

# **Iron and zinc bioaccessibility from African leafy vegetables: implications for nutrition**

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## ABSTRACT

**Objectives:** The aim of this study was to assess the bioaccessibility of iron and zinc in African leafy vegetables (ALV) and maize porridge composite dishes using an *in vitro* dialysability assay and to estimate the antinutrient content in ALV and maize porridge composite dishes.

**Methods:** ALV leaves were collected, cooked and mixed with either cooked fortified or unfortified maize porridge to simulate the way it is usually consumed. Mineral and antinutrient levels were determined using standard methods and the bioaccessibility of iron and zinc was determined using an *in vitro* dialysability assay.

**Findings:** The findings of the present study indicated that ALV dishes contain a reasonable amount of iron and zinc, but combining the ALV dishes with unfortified maize porridge resulted in dilution and hence a lower iron content. The amaranth-pumpkin dish contained most iron (24 mg/100 g). ALV dishes in the study had zinc contents ranging from 2.6 to 3.2 mg/100 g, with amaranth mixed with spider plant having the highest zinc content. Regarding antinutrients, the amaranth-cowpea dish had the highest phytate content of 2078 mg/100 g dry weight. ALV dishes also contained tannins and phenolic compounds. Iron percentage bioaccessibility was high in an amaranth-spider plant dish (25%), while other dishes had lower iron bioaccessibility of less than 11%. The percentage bioaccessibility of zinc in ALV dishes ranged from 7 to 8%. The amaranth-spider plant dish had higher zinc bioaccessibility when composited with fortified maize meal (13%). The percentage zinc bioaccessibility is negatively associated with phytate:zinc and phytate-calcium:zinc molar ratios.

**Conclusions:** ALV and maize meal composite dishes have a high iron and zinc content, though they also have a high antinutrient content that has some inhibitory effects. Despite the inhibiting factors, the amount of bioaccessible iron and zinc from ALV and maize porridge composite dishes could play a significant role in planning food security strategies. However, there is a need to understand the possible effects of consuming them in different combinations with other foods.

**Keywords:** African leafy vegetables, iron, zinc, bioaccessibility, nutrition

## **OPSOMMING**

**Doelwitte:** Die doel van hierdie studie was om die beskikbaarheid van yster en sink in saamgestelde disse van Afrika-blaargroente (ABG) en mieliepap te bepaal deur van 'n *in vitro* dialiseerbare toets gebruik te maak en om die antinutriëntinhoud in saamgestelde disse van ABG en mieliepap te ondersoek.

**Metodes:** Blare van ABG is versamel, gaargemaak en met óf gaar gefortifiseerde óf ongefortifiseerde mieliepap gemeng om die manier waarop dit gewoonlik ingeneem word, te simuleer. Mineraal- en antinutriëntvlakke is bepaal met behulp van standaardmetodes en die beskikbaarheid van yster en sink is met 'n *in vitro* dialiseerbare toets bepaal.

**Resultate:** The resultate van hierdie studie het aangedui dat ABG-disse 'n redelike hoeveelheid yster en sink bevat, maar kombinerend van die disse met ongefortifiseerde mieliepap het gelei tot verdunning en dus verlaagde ysterinhoud. Die amarant-pampoendis het die meeste yster bevat (24 mg/100 g). ABG-disse in hierdie studie het sinkinhoud van 2.6 tot 3.2 mg/100 mg bevat; amarant gemeng met spinnekopplant het die hoogste sinkinhoud gehad. Wat betref antinutriënte, het die amarant-swartbekboontjedis die hoogste fitaatinhoud van 2078 mg/100 droë gewig bevat. ABG-disse bevat ook taniene en fenoliese verbindings. Die persentasie biobeskikbaarheid van yster was hoog in die amarant-spinnekopplantdis (25%), terwyl ander disse laer biobeskikbaarheid van minder as 11% gehad het. Die persentasie biobeskikbaarheid van sink in ABG-geregte het gewissel van 7 tot 8%. Die amarant-spinnekopplantdis het hoër sinkbeskikbaarheid getoon wanneer dit gekombineer is met gefortifiseerde meliemeel (13%). Die persentasie beskikbare sink is negatief met fitaat:sink- en fitaat-kalsium:sink- molare verhoudings geassosieer.

**Gevolgtrekkings:** ABG-en-meliemeel- saamgestelde geregte het 'n hoër yster- en sinkinhoud, hoewel dit ook 'n hoë antinutriëntinhoud het wat bepaalde inhiberende effekte het. Ten spyte van die inhiberende faktore, mag die hoeveelheid yster en sink beskikbaar van ABG-en-mieliepap saamgestelde disse 'n betekenisvolle rol in die beplanning van voedselsekureiteitsstrategieë speel, maar daar is 'n behoefte om die moontlike effekte van die inname daarvan in verskillende kombinasies met ander voedsels te verstaan.

**Sleutelwoorde:** Afrika-blaargroente, yster, sink, beskikbaarheid, voeding

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## **LIST OF ABBREVIATIONS**

|                 |                                                                |
|-----------------|----------------------------------------------------------------|
| <b>AI</b>       | Adequate Intake                                                |
| <b>ALVs</b>     | African leafy vegetables                                       |
| <b>ANOVA</b>    | One-factor analysis of variance                                |
| <b>ASSAf</b>    | Academy of Science of South Africa                             |
| <b>DW</b>       | Dry weight                                                     |
| <b>EAR</b>      | Estimated average requirements                                 |
| <b>Hb</b>       | Haemoglobin                                                    |
| <b>ICP-OES</b>  | Ion coupled plasma-optical emission spectrometry               |
| <b>IDA</b>      | Iron deficiency anaemia                                        |
| <b>IPGRI</b>    | International Plant Genetic Resource Institute                 |
| <b>NaFeEDTA</b> | Sodium iron ethylenediaminetetraacetic acid                    |
| <b>NFCS</b>     | National Food Consumption Survey                               |
| <b>NFCS-FB</b>  | National Food Consumption Survey - Fortification Baseline      |
| <b>PA</b>       | Phytic acid                                                    |
| <b>RDA</b>      | Recommended dietary requirements                               |
| <b>SANHANES</b> | South African National Health and Nutrition Examination Survey |
| <b>SAVACG</b>   | South African Vitamin A Consultative Group                     |
| <b>THUSA</b>    | Transition, Health and Urbanisation in South Africa            |
| <b>UL</b>       | Tolerable upper intake level                                   |
| <b>WHO</b>      | World Health Organisation                                      |
| <b>WW</b>       | Wet weight                                                     |

## CHAPTER 1

### 1.1 BACKGROUND AND PROBLEM STATEMENT

Malnutrition is still a major public health issue globally. The main causes of malnutrition are inadequate food intake, poor nutritional quality of diets and poverty (Muyonga *et al.*, 2008; Lartey, 2008; Legwaila *et al.*, 2011). Globally studies have shown that people living in rural areas are faced with food insecurity and chronic malnutrition (Legwaila *et al.*, 2011). According to the World Health Organization (WHO) one out of three people in developing countries are affected by vitamin and mineral deficiencies (WHO, 2012). In areas where iron deficiency was reported, nutritional zinc deficiency was also common (Uusiku *et al.*, 2010). Malnutrition has been found to be widespread in Sub-Saharan Africa (Lartey, 2008; Muyonga *et al.*, 2008).

The prevalence of under-nutrition in South Africa had been associated with chronic food shortages (Misselhorn, 2004). The recent South African National Health and Nutrition Examination Survey (SANHANES-1) reported that 45.6% of the population were food-secure, while 28.3% were at risk of hunger and 26.0% experienced hunger (Shisana *et al.*, 2013). The black African population was mostly affected, having the highest rate of food insecurity (30.3%). In 1994 the South African Vitamin A Consultative Group (SAVACG) reported that 20% of children were anaemic and in 1999 iron and zinc intakes of children aged one to nine years were below two thirds of recommended dietary allowances (RDA) (SAVACG, 1995; Labadarios *et al.*, 2005). However, the 2012 survey (Shisana *et al.*, 2013) reported an improving nutritional iron situation concerning the prevalence of anaemia (10.5%), iron deficiency (11%) and iron deficiency anaemia (IDA) (2.1%) when compared to other years, nutrition remained a public health problem in South Africa.

The Transition, Health and Urbanization in South Africa (THUSA) project found that dietary intakes were shifting from the traditional high complex carbohydrate low fat diet to a diet high in fat and refined carbohydrates, which are associated with non-communicable diseases (MacIntyre *et al.*, 2002). In addition, Labadarios *et al.* (2011) reported that South Africans consumed a diet that lacked diversity and this trend was also observed in the SANHANES-1 survey (Shisana *et al.*, 2013). An adequately diverse diet may result in nutrient adequacy. In strategies that promote dietary diversity, people are encouraged to use locally available biodiversity and this may result in good nutrition and more diverse, balanced diets (Frison *et al.*, 2006). Therefore, strategies that may increase vegetable consumption in rural areas, such as the use of African leafy vegetables (ALVs), should be promoted. In Kenya, traditional vegetables have been embraced and encouraged, mainly to curb the rise in non-communicable diseases related to urban dietary practices (Oiyee *et al.*, 2009). The use of ALVs (spider plant, cowpea and pumpkin leaves) and exotic commercial vegetables (spinach, kale and cabbage) can contribute significantly to an increase in the consumption of vegetables and diversification of South African diets.

In South Africa, the consumption of ALVs is common in rural settings, as they are regarded as cheap and accessible (Uusiku *et al.*, 2010; Odhav *et al.*, 2007). ALVs can potentially be used to make an important contribution to combating micronutrient malnutrition as well as contributing to food security, as they are hardy, require less care and are a rich source of micronutrients compared to conventionally cultivated species of kale and cabbage (Flyman & Afolayan, 2006). ALVs contain higher levels of calcium, iron and zinc than introduced varieties of vegetables such as spinach, kale and cabbage (Orech *et al.*, 2007; Uusiku *et al.*, 2010) and they also contain high amounts of vitamins and antioxidants (Oiye *et al.*, 2009). If eaten abundantly, ALVs can provide both nutritional and medicinal properties to consumers (Van der Walt *et al.*, 2009; Oiye *et al.*, 2009). Studies done in some parts of Southern Africa have shown that the use of traditional food plants increases in times of food shortage and famine (Mojeremane & Tshwenyane 2004; Oiye *et al.*, 2009; Van der Walt *et al.*, 2009; Legwaila *et al.*, 2011). ALVs can be used as a food strategy to improve household food security and dietary diversity.

ALVs have been associated with people of low socioeconomic status and this has decreased the consumption of ALVs, as they are often regarded as inferior in taste and nutritional value compared to exotic vegetables, and associated with poverty (Odhav *et al.*, 2007; Uusiku *et al.*, 2010; Matenge *et al.*, 2012; van der Hoeven *et al.*, 2013). Lack of knowledge in the use and nutritional composition of ALVs resulted in them being underutilised and neglected (Van der Walt *et al.*, 2009; Matenge *et al.*, 2012). In both North West and KwaZulu-Natal provinces in South Africa it was reported that young people were ignorant about the existence of nutritionally rich ALVs (Odhav *et al.*, 2007; Matenge 2012). Nevertheless, ALVs were sensorily acceptable to children when used in a study in a feeding programme (van der Hoeven *et al.*, 2013).

ALVs are part of the traditional starch-based African diet (Van der Walt *et al.*, 2009). However, it is often very difficult to determine their nutritional contribution to total nutrient intake, because of lack of compositional data about these dishes in South Africa (Faber *et al.*, 2010). Flyman and Afolayan (2006) reported that there is generally insufficient information on the micronutrient contents of ALV dishes. In a review by Uusiku *et al.* (2010), the content of micronutrients in ALVs were summarised based on extracts of fresh ALVs, therefore the effects of cooking had not been taken into account, despite the fact that these vegetables are consumed cooked and combined into a composite dish. It is therefore important to know the nutritional contents of dishes.

The bioaccessibility of nutrients needs to be analysed in order to evaluate the contribution of food or meals to iron and zinc intake (Argyri *et al.*, 2011). The bioaccessibility of micronutrients, especially those of zinc and iron, is generally low in plant foods (Hemalatha *et al.*, 2007). It is therefore crucial to know the amount of iron and zinc ALVs can contribute. ALVs contain antinutrients (Uusiku *et al.*, 2010) and these can inhibit the accessibility of nutrients (Hemalatha *et al.*, 2007)

ALVs contain iron and zinc in varying degrees and these micronutrients are of public health importance in South Africa and many other African countries. However, it should be taken into consideration that ALV dishes are commonly consumed with staple stiff porridges made from maize meal and/or sorghummeal (Van der Walt *et al.*, 2009), which are natural high in phytate (Hotz & Gibson, 2001), which is an inhibitor of both iron and zinc. The Department of Health in 2003 enacted the mandatory fortification of maize meal and wheat flour with vitamin A, iron, zinc, folic acid, thiamine, niacin, vitamin B6 and riboflavin (South Africa, Department of Health, 2003). Vegetables are usually not taken as single dishes; therefore it is important to assess how starchy staple foods affect the bioaccessibility of iron and zinc from ALVs. Moreover, it will be important to know how the fortified and unfortified meal affects the bioaccessibility of iron and zinc from ALV dishes. Therefore, this research used both unfortified and fortified maize meal porridges to make composite ALVs porridges. ALVs contain high amount of antinutrients and studies that can evaluate how these antinutrients affect the bioaccessibility of nutrients from the vegetables are crucial.

Lack of information on the nutritional composition, nutritional value and health benefits of cooked ALV dishes (Orech *et al.*, 2007; Van der Walt *et al.*, 2009) limits their use in strategies aimed at alleviating particular iron and zinc deficiencies (Frison *et al.*, 2006). The nutritional composition of ALVs has mostly been studied in raw samples and the bioaccessibility of their vitamin and mineral content has not been studied adequately. More research is therefore needed on the bioavailability of iron and zinc in ALVs to determine their potential impact on micronutrient status in the human body and the way in which anti-nutrients affect their bioaccessibility.

## **1.2 PURPOSE AND IMPORTANCE OF STUDY**

This study is a first of its kind to be conducted in South Africa on the bioaccessibility of iron and zinc from ALV dishes and maize meal porridge composites. ALVs can play a potential role in the dietary patterns of ordinary South Africans living in rural areas and information on the nutritional composition on dishes will help researchers and policy makers to advocate the use ALVs in nutritional strategies. Information on the level of antinutrients in ALVs and the way in which they affect the bioaccessibility of iron and zinc will be generated in this study. This will be valuable information for planning food security programmes and determining the actual contribution ALVs can make in iron and zinc dietary intake. Combining ALVs with maize meal porridge into composite meals gives a representative meal and information on whether nutrient contents are affected when ALVs are eaten as a composite meal will be generated. The presence of antinutrients from both the ALV dish and composite meals will have an effect on nutrients absorption. The results generated will help to close the information gap on the nutrient and antinutrient content of ALV dishes and composite meals, the accessibility of iron and zinc from ALV dishes and the way in which it is affected by compositing the dishes with fortified and unfortified maize meal.

### 1.3 AIMS AND OBJECTIVES

#### 1.3.1 Overall aim

To evaluate the bioaccessibility of iron and zinc from ALV dishes and composite meals made from fortified and unfortified maize and its implications for nutrition.

#### 1.3.2 Specific objectives

1. To determine the iron and zinc content of *Amaranthus cruentus* (100%), and mixtures of *Amaranthus cruentus* (80%) and *Cleome gynandra* (20%), *Amaranthus cruentus* (80%) and *Cucurbita maxima* (20%) and *Amaranthus cruentus* (80%) and *Vigna unguiculata* (20%) ALV dishes, when combined with maize meal porridge.
2. To assess the bioaccessibility of iron and zinc in the ALV dishes listed above and when combined in dishes made from maize meal using the *in vitro* dialysability method.
3. To estimate anti-nutrient content in ALV dishes and to determine how it affects zinc and iron accessibility.
4. To evaluate how dishes that combine ALVs and unfortified or fortified maize-meal affect the bioaccessibility of zinc and iron in ALVs.
5. To evaluate if accessible iron and zinc in ALV dishes can potentially contribute to micronutrient nutrition.

### 1.4 METHODOLOGY

ALVs were harvested, combined and cooked in four dishes and representative samples were freeze-dried. The recipes used to cook and constitute each ALV dish was based on the results of studies conducted by Matenge *et al.* (2011). Each dish was combined with one sample of cooked fortified maize meal or unfortified maize meal to make two different composite meals. The mineral and antinutrient contents of ALVs and composites were evaluated; the bioaccessibility of iron and zinc from ALV dishes and composite dishes were evaluated using the *in vitro* bioaccessibility test. It is cheaper and easier to use *in vitro* dialysability method compared to use of animal models (Van & Glahn, 1998). Moreover, the method provides information on the efficiency of each digestion stage (Fernandez-Garcia *et al.*, 2009). However, the method has some limitations such as diffusion of iron into the dialysis bag and becoming insoluble at higher pH (Van & Glahn, 1998) which might give an inaccurate amount of iron absorbed. The method should be used with caution looking at its strength and limitations.

## 1.5 STRUCTURE OF THIS MINI-DISSERTATION

The mini-dissertation is divided into four chapters. Chapter 1 is the introductory chapter to the research. Chapter 2 is the literature review; it covers existing literature on iron and zinc, ALVs, *in vitro* studies on ALVs and the importance of ALVs in nutrition. Chapter 3 is an article prepared for publishing in the Journal of Global Food Security, titled “Mineral contents and bioaccessibility of iron and zinc in African leafy vegetables (ALVs) and maize meal composite dishes”. The last chapter (Chapter 4) states the main findings and makes further recommendations. A list of references for each chapter will follow at the end of the chapter. For chapters 1, 2 and 4 the North-West University reference guideline style will be used and chapter 3 will be referenced according to the Journal of Global Food Security reference style.

## 1.6 RESEARCH TEAM

| Name                      | Affiliation                                                                     | Role in the research                                                                                                |
|---------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| Prof. C.M. Smuts          | Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus | Supervisor for the whole research project, designed the study                                                       |
| Dr Marinka van der Hoeven | Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus | Co-supervisor - Assisted the supervisor in the research and guided the student                                      |
| Dr Namukolo Covic         | Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus | Co-supervisor - Assisted the supervisor in the research to guide the student, assisted in study design              |
| Dr Johanita Kruger        | Department of Food Science, University of Pretoria                              | Mentorship in laboratory analysis. Mentorship in data analysis and reporting, assisted in study design              |
| Mrs T. Mongwaketse        | Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus | Full-time MSc student, protocol writing, laboratory analysis, data interpretation and write-up of the dissertation. |

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## CHAPTER 2: LITERATURE REVIEW

### 2.1 INTRODUCTION

Malnutrition is a public health problem globally and includes both under- and over-nutrition (figure 1). Micronutrient deficiencies are mainly caused by inadequate food intake, poor nutritional quality of diets and poverty (WHO, 2012). Chronically malnourished individuals are usually faced with food insecurity and are mostly found in rural areas (Legwaila et al., 2011). Thirty-three percent of people in developing countries are affected by vitamin and mineral deficiencies (WHO, 2012). Iodine is one of the micronutrients of importance, which is being addressed by iodised salt and iodine capsules. Micronutrient deficiency can seriously affect the normal functioning of the body and makes the body susceptible to opportunistic infections and even increased death rates in women and children (Barasi, 2013). One of the highest levels of malnutrition in the world is found in Sub-Saharan Africa, particularly among rural children below five years of age (WHO 2012). Malnutrition is further explained in figure 2.1; the literature review will focus only on iron and zinc micronutrient deficiencies.

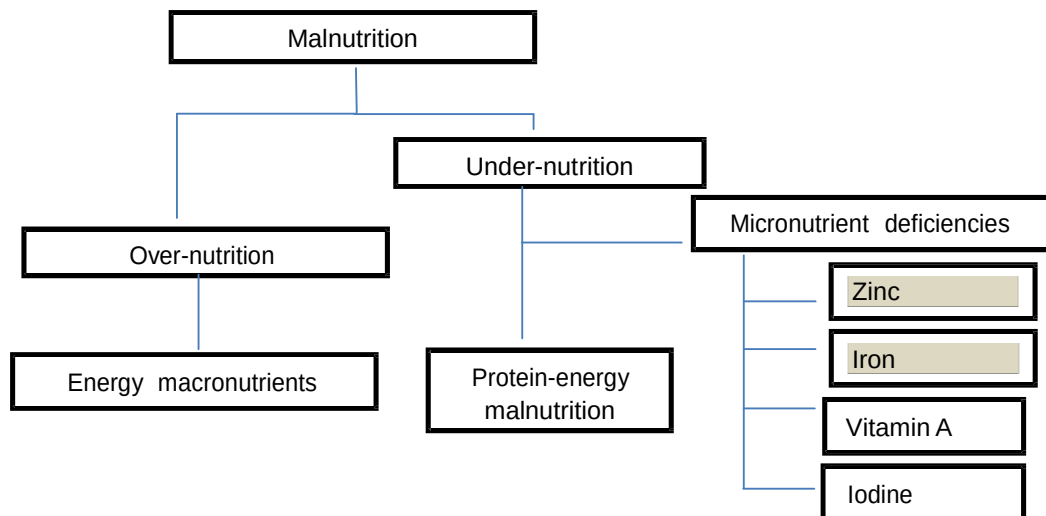


Figure 2.1: Classification of malnutrition (Adapted from Faber and Wenhold, 2007).

The aim of this chapter is to provide background information on iron and zinc in terms of functions, requirements, commonly used biomarkers and strategies used to address these deficiencies. It will

also focus on information available on ALVs in terms of nutrient composition, knowledge and use, accessibility of iron and zinc and factors that influence the bioavailability of iron and zinc.

## **2.2 BACKGROUND INFORMATION ON IRON AND ZINC**

Iron and zinc are crucial minerals in the body and are mostly assessed together, as they share the same food sources and inhibitors and their deficiencies occur simultaneously. These micronutrients are key in human growth, development and immune system maintenance (Fischer *et al.*, 2005). Iron is essential for binding and transporting oxygen and regulating cell growth (Formanowicz & Formanowicz, 2011). Most iron is present in the red blood cells as haemoglobin (WHO, 2004). Zinc is essential for growth, cell division, the immune system and fertility (WHO, 2004). Almost all good sources of iron also contain zinc, with the exception of milk products, which are good sources of zinc but poor sources of iron (Walker *et al.*, 2005; Lim *et al.*, 2013). The two micronutrients are very important in the human body and their deficiencies, biomarkers and requirements will be elaborated further.

### **2.2.1 Iron and zinc deficiency**

A deficiency occurs when an imbalance occurs between consumed bioavailable iron and zinc and utilisation of these minerals in the body, which may be due to inadequate intake or increased requirements (Lynch, 2011). Furthermore, Faber and Wenhold (2007) state that iron deficiency occurs when the amount of bioavailable iron is below the daily requirements or when excessive physiological or pathological losses of iron occur. The leading risk factors for disability and death worldwide is iron deficiency, affecting approximately two billion people (Zimmerman & Hurrell 2007). According to the WHO (2001), a decrease in levels of haemoglobin and serum ferritin in the body leads to iron deficiency. In clinical settings, anaemia is commonly used as an indicator of iron deficiency (Ramakrishnan & Semba, 2008). IDA is one of the most important forms of malnutrition worldwide, affecting 1.62 billion people globally (Balarajan *et al.*, 2011). The WHO defines IDA as “reduced erythropoiesis as a consequence of iron deficiency such that the haemoglobin levels fall two standard deviations below the mean haemoglobin (Hb) for that population and gender” (Rattehalli *et al.*, 2003). The WHO uses age-specific Hb cut-off points to define anaemia (Table 2.1). IDA results in reductions in Hb concentration, red cell count, packed-cell volume and subsequent impairments in meeting the oxygen demands of tissues (Balarajan *et al.*, 2011). Shortages of iron result in brain damage, which may result in cognitive and behavioural abnormalities (Rattehalli *et al.*, 2003; Zimmerman & Hurrell 2007). Children who are iron-deficient will also have low motor development and pre-menopausal women participating in sport may perform poorly and experience fatigue (Yip, 2001)). Hb

concentration can be affected by physiological characteristics such as age, sex, and pregnancy status, as well as environmental factors such as smoking and altitude (Lynch, 2011).

Table 2.1: World Health Organisation haemoglobin cut-off points for anemia

| <b>Anaemia</b>             |            |
|----------------------------|------------|
| Children under five years  | Hb<110 g/L |
| Non-pregnant women         | Hb<120 g/L |
| Pregnant women             | Hb<110g/L  |
| Men                        | Hb<130 g/L |
| <b>Severe anaemia</b>      |            |
| All age groups             | Hb<70 g/L  |
| <b>Very severe anaemia</b> |            |
| All age groups             | Hb<40 g/L  |

(Adapted from WHO, 2004).

Different biomarkers, such as serum ferritin, transferrin saturation, haemoglobin, haematocrit and erythrocyte zinc protoporphyrin, are used to measure the iron status of the body. Usually iron deficiency is defined by abnormality in one or more of these biomarkers, while a diagnosis of IDA will result from meeting the criteria of both iron deficiency and anaemia based on haemoglobin status (Ramakrishnan & Semba, 2008). Serum ferritin measures body iron stores, but it may give inaccurate iron store scores during inflammation and infections, therefore one needs to include some markers of inflammation, such as C-reactive protein for acute inflammation and alpha-1-acid glycoprotein for chronic inflammation (Mei *et al.*, 2005). Serum ferritin cut-off points are <12 µg/L for children of five years and younger, <15 µg/L for children older than 15 years and <30 µg/L for all age groups in the presence of infection (Mei *et al.*, 2005). The transferrin saturation biomarker of iron status is based on the amount of iron transported in the body, as iron binds to the iron-binding protein transferrin and values under 16% in adults, 12% in infants and 14% in children are indicative of iron deficiency (WHO, 2004). Haematocrit measures the percentage of whole blood made up of red blood cells (Ramakrishnan & Semba, 2008). A low haematocrit may indicate anaemia. Another measure is looking at the colour and size of the erythrocyte, which is usually in bi-concave shape. When the erythrocytes look smaller and paler than usual, these are indications of anaemia (WHO, 2004). All these biomarkers have limitations and need special consideration. Malaria and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) have an effect on the iron status of an individual and there have been debates on how biomarkers should be used in areas where malaria and HIV/AIDS occur, though this is outside the scope of this thesis.

Insufficient zinc intake is associated with poor growth in children, loss of appetite, skin lesions, delayed wound healing, delayed sexual maturation and an impaired immune system (Shrimpton & Shankar, 2008). Mild zinc deficiency can contribute to low birth weight, an impaired immune system,

maternal and infant mortality and morbidity and growth failure in infancy and childhood (Gibson, 2012). Zinc status is very important at every stage of life. More than one third of children in developing countries are stunted and it is believed that zinc deficiency is one of the underlying causes of stunting and delayed sexual maturation (WHO, 2004). In addition, high mortality rates in developing countries are linked to zinc deficiency (WHO, 2004). Infants and young children, as well as pregnant and lactating women, are at risk of zinc deficiency (Shrimpton & Shankar, 2008). In complementary feeding mixtures fed to infants, zinc is commonly deficient (Dewey & Brown, 2003). This places infants at risk of zinc deficiency. To measure zinc deficiency, different biomarkers will be discussed.

Zinc status is widely measured using serum zinc concentration (Davidsson *et al.*, 2007). It is believed that serum zinc concentration decreases within days of dietary zinc restrictions and rises when zinc is ingested (Hess *et al.*, 2007). However, this method has its own pitfalls, as there are metabolic conditions unrelated to zinc status such as pregnancy, chronic diseases, malnutrition and liver diseases, that can result in a decline of serum zinc concentration (Gibson, 2006). Nevertheless, several zinc metalloenzymes, zinc-binding proteins and hair zinc concentrations have been investigated as an alternative to serum zinc concentrations (Gibson *et al.*, 2008). More research is needed to determine sex-specific and cut-off points for hair zinc concentration for children.

### **2.2.2 Requirements for iron and zinc**

Requirements for iron and zinc are age and gender specific and other conditions, such as pregnancy and infections, are taken into consideration when determining the requirements of an individual. According to the Academy of Science of South Africa age, gender and physiological state influence the iron and zinc requirements of an individual (ASSAf, 2013). Children and women of child-bearing age are usually physiologically vulnerable because the demand for iron and zinc becomes high during periods of rapid growth, especially during infancy and pregnancy (Balarajan *et al.*, 2011; Gibson, 2012; ASSAf, 2013). Therefore, it is very important that diets of women of child-bearing age and pregnant mothers have adequate iron and zinc to avoid low birth weight and deficiencies. In addition, during pregnancy iron requirements range from 0.8 mg per day in the first trimester to 7.5 mg per day in the third trimester (Balarajan *et al.*, 2011). In a review conducted by Gibson (1994), male infants and children had higher zinc requirements than their female counterparts because of their high growth rates and greater proportion of muscle per kilogram of body weight. Countries such as Australia, the United States of America and Canada have set RDA and estimated average requirements (EAR) standards. RDA gives the intake level of a particular nutrient that is deemed sufficient to meet the requirements of 97-98% of a healthy population and the EAR is the estimated nutrient intake that will meet the requirements of 50% of the population (Gibney & Wolmarans, 2004). Figures 2.2 and 2.3 present the iron and zinc EAR and RDA (Lim *et al.*, 2013). EAR values are lower than RDA because it is assumed that since the exact requirements of an individual are unknown, the best estimate will be the mid-point (Lim *et al.*, 2013).

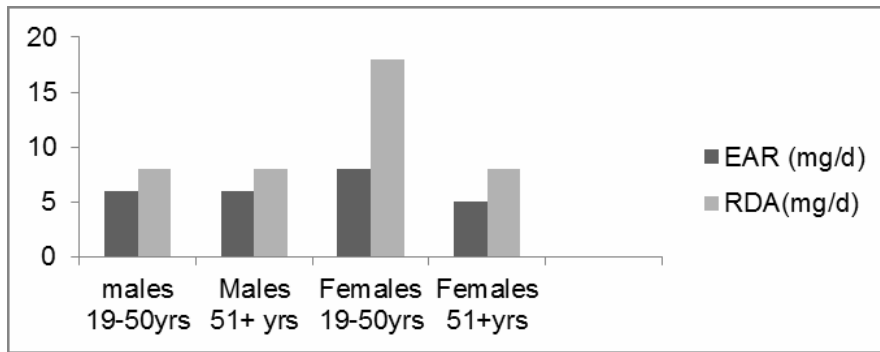


Figure 2.2: Recommended iron intake (Adapted from Lim *et al.*, 2013).

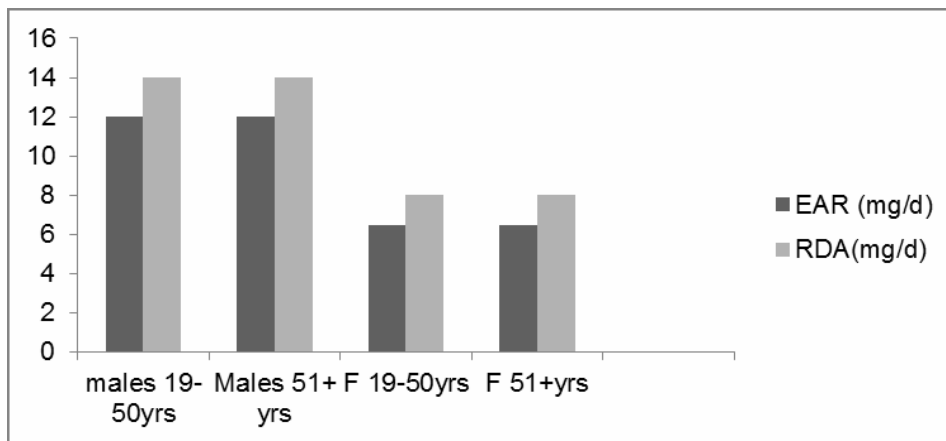


Figure 2.3: Zinc recommended daily intake (Adapted from Lim *et al.* 2013).

### 2.2.3 Summary of iron and zinc nutritional situation in South Africa

Several national surveys were conducted in South Africa to assess nutritional status. In 1994 the South African Vitamin A Consultative Group (SAVACG) reported that 20% of children were anaemic and in 1999 iron and zinc intakes of children aged one to nine years were below two thirds of RDA (SAVACG, 1995; Labadarios, 2000). The 1999 National Food Consumption Survey (NFCS) recommended the fortification of the most frequently consumed staple foods (maize and bread). In 2003, the mandatory fortification of maize meal and wheat flour with iron, zinc, vitamin A, folic acid, thiamine, niacin, vitamin B<sub>6</sub> and riboflavin was introduced to help eradicate vitamin and mineral deficiencies (Steyn *et al.*; 2007). In 2005, the National Food Consumption Survey-Fortification Baseline (NFCS-FB) on children aged one to nine years also reported levels of iron deficiency (14%),

anaemia (33%), zinc deficiency (45.3%), stunting (10%) and underweight (10%) (Labadarios, 2007). A study conducted on elderly South Africans in Sharpeville reported that 76.3% of the respondents were zinc-deficient (Oldewage-Theron *et al.*, 2008). From 1994 to 2005 the iron and zinc nutritional status of South Africans did not improve and stunting and underweight were still evident. However, a review conducted by Taljaad *et al.* (2013) on studies conducted since 2005 in South Africa reported an anaemia prevalence lower than that of the NFCS-FB and the recent SANHANES-1 conducted in 2012 reported an improving situation in terms of the prevalence of iron deficiency (11%), anaemia (10.5%) and IDA (2.1%) in children under five years of age (Shisana *et al.*, 2013). Stunting has been reported to be a proxy measure outcome of zinc deficiency (ASSAf, 2013). The 1994 South African Vitamin A Consultative Group (SAVACG) study on children aged six to 71 months reported stunting as the most prominent nutritional disorder (SAVACG, 1995), with a prevalence of 25%; in the 1999 survey it reduced to 20% (Labadarios, 2000). The NFCS-FB survey conducted in 2005 reported that 20% of children aged one to nine years were stunted (Labadarios, 2007) however, the SANHANES-1 survey reported decreased stunting rate (15.4%) for children aged 0-14 years, with a highest prevalence among boys and girls respectively of 26.9% and 25.9% (Shisana *et al.*, 2013). Moreover, studies conducted at provincial level in South Africa also reported patterns of stunting, i.e 48% of children aged three years residing in the central region of Limpopo province were reported to be stunted (Mamabolo *et al.*, 2005) and 25% of children aged one to four years in a study conducted in 2007 in Mpumalanga province were also reported to be stunted (Kimane-Murage *et al.*, 2010). Nationally the prevalence may be low but the situation may be higher in individual provinces. Although iron nutritional status has improved compared to previous years, it remains a public health concern in South Africa.

#### **2.2.4 Nutrition interventions strategies to prevent zinc and Iron deficiency with special focus on South Africa**

To address iron and zinc deficiencies, strategies such as fortification, supplementation and dietary diversification are very important. Evidence presented above on the iron and zinc situation shows that South Africa has a problem in respect of both iron and zinc deficiency. According to Witten *et al.* (2003) the South African Department of Health implemented strategies such as food fortification, iron and zinc supplementation and dietary diversification to eliminate micronutrient deficiencies.

##### **2.2.4.1 Fortification**

Fortification plays a crucial role in reducing micronutrient deficiencies in South Africa. In 2003, the national mandatory fortification of maize meal and wheat flour was introduced (South African Government notice, 2003). Maize meal and wheat flour were reported to be the most frequently consumed food in the 1999 National Consumption Survey, together with sugar, tea and whole milk (Labadarios, 2000).

Fortification is the most practical, sustainable, cost-effective long-term solution to control iron deficiency at national level (Zimmermann & Hurrell, 2007). The NFCS identified the staple foods consumed most often and these were chosen as vehicles for fortification. However, it is always more difficult to fortify with iron than any other nutrient because most bioavailable iron compounds are soluble in water and react with other food components to produce off-flavours, hence they result in undesirable colour changes and fat oxidation (Hurrell, 2002). Less soluble forms are always deemed the best choice to avoid unwanted sensory changes. Staple foods are mostly used in developing countries as a vehicle for fortification; however, iron is poorly absorbed from high-extraction flours because of inhibitory factors such as phytates and tannins (Hurrell *et al.*, 2004). This makes elementary iron powders the best option to use, as they are less reactive though they have lower bioavailability (Swain *et al.*, 2003). In South Africa, maize and flour are fortified with 35 mg/kg of electrolytic iron (South African Government notice, 2003).

Five forms of zinc compounds are considered safe by the United States of America Food and Drug Administration, namely zinc sulphate, zinc chloride, zinc gluconate, zinc oxide and zinc stearate (Robberstad *et al.*, 2004). When considering fortifying with zinc, some studies recommended zinc oxide as the ideal compound to use because it is easily absorbed, stable and cost-effective (Rosado, 2003), though some studies have found that the compound has lower solubility (Black *et al.*, 2003). According to Rosado (2003), the amount of compound to be used when fortifying staples should be approximately 20-50 mg/kg, but in South Africa the dosage for wheat flour and maize meal is much lower, i.e. 15 mg/kg of zinc oxide. Fortification with zinc has increased dietary zinc intake and total daily zinc intake (Hess & Brown 2009).

In South Africa fortified maize meal porridge with ferrous fumarate effectively lowered IDA and improved both the iron stores and motor development of infants in poor settings (Faber *et al.*, 2005). Fortifying curry powder with sodium iron ethylenediaminetetraacetic acid (NaFeEDTA) has also been effective in South Africa (Ballot *et al.*, 1989). There is a two to three times chance that NaFeEDTA will be absorbed better than ferrous sulphate from high phytic acid diets and will not promote fat oxidation in stored cereals. Unfortunately it is only approved for use as a food additive at 0.2 mg of iron a day as NaFeEDTA per kilogram of body weight (WHO, 1999). Beyond this level there are some health implications and at the level it is used as food additive it will not have any impact on the iron status. A national fortification evaluation survey should be conducted to evaluate the effectiveness of fortification with zinc and iron in South Africa.

#### 2.2.4.2 Supplementation

Iron and zinc can be consumed in the form of supplements to help prevent deficiencies of these nutrients. According to Graham *et al.* (2012), most anaemic children are also zinc-deficient, therefore supplementation with iron alone will not be ideal to treat anaemia effectively. For high-risk groups such as pregnant women and infants, iron supplementation should be a target and oral supplementation with ferrous iron salts is cost-effective, though the logistics of distribution and lack of compliance are always major limitations (Zimmermann & Hurrell 2007). However, when there is chronic inflammation, oral supplementation of iron is poorly absorbed in the gut (Rattehalli *et al.*, 2003). In South Africa, children aged six to 11 years were given supplementary iron for 8.5 months. Where children were overweight and obese or remained iron-deficient after supplementation, inflammation was cited as the cause of poor absorption of the supplement (Baumgarther *et al.*, 2013). The SANHANES-1 survey reported that 40.1% of women 15 years and older were obese (Shisana *et al.*, 2013) and this could hinder iron absorption during supplementation. There is evidence that children who received supplementary zinc had a lower rate of diarrhoea and pneumonia (Black *et al.*, 2004). Combined interventions of zinc and iron in treating IDA in women endurance runners, disabled patients, pregnant women and premature infants resulted in faster recovery (Graham *et al.*, 2012). Conversely, it is believed that iron only reduces zinc absorption when consumed in the form of supplements (Etcheverry *et al.*, 2012).

In a study conducted in Southeast Asia, iron and folic acid supplementation for a week improved iron nutrition and reduced IDA (Cavalli-Sforza *et al.*, 2005). A study conducted in the USA also showed that giving women supplementary iron increased birth weight and reduced the incidence of preterm delivery (Cogswell *et al.*, 2003). In most developing countries, where most pregnant women have low iron stores in their third trimester, iron supplementation is encouraged (Zimmermann & Hurrell 2007). In South Africa, a study conducted in 2009 in the Northern Cape province reported that the prevalence of iron deficiency decreased by 30% and zinc by 11.8% after school children received a supplementary multinutrient powder that contained phytase (Troesch *et al.*, 2010). A study in conducted in the Valley of a Thousand Hills, northwest of Durban, KwaZulu-Natal province in South Africa, found improved iron and zinc status for infants aged six to 12 months after multiple micronutrient supplementation (Smuts *et al.*, 2005). To improve the availability of nutrients, supplements can be added directly to meals immediately before consumption in the form of sprinkles, crushable tablets and fat-based spreads (Zimmermann & Hurrell 2007). The available evidence shows that zinc and iron supplementation is effective, but the high prevalence of obesity in South Africa may pose a challenge for iron absorption.

#### 2.2.4.3 Dietary modification/diversification

Fortification and supplementation are effective strategies in preventing and treating iron and zinc deficiencies; however, these strategies target one or specific nutrients and it is usually not cheap to implement these programmes (Zimmermann & Hurrell 2007). Therefore, other food-based strategies should be implemented. Dietary diversity encourages people to eat a variety of food and exposes them to more nutrients.

Dietary diversity results in increased access to a variety of foods, which results in good nutrition (Frison *et al.*, 2006). The strategy of dietary diversification results in long-term access to good nutrition, whereas supplementation is a short-term and fortification a medium-term measure. Fortification and supplementation focus on particular nutrients, while diversity is broader and aims at changing global patterns of diet and diseases by supporting the notion that micronutrient deficiencies rarely occur in isolation; it is a long-term strategy that assists households to gain access to a variety of sustainable food systems (Frison *et al.*, 2006; Labadarios *et al.*, 2011). In a study conducted in Bangladesh (Talukder *et al.*, 2000), growing a vegetable garden increased production and consumption of vegetables by 50%. Lack of dietary diversity has been linked to deficiencies such as iron and zinc (Chakravarty 2000; Grivetti & Ogle 2000).

Smith (2013) explains that people in West Africa are replacing local indigenous foods with maize, wheat or rice because these are easier to prepare than traditional foods, which may need some traditional processing that is tedious and time-consuming. Even in South Africa the THUSA study reported that people are moving from a diet consisting of high complex carbohydrates with low fat to a diet with refined carbohydrates and high fat (MacIntyre *et al.*, 2002). In a study conducted among South African people aged 16 years and older in 2009, low dietary diversity was reported, with dark green leafy vegetables being the least consumed food group (Labadarios *et al.*, 2011). These included ALVs eaten in South Africa. Strategies that may increase the dietary intake of fruit and vegetables, including the use of ALVs, should be encouraged in rural areas. To encourage the consumption of fruit and vegetables and dietary diversity, South Africa has dietary guidelines to encourage people to diversify their diets by eating a variety of foods from different food groups and mixed meals and to have at least five servings of fruit and vegetables per day (Steyn, 2013).

## 2.3 AFRICAN LEAFY VEGETABLES

ALVs may play a greater role in preventing iron and zinc deficiencies, as they may have a potential in diversifying the diet, thus improving the chances of access to other nutrients. The use of traditional wild vegetables has been found to be a very important strategy in improving food security and livelihoods (Frison *et al.*, 2006; Faber *et al.*, 2010)

According to Chadha and Oluoch (2003), high cost, seasonal variability and limited supply restrict the consumption of commercially grown vegetables in developing countries. ALVs play a major role as an accompaniment to staples in the diet of people living in rural areas. According to Jansen van Rensburg *et al.* (2007), the first people to settle in Southern Africa (Khoisanoid) 120 000 years ago depended heavily on the gathering of plants from the wild for their survival. Habwe and Walingo (2008) report that there are more than 45 000 species of plants in Sub-Saharan Africa, of which about 1000 can be eaten as ALVs. Some of these ALVs either grow wild or are cultivated, such as pumpkin and cowpeas (Faber *et al.*, 2010). According to Uusiku *et al.* (2010) ALVs are usually cooked and eaten as a relish together with starchy staple foods made from maize meal, millet and sorghum. In addition, condiments and spices, oil, butter, groundnuts, coconut, milk, bicarbonate of soda, tomatoes, potatoes, onions and peppers are sometimes added to them, depending on availability and preferences (Uusiku *et al.*, 2010). Some of the different types of ALVs found in Africa are presented in table 2.2.

Table 2.2: Common African leafy vegetables consumed in Africa and South Africa

| West/East and Central Africa | West and Southern Africa   | East/Central and Southern Africa | South Africa             |
|------------------------------|----------------------------|----------------------------------|--------------------------|
| <i>Basella alba</i>          | <i>Amaranthus caudatus</i> | <i>Solanum nigrum</i>            | <i>Amaranthus spp</i>    |
| <i>Citrullus lunatus</i>     | <i>Amaranthus hybridus</i> | <i>Bidens pilosa</i>             | <i>Cleome gynandra</i>   |
| <i>Colocasia esculenta</i>   | <i>Portulaca oleracea</i>  | <i>Cleome gynandra</i>           | <i>Bidens spinosa</i>    |
| <i>Hibiscus sabdariffa</i>   |                            |                                  | <i>Cucurbita maxima</i>  |
| <i>Moringa oleifera</i>      |                            |                                  | <i>Vigna unguiculata</i> |

(Adapted from Smith & Eyzaguirre, 2007 & Faber *et al.*, 2010).

Note: For the purpose of this literature review, common English names for each ALV will be used.

Although the consumption or use of ALVs has been associated with people of low socioeconomic status (Odhav *et al.*, 2007), a review by Uusiku *et al.* (2010) reported that ALVs contained micronutrient levels as high as or higher than those found in exotic leafy vegetables such as cabbage (*Brassica oleracea*) or spinach (*Spinacea oleracea*). Furthermore, a study in Kenya found that traditional leafy vegetables such as spider plant (*Cleome gynandra*), cowpea (*Vigna unguiculata*) and

amaranth (several species), both domesticated and wild, contained higher levels of calcium, iron and zinc, compared with introduced varieties such as spinach, kale and cabbage (Orech *et al.*, 2007). Nevertheless, many research and community extension personnel have treated ALVs as weeds and encouraged farmers to keep the weed population under control (Voster *et al.*, 2007). Odhav *et al.* (2007) encourages health workers to promote the use of locally consumed ALVs, especially for children to improve the quality of their diets. To address malnutrition, dietary modification strategies should focus on the nutritional quality of the diet, particularly the micronutrients of greatest concern, namely iron, zinc and vitamin A (Faber *et al.*, 2010).

Large proportions of households in Sub-Saharan Africa are poor and depend on diets that consist mainly of staple foods prepared from cereals that are low in micronutrients. ALVs can play a major role in enhancing the nutritional value of diets (Uusiku *et al.*, 2010). In addition, ALVs have been found to be well adapted to harsh weather and resistant to pests and pathogens, hence they can contribute positively to improve nutritional status (Chanda & Oluoch 2003). In addition, they may undergo many processing methods such as drying to ensure the availability of the vegetables when out of season (Voster *et al.*, 2007).

Studies reported that cultures that retain their traditional diets and consumption of ALVs were less likely to be affected by non-communicable diseases (Habwe & Walingo, 2008). Nutrition transition resulted in the decline of consumption of traditional vegetables and increasing consumption of refined and processed foods, fats, sugars and animal products (Weinberger & Swai, 2006). Moreover, increases in non-communicable diseases have been associated with shifts in dietary patterns in urban and rural areas from traditional food systems to western-type cereal-based high energy diets (Frison *et al.*, 2005).

### **2.3.1 African leafy vegetables found in South Africa**

In South Africa, different kinds of ALVs are available. Despite the different ALVs that are available, a study conducted in South Africa (Voster *et al.*, 2007) found that the ALVs consumed most often were amaranth and pumpkins leaves, with jute mallow (*chorcorus spp*) and spider plant (*Cleome gynandra*) (figure 2.4) being commonly used in the northern areas of South Africa. It was reported that cowpea was seen as the most important dried leafy product; it was used during drought seasons owing to its long shelf life (Voster *et al.*, 2007). Matenge *et al.* (2012) reported that cowpea leaves were the most acceptable ALV during a sensory evaluation conducted in the North West province of South Africa.



Figure 2.4: Common ALVs found in some parts of South Africa

The four common ALVs found in some parts of South Africa will be briefly discussed below to give a background insight. It is reported that *Amaranthus cruetus* (amaranth) is a herbaceous plant that is normally an annual and its leaves are eaten in most developing countries; in Nigeria it is used combined with other condiments for soup (Akubugwo *et al.*, 2007). *Vigna unguiculata* (cowpea leaves) belongs to the leguminosae family and is a leafy pulse crop (Jansen van Rensburg *et al.*, 2007). According to Vorster *et al.* (2002), cowpea leaves are indigenous to Africa and are cultivated annually throughout the continent. They are primarily grown for pulses, but the young leaves are used as a leafy vegetable (Jansen van Rensburg *et al.*, 2007). *Cleome Gynandra* (spider plant) is from the *capparaceae* family, is a branched plant and has a height that ranges from 0.5 m to 1.5 m (Jansen van Rensburg *et al.*, 2007). In the hot northern parts of South Africa spider plant is more popular than amaranth. It is sensitive to cold and grows best during summer (Jansen van Rensburg *et al.*, 2007). According to Vorster *et al.* (2002), when preparing spider plant, amaranth leaves are sometimes added to increase the volume. Spider plant is bitter and strategies to reduce its bitterness include changing the water when boiling it and cooking it in milk (Jansen van Rensburg *et al.*, 2007). According to Ndlovu and Afolayan (2008), *Corchorous olitorius* (wild jute) is found in the wild and grows naturally in South Africa. Its nutritive value and nutrient bioavailability have not been well investigated. ALVs can be eaten fresh and are sometimes cooked and sun-dried to be used when out of season. Evidence shows that ALVs are commonly used in South Africa (Matenge *et al.*, 2012) and therefore it is very crucial to study their nutritional composition (Uusiku *et al.*, 2010) as they might potentially contribute towards better nutrition.

### 2.3.2 Nutritional composition of African leafy vegetables with the focus on South Africa

Different types of ALVs have been investigated to determine their nutritional content by different researchers in different parts of Africa, including South Africa. A study conducted in Kenya reported that cultivated or wild amaranth contained higher levels of calcium, iron and zinc compared to spinach, kale and cabbage (Orech *et al.*, 2007). In a study conducted in Tanzania, amaranth and spider provided approximately 11% of the iron requirements of poor households (Weiberger & Swai, 2006). ALVs are good sources of fibre, vitamins and minerals (Chanda & Oluoch, 2003). A comparison of the nutritional value of amaranth with the RDA values proved that the leaves can contribute appreciable amounts of zinc and iron (Akubugwo *et al.*, 2007). According to Abukutsa-Onyango (2007), amaranth, spider plant, vegetable cowpeas and pumpkin leaves could potential meet the iron RDA needs of individuals.

In a study conducted on five traditional South African dark green leafy vegetables, it was determined that raw leaves of pigweed (*Amaranthus tricolor*), pumpkin leaves (*curcubita maxima*) and spider plant contain high levels of iron, both cooked and raw (figure 2.5), and can potentially contribute to iron intake in South Africa (Schönfeldt & Pretorius 2011). In a study conducted in Limpopo and KwaZulu-Natal provinces in South Africa, amaranth leaves were found to be rich in  $\beta$ -carotene content. The plant can potentially contribute to the vitamin A requirements of nutritionally vulnerable communities (Faber *et al.*, 2010). In addition, in KwaZulu-Natal a preliminary study was conducted on the nutritional content of raw ALVs (Table 2.3), which reported high zinc content in amaranth (56%).

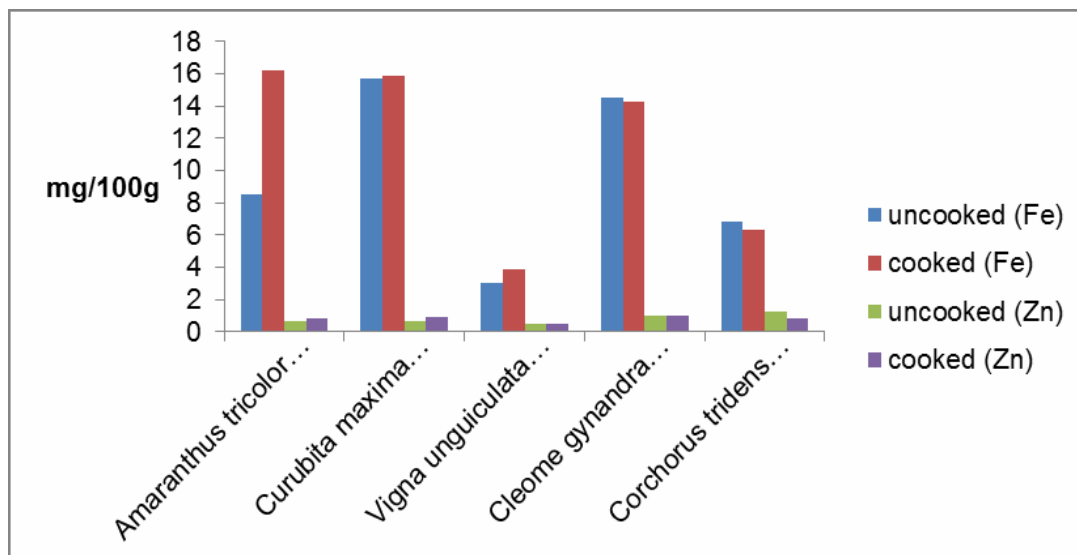


Figure 2.5: Zinc and iron content of five ALVs (mg/100g dry weight [DW]) (adapted from Schonfeldt & Pretorius, 2011).

Table 2.3: Iron and zinc content of raw vegetables found in KwaZulu-Natal (mg /100 g DW) (Adapted from Faber *et al.*, 2010).

| ALV            | Zinc (mg/100g) | Iron (mg/100g) |
|----------------|----------------|----------------|
| Amaranth       | 56             | 25             |
| Spider plant   | 5              | 24             |
| Pumpkin leaves | 11             | 20             |

According to Uusiku *et al.* (2010), the same species of ALVs in different studies yielded varied amounts of nutrients. This might have been due to factors such soil type, quantity and type of fertilizers used and age at harvesting. When comparing ALVs to legumes, they are not good protein sources (Uusiku *et al.*, 2010). In rural South Africa, ALVs are found to be the main accompaniment source of food in the maize-based subsistence farming sector, as they are eaten as relish to accompany maize meal porridge (Mavengahama *et al.*, 2013).

The nutritional composition of ALVs was reported only for raw and cooked vegetables on their own. No studies in South Africa reported the nutritional composition of combined ALV dishes and as composites with maize meal. There is insufficient nutritional knowledge of the composition of ALV dishes, ways of preservation and cooking methods (Flyman & Afolayan 2006). The nutritional composition of ALV dishes needs to be investigated, also when taken as part of a meal, i.e. compositing it with maize meal. It is recommended that the bioavailability of micronutrients in cooked vegetables should be investigated, as they contain antinutrients, which may hinder the absorption of nutrients (Uusiku *et al.*, 2010).

### 2.3.3 Antinutrient content of ALVs

It is believed that wild vegetables contain high levels of anti-nutrients, which relate to their function in plant protection (Olge *et al.*, 2001). ALVs contain antinutrients such as phytates, phenolics and tannins (Uusiku *et al.*, 2010). These antinutrients may inhibit the bioaccessibility of iron and zinc that should be absorbed. When ALVs are mixed with other dishes, there is also a possibility that the antinutrients present in them may hinder the absorption of iron and zinc from staples (Mavengahama *et al.*, 2013). There is insufficient evidence on how antinutrients in ALVs affect the accessibility of nutrients. Moreover, as previously reported, ALVs are taken with a starch food, mostly maize meal, which also contains phytate that has an effect on the availability of nutrients. Studies that can determine the bioaccessibility of nutrients in ALVs and in maize meal-ALV dishes are therefore needed to be able to provide erudite information on their nutrient value in food security and other nutrition programmes. This literature review will look at phytate and polyphenols.

### 2.3.3.1 Phytates

Salts of phytic acid, designated as phytates are regarded as the primary storage form of both phosphate and inositol in plants and seeds (Greiner & Konietzny, 2006). Phosphorus in many plant tissues is stored as phytic acid (Kumar *et al.*, 2010). Phytic acid chemical description is myoinositol hexakisphosphoric acid hence known as inositol hexakisphosphate (IP6) or phytate when in salt form and it is the most common constituent of plant derived food such as cereals and legumes (Kumar *et al.*, 2010). The structure of phytic acid has the ability to attract and binds cations from calcium, magnesium, zinc, copper, zinc, iron and potassium to form insoluble salts (Raboy, 2001) and this result in the negative effect on mineral uptake (Greiner & Konietzny, 2006; Kumar *et al.*, 2010). It also forms insoluble complexes with aminoacids where the phosphoric acid group of phytate bind with the cations in the group of the amino acid and only dissolves at pH below 3.5 (Kumar *et al.*, 2010). Human small intestinal gut has limited capacity to break down phytate due to lack of phytate degrading enzymes (phytases) (Iqbal *et al.*, 1994). There are about four possible phytase sources i.e plant phytase, microbial phytase, phytase in the small intestinal mucosa and gut associated microfloral phytase (Kumar *et al.*, 2010).

Zinc and iron deficiencies had been reported in populations consuming unrefined cereals and pulses as major dietary component (Lonnerdal, 2000). These are common diets of people living in developing countries. To reduce iron and zinc deficiency in diets containing phytates, dietary strategies should focus on reducing the content of phytate in the food product. Hurrell (2003) also emphasised that, phytate must be reduced in food products to increase mineral absorption especial iron.

Genetically engineering had been reported to increase the absorption of iron and zinc as it decrease the phytate content of food product. Phytate degradation during food processing could be activated by adding exogenous phytases or by creating favourable environmental conditions for the native plant or microbial phytases to function (Greiner & Konietzny, 2006). Addition of isolated phytase during food processing was reported to be an option in the optimisation of phytate dephosphorylation by enzyme already present in the raw food. Phytate content was reduced in black beans after treated with supplemental phytase (Greiner & Konietzny, 1999). In addition, desphosphorylation of phytate is very vital to improve the nutritional value of food as the removal of the phosphate group from the inositol ring decreases the mineral binding effect of phytate (Kumar *et al.*, 2010). In a study investigating how inositol phosphates with different number of phosphate groups influence iron absorption it was found that the less the phosphate group the increased the mineral availability (Sandberg *et al.*, 1999). Different food processing and preparation methods can reduce phytate content. In a study in

rural Malawi, soaking and pounding were effective in reducing phytate content of white maize (Hotz & Gibson, 2001).

However, phytate consumption does not seem to have only negative impact on human health, it was reported that dietary phytate prevented kidney stone formation in a study conducted on calcium oxalate stone formers and healthy people (Grases *et al.*, 2000). Also in mammalian cells, phytate exert its anticancer effect by affecting cell signalling mechanism (Vucenik & Shamsuddin, 2003). Also colon cancer cells, HT-29 were inhibited when subjected to phytate in *in vitro* study (Yang & Shamsuddin, 1995)

ALVs contains phytate which reduce mineral absorption and commercial and home based strategies are needed to reduce phytate content and improve mineral uptake. Positive benefits of phytate in the diet should therefore, not be ignored.

### **2.3.3.2 Polyphenols**

According to Gillooly *et al.*, (1983), polyphenols present in many vegetables include phenolic compounds tannins and flavonoids. Phenolic compounds are products of secondary metabolism of plants (Bravo, 1998). They range from simple molecules (phenolic compounds) to highly polymerised compounds (tannis)(Porter, 1989). Astringency and bitterness of foods and beverages depend on the content of polyphenolic compounds content of the food product (Bravo, 1998).

Polyphenols form complexes with mineral cations through their carboxylic and hydroxylic groups and interfere with absorption of minerals (Shahidi & Ho, 2005). Beverages such as tea, red wine, cocoa and coffee contains different kinds of phenolic compounds and they form insoluble complexes with iron and inhibit iron absorption (Brown *et al.*, 1990). Phenolics in vegetables may strongly inhibit iron absorption from a composite meal (Shahidi & Ho, 2005). In a study that investigated non-haem iron absorption in man consuming polyphenolic-containing beverages, it was reported that beverages containing polyphenolic compounds were potent inhibitors of iron absorption (Hurrell, Reddy & Cook, 1999). Polyphenols binds to iron to make it unavailable for absorption. A significant negative correlation had been reported between the total polyphenol content and absorption of iron content (Gillooly *et al.*, 1983).

Tannins are water soluble phenolic compounds and occur in all vascular plants (Shahidi & Ho, 2005). Tannins can form insoluble complexes with protein and carbohydrates (Porter, 1989). They are two groups of tannins i.e hydrolysable and condensed tannins. Condensed tannins have a more profound digestibility-reducing effect than hydrosable tannins (Gillooly *et al.*, 1983). Hydrolysable tannins are easily hydrolysed with acid, alkali, and hot water and enzymatic action which yield polyhydric alcohol and Phenylcarboxylic acid (Porter, 1989).

However, polyphenols also have a positive effect in the body as antioxidants, antimutagens and scavengers of free radicals and their major role in the prevention of cancer and cardiovascular diseases (Shahidi & Ho, 2005).

Large literature is available about the polyphenolic compounds and content of plant foods, however, because of their complexity many polyphenols had remained unidentified and these literatures are not only incomplete but also contradictory and difficult to compare (Bravo, 1998). Little is known about the absorption of polyphenols in the body (Shadidi & Ho, 2005). However, fermentation can play a major role in the metabolism of some phenolic compounds (Bravo, 1998).

Polyphenolic compounds are important antinutrients that need to be investigated in ALVs and how they affect mineral absorption. Also characterising polyphenolic present in ALVs is crucial.

#### **2.3.4 Knowledge and use of ALVs in South Africa**

Despite the promising good nutritional content and ready availability of ALVs, there may be lack of knowledge on the use of ALVs in South Africa, especially among the young and people living in urban areas. Elderly women are regarded as more knowledgeable about the use, preparation and preservation of ALVs (Dweba & Mearns, 2011; van der Hoeven *et al.*, 2013). Health workers need to be encouraged to promote the use of locally grown ALVs, especially for children, to improve the quality of their diets (Odhav *et al.*, 2007). There is still a need to transfer knowledge on the use of ALVs to the young generation.

In a study conducted by Voster *et al.* (2007), knowledge of ALVs was more predominant among women than men. The most popular ALVs listed by households in South Africa for home use include amaranth (45.8%), pumpkin (96.7%), jute mallow (44.4%), spider plant (64.4%) and cowpea (58.3%) (Voster *et al.*, 2007). There is also a decline in indigenous knowledge of ALVs in the new generation (Jansen van Rensburg *et al.*, 2007; Odhav *et al.*, 2007, Matenge *et al.*, 2011, van der Hoeven *et al.*, 2013).

In studies conducted by van der Hoeven *et al.* (2013) and Matenge (2012) on sensory evaluation of ALVs, positive results were reported on the acceptance of ALVs. ALVs were more often consumed in rural areas than in town (Matenge, 2011). Lack of exposure and availability to ALVs had resulted in children opting for familiar vegetables such as spinach in a study in the North West province of South Africa (van der Hoeven *et al.*, 2013). However, in rural Limpopo province amaranth was reportedly preferred to spinach (Faber *et al.*, 2010). It is nevertheless impossible to make a direct comparison because the study conducted in rural Limpopo province was conducted among adults and the North-West province study involved children. Lack of nutritional knowledge has resulted in a decrease in

consumption of ALVs and resulted in negative perceptions about ALVs' nutritional value compared to commercial vegetables (Van der Walt *et al.*, 2009; Uusiku *et al.*, 2010). Dried ALVs are used in winter when the vegetables are off-season (Voster *et al.*, 2007). However, Matenge *et al.* (2012) recommend that ALVs should be commercialised to ensure accessibility.

However, the cultivation of the ALVs is not very comprehensive (Voster *et al.*, 2007). Methods that can encourage more yields should be advocated for. Drought has also impacted on the availability of seeds of ALVs (Voster *et al.*, 2007; Matenge *et al.*, 2012).

Studies presented here indicate that in some parts of South Africa there is some knowledge about ALVs and that they are used, but additional work will be needed to evaluate the potential of ALVs to address micronutrient deficiencies.

### 2.3.5 Contribution of ALVs to food security

The trend of food security in South Africa has been reported by different national surveys conducted from 1999 to 2012 showing proportions of people with different levels of food security (figure 2.6). Although the number of people experiencing hunger has decreased, the country still has about 26% of its population experiencing hunger. To measure the level of food insecurity at household level in these surveys, several parameters were assessed, such as household food production, household income, level of hunger and food procurement patterns. Hunger was assessed using hunger scale questionnaires in all three surveys (Labadarios, 2000; Pillay *et al.*, 2006; Labadarios, 2007) except the SANHANES-1 survey, which used the Community Childhood Hunger Identification Project tool (Shisanar *et al.*, 2013).

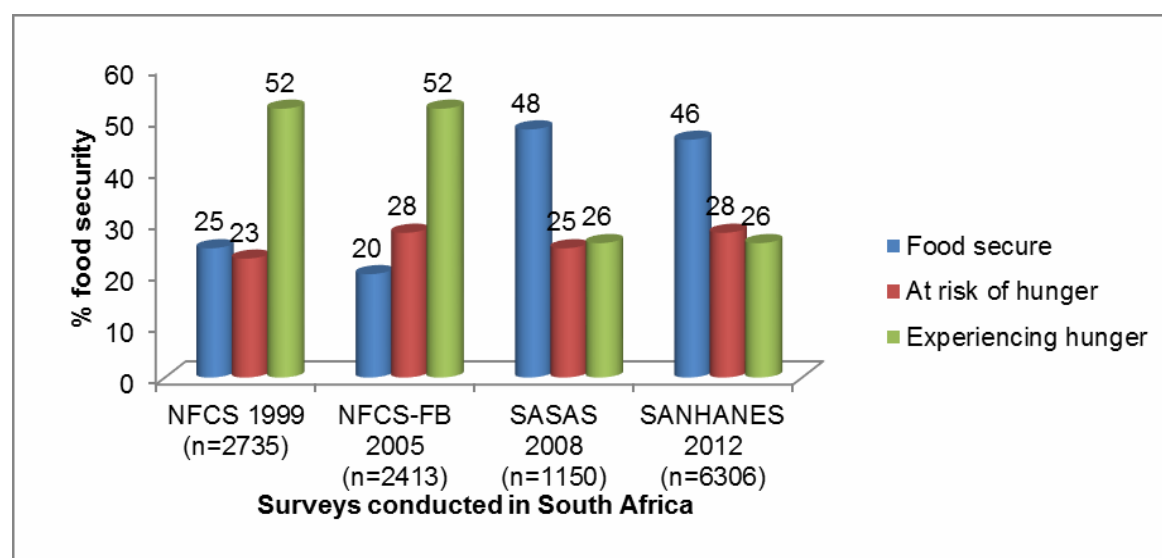


Figure 2.6: Food security patterns in South Africa from 1999-2012

NFCS-National food consumption survey; National food consumption survey-FB, SASAS-South African Social Attitudes Survey; SANHANES- South African National Health and Nutrition Examination Survey (Adapted from Labadarios, 2000; Pillay *et al.*, 2006; Labadarios; 2007; Shisanar *et al.*, 2013).

The black African population was reported to be most severely affected, having the highest rate of food insecurity (30.3%), followed by the coloured population (12%) (Shisana *et al.*, 2013). ALVs can play a major role in income generation, especially for the rural poor (Schönfeldt & Pretorius, 2011). The high content of micronutrients, medicinal properties and economic value of ALVs give them potential to alleviate poverty (Abukutsa-Onyango, 2007). Because of the nutritional content of raw ALVs, cooked and processed vegetables will have decreased antinutrients that enhancing nutrient availability and can potentially contribute to good nutrition. ALVs can contribute positively to world food production because many are adapted to harsh conditions and they highly resistant to pathogens, thus requiring fewer chemicals and pesticides compared to conventional vegetables (Habwe & Walingo, 2008).

Wild vegetables play a bigger role in diets in rural areas than in urban South Africa (Voster *et al.*, 2007). Schönfeldt and Pretorius (2011) emphasised that communities should be motivated to increase their household consumption of ALVs as a way of reducing food insecurity. Moreover, it has been reported that ALVs have a better shelf life than non-indigenous vegetables and can be preserved to be used in times of famine (Chanda & Oluoch 2003). ALVs play a role in mitigating micronutrient deficiencies, as they are always used as a complement to staple foods (Mavengahama *et al.*, 2013). In addition to addressing malnutrition, promoting ALVs can help in maintaining biodiversity in the food system, which is crucial in ensuring food security.

### **2.3.6 Composition data on ALV dishes**

ALVs are usually eaten as part of dishes. It is very important to know the nutritional composition of the dishes consumed with ALV. However, Faber *et al.* (2010) reported that it is often very difficult to determine the nutritional contribution of wild plant food to total nutrient intake, owing to lack of compositional data of these dishes in South Africa. According to Schönfeldt and Gibson (2009), South Africa needs a food composition table that includes traditional foods. Most literature available is of studies conducted on raw ALVs not dishes and composite dishes. ALVs are consumed as composite dishes and it is very crucial to know their nutrient content as they represent the actual meal consumed. Also antinutrients content and their effect on nutrients availability has never been investigated on dishes.. Therefore, this is an important gap in the research.

## 2.4 IRON AND ZINC BIOACCESSIBILITY AND BIOAVAILABILITY

Available evidence attests to it that ALVs are good sources of zinc and iron and can be used as a strategy to support nutrition programmes. However, it is uncertain whether these nutrients are available to be used by the body. The availability of nutrients is crucial, as it determines the proportion of nutrient that can be absorbed and used by the body, and should be evaluated.

It is very important to know the amount of micronutrients in the food product available for absorption and utilisation (Cillia *et al.*, 2009), as the mineral content of the food product does not always guarantee that the full amount will be available for absorption into the body (Watzke, 1998). Bioaccessibility is the amount of nutrient that is potentially available for absorption by the body, while bioavailability is the amount of nutrient that is absorbed and used by the body (Etcheverry *et al.*, 2012). According to Salovaara *et al.* (2002), solubility in the intestinal tract is bioaccessibility. Bioavailability can be subdivided into three phases, i.e. the amount of nutrient available in the intestinal lumen for absorption, amount of nutrient absorbed by the body and amount of nutrient used by the body (Watzke, 1998). The bioavailability of nutrients in food influences the nutritional status of man (Luten, 1996). Bioavailability of nutrients from food is very important in developing and computing the RDA, even in designing strategies to improve nutrition (Hemalatha *et al.*, 2007). Several diet-related and physiological factors influence the bioavailability of minerals (Table 2.4)

Table 2.4: Common inhibiting and enhancing factors that influence the bioavailability of iron and zinc

| Inhibiting Factors                                                                       | Enhancing Factors                                                               |
|------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Presence of antinutrients in the food product (e.g. phytates, tannins and dietary fibre) | Availability of enhancers in the food (e.g. vitamin C enhances iron absorption) |
| Mineral interaction (e.g. calcium reduces zinc absorption)                               | Favourable pH in the gut.                                                       |
| Infections and stress                                                                    | Good nutritional status of the body                                             |

(Adapted from Watzke 1998; Gibson *et al.*, 2006 Lucanni *et al.*, 2000)

The body cannot produce its own zinc and iron, therefore it depends on dietary intake of foods rich in these minerals. The amount of iron and zinc the body can absorb with ease from food depends on the kind of food eaten and its constituents (Lim *et al.*, 2013). Zinc is found in food as organic complexes with protein meats or inorganic salts in plant food, but no studies have been conducted to compare the bioavailability of these different forms (Lim *et al.*, 2013). Absorption of iron and zinc from food to be used by the body helps to reduce deficiencies. The form in which iron and zinc are available in that particular food determines their absorption through the gut and into the bloodstream.

The amount of iron and zinc and their bioavailability in the diet determines the nutritional adequacy of dietary zinc (Roohani *et al.*, 2013). Meat products are good sources of zinc and iron, which are readily available for absorption into the body, compared to zinc and iron in plant-based foods (Roohani *et al.*, 2013). Many people living in rural areas in developing countries follow diets low in meat products and depend mostly on plant-based diets, which have low zinc and iron content (Watzke, 1998; Gibson, 2012; Walingo, 2009; De Oliveira Otto *et al.*, 2012). Iron found in meat is 30-70% haem iron and 15-35% is absorbed, while in plant-based diets it is non-haem iron and often less than 10% is absorbed (Zimmerman & Hurrell 2007). Complementary feeding should be introduced well in time because breast milk has low levels of iron, therefore infants run the risk of developing iron deficiency after six months, as their neonatal stores are rapidly used and depleted (Ratthalli *et al.*, 2003). Therefore, complementary foods should be rich in bioavailable iron and zinc to avoid deficiencies in infants and children. Plant-based food products contain zinc and iron, of which the availability is low. Diets containing high levels of dietary fibre, phytate and polyphenols, mainly from plants, are associated with zinc and iron deficiency (Gibson, 2005). Diets that have maize meal as staple have a high phytate content and hence lower zinc availability (Mazariegos *et al.*, 2006). Most vegetables are eaten as part of a composite meal; therefore, including vegetables in a composite meal has a significant effect on the availability of non-haem iron and zinc because of the presence of antinutrients (Lucanni *et al.*, 2000). The inhibitory effects of phytic acid (PA) on zinc and iron can be predicted by molar ratios, e.g. phytate:zinc and phytate:iron (Roohani *et al.*, 2013). Diets have been categorised into three nominal categories according to the potential availability of zinc based on the phytic acid:zinc ratio of items; those with  $<5$  = high,  $5-15$  = moderate and  $>15$  = low availability (WHO, 1996).). However, consuming diets containing meat products along with plant-based products can help in the absorption of zinc and iron despite the phytate content. The consumption of haem sources together with non-haem sources, (e.g eating maize meal with meat, and ALV dish) and consuming non-haem sources together with ascorbic acid increases the absorption of iron (Hurrell & Egli, 2010). It has been reported that traditional food-processing and preparation methods have the potential to improve the bioavailability of micronutrients in plant-based diets (Hotz & Gibson 2007). To reduce these factors that hinder iron and zinc absorption in plant-based diets, strategies such as soaking and fermentation should be practised. These strategies partially hydrolyse phytate to other metabolites with a lower capacity to bind iron (Gibson *et al.*, 2008). More in-depth knowledge is needed on the ability of ALVs to contribute nutrients to the body, as most of the literature noted and suggested that the nutrients in the ALVs may not be available because of high antinutrient contents (Mavengahama *et al.*, 2013). Furthermore, there is enough evidence that the bioavailability of nutrients in ALVs has not been widely characterised (Mavengahama *et al.*, 2013). Different foods have different bioavailability, with plant-based products having lower availability.

## 2.5 METHODS USED TO ESTIMATE ALVS' BIOACCESSIBILITY AND BIOAVAILABILITY

Methods that are used to evaluate the bioavailability and/or bioaccessibility of nutrients include human or animal studies (*in vivo*) or simulated laboratory experiments (*in vitro*) (Parada & Aguilera 2007). According to Gibson *et al.* (2006), both *in vitro* and *in vivo* methods have been used to determine the bioavailability of iron and zinc. According to Yeum and Russel (2002), *in vivo* experiments involve measuring the level of nutrient absorbed after consumption of a pure nutrient and comparing it to an equivalent nutrient dose found in a food source. The advantages of *in vivo* experiments are that they provide direct data about bioavailability (Parada & Aguilera, 2007). However, *in vivo* methods are time-consuming, expensive and very difficult to perform (Luten, 1996).

In contrast, *in vitro* methods are based on a two-stage simulated digestive process (gastric and intestinal) of the food product in the gut (Gibson *et al.*, 2006). There are some exceptions involving a third step, which is digestion by lingual alpha-amylase, the enzyme that breaks down the glycosidic bonds of starch molecules (Etcheverry *et al.*, 2012). This step precedes the gastric stage. The technique of the *in vitro* system is based on simulated gastric extraction with pepsin, pancreatic amylase and bile salt in the final digestion stage (Velasco-Reynold *et al.*, 2008). There are four *in vitro* methods for bioaccessibility and/or bioavailability measurements, namely solubility, dialysability, the gastrointestinal model and the Caco-2 model of bioavailability (Etcheverry *et al.*, 2012). The *in vitro* methods measure the mineral fraction that has solubilised from the sample and is available for dialysis through the dialysis membrane under simulated gut conditions (Velasco-Reynold *et al.*, 2008; Etcheverry *et al.*, 2012).

Solubility, dialysability and Caco-2 uptake methods have all been used to evaluate iron and zinc bioaccessibility, though more studies are needed to validate *in vitro* methods in measuring zinc bioavailability (Etcheverry *et al.*, 2012). Animal studies had been used to validate studies on zinc bioaccessibility using *in vitro* techniques but little studies on comparing with human studies (Van & Glahn, 1999). *In vivo* studies are needed to validate *in vitro* studies and this is usually time consuming and expensive. Bioaccessibility is a key factor when designing foods and formulas that claim a health benefit measuring (Fernandez-Garcia *et al.*, 2009). ALVs are still perceived as nutritional valuable and investigating their nutritional potential using *in vitro* dialysability will give more insights on the bioaccessibility of iron and zinc. Also *in vitro* studies gives a chance of formula modification if the food product have a negative effect than when using animal or human studies which might have detrimental consequences (Fernandez-Garcia *et al.*, 2009).

The dialysability method has limitations: during the intestinal stage when the pH is high, iron becomes insoluble and may not diffuse into the dialysis bag (Van & Glahn, 1999). These methods can help to provide useful information on the bioaccessibility and bioavailability of iron and zinc in ALVs, taking into consideration several factors that can inhibit their absorption from ALVs.

## 2.6 CONCLUSION

Zinc and iron play a crucial role in the body and deficiencies should be prevented. Plant-based food provides iron and zinc with low bioavailability because of the presence of inhibitors. Fortification, supplementation and dietary diversity are important strategies in the prevention of iron and zinc deficiency. Generally there is an information gap on the nutritional composition of cooked ALV dishes, as well as on the bioavailability and bioaccessibility of iron and zinc from these dishes. ALVs are widely used in some parts of South Africa and the younger generation should be provided with knowledge on the use of ALVs. ALVs contain phytates, phenolics and tannins, which inhibit zinc and iron absorption. *In vitro* dialysability study results may differ from results obtained in human studies and therefore limitations of the dialysability assay should be taken into consideration when interpreting the results. This literature review indicates that there are gaps in the research done on ALV dishes in terms of their potential contribution to addressing micronutrient intakes

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## CHAPTER 3: ARTICLE

### Iron and zinc bioaccessibility in African leafy vegetable and maize meal porridge composite dishes

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#### Abstract

**Objectives:** The aim of this study was to assess the bioaccessibility of iron and zinc in African leafy vegetable (ALV) and maize porridge composite dishes using an *in vitro* dialysability assay.

**Methods:** Cooked ALV dishes, on their own and mixed with porridge made with either unfortified or fortified maize meal, were analyzed for mineral and antinutrient content using standard methods and the bioaccessibility of iron and zinc, using *in vitro* dialysability assay.

**Findings:** The amaranth-pumpkin dish contained most iron (24 mg/100 g) per dry weight (DW). The zinc content of ALV dishes ranged from 2.6 to 3.2 mg/100 g (DW). The amaranth-cowpea dish had the highest phytate content of 2078 mg/100 g (DW). Iron percentage bioaccessibility was high in the amaranth-spider plant dish (25%), while other dishes had lower iron bioaccessibility of less than 11%. The percentage bioaccessibility of zinc in ALV dishes ranged from 7-8%. The amaranth-spider plant dish with fortified maize meal had the highest zinc bioaccessibility (13%). The percentage zinc bioaccessibility is negatively associated with phytate:zinc and phytate-calcium:zinc molar ratios.

**Conclusions:** Antinutrient contents in ALV dishes and maize meal composites dishes do have an influence on the bioaccessibility of iron and zinc.

**Keywords:** African leafy vegetables, iron, zinc, bioaccessibility, nutrition

## Introduction

Iron deficiency anaemia (IDA) is one of the major forms of malnutrition worldwide, affecting 1.62 billion people globally (Balarajan *et al.*, 2011). Zinc deficiency has also been identified as a public health problem in both developing and developed countries (Shrimpton & Shankar, 2008). Large proportions of households in Sub-Saharan Africa, where iron and zinc deficiencies are prevalent, depend on monotonous cereal-based diets for energy as well as micronutrients (WHO, 2004). These diets contain high levels of phytate (Gibson, 2006) and sometimes tannins (Hemalatha *et al.*, 2007), which reduces the already low bioavailability of the non-haem iron and zinc in the diet even further (Zimmermann & Hurrell, 2007).

Fortification is the most practical, sustainable, cost-effective long-term solution to control iron and zinc deficiency at national level (Zimmermann & Hurrell, 2007). Staple foods as a vehicle for fortification are mostly used in developing countries, though inhibitory factors such as phytates and tannins in cereal-based staples may result in low bioaccessibility of iron and zinc (Hurrell *et al.*, 2004). Fortification is an effective strategy, but other strategies such as supplementation and dietary diversity also play a crucial role in eradicating zinc and iron deficiency (Zimmermann & Hurrell, 2007). In South Africa, after the recommendations made by the 1999 National Food Consumption Survey (Labadarios *et al.*, 2005), the Department of Health in 2003 enacted the mandatory fortification of maize meal and wheat flour with vitamin A, iron, zinc, folic acid, thiamine, niacin, vitamin B6 and riboflavin (South Africa, Department of Health, 2003).

It has been argued that African leafy vegetables (ALVs) can play a major role in enhancing the nutritional value of the diets of people living in sub-Saharan Africa (Smith & Eyzaguirre, 2007; Uusiku *et al.*, 2010) and improving household food and nutrition security (Chadha & Oluoch 2003). ALVs have been found to be adapted well to harsh weather conditions such as drought, are resistant to pests and pathogens, and have a better shelf life than non-indigenous vegetables (Chadha & Oluoch 2003). The micronutrient content of ALVs such as amaranth (*Amaranth spp*), spider plant (*Cleome gynandra*), cowpea leaves (*Vigna unguiculata*) and pumpkin leaves (*Cucurbita maxima*) is as high as or higher than that of cabbage, spinach and kale (Abukutsa-Onyango, 2005; Uusiku *et al.*, 2010).

There is insufficient knowledge on the nutrient composition of ALV dishes (Flyman & Afolayan 2006), as well as the effect of cooking, on the bioavailability of nutrients (Uusiku *et al.*, 2010; Mavengahama *et al.*, 2013). This lack of knowledge has contributed to ALVs being neglected by researchers and policy makers (Flyman & Afolan, 2006) and has influenced people's perception of ALVs negatively (Voster *et al.*, 2007). ALVs are eaten mostly in composite meals with staple grains such as maize. It is important to assess the mineral bioavailability from these composite dishes, as both the staple grains and the ALVs contain antinutrients, which will reduce mineral bioavailability (Mavengahama *et al.*, 2013). It has been emphasised that for *in vitro* analyses to be applicable to human bioavailability, food should be prepared as closely to how it is normally eaten as possible (Fairweather-Tait *et al.*, 2005). To our knowledge, no research has been published on the bioaccessibility of iron and zinc in ALVs when eaten with grains rich in antinutrients in composite meals.

This research is a follow-up study of a project that focused on the potential effect of ALVs on the micronutrient status of school children in the North West province of South Africa (van der Hoeven, 2014). The current study examined the bioaccessibility of iron and zinc in blanched ALV dishes. This study was therefore conducted to address the following objectives: to determine the mineral content of ALV and maize porridge composite dishes; to assess the bioaccessibility of iron and zinc in ALV and maize porridge composite dishes using an *in vitro* dialysability assay; to estimate the antinutrient content in ALV and maize porridge composite dishes and how they relate to iron and zinc dialysability and to evaluate the potential contribution of ALV and maize composite dishes to iron and zinc requirements.

## **MATERIALS AND METHODS**

### **ALV dishes**

The recipes used to cook the dishes were based on studies conducted by Matenge *et al.* (2011 and 2012). Ingredients added to the ALVs (49%) were tomato and onion mix (22%), sunflower vegetable oil (3%) and commercial gravy powder (5%) and water (21%), all on a wet basis. The combination of the leaves in the dishes varied (Table 3.1).

Table 3.1: Combinations of ALVs used in the different dishes on wet weight basis

| Leafy vegetables used in dish (% proportions)                            | Common name               |
|--------------------------------------------------------------------------|---------------------------|
| ALV: <i>Amaranthus cruentus</i> (100%)                                   | Amaranth                  |
| ALV: <i>Amaranthus cruentus</i> (80%) and <i>Cleome gynandra</i> (20%)   | Amaranth and spider plant |
| ALV: <i>Amaranthus cruentus</i> (80%) and <i>Cucurbita maxima</i> (20%)  | Amaranth and pumpkin      |
| ALV: <i>Amaranthus cruentus</i> (80%) and <i>Vigna unguiculata</i> (20%) | Amaranth and cowpea       |

ALVs were grown, harvested and prepared in dishes as previously described by van der Hoeven *et al.* (2013). To ensure representative samples for all the ALV dishes, three random samples from dishes prepared with leaves from initial, mid- and end-harvest (total nine samples) for each dish were included in the current study. The three random ALV dish samples for each harvesting period were mixed to make one sample for each ALV dish for that particular period, which was then frozen until analysis. Before being analysed for mineral content and *in vitro* dialysability, the sample of each ALV dish per harvest period was freeze-dried and milled. The samples for each ALV dish per harvest period were then mixed in equal parts (30 g each) to make a mixture for that specific ALV dish, which was then analysed.

### Porridge preparation

Two maize meal porridge samples were prepared separately, one using unfortified maize meal and the other fortified maize meal. A paste made from 15 g maize meal and 10 ml cold deionised water was added to 200 ml of boiling deionised water; this mixture was cooked for 10 minutes, then 30 g of maize meal was added and cooked for another 10 minutes to prepare a stiff porridge. The two cooked porridge samples were freeze-dried and milled into a fine powder, using a grinder.

### ALV-maize meal porridge composites dish preparation

ALV-maize meal composites were prepared according to the proportions eaten by school children in the study conducted by van der Hoeven (2014). In the van der Hoeven study school children consumed 125 g maize meal porridge served with 300 g of an ALV dish. In our study two composites for each ALV dish were made by adding either fortified or unfortified milled freeze-dried maize meal

porridge to milled ALVs in the ratio of 1:2.4 (dry weight basis (DW)), taking into account moisture calculations for representation of the wet meal situation. This was done to simulate the dishes that were consumed in the van der Hoeven (2014) study.

#### **Sample analyses: Total phenols, tannins, phytate, mineral content and mineral bioaccessibility**

Total phenols were determined using a modified Folin Ciocalteu method (Kaluza *et al.*, 1980). Tannin content was determined by the modified Vanillin HCl assay (Price *et al.*, 1978). Reagent blanks that corrected for the colour of the flour extracts were included. Phytate was determined through anion exchange chromatography, indirect quantitative analysis by measuring the organic phytate phosphorus (inositol -1 to 6- phosphate) (Frubeck *et al.*, 1995). The resin used was Dowex 1; anion-exchange resin-AG 1 x 4, 4% cross-linkage, chloride form, 100-200 mesh (Sigma, Johannesburg, South Africa). Iron, zinc, magnesium, calcium and phosphorous contents were analysed by ion-coupled plasma-optical emission spectrometry (ICP-OES, SPECTRO Analytical Instruments, and Kleve, Germany).

Mineral bioaccessibilities were determined according to the dialysis method developed by Luten *et al.* (1996). The iron and zinc contents of the dialysate were analysed by ICP-OES as described above. Results are presented as the percentage of the mineral in the dialysate to the total mineral content. Pepsin (P-7000), pancreatin (P-1750), and bile extract (B-8631) were from Sigma. The dialysis tubing used was Spectra/Por 7 ( $\varnothing = 20.4$  mm) with a molecular weight cut-off of 10 kDa (G.I.C. Scientific, Johannesburg South Africa). [Phytate]:[zinc], [phytate]:[iron] and [calcium] [phytate]:[zinc] molar ratios were calculated and derived as described by Wyatt and Triana-Teja (1994). For quality control, all analyses were conducted on two samples, mineral and antinutrients were analysed in duplicate and dialysability was conducted in five replicates.

#### **Statistical analyses**

IBM SPSS statistics 22 version program was used for data analysis and normality testing. One-factor analyses of variance (ANOVA) were used to test for differences among means for the dishes. Tukey's post hoc multiple test was applied to determine significant differences between specific means and Pearson's correlation analysis was used for associations between antinutrients and accessible zinc

and iron in ALV dishes. Values of  $P < 0.05$  were considered statistically significant for all analyses except for correlations, where a significance level of  $P < 0.01$  was used.

## RESULTS AND DISCUSSION

### Mineral and antinutrient contents of ALV dishes and ALV-maize porridge composite dishes

There were significant differences in mineral composition among the different vegetable combinations (Table 3.2). As expected, unfortified maize porridge had a significantly lower content of all the analysed minerals compared to fortified maize porridge. The mixtures of ALV dishes combined with unfortified maize porridge had a significantly ( $p < 0.05$ ) lower iron content than the specific ALV dish on its own because of a dilution effect. The amaranth-pumpkin dish contained most iron (24 mg/100 g DW) and when mixed with fortified and unfortified maize meal porridge a dilution effect was observed. No related studies were conducted on ALV dishes to use in comparison; nevertheless studies that investigated iron content on raw single ALVs reported iron contents ranging from 0.2 to 15.7 mg/100 g (DW), with pumpkin leaves having the highest iron content (Uusiku *et al.*, 2010; Schönfeldt & Pretorius, 2011).

The zinc content of ALV dishes in the study ranged from 2.6 to 3.2 mg/100 g, with amaranth mixed with spider plant having the highest zinc content. Studies on raw spider plant, pumpkin and cowpea leaves on their own found zinc contents ranging from 0.02-3.8 mg/100 g (DW) (Akubugwo *et al.*, 2007; van der Walt *et al.* 2009; Uusiku *et al.*, 2010; Schönfeldt & Pretorius, 2011).

The magnesium, calcium and phosphorous contents of the ALV dishes ranged from 213-442 mg /100 g, 591-947 mg/100 g and 110-139 mg/100 g respectively. A study done by van der Walt *et al.* (2009) on raw single ALVs reported higher ranges of calcium and magnesium. Sometimes differences in nutrient contents may be due to factors such as soil type, pH, availability of water, climate conditions, variety of species used and the use of fertilisers during the growth phase of the ALVs (Odhav *et al.*, 2007). However, in this study other factors, such as ingredients added when preparing the ALV dishes, should be taken into consideration as possible reasons for the differences.

Regarding the antinutrients, the phytate content differed significantly ( $p < 0.05$ ) between the dishes on a DW basis (Table 3.3), but not between unfortified and fortified maize porridges. The amaranth-

cowpea dish had the highest phytate content (2078 mg/100 g DW), with the amaranth-pumpkin dish containing the lowest amount of phytate (1420 mg/100 g DW). Considering the moisture content factor, the phytate content of ALV dishes in our study compared to the phytate content reported in a review by Uusiku *et al.* (2010). There was no significant difference between the phenolic content of different ALV dishes (Table 3.4). Both maize meal porridges were essentially free from tannins (0.04 mg/100 mg and 0.09 mg/100 mg respectively). Food processing and cooking affect the phenolic content of ALVs; both can increase the phenolic content by breaking down the cell wall during heating, releasing flavonoids, or it can reduce the phenolic content through leaching, hence reducing the inhibitory effect of the phenolics (Uusiku *et al.*, 2010). ALV dishes in our study were processed into a dish and underwent cooking; this may have resulted in lower phenolic contents.

Table 3.2: Mean mineral contents and standard deviations for African leafy vegetable dishes and composite maize meal dishes (DW basis).

| Dish                                                  | Iron<br>(mg/100g)     | Zinc<br>(mg/100g)      | Magnesium<br>(mg/100g) | Calcium<br>(mg/100g)   | Phosphorous<br>(mg/100g) |
|-------------------------------------------------------|-----------------------|------------------------|------------------------|------------------------|--------------------------|
| Amaranth (100%)                                       | 18±1.4 <sup>f</sup>   | 2.8±0.0 <sup>fg</sup>  | 442±41.7 <sup>d</sup>  | 947±110 <sup>g</sup>   | 140±3.5 <sup>ef</sup>    |
| Amaranth-cowpea (80:20)                               | 17 ±0.0 <sup>f</sup>  | 2.6±0.7 <sup>def</sup> | 267±16.3 <sup>bc</sup> | 727±55.9 <sup>ef</sup> | 128±0.0 <sup>de</sup>    |
| Amaranth-pumpkin (80:20)                              | 24±0.7 <sup>g</sup>   | 2.7±0.3 <sup>ef</sup>  | 340±30.4 <sup>cd</sup> | 864±81.3 <sup>fg</sup> | 125±8.5 <sup>cde</sup>   |
| Amaranth-spider plant(80:20)                          | 13±0.7 <sup>d</sup>   | 3.2±0.1 <sup>gh</sup>  | 213±21.9 <sup>c</sup>  | 591±0 <sup>de</sup>    | 110±11.3 <sup>bcd</sup>  |
| Unfortified maize porridge                            | 1.0±0.0 <sup>a</sup>  | 1.4±0.0 <sup>a</sup>   | 17±0.0 <sup>a</sup>    | ND                     | 72±0.0 <sup>a</sup>      |
| Amaranth with unfortified maize porridge              | 13±0.0 <sup>de</sup>  | 2.0±0.1 <sup>bc</sup>  | 281±11 <sup>bc</sup>   | 669±2.8 <sup>de</sup>  | 107±0.7 <sup>bc</sup>    |
| Amaranth-cowpea with unfortified maize porridge       | 8.0±0.0 <sup>bc</sup> | 2.2±0 <sup>bcd</sup>   | 84±0 <sup>a</sup>      | 212±1.4 <sup>b</sup>   | 53±0 <sup>a</sup>        |
| Amaranth-pumpkin with unfortified maize porridge      | 17±0.7 <sup>ef</sup>  | 2.1±0 <sup>bcd</sup>   | 230±11 <sup>b</sup>    | 500±28.3 <sup>c</sup>  | 100±4.2 <sup>b</sup>     |
| Amaranth-spider plant with unfortified maize porridge | 13±0.7 <sup>d</sup>   | 3.3±0.1 <sup>h</sup>   | 274±2.8 <sup>bc</sup>  | 629±8.4 <sup>cd</sup>  | 132±4.2 <sup>e</sup>     |
| Fortified maize porridge                              | 4.7±0.1 <sup>b</sup>  | 2±0.0 <sup>bc</sup>    | 11±0.4 <sup>a</sup>    | ND                     | 72±5.6 <sup>a</sup>      |
| Amaranth with fortified maize porridge                | 13±1.0 <sup>d</sup>   | 2.3±0.1 <sup>cde</sup> | 716±67.4 <sup>f</sup>  | 860±42.4 <sup>fg</sup> | 183±6.0 <sup>h</sup>     |
| Amaranth-cowpea with fortified maize porridge         | 13±0.8 <sup>de</sup>  | 1.8±0.2 <sup>ab</sup>  | 385±19.0 <sup>d</sup>  | 395±9.4 <sup>c</sup>   | 159±3.3 <sup>fg</sup>    |
| Amaranth-pumpkin with fortified maize porridge        | 18±2.2 <sup>f</sup>   | 2.1±0.1 <sup>bcd</sup> | 550±13.1 <sup>e</sup>  | 589±29.1 <sup>de</sup> | 168±6.0 <sup>gh</sup>    |
| Amaranth-spider plant with fortified maize porridge   | 11±1.0 <sup>cd</sup>  | 2.9±0.2 <sup>fgn</sup> | 423±30.0 <sup>d</sup>  | 512±33.9 <sup>cd</sup> | 167±1.2 <sup>gh</sup>    |

Values expressed as mean of duplicates in mg/100g ±SD on a DW basis, ND - Not detectable. Values with different superscript in the same column are statistically significant (p<0.05) based on the post hoc Tukey test.

**Table 3.3:** Phytate content and calculated phytate to mineral molar ratios of African Leafy Vegetables and maize composite dishes (DW basis).

| ALV                                                   | phytate<br>(mg/100g)     | [Phytate]:[Zn]        | [Phytate]:[Fe]         | [Ca]:[Phytate]:[Zn]    |
|-------------------------------------------------------|--------------------------|-----------------------|------------------------|------------------------|
|                                                       |                          | Molar Ratios          |                        |                        |
| Amaranth (100%)                                       | 1822±168 <sup>h</sup>    | 64±0.27 <sup>de</sup> | 9.4±0.62 <sup>cd</sup> | 1520±171 <sup>de</sup> |
| Amaranth-cowpea (80:20)                               | 2078±202 <sup>igh</sup>  | 80±1.23 <sup>ig</sup> | 10±0.16 <sup>cde</sup> | 1452±90 <sup>d</sup>   |
| Amaranth-pumpkin (80:20)                              | 1420±142 <sup>de</sup>   | 52±5.15 <sup>bc</sup> | 5.1±0.17 <sup>a</sup>  | 1114±5 <sup>c</sup>    |
| Amaranth-spider plant (80:20)                         | 1543±95 <sup>cdef</sup>  | 48±2.64 <sup>bc</sup> | 10±0.98 <sup>cde</sup> | 711±39 <sup>b</sup>    |
| Unfortified maize porridge                            | 648±64 <sup>a</sup>      | 48±2.40 <sup>b</sup>  | 54±1.26 <sup>g</sup>   | ND*                    |
| Amaranth with unfortified maize porridge              | 1712±92 <sup>etg</sup>   | 87±4.34 <sup>g</sup>  | 11±0.02 <sup>de</sup>  | 1454±79 <sup>d</sup>   |
| Amaranth-cowpea with unfortified maize porridge       | 1629±97 <sup>detg</sup>  | 73±0.45 <sup>ef</sup> | 18±0.04 <sup>f</sup>   | 387±4 <sup>a</sup>     |
| Amaranth-pumpkin with unfortified maize porridge      | 1267±104 <sup>bc</sup>   | 60±0.36 <sup>cd</sup> | 6.5±0.27 <sup>ab</sup> | 752±38 <sup>b</sup>    |
| Amaranth-spider plant with unfortified maize porridge | 1533±113 <sup>cdef</sup> | 46±1.88 <sup>b</sup>  | 12±0.29 <sup>de</sup>  | 715±20 <sup>b</sup>    |
| Fortified maize porridge                              | 488±45 <sup>a</sup>      | 24±0.03 <sup>a</sup>  | 8.8±0.19 <sup>bc</sup> | ND*                    |
| Amaranth-cowpea with fortified maize porridge         | 1348±66 <sup>bc</sup>    | 74±6.32 <sup>ef</sup> | 8.7±0.53 <sup>bc</sup> | 732±45 <sup>b</sup>    |
| Amaranth with fortified maize porridge                | 1842±189 <sup>gh</sup>   | 79±4.72 <sup>ig</sup> | 13±1.03 <sup>e</sup>   | 1703±17 <sup>e</sup>   |
| Amaranth-pumpkin with fortified maize porridge        | 1088±80 <sup>b</sup>     | 51±1.60 <sup>bc</sup> | 5.1±0.61 <sup>a</sup>  | 754±14 <sup>b</sup>    |
| Amaranth-spider plant with fortified maize porridge   | 1270±0 <sup>bc</sup>     | 43±2.33 <sup>b</sup>  | 9.6±0.87 <sup>cd</sup> | 554±7 <sup>ab</sup>    |

Values expressed as mean of quadruples in mg/g ±SD on a DW basis. \*Not detectable. Values with different superscript in the same column are statistically significant differences (p<0.05) based on the post hoc Tukey test.

Table 3.4: Phenolic and tannin contents of ALV dishes and maize meal porridge

| Dish                         | Phenolic compounds                  | Tannins                 |
|------------------------------|-------------------------------------|-------------------------|
|                              | mg catechin equivalents (CE) /100 g |                         |
| Amaranth (100%)              | 1234±40 <sup>a</sup>                | 0.24±0.12 <sup>b</sup>  |
| Amaranth-cowpea(80:20)       | 1170±77 <sup>a</sup>                | 0.21±0.06 <sup>ab</sup> |
| Amaranth-pumpkin(80:20)      | 1244±79 <sup>a</sup>                | 0.16±0.03 <sup>ab</sup> |
| Amaranth-spider plant(80:20) | 1297±86 <sup>a</sup>                | 0.88±0.04 <sup>ab</sup> |
| Unfortified maize meal       | ND                                  | 0.04±0.01 <sup>a</sup>  |
| Fortified maize meal         | ND                                  | 0.09±0.01 <sup>ab</sup> |

Values expressed as mean of quadruples in mg/g ±SD on a DW basis. ND - Not detectable. Values with different superscript in the same column are statistically significant differences (p<0.05) based on the post hoc Tukey test.

### Bioaccessibility of iron and zinc in ALVs and composite maize meal dishes

Iron percentage bioaccessibility was high in the amaranth-spider plant dish (25%), while other ALV dishes had an iron bioaccessibility of less than 11% (figure 3.1). The phytate content in the amaranth-spider plant dish was lower than in other dishes (Table 3.3). Unfortified maize meal porridge had the highest percentage of bioaccessible iron (45%) compared to ALV dishes (6.8-25%) and fortified maize meal porridge (6.2%) (Figure 3.1). The percentage of iron bioaccessibility was significantly (p<0.01) negatively related to the phytate and tannin contents of ALV dishes with coefficients of  $r=-0.415$  and  $r=-0.726$  respectively (Table 3.5). Although the phytate content was negatively correlated to the percentage of iron bioaccessibility, it can only account for up to 22% of the variability in iron absorption, while the tannin content explained 43% of the variation. In this study ALV dishes had high antinutrient contents compared to both unfortified and fortified maize meal, which had lower phytates, phenolic and tannin contents (Tables 3.3 and 3.4). These factors are known to lower iron bioaccessibility (Hemalatha *et al.*, 2007; Etcheverry *et al.*, 2012). However, the influence of phytate on the availability of iron and zinc does not depend on the phytate contents in the dish, but on phytate-to-mineral ratios (Ma *et al.*, 2007). When the phytate:iron molar ratio is more than one, the phytate

inhibitory role has been found to be substantial (Hurrell et al., 2004). The phytate:iron molar ratios of all ALV dishes and ALV-maize composite dishes in this study were more than the critical value and ranged from 5 to 54, which thus explains their lower iron bioaccessibility (Table 3.3). The lower iron accessibility in fortified maize meal porridge may be due to the type of iron fortificant that was used. In South Africa maize meal is fortified using elemental iron (South Africa, Department of Health, 2003), which has been reported to have low availability, especially in products containing phytate such as maize meal (Hurrell & Egli, 2010; Moretti *et al.*, 2014). Compositing the ALV dishes with fortified maize meal increased iron dialysability in all the dishes except in the amaranth-pumpkin dish. The phytate:iron molar ratio in this dish stayed the same when composited with fortified maize meal, while others showed a decrease. Phytate contents in composite dishes were lower compared to ALV dishes.

The percentage bioaccessibility of zinc in ALV dishes ranged from 7-8%, while unfortified and fortified maize meal had bioaccessibilities of 12% and 7.2% respectively (figure 3.2). The amaranth-spider plant dish had higher zinc accessibility when composited with fortified maize meal (13%). Zinc bioaccessibility appears to be negatively associated with phytate content,  $r=-0.413$ ,  $p<0.01$  and tannin content,  $r=-0.709$ ,  $p<0.01$ . Phytate and tannin contents can account for up to 24% and 36% respectively of the variation in zinc bioavailability (Table 3.6). Phytate:zinc molar ratios and phytate-calcium:zinc molar ratios are considered more appropriate indicators in determining the availability of zinc than using phytate levels only (Igwe *et al.*, 2013). Diets have been categorised according to the potential availability of zinc based on phytic acid:zinc ratio i.e. those with  $<5$  = high,  $5-15$  = moderate and  $>15$  = low availability (WHO, 1996). In the current study the ALV dishes and composite dishes had phytate:zinc molar ratios above 15 with values ranging from 24 to 80 (Table 3.3). These dishes are described as having low zinc bioaccessibility and therefore may explain the lower zinc bioaccessibility percentages in our study. The percentage zinc bioaccessibility is negatively associated with phytate:zinc and phytate-calcium:zinc molar ratios (Table 3.7). In addition, values that are higher than 200 for phytate-calcium:zinc molar ratios affect zinc bioaccessibility negatively (Etcheverry *et al.*, 2012; Igwe *et al.*, 2013). All ALV and maize porridge composite dishes had ratios above the critical value, hence had a negative effect on zinc bioaccessibility. Therefore, phytate plays a major role in zinc bioaccessibility in ALV dishes and composite dishes. ALV dishes that had a higher zinc content had a low bioaccessibility percentage. This could be because of the generally low

bioavailability of zinc in plant-based foods. When comparing the fortified and unfortified composite dishes, the amaranth-spider plant with fortified maize porridge had an increase in zinc bioaccessibility; this might be due to the lower phytate:zinc ratio compared to other composite dishes.

Higher phytate and tannin contents of ALV dishes were associated with a lower bioaccessibility percentage of zinc and iron. However, the information presented on bioaccessibility suggests that it is important to make sure that when bioaccessibility assays are done the ALVs are assessed in combination with the foods they are normally consumed with to get a more realistic and complete picture of bioaccessibility. We were unable to find literature reporting where this has been done before, which makes the current study quite novel at this stage.

Table 3.5: Correlations between iron bioaccessibility percentage and antinutrients

|                    | Iron bioaccessibility (%) |       |                |
|--------------------|---------------------------|-------|----------------|
|                    | r                         | p     | R <sup>2</sup> |
| Phytate            | -0.415**                  | 0.002 | 0.222          |
| Phenolic compounds | 0.315                     | 0.235 | 0.177          |
| Tannin             | -0.726**                  | 0.000 | 0.433          |

\*\* Correlation is significant at the 0.01 level

Table 5: Correlations between zinc bioaccessibility percentage and antinutrients

|                    | Zinc bioaccessibility (%) |       |                |
|--------------------|---------------------------|-------|----------------|
|                    | r                         | p     | R <sup>2</sup> |
| Phytate            | -0.413**                  | 0.002 | 0.242          |
| Phenolic compounds | 0.312                     | 0.247 | 0.075          |
| Tannin             | -0.769**                  | 0.000 | 0.358          |

\*\* Correlation is significant at the 0.01 level

Table 6: Correlations between phytate:mineral ratios and bioaccessibility percentage

|                       | Percentage bioaccessibility (%) |       |                |
|-----------------------|---------------------------------|-------|----------------|
|                       | r                               | p     | R <sup>2</sup> |
| Phytate: Iron         | 0.570*                          | 0.017 | 0.325          |
| Phytate: Zinc         | -0.508*                         | 0.032 | 0.258          |
| Phytate-Calcium: Zinc | -0.663**                        | 0.005 | 0.440          |

\* Correlation is significant at the 0.05 level, \*\* Correlation is significant at the 0.01 level

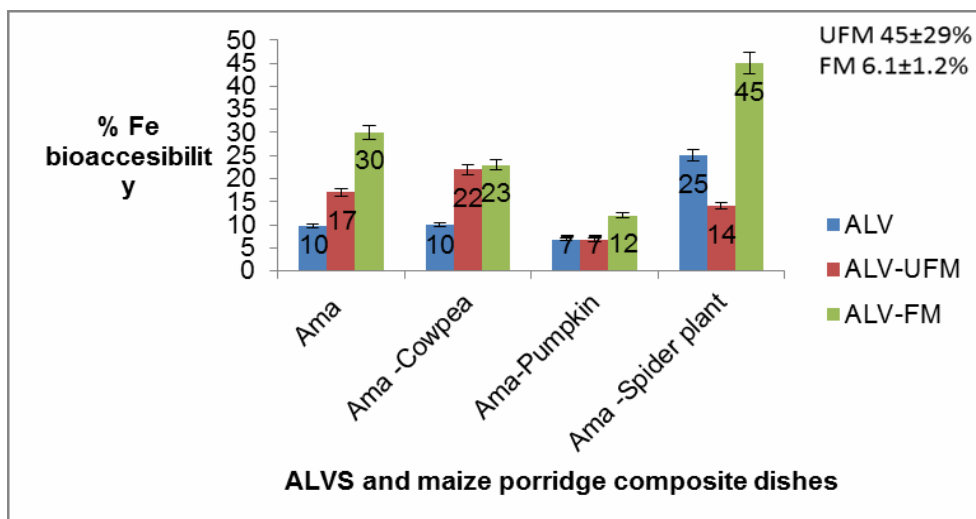


Figure 3.1: Iron bioaccessibility in ALV, fortified and unfortified maize porridge

ALV - African leafy vegetable dish; ALV-UFM: African leafy vegetable-unfortified maize porridge composite dishes; ALV-FM: African leafy vegetable-fortified maize porridge composite dishes; Ama = Amaranth; UFM = Unfortified maize porridge; FM = Fortified maize porridge

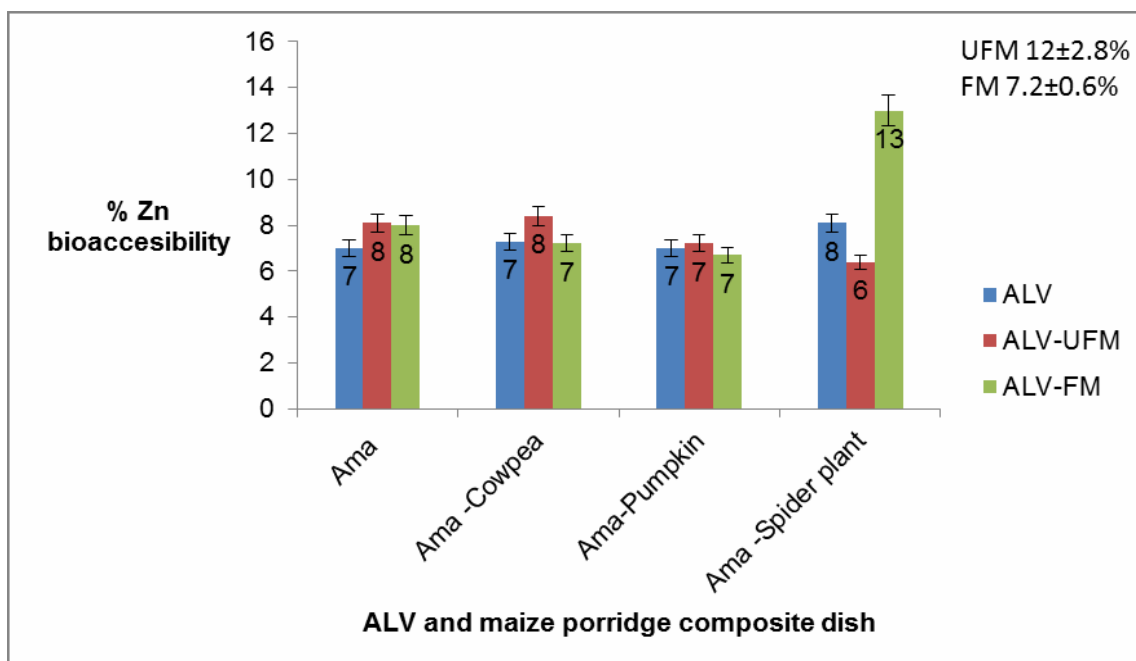


Figure 3.2: Zinc bioaccessibility in ALV, fortified and unfortified maize porridge

ALV - African leafy vegetable dish; ALV-UFM: African leafy vegetable-unfortified maize porridge composite dishes; ALV-FM: African leafy vegetable-fortified maize porridge composite dishes; Ama = Amaranth; UFM = Unfortified maize porridge; FM = Fortified maize porridge

### **Potential contribution of ALV to daily iron and zinc requirements**

The contribution of iron and zinc by ALV and composite dishes in a diet of 300 g ALV dish and 125 g maize meal porridge was calculated based on the percentage mineral bioaccessibility. Almost all the ALV dishes and unfortified and fortified composite dishes had the potential of contributing more than 100% of the USA iron recommended daily allowances (RDA) for children aged between nine and 13 years (Food and Nutrition board, 2003), except the amaranth-cowpea:unfortified maize meal dish, which could only contribute 79% (Table 3.8). RDA is estimated as the daily dietary intake level sufficient to meet the nutrient requirement of almost 98% of healthy individuals (Murphy & Barr, 2006). Though the RDA values are high, it should be taken into consideration that these have to compensate for bioavailability. In our study it was found that the dishes can provide more than 100% of iron absolute requirements.

Regarding zinc, ALV dishes can provide between 18.5% and 35%, fortified composite dishes 17-31% and unfortified composites 21-35% of the US RDA for female and male children aged nine to 13 years (Food and Nutrition Board, 2003) (Table 3.9). Though the ALV dishes and composite dishes contain some inhibitory factors for iron and zinc absorption, they can potentially contribute to the zinc and iron needed by the body.

Table 7: Contributions of iron by consuming 300 g ALV dish, and consuming 300g ALV dish mixed with 125g maize porridge (wet weight)

| ALV dish                                                 | Moisture<br>(% wet<br>weight) | Fe<br>(mg) | %<br>RDA* | %<br>bioaccessible<br>Fe | Bioaccessible<br>Fe (mg) | %<br>Absolute<br>value** |
|----------------------------------------------------------|-------------------------------|------------|-----------|--------------------------|--------------------------|--------------------------|
| Amaranth (100%)                                          | 78±0.4                        | 11.9       | 149       | 9.7±3.6 <sup>ab</sup>    | 1.2                      | 129                      |
| Amaranth-cowpea (80:20)                                  | 81±0.5                        | 9.7        | 121       | 10.1±1.3 <sup>ab</sup>   | 1.0                      | 110                      |
| Amaranth-pumpkin (80:20)                                 | 77±0.5                        | 16.2       | 203       | 6.8±0.9 <sup>a</sup>     | 1.1                      | 124                      |
| Amaranth-spider plant<br>(80:20)                         | 78±0.4                        | 8.3        | 103       | 25.0±3.5 <sup>abc</sup>  | 2.1                      | 232                      |
| Amaranth with unfortified<br>maize porridge              | 82±0.2                        | 11.1       | 138       | 22±2.5 <sup>ab</sup>     | 1.8                      | 207                      |
| Amaranth-cowpea with<br>unfortified maize porridge       | 80±0.2                        | 6.3        | 79        | 17±4.5 <sup>ab</sup>     | 1.4                      | 154                      |
| Amaranth-pumpkin with<br>unfortified maize porridge      | 80±0.1                        | 14.8       | 185       | 6.7±0.98 <sup>a</sup>    | 1.0                      | 112                      |
| Amaranth-spider plant with<br>unfortified maize porridge | 80±0.4                        | 11.1       | 138       | 14±1.5 <sup>ab</sup>     | 1.6                      | 175                      |
| Amaranth:fortified maize                                 | 80±0.2                        | 11.1       | 138       | 23±2.5 <sup>ab</sup>     | 3.3                      | 374                      |
| Amaranth-cowpea with<br>fortified maize porridge         | 82±0.5                        | 10.2       | 128       | 30±6.0 <sup>bc</sup>     | 2.4                      | 264                      |
| Amaranth-pumpkin with<br>fortified maize porridge        | 80±0.4                        | 15.7       | 196       | 12±2.0 <sup>ab</sup>     | 1.9                      | 213                      |
| Amaranth-spider plant with<br>fortified maize porridge   | 80±0.5                        | 9.4        | 117       | 45±1.8 <sup>c</sup>      | 4.2                      | 476                      |

\* 8 mg/d as RDA for both female and male children aged nine to 13 years (USA Food and nutrition board, 2003) WW (Wet Weight) \*\* Total absolute iron requirements for children 7-10 years (0.89 mg/d) (FAO/WHO technical report 2003). Values with different superscript in the same column are statistically significant differences (p<0.05) based on the post hoc Tukey test. Moisture values are the mean of triplicates expressed in % wet weight ± standard deviation

Table 8: Contributions of zinc by consuming 300 g ALV dish, and consuming 300g ALV dish mixed with 125 g maize porridge (wet weight)

| ALV                                                   | Zn (mg) | % RDA* | % accessibility       | Bioaccessible Zn (mg) |
|-------------------------------------------------------|---------|--------|-----------------------|-----------------------|
| Amaranth (100%)                                       | 1.8     | 23     | 7.0±0.38 <sup>a</sup> | 0.1                   |
| Amaranth-cowpea (80:20)                               | 1.5     | 19     | 7.3±0.44 <sup>a</sup> | 0.1                   |
| Amaranth-pumpkin (80:20)                              | 1.9     | 23     | 7.0±0.44 <sup>a</sup> | 0.1                   |
| Amaranth-spider plant (80:20)                         | 2.1     | 26     | 8.1±0.31 <sup>a</sup> | 0.2                   |
| Amaranth with unfortified maize porridge              | 1.7     | 21     | 8.1±1.02 <sup>a</sup> | 0.1                   |
| Amaranth-cowpea with unfortified maize porridge       | 1.7     | 22     | 8.4±0.34 <sup>a</sup> | 0.1                   |
| Amaranth-pumpkin with unfortified maize porridge      | 1.8     | 23     | 7.2±0.15 <sup>a</sup> | 0.1                   |
| Amaranth-spider plant with unfortified maize porridge | 2.8     | 35     | 6.4±0.35 <sup>a</sup> | 0.2                   |
| Amaranth with fortified maize porridge                | 2.0     | 24     | 8.0±0.9 <sup>a</sup>  | 0.2                   |
| Amaranth-cowpea with fortified maize porridge         | 1.4     | 18     | 7.2±0.6 <sup>a</sup>  | 0.1                   |
| Amaranth-pumpkin with fortified maize porridge        | 1.8     | 23     | 6.7±0.6 <sup>a</sup>  | 0.1                   |
| Amaranth-spider plant with fortified maize porridge   | 2.5     | 31     | 13±0.2 <sup>b</sup>   | 0.3                   |

\* 8 mg/d as zinc RDA for both female and male children aged nine to 13 years (USA Food and nutrition board, 2003), WW (wet weight). Values with different superscript in the same column are statistically significant differences ( $p < 0.05$ ) based on the post hoc Tukey test.

## CONCLUSION

In the context of dietary requirements, ALV and maize meal composite dishes have a high iron and, to a lesser degree, zinc content, though they also have high antinutrient contents, which can have some inhibitory effects. The bioaccessibility of iron is higher in ALV dishes compared to zinc. In the presence of fortified maize meal the dialysability of iron is higher. ALV dishes have high [phytate]:[zinc] and [phytate]:[iron] molar ratios, which resulted in low bioaccessibility of these minerals. Despite the inhibiting factors, the amount of iron and to a lesser degree zinc bioaccessible from ALV and maize porridge composite dishes could play a significant role in planning food and nutrition security strategies. However, there is a need to understand the possible effects of consuming them in different combinations with other foods. *In vitro* dialysability study results may differ from results obtained in human studies and therefore limitations of the dialysability assay should be taken into consideration when interpreting the results. However, in this study dishes were used in a similar way to how they would be consumed in real life, making it more applicable to the human situation in

terms of bioaccessibility. Future studies should explore the use of the Caco-2 cell methods to evaluate bioaccessibility and bioavailability, as the methodology can identify other undiscovered factors that may influence mineral absorption. Other methods such as isotope dilution and whole body counting can be used to validate the results. A limitation of this study is that other cereals usually taken with the ALV dishes were not investigated, such as sorghum and millet. Irrespective of this, results from this follow-up study may explain the lack of intervention effects reported by van der Hoeven (2013) on iron and zinc status in school children. The consumption of ALVs should be used to diversify diets and individuals encourage to consume a diet with variety to enhance nutrients accessibility.

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## CHAPTER 4: CONCLUSIONS AND RECOMMENDATION

### 4.1 INTRODUCTION

The aim of this study was to determine the mineral content of ALV dishes and ALV maize porridge composite dishes; to assess the bioaccessibility of iron and zinc in ALV and maize porridge composite dishes using an *in vitro* dialysability assay; to estimate the antinutrient content in ALV and maize porridge composite dishes, to determine how they affect iron and zinc dialysability and to evaluate the potential of ALV and maize composite dishes in addressing nutrient intake. This chapter presents the general findings and makes recommendations.

### 4.2 MAIN FINDINGS

The study confirmed that combining ALV dishes with unfortified maize porridge resulted in significantly lower iron content due to dilution. The amaranth-pumpkin dish contained most iron (24 mg/100 g) and the content per 100 g decreased when mixed with both fortified and unfortified maize meal porridges, because of a dilution effect. ALV dishes in the study had zinc contents ranging from 2.6 to 3.2 mg/100 g, with amaranth mixed with spider plant having the highest zinc content. Regarding antinutrients, the amaranth-cowpea dish had the highest phytate content of 2078 mg/100 g, with the amaranth-pumpkin dish containing the lowest amount of phytate (1420 mg/100 g DW). ALV dishes also contained tannins and phenolic compounds.

Iron percentage bioaccessibility was high in the amaranth-spider plant dish (25%), while other dishes had lower iron bioaccessibility of less than 11%. The phytate: iron molar ratios of all ALV dishes and ALV-maize composite dishes in this study were higher than 1, which is the critical value, and ranged from 5 to 54. This explains their lower iron percentage bioaccessibility. The percentage bioaccessibility of zinc in ALV dishes ranged from 7-8%. The amaranth-spider plant dish had higher zinc bioaccessibility when composited with fortified maize meal (13%). The percentage zinc bioaccessibility is negatively associated with phytate: zinc and phytate-calcium:zinc molar ratios.

Almost all the ALV dishes and unfortified and fortified composite dishes have the potential to contribute more than 100% of the US iron RDA for children aged between nine and 13 years (Food and Nutrition Board, 2003) if 300 g of an ALV dish is eaten with 125 g of fortified maize porridge, except the amaranth-cowpea: unfortified maize meal dish, which could only contribute 79%. Regarding the iron percentage absolute value, ALV dishes and maize composites dishes can provide more than 100% of the requirements if 300 g of an ALV dish is eaten with 125 g of either fortified or unfortified maize porridge. Regarding zinc, ALV dishes can provide between 18.5% and 35%, fortified

composites dishes 17-31% and unfortified composites 21-35% of the US RDA for female and male children aged nine to 13 years if 300 g of an ALV dish is served with 125 g of either fortified or unfortified maize porridge.

### **4.3 CONCLUSIONS**

ALV and maize meal composite dishes have a high iron and zinc content, though they also have a high antinutrient content, which may have some inhibitory effects. The bioaccessibility of iron is higher in ALV dishes compared to zinc. In the presence of fortified maize meal the dialysability of iron was higher compared to unfortified composites. ALV dishes had high phytate:zinc and phytate:iron molar ratios, which resulted in low bioaccessibility of these minerals. Despite the inhibiting factors, the amount of iron and zinc bioaccessible from ALV and maize porridge composite dishes could play a significant role in planning food security strategies, but there is a need to understand the possible effects of consuming them in different combinations with other foods.

When planning nutritional strategies, zinc and iron content should not be treated as the only option to determine the implementation of the programme, but their bioaccessibility should also be taken into consideration. Therefore, promotion of ALV dishes can be an excellent potential strategy to help improve zinc and iron nutrition status. *In vitro* dialysability study results may differ from results obtained in human studies and therefore limitations of the dialysability assay should be taken into consideration when interpreting the results. *In vivo* methods should be conducted to validate the results.

### **4.4 RECOMMENDATIONS FOR IMPLEMENTATION**

ALV dishes and maize meal porridge composite dishes can provide a viable option for nutrition and their consumption should be encouraged. For ALV dishes to be nutritional viable, they should be eaten accompanied with other food groups. ALV dishes should be advocated to be eaten in accompaniment with meat products as combining haem and non-haem iron had been shown to improve iron and zinc availability.

### **4.5 RECOMMENDATIONS FOR FURTHER RESEARCH**

More research is needed on ALV dishes that have undergone food-processing methods such as sun drying and blanching and their iron and zinc bioaccessibility. Methods of reducing antinutrients, especially phytates, in ALV dishes should be exploited to increase their iron and zinc availability. Future studies should also explore the use of the Caco-2 cell methods to evaluate bioaccessibility and bioavailability, as the methodology can identify other undiscovered factors that may influence mineral absorption.

## 5.ADDENDUMS

### ADDENDUM A: AUTHORS GUIDELINES

#### GUIDE FOR AUTHORS

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##### INTRODUCTION

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