labadarios et al., 2000). Children living in the Eastern Cape, Limpopo, North-West, Free State and Northern Cape provinces were the most vulnerable nutritionally (Shisana et al., 2013). Further, the 2013 South African National Health and Nutrition Examination Survey (SANHANES-1) found that 34% of the children surveyed did not have enough food at home for breakfast, 37% did not have enough food the whole day and 30% had nothing at home to take to school for lunch (Shisana et al., 2013).

It is against this background and the high consumption of predominantly cereal-based nutrient deficient infant and young children's foods in developing countries that this study was formulated. The study therefore investigated the use of locally produced grains (sorghum and cowpea) to develop a formulation that could be a nutritious ready-to-eat (RTE) meal comparable to a commercial fortified RTE meal. The formulated meal would be suitable for inclusion in the diet of children living in sorghum and cowpea producing and consuming areas such as Sekhukhuneland, in the Limpopo province of South Africa.

The study considered different technologies for developing a nutritious RTE meal for young children living in resource poor areas. The formulated meal was made from local grains (sorghum and cowpea) processed using high temperature short time (HTST) technologies to enhance the nutritional value and reduce the antinutrient content of the grains. The rationale was based on recommendations that advocate using indigenous foods to improve the nutritional quality of diets in rural communities (Dewey & Brown, 2003). Using local ingredients has been successfully implemented in areas such as the Iringa region of Tanzania where maize, sorghum and finger millet are used as staples and common beans, cowpeas and green peas are used as protein supplements (Muhimbula, Issa-Zacharia & Kinabo, 2011). In addition to improving the nutritional quality of the rural diets, the technologies were also selected with the aim of creating business and employment opportunities for the targeted communities. With assistance from industry, government and no-governmental organisations, manufacturing plants in the targeted areas where these grains are grown could be set up.

The outline of the discussion that follows firstly critically discusses the methodologies used in this study and gives supporting evidence for and against the main findings of the study. The limitations and strong points the methodologies used and comparisons with other alternative methods that can be used are discussed. The discussion examines the integration of the formulated product in terms of its application and use as part of food-based nutrition interventions. Lastly the discussion examines the possibilities of doing an in vivo study, a consumer acceptance test and an effectiveness trial with the product.

5.2 Analysis of methodologies applied

5.2.1 Commercial ready-to-eat meal product

As stated, one of the objectives for the study was to formulate a composite RTE meal using raw and processed (extruded and micronised) sorghum and cowpea grains together with a cooked cowpea leaf relish. The sorghum and cowpea formulation's protein, iron and zinc contents and bioaccessibilities would then be compared against a commercially fortified RTE meal. Sourcing a commercially fortified RTE meal targeted at infant and young children in rural communities was a challenge. Products that are produced and made commercially available for low socioeconomic populations are, in most cases, specially formulated foods intended for treating specific conditions. For example, a specially formulated food was used to correct retarded linear growth and anaemia in stunted children in Ghana (Lopriore, Guidoum, Briend & Branca, 2004).

Specially formulated meals were first produced and distributed by international organisations such as the World Food Programme and the United States Agency for International Development as food donations for use in low and middle income countries (Briend & Prinzo, 2009). The specially formulated meals are largely corn (maize)-soy blends (CSB), wheat-soy blends (WSB) (Ruel, Menon, Loechl & Pelto, 2004) and lipid-based nutrient supplements (LNS) (Chaparro & Dewey, 2010). Such foods have been used for emergencies in countries such as India, Haiti, Ethiopia and Malawi (Annan, Webb & Brown, 2014). Typically, these are manufactured for use as ready-to-use-foods (RUFs) to manage severe acute malnutrition

(Chaparro, & Dewey, 2010; Lazzerini, Rubert & Pani, 2013; Lenters, Wazny, Webb, Ahmed & Bhutta, 2013).

The commercially fortified RTE meal selected as the reference food for the present study was chosen because it was designed for young children aged between one to four years, the target age for the study. The selected commercially fortified RTE meal is manufactured in South Africa (Futurelife®, 2012). The limitation was that the price of the product is relatively high for consumers on a limited budget in low socioeconomic settings.

Information provided about the production of the commercially fortified RTE meal was given on the packaging. The ingredients used included maize (exfoliated), soy (micronised), sucrose, vegetable oil (sunflower seed), soy isolate, salt, vanilla, lecithin (soya) and inulin (Futurelife®, 2012). Nutrition information of the proportions of the corn (maize) and soy (70:30) and the amounts of added vitamins and minerals was provided. Further information is that the product underwent a patented cooking process (Futurelife®, 2012).

Even though the nutrition information of the commercially fortified RTE meal was provided on the products label, the commercially fortified RTE meal was analysed for all the required nutrients in our laboratory. Variations in the results between the analysed values and the nutrition information on the products label could have been caused by not evenly mixing the flour inside the package.

A simulation of the raw form of the corn (maize): soy blend was made in our laboratory using maize flour (70%) and DFS (30%). This was done to create a raw composite that would represent the raw form of the commercially fortified RTE meal. Even though no micronutrients were added to the raw composite, the maize flour used was a commercial super maize meal flour.

5.2.2 Preparing the sorghum and cowpea ready-to-eat meal product

Two HTST processing technologies to be used for the two grain types were selected for this study. The aim was to assess the effectiveness of suitable HTST processing technologies that could potentially be used in resource poor communities. The intention was to use technologies that could, when introduced, maintain a supply of the ESMC RTE meal with cooked cowpea leaf relish within the community. While extrusion cooking has been used for cooking both sorghum and cowpea (Pelembe et al., 2002), Mwangela (2006) proposed that micronisation could be used to substantially reduce the long cooking time required for cowpea. However, micronisation has not previously been used as a full cooking method for legumes. There was therefore a need to explore the potential of micronisation as a HTST full cooking method.

As suggested by Pelembe et al. (2002), extrusion cooking can be a fuel saving technology. Extrusion cooking is also a labour and energy saving operation which increases productivity (Heldman & Hartel, 1997). Micronisation denatures protein and decreases its solubility (Mwangwela, Waniska & Minnaar, 2007) which results in increased protein digestibility (Arntfield et al., 2001). Arntfield et al. (2001) also reported a decrease in the phytic acid and phenolic acid contents, which resulted in increased availability of nutrients such as iron, zinc and protein.

As a HTST technology, micronisation can save time and energy, which could bring about increased production (Krishnamurthy et al., 2008). Micronisation and extrusion cooking methods were selected over traditional processing methods such as lactic acid fermentation (Egounlety & Aworh, 2003), soaking (Alonso et al., 2000) and malting/sprouting (Kayembe & Jansen van Rensburg, 2013) because of their ability to create fully cooked instant products within a short time, which was part of the aim of the study.

As mentioned, micronisation has been used to reduce the cooking time of hard-to-cook legumes such as cowpea (Mwangwela et al., 2006; Kayitesi, Duodu, Minnaar & de Kock, 2013) and lentils (Arntfield, Scanlon, Malcolmson, Watts, Cenkowski, Ryland & Savoie, 2001). Micronisation has been used to reduce the cooking time of cereals such as wheat, barley, rye, triticale, millet, wild rice (Zheng, Fasina, Sosulski & Tyler, 1998) and sorghum (Shiau & Yang, 1982).

The choice to allocate extrusion and micronisation as cooking technologies for the sorghum and the cowpea respectively was based on the sizes of the individual sorghum and cowpea grains. Grain size affects the amount of water that the grain can absorb during preconditioning prior to the actual micronisation (Zheng et al., 1998). If the grain is able to absorb a relatively large amount of water it can withstand the heating process, allowing the grain to cook without burning. Since sorghum is a much smaller sized grain (up to 2.4 mm) (Lee, Pedersen & Shelton, 2002) than cowpea (5.0-6.8 mm) (Demooy & Demooy, 1990), subjecting the former to the intense infrared heating of the micronisation process could result in over cooking and burning. To demonstrate the effect of grain size on the cooking duration, a study investigating the effects of a conventional cooking method (boiling) reported that the cowpea size and seed coat thickness had a direct influence on the duration of cooking that it could withstand (Penicela, 2010).

Sorghum was processed using extrusion cooking, which can be a relatively low-cost food processing technology (WFP, 2010) if the equipment is utilised optimally. In fact, compared to other large scale processing options (such as the high pressure processing used to produce all natural, extended shelf life entrées or meals), extrusion cooking is energy efficient and low cost (Guy, 2001). It can be used to improve the quality, taste, convenience and safety of food (Riaz et al., 2007). As stated, extrusion cooking has been used to develop a number of sorghum RTE products (Youssef, Moharram, Moustaffa, Bolling, El-Baya & Harmuth, 1990; Pelembe et al., 2002; Devi, Shobha, Tang, Shaur, Dogan & Alavi, 2013).

Prior to extrusion cooking, the sorghum grains were decorticated, removing the outer parts of the grain that contain higher concentrations of antinutrients such as phytate. For the current study, an abrasive dehuller was used for the decortication. In terms of extending the innovation in rural communities, manual dehulling could be effected. Manual decortication is a traditionally used method in resource-poor areas (Saleh, Zhang, Chen & Shen, 2013). However, even though manual decortication could be an option, it has the limitation of being a low throughput and highly laborious process (Taiwo, 1998). Thus, the time required to manually dehull the grain could pose logistical challenges in meeting production demands.

In order to determine the most effective approach to follow for micronising the cowpeas, two treatments were used. Half of the cowpeas were preconditioned and manually dehulled prior to micronisation, and the other half was manually dehulled after the micronisation process. As can be seen in Chapter 4.1, the two approaches had the same effect on the level of reduction on the trypsin inhibitor and tannin contents and not affecting the total phenolics (Table 4.1.3) and lysine contents (Table 4.1.1) and slightly increasing the protein contents (Table 4.1.1).

As micronutrients were not added to the ESMC RTE meal, the commercially fortified RTE meal used was not directly comparable. To compensate for the difference between the commercial product and the ESMC RTE meal, a cooked cowpea leaf relish was used as a food to food fortificant to improve the ESMC RTE meal. Food to food fortification, which is discussed later, is one of the food-based nutrition intervention strategies that can enhance nutrient content and availability (Ruel, 2001).

Analyses were done on the micronised cowpea to measure the degree to which the products had been cooked. The ability of the flour to absorb water, swell and go into solution as a result of starch gelatinisation exerted by the heat treatments was measured. The parameters used were the water absorption index (WAI) and water solubility index (WSI). The findings from these analyses were a very positive outcome of this study; they showed that through micronisation, an acceptable level of cookability of the cowpea was achieved (Table 4.1.4). This finding, to the best of the author’s knowledge, has not been previously reported on micronised products.

Because the grains were processed using different technologies, it was not possible to compare the effects of extrusion cooking on sorghum and cowpea or the effects of micronisation on sorghum and cowpea. As explained, micronisation (as applied) was not a viable heat treatment option for sorghum. It would have been possible to assess if extrusion cooking had a different effect than micronisation on the cowpea. Comparing micronisation and extrusion cooking effects on cowpea could have been valuable as no study has compared the effect of the two heat treatment methods on antinutrients and nutrients in cowpea. In terms of introducing the product to the target community, the practicability of using both extrusion cooking and micronisation to develop one product may not be an economically viable option.

5.3 Nutrient content and in vitro digestibility analyses

This study used various in vitro assays to measure the bioaccessibility of protein, iron and zinc of the formulated product and a commercially fortified RTE meal for children. Bioaccessibility is a component of bioavailability and is defined as the amount of a nutrient that is potentially released from a food matrix and made available for absorption (Fernández-García, Carvajal-Lérida & Pérez-Gálvez, 2009). In addition to including bioaccessibility, bioavailability measures the amount of a consumed nutrient absorbed into the circulation (Heaney, 2001).

5.3.1 Protein

The use of two different IVPD assays (pepsin and multienzyme assays) was a strength of the study. The pepsin IVPD assay is a commonly used method for cereals (Hamaker et al., 1986) and has been modified for sorghum (Taylor & Taylor, 2002). The multienzyme IVPD assay is another commonly used method which has been used in many studies, especially with legumes (Hsu et al.; 1977; Wolzak, Bressani & Brenes, 1981; Hahn, Faubion, Ring, Doherty & Rooney, 1982).

Both IVPD assays were used in the present study to simulate digestion in humans. Both methods assessed the susceptibility of protein in the sample to the digestive enzyme used. The methods have provided results that correlate with in vivo protein digestibility studies in children (MacLean Jr, Lopez, Placko & Graham, 1981) and a suckling rat pup model (Wolzak et al., 1981). Even though the measured digestibility differed between the assays, the trends observed were similar (Chapter 4.1, Table 4.1.2). Using the two assays further increased the validity of the results. Validation is important as it provides objective evidence that the results are relevant for the intended purpose (Czichos, Saito & Smith, 2006).

In the study, the pepsin IVPD assay, which used a single enzyme, gave higher percentage digestibility values than the multienzyme IVPD assay, which used a cocktail of enzymes (pancreatic trypsin, chymotrypsin, and peptidase). It has been suggested that the reason for this difference might be the number of peptide bonds of the protein that the single enzyme (pepsin) and the cocktail of enzymes (pancreatic trypsin, chymotrypsin, and peptidase) were able to hydrolyse (Boisen & Eggum, 1991). According to Boisen & Eggum (1991), using a single-enzyme to simulate digestibility of a single nutrient is more effective than using a cocktail of enzymes. However, though deemed effective, using a single enzyme does not precisely simulate human protein digestion which involves multiple enzymes (Boisen & Eggum, 1991).

Some problems associated with using the multienzyme method have been identified. One study suggested that different samples could respond differently to the cocktail of enzymes used in the multienzyme assay (Wolzak et al., 1981). The expected action would be a drop in pH which serves as a sign of hydrogen ion release from the protein being hydrolysed. The composition of some food samples may require more time for the enzymes to act effectively. Another problem identified was the buffering capacity of some food components which could affect the ideal pH of the environment required by the enzyme for effectiveness. The effects of buffering capacity were seen with a germinated pea meal which had a buffering capacity that interfered with the drop in pH, resulting in low apparent protein digestibility values (Urbano, Lopez-Jurado, Frejnage, Gómez-Villalva, Porres, Frías, Vidal-Valverde & Aranda, 2005).

In addition to the protein digestibility, protein quality was assessed using the PDCAAS measure. PDCAAS involves a simple calculation that is suitable for routine assessment of protein quality (FAO/WHO, 2001). Hence it was an appropriate method to use in this study.

Although the IVPD methods give good estimates for protein bioavailability and utilisation by humans (FAO Expert Consultation, 2013), in vivo methods could provide more realistic estimates of protein digestibility. In vivo assays are able to simulate human digestion and show how physiological factors affect protein bioavailability. Generally in vitro assays can be used to determine the trend while, in vivo assays measure the degree of the effect (Fairweather-Tait et al., 2005). In vivo methods using animals such as pigs and rats or human models could provide more realistic results of the extent of the effects.

The presence of tannins from the cowpea leaves was found to adversely affect protein digestion (Chapter 4.1). Tannins are polyphenolic compounds that bind to dietary protein and enzymes and inhibit the digestion of protein (Chung, Wong, Wei, Huang & Lin, 1998; De Bruyne et al., 1999). The presence of tannins in the cowpea leaf relish reduced bioavailability of the protein in the ESMC RTE meal. Further research is needed to determine the degree of the effect and how cooked green leafy vegetables could affect protein digestibility when included as part of a meal. It should, however, be borne in mind that the reason for including the cooked cowpea leaf relish in the meal was to use a locally grown plant food to increase the iron and zinc content and the dietary diversity of the meal.

From the study, therefore, the consumption of absorption enhancers together with the product is recommended. For example ascorbic acid rich foods such as tomatoes can increase the absorption of non heme iron from the ESMC RTE meal (Ruel & Bouis, 2004).There are however, reports that have indicated that including traces of animal proteins in a meal can reduce inhibitory effects of tannins on protein digestion (Chung et al., 1998). Instead of using the more expensive animal protein sources, locally available small animals such as insect foods that have a high protein content can be used as was done in a study in Southern Nigeria (Ekpo, 2011). Adoption of such ideas would add to the dietary diversity using locally grown food components and could improve the protein quality of locally produced meals.

5.3.2 Minerals

As stated, mineral bioaccessibility, but not the bioavailability, of the products was assessed in this study. The dialysability assay used only estimated bioaccessibility, that is, the amount of a nutrient released from the food matrix upon digestion (Etcheverry, Grusak, & Fleige 2011). The assay measured the solubility of the minerals through a two-step process which simulated the action of the gastric and intestinal phases of the digestion process. Solubility, which is the principle of the dialysability assay, assumes that the substance released from the food matrix will be available for absorption in the small intestine (Etcheverry et al., 2011).

It was observed, however, that when the dialysability assay was used, samples with lower total iron and zinc contents gave higher percentages of the total dialyzable mineral (iron or zinc) indicating that the minerals are more dialyzable (available) compared to the samples that had higher total mineral contents. This was a concern in terms of the reliability of the estimates from the in vitro method (Chapter 4.2, Table 4.2.3). Hence, the dialysability assay can only be regarded as a relative but not the actual measure of mineral bioavailability (Fairweather-Tait et al., 2005).

Multiple stage assays which require a lot of handling, as was the case with the dialysability bioaccessibility assay, increase the likelihood of errors during the assay (Coles, Moughan & Darragh, 2005). For example, the percentage coefficient of variation for the results of an interlaboratory trial between nine laboratories using a dialysability assay to assess iron bioaccessibility in three different meals varied between 2 and 55% between the laboratories (Luten et al., 1996).

Thus in vitro assay can be considered as a useful tool for determining the direction of the effects of either an inhibitor or enhancer to the nutrient bioavailability (Fairweather-Tait et al., 2005). The dialysability assay, as was used in the present study, is a useful assay at the initial phase of product development to test if nutrients in the product can be released from the food matrix and made available for absorption (Carbonell-Capella, Buniowska, Barba, Esteve & Frígola, 2014).

A useful ex vivo bioavailability assay is the Caco-2-cell culture method which uses cells from a human adenocarcinoma which are similar to normal absorptive epithelial cells (Carbonell-Capella et al., 2014). Bioaccessibility assays using Caco-2-cell culture might be sufficient as an estimation of the mineral bioavailable in a sample because they simulate the digestion and absorption processes in the human gut and can include the third stage of the digestion process to simulate metabolism (O'Sullivan, Jiwan, Daly, O'Brien & Aherne, 2010). The Caco-2-cell culture model gives better human iron absorption estimates, than the solubility assay (Au & Reddy, 2000).

Features that are important for in vitro assay that are used to reflect human conditions include pH gradients for the stages of the digestion process and the temperature of the environment. The correct pH is very important in the simulation of food digestion (reviewed by Miller & Berner, 1989). The pH must be maintained to achieve the expected enzyme action (Wolfgor, Drago, Rodriguez, Pellegrino & Valencia, 2002). Digestive enzymes can only function at the appropriate pH ranges of the different regions of the digestive process, as seen in the human gut (Miller & Berner, 1989). For example, pepsin functions in an acidic environment in the stomach and pancreatic enzymes function best in the more basic pH environment of the small intestine (Fox, 2004). Deviation from the right pH decreases enzyme activity and can hinder the digestion and the mineral availability.

Other factors include the buffering capacity and the consistency of the sample (thin or thick) and knowing the behaviour of the minerals in solution (Miller, Schricker, Rasmussen & Van Campen, 1981). Researchers also need to consider the molecular weight of the mineral when using a dialysability assay. The molecular weight of a soluble mineral must be thought about together with the molecular weight cut off (MWCO) of the dialysis tubing which will allow the soluble mineral to pass through (Etcheverry et al., 2011). Notwithstanding the limitations in the present study, it has been stated that, in vitro assays are good predictors to use for the simulation of digestion and estimation of the amount of a mineral that can be released and made potentially available from a food sample (Das, Raghuramulu & Rao, 2005).

Even though the iron, zinc and calcium contents of the micronised cowpea flour were higher than those of the extruded sorghum alone, adding the micronised cowpea to the extruded sorghum flour did not improve mineral content of the ESMC RTE meal (Chapter 4.2, Table 4.2.1). Using different proportions of sorghum and cowpea to make a composite porridge with the desired level of improvement of the iron and zinc content could be explored, while observing the Codex standards. The Codex standard for processed cereal-based foods for infants and young children (Codex Alimentarius Commission, 2006) states that composition of the cereal-based foods must contain legumes (pulses), starchy roots (such as arrowroot, yam or cassava) or starchy stems or oil seeds in smaller proportions than the main cereal component.

As already highlighted, adding a cooked cowpea leaf relish improved the mineral content and the amount of bioaccessible iron but not the percentage bioaccessibility (Chapter 4.1, Table 4.2.2). Together with the high level of the minerals, there was, however, a high level of antinutrients (phytate and tannins) in the cooked cowpea leaf relish. It is therefore possible that the antinutrients exerted their inhibiting effects and adversely affected the mineral bioaccessibility.

More investigation is needed regarding the use of iron-rich vegetable condiments, such as cowpea leaves, which are strongly recommended for increasing dietary iron intakes especially in low income settings (Uauy, Hertrampf & Reddy, 2002). Clearly, the high antinutrient content in such leafy vegetables could adversely affect mineral bioavailability. An alternative to the addition of cooked cowpea leaf relish with high tannin content is to use staple foods that have been biofortified to have improved micronutrient contents and reduced antinutrient contents (Bouis, 2000; Harvest Plus, 2011). Some biofortification strategies that have been implemented or that are still being investigated include iron rich cowpea, iron and zinc rich sorghum, vitamin A rich cassava, iron rich beans and vitamin A rich maize in Africa; while in South Asia and parts of Africa iron pearl millet and zinc rich rice and wheat have been introduced (Harvest Plus, 2014). Increasing the use of foods with enhancers such as ascorbic acid is suggested when making food combinations for non heme iron (Ruel & Bouis, 2004). For example, foods with high levels of ascorbic acid (such as oranges and tomatoes), meat, fish and poultry can be used as enhancers to increase mineral absorption (Gillooly, Bothwell, Torrance, MacPhail, Derman, Bezwoda, Mills, Charlton & Mayet, 1983; Zijp, Korver & Tijburg, 2000; Teucher, Olivares & Cori, 2004).

To conclude, it must be highlighted that not all factors involved in human digestion can be simulated in vitro. In cases when critical human factors such as the health status need to be considered, in vivo assays will have to be applied (Lestienne, Caporiccio, Besancon, Rochette & Treche, 2005). The status of an individual consuming a food is very important because it has a strong influence on mineral absorption from a meal (Collings, Harvey, Hooper, Hurst, Brown, Ansett, King & Fairweather-Tait, 2013). Individuals with infections and in a state of inflammation, for example, have lower iron absorption (Hurrell, 2012). This may be a concern especially in developing countries where iron deficiency and infections among children are very prevalent. In such cases, even though a food may be a high source of iron, it is likely not to be able to improve the iron status as expected. If finances and time are available, in vivo assays could be used simultaneously with in vitro assays to improve or confirm what has been reported by the in vitro assays (Schricker, Miller, Rasmussen & Van Campen, 1981).

5.4 Future studies

5.4.1 What type of in vivo studies should be used?

As explained, although the in vitro methods used in the present study demonstrated the availability of protein, iron and zinc, it needs to be highlighted that in vitro data can only be interpreted as an estimate and not as an actual value.

The study showed a high level of iron in the cooked cowpea leaf relish, similar to the high level (46 mg/100 g) reported by Oulai, Zoue, Megnanou, Doue & Niamke (2014). From the bioaccessibility data, it can be assumed that the cooked cowpea leaf relish was a good source of iron and hence would be expected to somewhat improve iron bioavailability. The Food Based Dietary Guidelines of South Africa (FBDGSA) advocate that children's (aged one to seven years) diets should include plenty of vegetables and fruits (Bowley et al., 2007). It could therefore be assumed that intake of the cooked cowpea leaf relish (33 g and 42 g for children aged two to three and four to five years respectively) should meet the iron RDA for children aged two to three and four to five years of 7 and 10 mg/day, respectively (Institute of Medicine, 2001). In vivo assays need therefore be used to clarify the interaction that was seen when the cowpea leaf relish was added to the ESMC RTE meal caused by the tannins in the cooked cowpea leaf relish.

The in vivo assay models could be necessary for investigating the effect of different forms of the iron and zinc (due to molecular weight, mineral complexes and presence of organic acids). The in vivo methods can therefore assess the overall bioavailability (gastrointestinal digestion, absorption, metabolism, tissue distribution and bioactivity) using either an animal or human model (Carbonell-Capella et al., 2014).

Even though human clinical studies provide the most accurate assessment of human requirements and bioavailability (Boutrif, 1991), an animal model could be used instead for this study. Animal models that have been used successfully in studying nutrition in humans for example include mice, rats, rabbits, guinea pigs, dogs, pigs, and nonhuman primates (Puiman & Stoll, 2008).

While rat bioassays have been used to determine non heme iron absorption from plant sources, with results showing similarities to humans (Buchowski, Mahoney, Kalpalathika & Hendricks, 1991), animal models such as the pig are used because they have developmental characteristics of the gastrointestinal tract similar to humans (Sangild, 2006).

It is, however, worth mentioning, that cost implications of in vivo studies influence the decision on the type of assay to use. In addition to the cost considerations, the in vivo assay will require ethical approval which is necessary when doing investigations using animals or humans. Ethical principles must be maintained at all time, for example children should be used only if important scientific and public health objective about children is essential (Roth-Cline, Gerson, Bright, Lee & Nelson, 2011). Apart from using an in vivo study, an algorithm or mathematical model could also be considered for modelling bioavailability of the ESMC RTE meal with or without the cooked cowpea leaf relish.

Algorithm or mathematical models determine future outcomes in order to have a better understanding of a problem (Mapoka, Masebu & Zuva, 2013). An example of an algorithm is one that was used to predicting non-haem iron absorption in adults (Armah, Carriquiry, Sullivan, Cook & Reddy, 2013). Simulation of a similar algorithm as the one that was used for the adult study could be suitable for predicting bioavailability in young children aged 2-5 years old because it considers the iron status and the dietary factors within the population being investigated. The ESMC RTE meal with or without the cooked cowpea leaf relish is plant based and could result in low bioavailability when consumed. Importantly, the proposed algorithm would be suitable for this study because it was developed from a complete diet database, which included a variety of dietary factors.

The following section looks at further investigations that would be performed regarding the ESMC RTE meal with cooked cowpea leaf relish so it can be introduced to the community.

5.4.2 Can the product be used as part of food-based nutrition interventions?

The present study investigated the potential of non-traditional processing methods to eliminate antinutrients and deliver a nutritionally adequate meal. The intention was to create a RTE meal product at economic processing and production costs using sorghum and cowpea.

Formulated using HTST processed (micronised and extruded) sorghum and cowpea grains together with a cooked cowpea leaf relish, the RTE meal had a light brown colour, with a green looking relish. The colour and the taste were characteristic of sorghum and cowpea dishes that have a green leafy vegetable component. The meal was created to form part of the daily dietary intakes to meet the protein, iron and zinc RDA's for young children aged two to five years.

The product created had to address the critical aspects which a food-based nutrition intervention aims to achieve in rural community. It must be emphasized that the product was intended to supplement nutrition programmes already in place in the targeted community such as the mandatory national maize meal and wheat flour fortification programme in South Africa (The Department of Health South Africa and UNICEF South Africa, 2007).

Community food-based nutrition interventions have in general been successful in feeding poorly resourced communities (Johnson-Welch, 2002). Community food-based nutrition interventions make use of local foods that are found within the natural environment, either from farming or wild harvesting. The success of the formulated product as a community food-based nutrition intervention is founded on its ability to use local foods that can be accessed locally without having to procure foods externally as reviewed by Kuhnlein, Erasmus & Spigelski (2009). Community food-based nutrition interventions have to include components of production, education and community participation in order to ensure that they will be effective and sustainable (Kuhnlein et al., 2009).

Since the intervention must be relatively easy to implement, the production process must be accessible and suitable for the target community (Kuhnlein et al., 2009). A collaborated effort between industry, government and non-government organisations can help small scale farmers become economically active by empowering them to become producers of the meal. Due to the cost implication attached to acquiring the microniser and the extruder, funding could be sourced and obtained through the collaborated efforts of industry, the government or non-governmental organisation's funding sources. Funding is important for setting up the initial infrastructure, operations costs as well as any unexpected opportunities that require financing (Falkena, Abedian, Von Blottnitz, Coovadia, Davel, Magungandaba, Masilela & Rees, 2002). Since production of the ESMC RTE meal with cooked cowpea leaf relish is intended for the community where the grains are grown, farmers will be encouraged to consider initially self-financing which can be supplemented by the funding from either industry, government or non-government sources who are interested in community development. Assistance with a suitable business model in order to get the funding will have to also be provided.

The commonly used food-based nutrition interventions i.e. supplementation, dietary diversification and modification, fortification, agricultural interventions (biofortification) and education are more effective as a coordinated effort rather than as a single intervention strategy (Ruel, 2001; Gibson, 2011). The following sub-sections will discuss the important characteristics of some of the food-based strategies where the developed product (ESMC RTE meal with cooked cowpea relish) meets those characteristics of being a food-based nutrition intervention strategy.

5.4.2.1 Dietary diversification and modification

Dietary diversification and modification as an intervention can be used to produce, utilise and lead to the consumption of micronutrient rich foods especially in rural communities (FAO/ILSI, 1997). Dietary diversification strategies are more likely to be sustainable as they make use of the local foods that are already being consumed. The additional benefits of dietary diversification and modification as an intervention approach are that it can be used as a way to increase household income through the sale of products especially for women farmers, who are among the most vulnerable groups. For this reason, dietary diversification and modification involves the use of either traditional or non-traditional food processing methods which can change the way in which food is used (Gibson, 2011). Increasing variety in the way a food can be prepared helps as a form of increasing diversity as it equally modifies a diet (Webb, 2011).

The ESMC RTE meal with cooked cowpea leaf relish appears to meet the criteria considered for dietary diversification and modification intervention approaches. Instead of using conventional cooking methods, the unique processing methods (extrusion cooking and micronisation) changed the form in which both grains are prepared. Importantly, the modification of the grains created a RTE meal which could be quickly prepared/reconstituted. The new product development was a way of creating diversity in the choices available to consumers within the target communities.

5.4.2.2 Food to food fortification and supplementation

Food to food fortification is described as the selection of foods and using them in combinations that either improve bioavailability of a nutrient or reduce the effects of antinutrients on the main food component (Ruel, 2001). Food to food fortification can be used to add nutrients of concern that may be in short supply in the staple food. Food to food fortification was addressed in the current study through using cowpea grains to supplement protein, iron and zinc which were in short supply in the sorghum grain. Supplements are added to complete a meal which might be deficient in specific nutrients (Ruel, 2001).

Promoting the use of micronutrient rich foods in order to improve bioavailability of micronutrients such as iron and zinc is a viable option in rural communities (Thompson, 2011). An example of food to food fortification is used in developing countries such as Papua New Guinea where protein rich foods such as insects (palm weevil larvae) which are lysine and leucine rich are mixed with tubers poor in lysine and leucine (Van Huis, Van Itterbeeck, Klunder, Mertens, Halloran, Muir & Vantomme, 2013). In West Africa, dried fish powder is added to dehydrated fermented maize to make a traditional complementary food for infants (Lartey, Manu, Brown, Peerson & Dewey, 1999).

Supplementation as a food-based nutrition intervention strategy in this study was implemented by incorporating the cooked cowpea leaf relish as ingredient to supplement the ESMC RTE meal's nutritional value. Supplementation using local foods in developing countries can be a more viable intervention than using micronutrient supplements. The ESMC RTE meal with cooked cowpea leaf relish can be used to aid short term supplementation programmes. For example, boiled and mashed orange fleshed sweet potatoes (OFSP) is consumed to improve the vitamin A status of primary school children in Durban, KwaZulu-Natal province (van Jaarsveld, Faber, Tanumihardjo, Nestel, Lombard & Benade, 2005). The OFSP can be used together with the current vitamin A supplementation programme given to all children aged 12-59 months in health facilities in South Africa launched in 2001 (The Department of Health South Africa 2012). The OFSP was found to be a sustainable long-term strategy that complemented the on-going vitamin A supplementation programme by the government.

The potential of the product as a source of micronutrients needs to be investigated. For example, the potential of using the cooked cowpea leaf relish as a vitamin A rich source in children's diets. Other studies have demonstrated that green leafy vegetables have the potential of improving vitamin A intake (Ruel, & Levin, 2000). The likelihood of the product being a good source of other nutrients whose deficiencies are prevalent in the community is worth further investigation.

5.4.2.3 Nutrition education

For any food-based intervention (dietary diversification and modification, food to food fortification and supplementation) to be effective, it has to go together with a nutrition education component which leads to behaviour change (Brown, Peerson, Kimmons & Hotz, 2002). For this study, nutrition education is part of the future work to be done before the product can be introduced to the target community. Nutrition education is important for the translation of information to ensure that the community is made aware what the product is intended for nutritionally. For example, the knowledge about the health benefits of cowpeas was influential in ensuring attitude and behaviour change towards positive use of cowpeas in a school-feeding scheme (Abizari, Pilime, Armar-Klemesu & Brouwer, 2013).

It is important that the local farmers, mothers and caregivers who are interested in becoming producers are educated on how to produce the meal and on the nutritional and economic benefits they can gain from producing the ESMC meal with cooked cowpea leaf relish. The education will encourage ownership and responsibility for the production of the meal in the appropriate way for their local community. The education will include training and on-going support for the farmers on how to operate the extruder and microniser. The training will create more interest and the buy in of all the stakeholders needed for the success of the production of the meal. Community food-based nutrition interventions require some level of ownership from the community for which it is intended (Howson, Kennedy & Horwitz, 1998).

A multidisciplinary approach can be used to implement the food-based strategy by combining supplementation, food to food fortification and dietary diversification. The combined use of the interventions with a nutrition education component could have the desired effect for the product, therefore more investigations in this area is needed. As a future study, integrating dietary diversification, food to food fortification, supplementation and nutrition education can be explored.

Based on the above discussion of the elements that make food-based nutrition interventions effective, it appears that the product could be produced in the targeted community. The processing technologies (extrusion cooking and micronisation) can be used in rural community settings to create culturally acceptable RTE meal suitable for young children. The product could be easily accessible when produced by local farmers. As a non-nutritional benefit, the meal can be produced by local women farmers as a way of women empowerment and income generation (Gibson, 2011).

Part of the successful acceptance and use of the introduced food product include conducting consumer acceptance and preference testing of the product. The next section will give a brief outline of why acceptance and preference testing needs to be done.

5.5 Consumer acceptance and preference testing

Consumers' food preferences are influenced by both western culture and tradition (Viljoen & Gericke, 2001). Foods are consumed by different cultures for health, nutritional and religious purposes (Boyle & Holben, 2013). Local foods in rural communities have been found to be acceptable to older people, especially women responsible for the family's food preparation (Maanda & Bhat, 2010). Interest among the younger generations can be aroused if the general knowledge and skills for preparing local foods are filtered down to the younger generations by the older family members.

Due to the use of sorghum and cowpea which the people in the target community are familiar with, the ESMC RTE meal with cooked cowpea leaf relish stand a chance of being accepted by the community. Even though the sorghum and cowpea are local foods, a consumer acceptance and preference testing is required for the formulated ESMC RTE meal with cooked cowpea leaf relish. The product's taste, appearance, smell and texture will be assessed. The consumer acceptance and preference testing will be used to determine the consumers' preferences for the quantity served, the consistencies for children of different ages, the ease of preparation and the shelf life.

As part of conducting the future study, the acceptance and preference test will be done using mothers and caregivers from the target community who are the main decision makers for food preparation in the household. The mothers will be required to test the product themselves and to give it to their children who are in the target aged group for the product. Depending on the logistics, testing a new food innovation over several days is useful in determining long term acceptance (Wiejzen, Zanstra, Alfieri & de Graaf, 2008), which may be considered if the product is unknown.

Planning the sensory evaluation study will have to take economic, social and food factors into consideration. A central location in the community should be used as in a study conducted in Mthatha region in the Eastern Cape province of South Africa testing acceptability of selected maize meal types (Ngqaka, 2009). A central location testing would allow for longer exposure of the product to the intended users and it can be beneficial in resource poor settings (Stone & Sidel, 1993). The ESMC RTE meal with a cooked cowpea leaf relish would be tested in a central location that can easily be accessed in a rural community where the sorghum and cowpea are staple foods. A potential area identified is Sekhukhuneland in the Limpopo province of South Africa. Sorghum production and use in the Limpopo province of South

Africa is relatively high compared to other parts of the country (Bichard et al, 2005). Sekhukhuneland, a district in the Limpopo province, is among the leading growers of sorghum (19 033 ha) (Department of Agriculture, Forestry and Fisheries, 2010). Sorghum was reported as being the main crop that is cultivated by emerging farmers mainly for their own consumption in the arid area of Sekhukhuneland (NAMC, 2007), hence it has been identified as a potential study location. Because the extruder and the microniser cannot be transported to the study location, the product should come pre-prepared, which would be done at the University of Pretoria's Department of Food Science, packaged into individual serving portions and then transported for testing at the central test location where the participants will be served.

A follow-up phase will be considered as it is necessary to capture opinions about how the product can be improved or used with other available plant food products commonly consumed and deemed as nutritious in the area. Using common foods such as sorghum supplemented using other food components has previously been reported to be acceptable to consumers where these foods are staples (Keregero & Mtebe, 1994). For example, one study found that sorghum biscuits were accepted in the same way as the traditional whole wheat biscuits by school children (Serrem, de Kock & Taylor, 2011).

5.6 Effectiveness trial

As a future study, an effectiveness trial has been considered in order to find out if the product will have the expected outcome when used in the community. An effectiveness trial is a study done to test an intervention under usual conditions (Revicki & Frank, 1999). An effectiveness trial can be a useful tool for assessing outcomes of a trial such as the improvement of the health and nutritional status of the children consuming the ESMC RTE meal with cooked cowpea leaf relish during an intervention. Because effectiveness trials can be applied in a natural setting to a large population with fewer restrictions than an efficacy trial; it is also called a real world study (Bombardier & Maetzel, 1999; Möller, 2011). For this reason, an effectiveness trial will be employed as the future study to test effectiveness of the product. Unlike efficacy trials which may be conducted under controlled settings, effectiveness trials are used within a broader target population in a natural environment and the results can be used to generalize to other situations (Möller, 2011). The effectiveness trials will be used because it is cost effective and it does not require expert opinion but instead employs the use of the usual providers to administer (Singal, Higgins & Waljee, 2014). Effectiveness trials can be extended to run over a relatively long period of time to do a proper assessment of the effectiveness of using the trial product, which will be the ESMC RTE meal with cooked cowpea leaf relish.

Application of an effectiveness trial prior to nutrition interventions as a measure to address the nutritional need of the target population will help to bridge the gap between what the situation currently is to what it is supposed to be. The effectiveness trial that could be conducted with the ESMC RTE meal with cooked cowpea leaf relish will provide more information and insight of whether the product can improve the nutrition of children aged two to five years over the duration of its use.

Two cross-sectional studies will be planned to assess the effectiveness of the implementation of the ESMC RTE meal with cooked cowpea leaf relish. Cross sectional studies are an important tool for assessing the prevalence of a disease in a population (Mann, 2013). They are easy, economical and important in assessing the relationship between an exposure and a disease (Morroni & Myer, 2007). The first cross sectional study will be conducted prior to the implementation of the intervention (ESMC RTE meal with cooked cowpea leaf relish) to collect baseline data about the health and nutritional status of the children (anthropometry and biochemical testing) aged two to five years in the community. The second cross-sectional study will be done at the end of one year after implementing the intervention. Data collected after the intervention will be of the nutritional and health status of children aged two to five years. The data will be used to determine if using the product was effective in changing the children's nutritional and health status. Anthropometry (weight, mid-upper arm circumference (MUAC) and height) and biochemical testing for iron, protein and zinc deficiencies (micronutrient deficiency indicators i.e. haemoglobin levels) will therefore be used as the main indicators for evaluating the effectiveness of the product.

The duration of the intervention will be based on the duration that was used for an intervention using whole cowpea meal fortified with NaFeEDTA given to the school children in a Ghana study which proceeded over a seven month duration (Abizari, Moretti, Zimmermann., Armar-Klemesu & Brouwer, 2012). As a future study, it will have to run for a year in order to control any outcomes that could possibly be as a result of the effects of seasonality.

The product will be distributed together with instructions on how the meal should be prepared before consumption. The instructions will go together with an education component to make the necessary behaviour adjustments, hence the need for nutrition education.

6 Conclusions and recommendations

A ready-to-eat porridge meal product developed using non-conventional cooking methods, by extrusion cooking of decorticated sorghum and micronisation of dehulled cowpea and adding a cooked cowpea leaf relish improves the protein, iron and zinc contents compared to sorghum alone. This fulfils the objective of formulating and developing a RTE composite meal using raw and processed (extruded) decorticated sorghum flour, and (micronized) dehulled cowpea flour with and without a cooked cowpea leaf relish to create a RTE composite meal with improved protein, iron and zinc nutritive value. In chapter 4.1, it is shown that the meal improves protein quality to a level similar to that of a commercial fortified ready-to-eat meal. The developed product had iron and zinc bioaccessibility that was higher than that of the commercial product according to the in vitro method used (discussed in detail in chapter 4.2).

The second objective addresses the effects of extrusion cooking on decorticated sorghum (ES), micronisation heat treatment on dehulled cowpea (MC) and compositing to form an ESMC RTE meal and adding a cooked cowpea leaf relish on the nutrient (protein, iron and zinc) and antinutrient (trypsin inhibitors, total phenolics, tannins and phytates) contents. Micronisation reduced trypsin inhibitor activity in cowpeas (chapter 4.1). Because trypsin inhibitors are protein in nature, the heat caused their denaturation. Adding the cooked cowpea leaf relish high in total phenolic and tannins significantly increases the overall content in the product. Total phenolics and tannins decreased protein digestibility (chapter 4.1) and iron and zinc bioaccessibilities (chapter 4.2) of the product. As a suggestion, a meal with green leafy vegetables must be consumed with commonly consumed local food components that are enhancers such as tomatoes and oranges, especially in areas with high protein, iron and zinc deficiency prevalence.

Objective third investigates effects of extrusion cooking, micronisation, compositing and adding a cooked cowpea leaf relish on in vitro protein digestion and in vitro iron and zinc bioaccessibilities compared to a commercial fortified corn (maize): soy RTE porridge for children. While in vitro digestibility and bioaccessibility assays indicated that the extruded sorghum, micronised cowpea ready-to-eat meal has protein digestibility comparable to the commercially fortified ready-to-eat porridge (chapter 4.1) and iron and zinc bioaccessibilities that were better than the commercial porridge (chapter 4.2), further research is needed. The extent of the possible negative effects of the phenolics and tannins present in the cooked cowpea leaf relish on protein digestibility and the iron and zinc bioaccessibilities should be assessed using an in vivo or algorithm method.

A serving of the composite porridge meal made from the sorghum and cowpea with the cooked cowpea leaf relish can make good contributions to the protein, iron and zinc intake of children aged two to five years. For this reason, the fourth objective that determines the contribution of the ESMC RTE meal with and without cooked cowpea leaf relish to the protein, iron and zinc RDA for children aged two to five years and compare it to the contribution of the commercially fortified corn (maize): soy RTE porridge is addressed. In chapter 4.1, it is shown that a serving could meet approximately 40% of the protein and lysine requirements and in chapter 4.2 a serving contributes almost 85 and 18% towards children's iron and zinc recommended dietary allowances (Institute of Medicine, 2001; 2005).

The use of the sorghum and cowpea grains and the cowpea leaves can improve the diversity of the children's diet and provide a form of sorghum and cowpea foods which is different from those produced by conventional cooking methods commonly used such as boiling. Using micronisation and extrusion cooking can contribute specifically to a food-based intervention of dietary diversification and modification. However the cost of using both technologies might be out of reach for resource poor communities. Funding from the government or non-governmental organisation in order to help finance the buying of the equipment has to be considered. Producing the product with one of the two technologies with the ability to process both grains is another option that has to be considered.

In terms of the extruded sorghum, micronised cowpea ready-to-eat meal improving the protein, iron and zinc status in the target population, it needs to be implemented as a food-based intervention strategy together with programmes already being implemented such as food fortification of staple foods (maize meal and wheat flour fortification programme). Of paramount importance, for the product to be part of an effective food-based intervention strategy to improve nutrition, it must increase availability of, accessibility to and overall bioavailability of the nutrients in the product.

It is recommended that future work includes an in vivo study using a rat bioassay to determine the overall digestion, absorption, metabolism and bioavailability of the protein, iron and zinc. Consumer sensory evaluation together with a focus group is recommended to determine the target group's acceptance of the product in terms of sensory acceptability and to make suggestions of portion sizes and other local food products that could be added to further enhance the nutritional quality of the meal. An effectiveness trial is recommended in order to ensure that the product improves nutrition of the children aged two to five years in the target communities.

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