Pearl millet porridge: improvement in iron and zinc bioaccessibilities through fortification with micronutrient-rich plant food components

By

Reneè van der Merwe

Dissertation

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DECLARATION

I hereby declare that this dissertation submitted at the University of Pretoria for the degree MSc Nutrition is my own work and has not previously been submitted by me for a degree at this or any other university or institution of higher education.

Reneè van der Merwe

November 2017

DEDICATION

I would like to dedicate this dissertation:

To my loving and caring husband, Halmar, who, even through the most difficult challenges, never stopped encouraging and supporting me. Without him, this dissertation would not have been possible.

To my Lord and saviour, Jesus Christ, in whom all the treasures of wisdom and knowledge are hidden (Colossians 2:3).

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ABSTRACT

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Supervisor: Dr J. Kruger

Co-supervisor: Prof J.R.N. Taylor

The most prevalent micronutrient deficiencies in sub-Saharan Africa are iron, zinc and vitamin A. This is partly due to staple diets composed of mainly cereals, containing high levels of iron and zinc bioavailability inhibitors. In this study, pearl millet porridge was food-to-food fortified with dried micronutrient-rich plant foodstuffs (moringa leaves, hibiscus calyces, baobab fruit pulp), and a mango-carrot premix (plus sunflower oil) as a provitamin A source, and the effect on iron and zinc bioaccessibilities evaluated. The foodstuffs were analysed for mineral and antinutrient contents. The effects of adding 5 and 15 g/100 g, dry basis (db) pearl millet plus provitamin A source of dried moringa leaves, hibiscus calyces or baobab fruit pulp on iron and zinc bioaccessibilities (*in vitro* dialysability assay) were determined.

Baobab fruit pulp, despite containing high levels of tannins (2286 mg CE/100 g, db), increased the iron and zinc bioaccessibilities the most, when added as food-to-food fortificants to pearl millet. This could contribute >200% and >180%, respectively, more to the iron and zinc absolute requirements (defined as the sum of the daily basal losses of the mineral plus the amounts of the mineral needed for growth) than the pearl millet plus provitamin A source porridge, for 2–5-year-old children. Fortification with hibiscus calyces also resulted in substantial increases in iron and zinc bioaccessibilities. This is because baobab fruit pulp and hibiscus calyces contain substantial levels of iron and zinc bioavailability enhancing organic acids. The addition of moringa leaves generally resulted in the lowest increases and, in some cases, even reduced the iron and zinc bioaccessibilities, even though it had the highest level of iron (58.4 mg/100 g, db) of all the plant foodstuffs. Dried moringa leaves had the highest levels of calcium and total phenolics, and substantial levels of phytate, as well as possible low levels of organic acids, all which contributed to the low iron and zinc bioaccessibilities.

Including baobab fruit pulp and possibly hibiscus calyces in a cereal based meal, show potential to increase both the iron and zinc bioaccessibilities. The iron and zinc status of people consuming a cereal-based diet may be improved by the inclusion of baobab fruit pulp or possibly hibiscus calyces, as food-to-food fortificants, to cereal-based porridges.

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Chapter 1: Introduction

This dissertation includes an in-depth literature review where the prevalence and consequences of iron, zinc and vitamin A deficiencies in sub-Saharan Africa is explored, followed by the nutrition offered from cereals and cereal-based porridges as staple foods. The intestinal mechanisms for iron and zinc absorption is explored next, followed by the potential of micronutrient-rich plant foodstuffs, used in food-to-food fortification of cereal-based porridges, to combat iron and zinc deficiencies. Iron and zinc bioavailability modifiers which are present in plant foods are discussed, followed by a brief review on the bioaccessibility of calcium and phosphorus. Lastly, some plant foodstuff candidates, available in Africa, are explored for their potential properties of enhancing iron and zinc bioavailabilities of pearl millet, as well as provitamin A contents. The literature review is followed by the hypotheses and objectives and then the effect of food-to-food fortification of pearl millet porridge with micronutrient-rich plant foodstuffs on the iron and zinc contents and bioaccessibilities is studied. The general discussion evaluates some of the research methodology used as well as suggesting some applications of the research findings and the possible impact it could have on contributions to recommended dietary allowances (RDA) as well as absolute physiological requirements (AR) of iron and zinc, with the focus on children. Possibilities of future research are also explored in the general discussion. The dissertation ends with the conclusions of the research work.

Iron deficiency is the most prevalent micronutrient deficiency in the world, and is the main cause of anaemia (WHO/FAO 2006). Approximately 50% of anaemia cases are estimated to be due to iron deficiency (WHO 2015). Of all the World Health Organization (WHO) regions, Africa has the lowest mean haemoglobin concentration (104 g/L) and the highest prevalences of anaemia (62.3%) and severe anaemia (3.6%) for children aged 6 months to 5 years (WHO 2015), some 89 million children. Iron deficiency anaemia is associated with low birth weight and increased risk of maternal and perinatal mortality, adversely affecting cognitive and motor development, and causing fatigue and low productivity (WHO/FAO 2006).

Information on zinc deficiency prevalence is lacking. However, it is believed that where iron deficiency persists, zinc deficiency may also occur (Bailey et al. 2015). In 2012 it was estimated that 17.3% of the world's population was at risk of inadequate zinc intake, with the highest estimate of 25.6% in sub-Saharan Africa (Wessells and Brown 2012). Africa has the

highest prevalence of zinc deficiency in school-aged children, where some 54% of children are estimated to be affected (Best et al. 2010). Zinc deficiency contributes especially to the morbidity and mortality of infants, young children, and suboptimal pregnancy outcomes (IZiNCG 2004).

About 1 in every 3 children, in developing countries, is affected by vitamin A deficiency (WHO 2009). Thus, it is considered a severe public health concern. Research recommendations are shifting from the high-dose periodic capsule distribution programmes towards frequent intakes of vitamin A at physiological levels (Mason et al. 2015). Food-based approaches have been found to be highly effective in increasing serum retinol and reducing vitamin A deficiency (Greiner 2013).

A large proportion of the population of sub-Saharan Africa, especially in the lower socio-economic group, rely on monotonous cereal-based diets, as their major source of nutrition (WHO 2014). While cereal-based diets may contain adequate amounts of iron and zinc to meet daily requirements, the bioavailabilities of these essential minerals are low (Zimmermann and Hurrell 2007). Cereal staple diets are low in enhancers, and high in inhibitors of iron (Glahn et al. 2002) and zinc (Lönnerdal 2000) bioavailabilities, with approximately only 5% of iron, and 10% of zinc bioavailable for absorption (WHO/FAO 2006).

Food-based approaches, such as dietary diversification and food-to-food fortification using micronutrient-rich foods, have been emphasised as cost-effective and sustainable ways to reduce the prevalence of micronutrient deficiencies in low socioeconomic groups (Olney et al. 2012). Fortification, according to the WHO/FAO (2006) is "the practice of deliberately increasing the content of an essential micronutrient, in a food, so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health." Food-to-food fortification is where micronutrient-rich food combinations are used to promote the bioavailability of essential micronutrients by increasing the micronutrient levels, as well as increasing the levels of enhancers, and decreasing the levels of inhibitors of micronutrient bioavailabilities (Thompson 2007). Food-to-food fortification (as an intervention) has the potential to alleviate iron, zinc, and vitamin A nutritional deficiencies and can lead to self-sustained success in improving the nutritional status of various at-risk populations.

Pearl millet (*Pennisetum glaucum* L.) is the most widely grown millet grain and accounts for approximately 10% of the total cereal production in Africa. Pearl millet can form more than

50% of the total cereal production in the Sahel region of West Africa (FAOSTAT 2017). The crop's ability to withstand difficult growing conditions, such as low rainfall and low soil fertility, makes it especially suitable for cultivation in sub-Saharan Africa. Pearl millet provides a major food staple for millions of people in sub-Saharan Africa and is consumed as thick or soft porridges or gruels (Svanberg et al. 1993). Like other cereals, the bioavailability of iron and zinc from pearl millet is low, due to inhibiting substances such as fibre, phytate and phenolics (Léder 2004).

Evaluations which demonstrate the efficacy of food-to-food fortification, specifically concerning iron and zinc are lacking. This is partly due to the complexity of interactions between different components in the complementary foods promoted (Thompson 2007). Thus, the purpose of this study is to determine the effects of various micronutrient-rich plant foodstuffs on the iron and zinc bioaccessibilities of pearl millet porridge.

Chapter 2: Literature review

2.1 Overview

In this review, the prevalence and consequences of iron, zinc and vitamin A deficiencies in sub-Saharan Africa are described. Then, nutrition from cereals, especially pearl millet, and cereal porridges as staple foods are reviewed. Next, intestinal iron and zinc absorption is discussed. This is followed by a discussion on the potential of micronutrient-rich plant foodstuffs to combat iron and zinc deficiencies when used in cereal-based porridges as a form of food-to-food fortification. Modifiers which act as enhancers and inhibitors of iron and zinc bioavailabilities such as organic acids, phenolics and phytate are also described. Lastly, some plant foodstuffs as possible candidates for food-to-food fortification of a pearl millet porridge are described.

2.2 The prevalence and consequences of iron, zinc and vitamin A deficiencies in sub-Saharan Africa

Micronutrient deficiencies, often referred to as 'hidden hunger' affects some two billion people worldwide, of whom women of reproductive age, young children and the elderly are most susceptible (Muthayya et al. 2013). Sub-Saharan Africa has an alarmingly high prevalence of micronutrient malnutrition, with the highest hidden hunger rates in the world, 18 of the 20 countries with the highest hidden hunger scores are in this region (WHO 2009).

The most critical and widespread micronutrient deficiencies are iron, zinc, vitamin A, iodine, and folate; however, vitamin B12 and other B vitamin deficiencies are also common (WHO/FAO 2006). Multiple micronutrient deficiencies often occur together in the same population. Even mild to moderate micronutrient malnutrition, although often not apparently visible in those affected by it, has significant negative and lifelong consequences for health (Muthayya et al. 2013). Micronutrient malnutrition leads to impaired mental and physical development, reduced work productivity, increased risk for perinatal complications and morbidity from infectious disease and mortality (Muthayya et al. 2013). Of greatest concern is the detrimental consequences of micronutrient malnutrition extending across generations (Bailey et al. 2015).

Diets based on low-micronutrient-containing staple foods, as well as frequent infections, are likely to be contributory factors, further aggravated by poor economic conditions and repressive political systems (Muthayya et al. 2013).

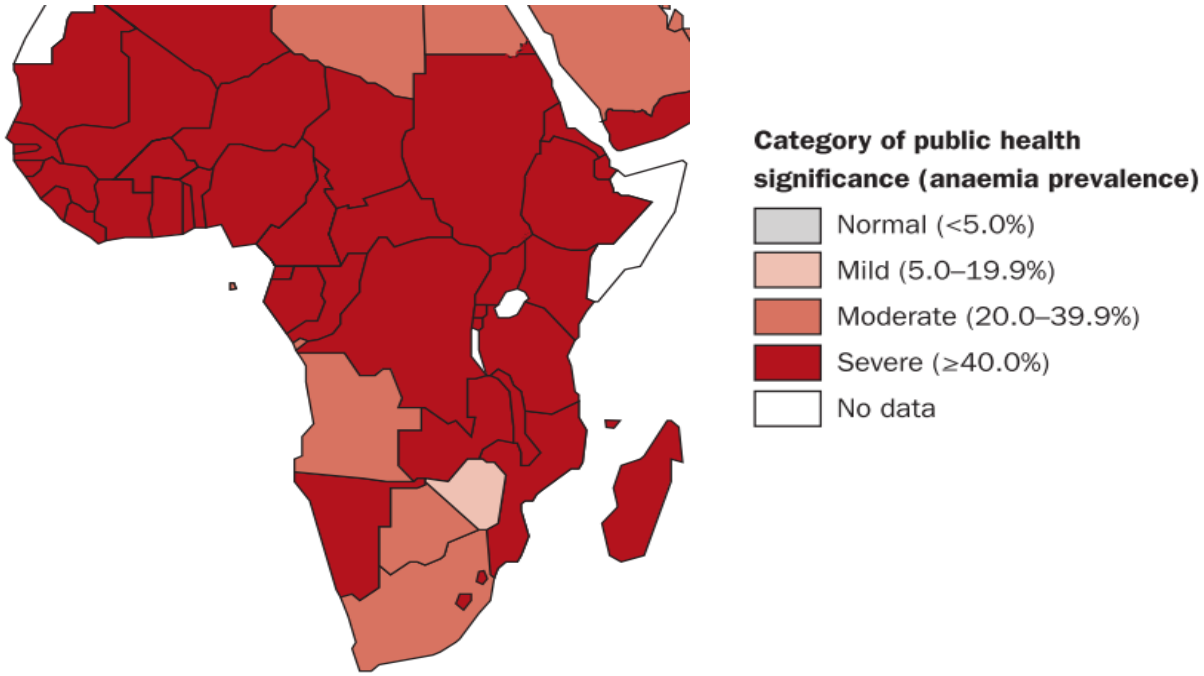

Figure 2.2-1: Anaemia prevalence of pre-school aged children in sub-Saharan Africa (WHO 2008)

Iron deficiency, the most prevalent micronutrient deficiency in the world, affects more than 30% of the world population, an estimated 2 billion people (Bailey et al. 2015). Iron deficiency prevails in two forms, namely with or without anaemia, where the more severe form is associated with anaemia (WHO/UNICEF/UNU 2001). The prevalence of anaemia in Africa is classified as a severe public health problem as more than 40% of the population is affected (WHO 2008). The estimated population percentages affected by anaemia and iron deficiency include 64.6% of children under the age of 5 years (Figure 2.2-1), 55.8% of pregnant women, and 44.4% of non-pregnant women, all of which are the highest prevalences in the world (WHO 2008). It is estimated that if iron fortification reached 50% of the African population, 570 000 disability adjusted life years (DALYs) would be averted every year (Zimmermann and Hurrell 2007). Several vital functions in the body are dependent on iron. Iron is essential for sufficient growth, physical and mental development, cell maintenance, and an effective immune response (Thompson 2007).

Zinc deficiency is also a major public health concern as country-specific prevalence of inadequate zinc intake in the whole of sub-Saharan Africa is either at high (prevalence >25%) or medium (prevalence 15-25%) risk for zinc deficiency (Figure 2.2-2) (Wessells and Brown 2012). High risk of zinc deficiency is classified as a stunting prevalence of more than 20% and estimated prevalence of inadequate zinc intake of more than 25%. It has been estimated that zinc deficiency in Africa accounts for more than nine million DALYs (Fischer Walker et al. 2009). In 2004, zinc deficiency was the underlying cause for some 260 000 deaths in Africa due to diarrhoea, malaria and lower respiratory infections (Fischer Walker et al. 2009). Adequate zinc nutrition is of critical importance to support normal growth and development, immune-competence, reproductive function, and to improve other aspects of human health and function (IZiNCG 2004).

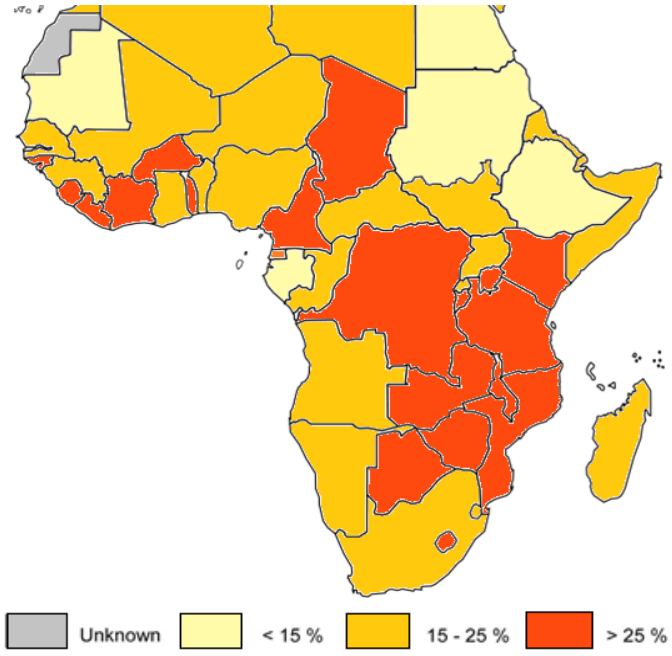

Figure 2.2-2: Estimated country-specific prevalence of inadequate zinc intake in sub-Saharan Africa (Wessells and Brown 2012)

Vitamin A deficiency is of another major public health concern in African countries. Prevalences of preschool aged children with low serum retinol concentrations (<0.7 µmol/L) are highest in countries of Eastern and Southern Africa (37%), followed by Western and Central Africa (33%) (Mason et al. 2001). Vitamin A deficiency can limit growth, weaken immunity, cause xerophthalmia leading to blindness, and increase mortality (Low et al. 2007). Vitamin A deficiency arises when a habitual diet contains too little bioavailable vitamin A to meet physiological needs. Such diets are usually poor in foods from animal

origin and plant sources rich in provitamin A carotenoids (Parker 1996). Beta-carotene is the major provitamin A component of most carotenoid-containing foods, smaller amounts of alpha-carotene and beta-cryptoxanthin can also be found. These are the only three natural important precursors for Vitamin A in humans.

Suboptimal iron and zinc status may be caused by an inadequate dietary intake of iron and zinc and/or low bioavailable iron and zinc. Low bioavailability, caused by inhibitors of iron and zinc absorption, are the most common causative factor (Lönnerdal 2000). In many African countries, cereals are consumed as staple foods, which have poor bioavailable non-haem iron and zinc due to inhibitory factors such as phytate, fibres, phenolic compounds and tannins (WHO/FAO 2006). The inhibitory effect of phytate on iron and zinc absorption may be further potentiated by calcium (Lönnerdal 2000). Thompson (2007) stated that the scale and magnitude of iron and zinc deficiencies, combined with its functional impact on the physiological and socioeconomical quality of life, require urgent attention and implementation of known and effective interventions. The lowest socioeconomic population groups are at greatest risk for iron and zinc deficiencies (WHO/UNICEF/UNU 2001) and should be an immediate target for intervention strategies.

2.3 Nutrition from cereals

Cereals are the largest single plant foodstuff in most diets, but especially in developing countries where up to 90% of the total diet can be cereals (Bender 1999). Cereal-based plant foodstuffs are considered the most important source of carbohydrates, proteins, certain vitamins, minerals, and dietary fibre, and can also provide vitamins E and B (thiamine, riboflavin and niacin) for the African population (McKevith 2004). However, apart from orange maize, which contains elevated levels of provitamin A, cereals are deficient in vitamins C, B12, and A (Poutanen et al. 2009). A major problem with cereals is that they can contain relatively high levels of antinutrients such as fibre, phytate, oxalate, phenolics, trypsin inhibitors, and sometimes tannins (McKevith 2004) (Table 2.3.1). Phytate, in particular, strongly binds minerals such as calcium, iron and zinc which renders them insoluble and reduces the bioavailability of these minerals for absorption, thus, iron and zinc deficiencies may result (Reale et al. 2007).

Table 2.3.1: Nutrient contents (mg/100 g, db) of important cereals in Africa, compared to the recommended nutrient intake (mg/day) for 1–3 and 4–6 year-old children, adult females and lactating women

Nutrient	**Recommended nutrient intake** (mg/day)				**Nutrient content** (mg/100 g, db)			
	Children[1]		Adult females, 18+ years[1]	Lactating women[1]	Yellow maize	Wheat	Sorghum	Pearl millet
	1–3 years	4–6 years						
Iron[d]	11.6	12.6	27.4	30	1.4–2.7 [a] (2.0)[2,3,4,6,16]	1.1–7.3 (3.9)[10,19,20]	1.1–8.8 (5.2)[6,10,23,24]	11.0–32.0 (20.3)[11,12]
Zinc[e]	8.3	9.6	9.8	19	1.6–3.4 (2.3)[2,3,4,6,16]	0.7–8.8 (3.8)[10,19,20]	0.3–2.5 (1.5)[6,10,23]	7.5–15.0 (10.8)[11,12]
Calcium	500	550	750	750	0–43 (14)[2,3,4,16]	16–20 (18)[10]	3–22 (16)[10,23, 24]	20–40 (28)[11,12]
Vitamin A (RAE[c])	0.40	0.45	0.50	0.85	61.2–187.0 (145.4)[15,16]	0.0–10.6 (2.1)[19,22]	0.6–1.4 (1.0)[5]	None detected
Total Phenolics	N.A.[b]		N.A.	N.A.	523–581 (554)[17,18]	35–82 (57)[10,22]	109.2–413 (213)[10,5]	148–790 (495)[10,11,13, 14]
Phytate	N.A.		N.A.	N.A.	215–8700 (2464)[2,3,4,17, 6,7]	20–1290 (522)[7,8,9,21]	439–1173 (846)[6,7,8]	500–690 (607)[12,13]

[a]Nutrient content values are reported as minimum–maximum (mean)
[b]N.A.: Not applicable
[c]RAE: Retinol activity equivalent
[d]For a dietary iron bioavailability of 5%
[e]For a low bioavailability of zinc

[1](WHO/FAO 2004), [2](Mendoza et al. 1998), [3](Beiseigel et al. 2007), [4](Hambidge et al. 2004), [5](Afify et al. 2012), [6](Lestienne et al. 2005), [7](Gibson and Hotz 2001), [8](Frontela et al. 2008), [9](Dost and Tokul 2006), [10](Ragaee et al. 2006), [11](Ravindran 1991), [12](Gwamba 2016), [13](Saharan 2015), [14](Jukanti et al. 2016), [15](Muzhingi et al. 2011), [16](Ejigui et al. 2005), [17](Lopez-Martinez et al. 2009), [18](Žilić et al. 2012), [19](Ortiz-Monasterio et al. 2007), [20](Cakmak et al. 2004), [21](Febles et al. 2002), [22](Hidalgo and Brandolini 2014), [23](Glew et al. 1997), [24](Radhakrishnan and Sivaprasad 1980)

As stated, pearl millet is a staple food for millions of people in sub-Saharan Africa, especially populations below the poverty line (Suma and Urooj 2014). Pearl millet has the ability to withstand difficult ecological conditions; it can tolerate poor soil quality and can be grown in semi-arid and sub-tropical conditions. It can grow where annual rainfall is variable, unpredictable or very low (200-500 mm) and daily temperatures reach 30˚C and outperforms other cereal crops under these conditions (Suma and Urooj 2014). Pearl millet is thus, especially suitable for growth in sub-Saharan Africa and can contribute more than 50% of the total cereal production in the Sahel region of West Africa (FAOSTAT 2017). Pearl millet is nutritionally comparable and even superior to most other cereals as it is rich in iron, zinc, calcium, lipids, high quality protein and has a high energy value (Klopfenstein and Hoseney 1995). However, pearl millet is rich in antinutrients such as phytate, phenolics, and may contain traces of oxalic acid, all of which reduce mineral bioavailability and also inhibit proteolytic and amylolytic enzyme activities (Jukanti et al. 2016).

Hemalatha et al. (2007) analysed the iron bioaccessibility (dialysability assay) from rice, wheat, finger millet, sorghum and maize, with iron contents ranging from 1.32 to 6.51 mg/100 g, dry basis (db). They reported only 4.13–8.05% bioaccessible iron. Suma and Urooj (2014) analysed the bioaccessibilities of two pearl millet varieties, namely Kalukombu (KK) and Maharashtra Rabi Bajra (MRB). The amount of bioaccessible iron was 0.16 mg/100 g and 0.44 mg/100 g, respectively, and the percentage bioaccessible iron was 2.5% and 7.1%, respectively. MRB had more than double the percentage and the amount of bioaccessible iron than KK. Tripathi et al. (2010) found percentage and amount of bioaccessible zinc in pearl millet flour of 17% and 0.69 mg/100 g, respectively, which was partially decorticated before milling. The differences in mineral bioaccessibilities between the various pearl millet varieties were likely due to varying levels of phytate, phenolics and other inhibiting substances (Suma and Urooj 2014). Hemalatha et al. (2007) also analysed the zinc bioaccessibility (dialysability assay) from various cereals, with zinc contents ranging from 1.08 to 2.24 mg/100 g, db, and found 5.51–21.4% bioaccessible zinc.

2.4 Cereal-based porridges as staple food

The staple diet of adults and children in Africa is usually thick or soft porridges or gruels prepared from a local cereal (Svanberg et al. 1993). Cereal-based porridges are culturally acceptable products, for which the raw materials are readily bioavailable, and are

economically feasible to produce. Cereal-based porridges would thus, serve as an appropriate vehicle to which other foods can be added in order to improve their nutritional quality.

Complementary foods with sufficient energy and nutrient density must be provided to infants at about six months of age, as the supply of energy and nutrients, especially iron, from breast milk is no longer adequate to meet the growing infant's needs (Gibson et al. 1998). Soft cereal porridges are usually the first complementary foods introduced to infants as weaning foods in most of Africa, from as early as three months (Onofiok and Nnanyelugo 1998). As these cereal porridges are usually prepared as thin, low viscosity gruels, infants and small children may not be able to ingest a large enough volume to cover their energy and nutrient requirements (Onofiok and Nnanyelugo 1998). It has been estimated that a baby aged four to six months would need an impossible 920 g of thin cereal porridges to meet their daily energy (3000 kJ), iron (0.76 mg/kg body weight) and protein (13 g) requirements (Domellöf et al. 2014). Thus, complementary porridges made from cereals are not sufficiently rich in energy and also lack proteins, essential vitamins and minerals (Oniang'o et al. 2003). It has been found that in most traditional settings, the same cooking pot or meal dishes laid out for the whole family are also used for complementary feeding of infants (Solomons 1999).

Gibson et al. (1998) found that traditional cereal-based complementary foods in Africa and other developing regions consistently yielded less than the required 10.8 mg bioavailable iron per day for 9–11-month-old infants, when intake of the complementary food was between 210 and 240 g, as eaten, per day. Thus, infants received insufficient iron (20.8 mg/day at 5% bioavailability), even when the basal requirement (10.8 mg/day) in association with moderate bioavailability (10%) was assumed. For zinc, none of the cereal-based (maize, wheat and sorghum) complementary foods provided the basal requirement (7.26 mg/day) when low bioavailability (15%) was assumed. They also found that the phytate content for the cereal-based complementary foods varied from 50 to 221 mg/100 g, as eaten. Phytate:iron molar ratios were in the range of 4.7:1 to 28.5:1 (the critical level being 1:1), and most phytate:zinc molar ratios were above the critical level of 15:1, which is associated with suboptimal zinc status (Lopez et al. 2002).

2.5 Intestinal iron and zinc absorption

The process of iron absorption is complex and involves at least three steps: (i) digestion and release from the diet, (ii) active absorption into the enterocytes, and (iii) transport from the

enterocytes to the circulation (Salovaara et al. 2002). Non-haem iron is absorbed as an ion and needs to be in a soluble state. Iron from plant foods is dissociated and made soluble by the low pH of the stomach which maintains iron in the ferric (Fe^{3+}) state (von Drygalski and Adamson 2012). Thereafter the free iron comes into contact with absorptive cells of the proximal small intestine where most iron absorption takes place (Figure 2.5-1). Ferric iron is converted to ferrous iron (Fe^{2+}) by a reductase which is then taken into the absorptive cell. Haem iron is much more bioavailable and absorbed intact and more efficiently (by a factor of 5–10 times) than elemental iron; however, the pathway of absorption for haem iron has not yet been clarified (von Drygalski and Adamson 2012).

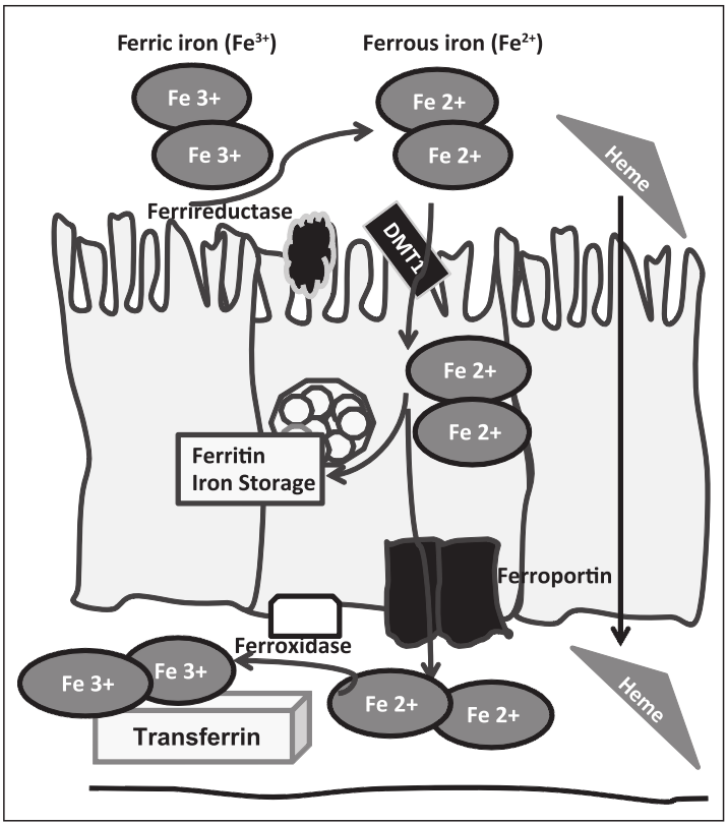

Figure 2.5-1: Haem and non-haem iron absorption by absorptive cells of the proximal small intestine (von Drygalski and Adamson 2012)

Zinc absorption is concentration dependent and occurs throughout the proximal small intestine, with the jejunum having the highest absorption rate (WHO/FAO 2004). It is thought that the subcellular mechanisms of exogenous zinc absorption involve both saturable and unsaturable processes (Krebs 2000). Several transport proteins have been identified; however, the exact absorption mechanisms still remain to be elucidated.

Due to the complex absorption mechanisms of iron and zinc, even though adequate amounts of iron and zinc to meet daily requirements may be consumed, not all that is consumed is bioavailable for absorption (WHO/FAO 2006). The term 'bioavailability' is thus used to describe the proportion of the ingested nutrients that is actually absorbed and metabolised through the normal digestive pathways (Heaney 2001). The 'bioaccessibility' of nutrients is used as an estimate of their bioavailability, using *in vitro* methods. Bioaccessibility is the amount of a compound that is released from the food matrix and is considered to be available for absorption through the gut wall (Minekus et al. 2014).

2.6 Potential of micronutrient-rich plant foodstuffs, used in food-to-food fortification of cereal-based porridges, to combat iron and zinc deficiencies

As indicated, traditional cereal-based complementary foods can be improved by combining locally available plant foodstuffs which complement each other in such a way as to provide improved nutrient requirements (Onofiok and Nnanyelugo 1998). Such food-to-food fortification may reduce the inhibitory effects of antinutrients found in cereals. Icard-Vernière et al. (2015) improved the iron and provitamin A levels of sauces consumed in Burkina Faso, by increasing the amount of leafy vegetables, such as amaranth, sorrel, and spider plant leaves, and reducing the amount of ingredients rich in mineral absorption inhibitors, such as legumes. By means of the dialysability assay, Gautam et al. (2010) found that the addition of carrot or amaranth leaves (2.5 g and 5 g per 10 g, fresh weight (fw) of grain) significantly increased iron and zinc bioaccessibilities from rice and sorghum. The addition of carrot resulted in a 14–86% increase in iron and zinc bioaccessibilities and amaranth leaves yielded 11–193% increases. In apparent contradiction, Cercamondi et al. (2014b) found that the maize paste-type porridge 'tô' accompanied by sauces made with amaranth or jute did not provide additional bioavailable iron, which the authors attributed to the high phenolic levels of their leaves.

2.7 Modifiers of iron and zinc bioavailabilities present in plant foodstuffs consumed in Africa

2.7.1 Provitamin A

It has been found that various provitamin A carotenoids are able to increase iron absorption from cereal based meals. Garcıa-Casal et al. (1998) studied the effect of varying beta-carotene levels on iron absorption (as radioactive labelled ^{59}Fe or ^{55}Fe) from cereal-based meals fed to adults. They found that iron absorption increased 4.2-fold for rice (beta-carotene: 0.95 μmol/100 g, fw), and 2.8-fold for wheat (beta-carotene: 0.67 μmol/100 g, fw) and 2.8-fold for maize (beta-carotene: 0.85 μmol/100 g, fw). Garcıa-Casal et al. (1998) also found that carotenoids promote iron absorption, from cereal-based meals, to the extent of counteracting the negative effects of tannins on iron absorption. The authors showed that the addition of coffee, contributing 200 mg tannin content, to beta-carotene fortified cereal-based meals did not significantly affect iron absorption in humans. They proposed that beta-carotene may form a complex with iron which keeps the mineral soluble in the intestinal lumen and thus, prevents the inhibitory effects of phytates and phenolics on iron absorption. These findings were confirmed by García-Casal (2006), who found that coffee had no effect on iron absorption when added to wheat and maize-based breakfast cereals, containing different concentrations of lycopene, lutein, and zeaxanthin-carotenoids, without provitamin A activity.

Green leafy vegetables such as amaranth and sweet potato leaves are traditionally grown and consumed in sub-Saharan Africa, and have been found to contain provitamin A (Odhav et al. 2007) (Table 2.7.1). Other commonly available plant foodstuffs in sub-Saharan Africa, including moringa leaves, orange fleshed sweet potatoes, carrots and so forth, also contain provitamin A. Consumption of these plant foodstuffs could possibly improve iron bioavailability.

Table 2.7.1: Provitamin A contents (RAE/ 100 g, db) of plant foodstuffs, available in sub-Saharan Africa

Plant foodstuff	**Provitamin A content**[1] (RAE/ 100 g, db)
Amaranth leaves	1.76
Sweet potato leaves	1.43
Moringa leaves	1.77
Orange fleshed sweet potatoes	3.12
Carrots	7.13

[1]Values from USDA Nutrient Database (2015).

2.7.2 Organic acids

Organic acids, such as those found in plant foodstuffs, are able to increase, and some to decrease, iron (Hurrell et al. 2004) and zinc (Pabón and Lönnerdal 1993) bioavailabilities. Ascorbic acid is a strong reducing agent and has been shown to increase dietary iron absorption by reducing ferric iron to the ferrous state (Teucher et al. 2004). Iron is absorbed in the divalent (Fe^{2+}) state as it is more soluble at the relatively high pH of the duodenum and small intestine (Lopez et al. 2002).

Ascorbic acid has been shown to be the most effective promoter of non-haem iron absorption from the diet (Teucher et al. 2004). However, the impact of ascorbic acid on iron status is apparently dependent on the characteristics of the habitual diet (Sandström 2001). High levels of vitamin C supplementation (2 g) in subjects consuming meat had no significant effect on iron status (Cook et al. 1984), whereas 500 mg ascorbic acid given to strict vegetarians improved iron status after 2 months (Sharma and Mathur 1995). Salovaara et al. (2002) found that ascorbic acid had only a moderate effect on ferrous iron (Fe^{2+}) absorption (human epithelial cell line Caco-2), with a 2-fold increased absorption in an almost linear manner. The effect of ascorbic acid on ferric iron (Fe^{3+}) absorption was much larger, even at low acid concentrations, with 70-fold increased iron absorption at an ascorbic acid concentration of 80 μml/L.

Ascorbic acid has been shown to promote absorption of non-haem iron from the diet, to the extent of counteracting the negative effects of dietary phytate, phenolics, tannins (Siegenberg et al. 1991), calcium and the milk protein, casein (Stekel et al. 1986) on iron absorption. A study done with maize bread found that a dose of 30 mg ascorbic acid doubled the iron absorption in the presence of 58 mg phytate (Siegenberg et al. 1991). They found that a dose

of ~50 mg ascorbic acid restored iron bioavailability to normal values from any meal containing more than 100 mg tannic acid. For low phytate containing foods and powdered milk, a molar ratio of 2:1 ascorbic acid to iron is recommended and a ratio of 4:1 for high phytate containing foods (Hurrell 2002). It must be noted, however, that ascorbic acid is limited by its instability in aqueous solutions, during storage of powdered foods and during prolonged heat processing or cooking (Hurrell and Egli 2007).

It has been found that citric, tartaric and malic acids can enhance or reduce iron absorption (Hurrell et al. 2004). However, these acids have not been studied as thoroughly as ascorbic acid and furthermore are only effective enhancers of iron bioavailability at molar ratios in excess of 100:1 (Gillooly et al. 1983). In a human subject study, Gillooly et al. (1983) found that in order to achieve an increase in iron absorption of two to threefold, more than one gram of citric, malic or tartaric acid was necessary, in a rice meal fortified with 3 mg ferrous sulphate. This is equivalent to molar ratios of 264:1, 378:1 and 337:1 (acid:fortified iron) for citric, malic and tartaric acids, respectively.

Salovaara et al. (2002) studied the influence of organic acids on iron absorption using the Caco-2 human epithelial cell line assay. They found contradictory results to other studies and showed that 0.1 mmol/L citric acid and 0.5 mmol/L lactic acid additions reduced ferrous iron (Fe^{2+}) absorption by 83 and 85%, respectively. The lowest absorption was with 4 mmol/L lactic acid and 0.8 mmol/L citric acid, which resulted in reduced iron absorption of 96%, and 94%, respectively. With ferric iron (Fe^{3+}) absorption on the other hand, there was increased absorption with citric acid concentrations of 0.01–0.5 mmol/L, which disappeared at concentrations between 0.5–2.5 mmol/L. Citric acid concentrations exceeding 2.5 mmol/L also resulted in increased absorption of ferric iron, with a maximum increase of 3.6-fold with 4 mmol/L citric acid. The seeming contradictory findings of the effect of citric and lactic acids on iron absorption can partly be explained by ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) being affected differently by the acids. The concentration of the acid used also plays a role in the positive or negative effect on iron absorption.

Vegetables rich in oxalic acid have been found to reduce iron absorption (Gillooly et al. 1983). Oxalic acid can inhibit ferric iron (Fe^{3+}) absorption, even at very low concentrations (0.1 mmol/L); however, at concentrations below 0.5 mmol/L it has been found to enhance ferric iron (Fe^{3+}) absorption (Salovaara et al. 2002). Very strong complexes are formed between ferrous iron (Fe^{2+}) and citric or oxalic acids, which may cause substantial blocking

of ferrous iron (Fe^{2+}) absorption (Salovaara et al. 2002). The dominating association between ferric iron (Fe^{3+}) and citric acid is believed to be ferric dicitrate complexes which slow the transfer of iron to absorption proteins as these strong chelates can cause a reluctance to donate iron to the epithelial cells.

Ascorbic acid has been found to have no influence on dietary zinc absorption, as demonstrated by Sandström and Cederblad (1987). They studied zinc absorption in a human subject study, a solution containing 100 mg ascorbic acid and 40 or 200 μmol zinc was administered and found to have no effect on zinc absorption. Likewise, 1000 mg ascorbic acid consumed with a high phytate meal, composed of wholemeal bread flour, did not affect zinc absorption. Solomons et al. (1979) found that ascorbic acid administered to human subjects in doses of 0.5, 1.0, and 2.0 g in combination with a 110 mg aqueous dose of $ZnSO_4.7H_2O$ (containing 25 mg elemental zinc) had no demonstrable effect on the absorption of inorganic zinc. Ascorbic acid probably has no effect on zinc absorption because zinc has a filled d^{10} orbital in its third electron shell, and thus, has virtually no tendency to undergo oxidation or reduction (Solomons et al. 1979).

Citric and acetic acids have been reported to facilitate zinc absorption (animal study) as it is able to form soluble ligands with zinc in the gastrointestinal tract (Pabón and Lönnerdal 1993). This also prevents the formation of insoluble zinc-phytate complexes, although the magnitude of this effect has not been extensively studied (Gibson 2006). The effect citric acid has on zinc absorption is thought to be partly due to a ligand competition between phytate and citric acid for divalent cations (Walter et al. 1998). Citric acid mineral complexes are able to easily break down in the small intestine, thus, releasing cations bioavailable for absorption. Walter et al. (1998) found that zinc was the element with the highest enhancement of bioaccessibility by dialysability assay with citric acid supplementation. An increase in zinc bioaccessibility greater than 10 times was evident with the addition of 4% citric acid to the diet.

Plant foodstuffs contain a wide variety of organic acids such as citric, acetic, malic, oxalic, succinic, fumaric, quinic, tartaric, and ascorbic acids, amongst others (Hounsome et al., 2008). Malic and citric acids are the most predominant; succinic, fumaric, and quinic acids are also prevalent. Ascorbic acid has strong antioxidant properties and is commonly found in plant foodstuffs, common in sub-Saharan Africa, such as spinach, spring onions, cabbage,

broccoli, sweet peppers, peas, beans, tomatoes, citrus fruits, mango and papaya (USDA Nutrient Database 2015) (Table 2.7.2).

Table 2.7.2: Total ascorbic acid contents (mg/ 100 g, db) of plant foodstuffs, available in sub-Saharan Africa

Plant foodstuff	**Total ascorbic acid content**[1] (mg/ 100 g, db)
Spinach	327
Spring onions	185
Cabbage	509
Broccoli	834
Peas	189
Tomatoes	250
Mango	220
Papaya	510
Oranges	402

[1]Values from USDA Nutrient Database (2015).

2.7.3 Phenolics

Phenolic compounds are one of the most highly diversified group of phytochemicals present in plants (Shahidi and Chandrasekara 2013). Pearl millet contains mainly free and conjugated forms of phenolic acids, as well as several flavonoids. The levels and types of phenolics in fruits and vegetables can vary greatly.

Phenolics are multifunctional antioxidants which have metal-chelating and chain-breaking activities in the same molecule (Khokhar and Apenten 2003). Phenolics are divided into three main groups, namely the phenolic acids, the flavonoids, and tannins (Hurrell et al. 1999). Phenolic acids and their derivatives are widely distributed in plants where they are linked to cell wall components such as hemicellulose and proteins (Siegenberg et al. 1991). Phenolics (flavonoids and tannins) are major antinutritional factors as they inhibit several hydrolytic enzymes, form tannin-protein complexes which limit starch and protein utilisation, and they can reduce the availability of minerals and vitamins (Saharan 2015).

Phenolics with galloyl and catechol groups are able to form insoluble complexes with iron which render the mineral unavailable for absorption (Towo et al. 2006). It has been suggested that the amount of iron binding phenolic galloyl groups in foods roughly correspond to the degree of inhibition of iron absorption (Brune et al. 1989).

The inhibitory effect of phenolics on iron absorption is likely due to the polymerisation with iron, which results in the formation of insoluble complexes (Siegenberg et al. 1991). It has been proposed that iron binds to phenolics via ortho-dihydroxy (catechol) or trihydroxy-benzene (galloyl) groups (Brune et al. 1991). It has been found that iron binding by phenolics increases with increasing numbers of OH-groups (Khokhar and Apenten 2003).

Hurrell et al. (1999) studied the effect of different phenolic-containing beverages on iron absorption from a bread meal in adult human subjects. It was found that iron absorption was reduced in a dose-dependent fashion, depending on total phenolic levels. Beverages containing 20–50 mg total phenolics per serving reduced iron absorption from the bread meal by 50–70%. Beverages containing 100–400 mg total phenolics per serving resulted in a 60–90% iron absorption reduction.

Khokhar and Apenten (2003) found that only phenolics possessing an ortho-dihydroxy (catechol) group reacted with an iron containing reagent. Compounds which lacked the catechol group did not react. They found that the relative order of decreasing iron-binding efficiency (at 0.2 mg/ml) was catechin > epicatechin (EC) > epicatechingallate (ECG) > epigallocatechin (EGC) > epigallocatechingallate (EGCG) ~ quercetin = gallic acid. In order for phenolics to bind to iron, a flavonoid ring *B* and a 3',4'-dihydroxy group is required (Figure 2.7-1), which is transformed into a 3',4',5'-trihydroxy (galloyl) group. Thus, a vicinal di-hydroxyl group is required for efficient iron-binding (Khokhar and Apenten 2003).

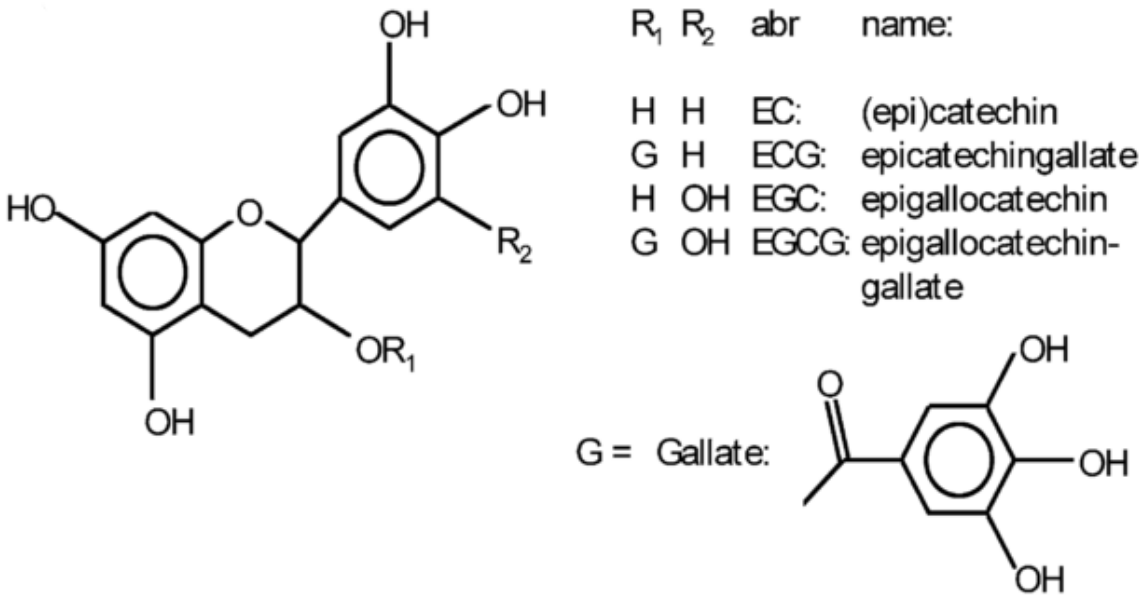

Figure 2.7-1: Structures of catechins (Khokhar and Apenten 2003)

Matuschek et al. (2001) studied the effect of oxidation of phenolics on *in vitro* iron accessibility (solubility). They found that bioaccessible iron increased with the addition of phenolic oxidase to phytate-reduced sorghum and finger millet, this was accompanied by a reduction in total phenols, as well as in the amount of catechol and resorcinol groups.

Condensed tannins

Some cereals, in particular sorghum and finger millet, can contain condensed tannins, namely proanthocyanidins, which are high molecular weight phenolics, consisting of polymerized flavan-3-ol and/or flavan-3,4-diol units (Devi et al. 2014).

Tannins are biologically active and adversely affect the nutritional quality of such cereals by decreasing iron bioavailability. Tannins also complex proteins and carbohydrates and have been found to inhibit digestive enzymes which results in a lowered digestibility of most nutrients (Shashi et al. 2007). Tannins (Figure 2.7-2) form complexes with ferrous iron, [Fe^{2+}n-tannic acid], which render the iron unavailable for absorption (Khokhar and Apenten 2003).

Iron binding by phenolics increases with increasing numbers of OH-groups (Khokhar and Apenten 2003). It is thought that the large size of tannin molecules may allow iron binding by mechanisms other than the galloyl group as catechin with no galloyl groups has a higher relative iron binding efficiency (121%) than epigallocatechin (45%) with 1 galloyl group, which in turn has higher relative iron binding efficiency than epigallocatechingallate (26%) with 2 galloyl groups (Khokhar and Apenten 2003).

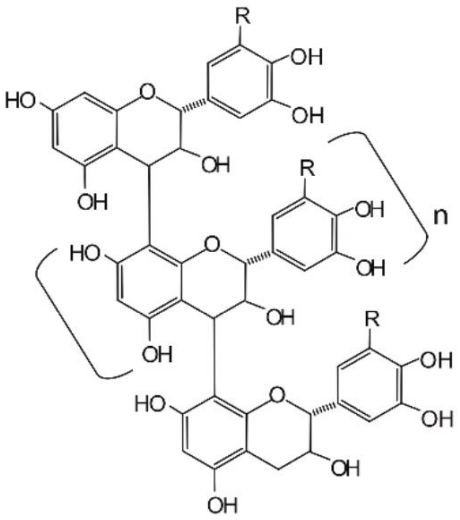

Figure 2.7-2: Condensed tannin structure, with R=H: catechin or epicatechin, R=OH: gallocatechin or epigallocatechin (Tsuruta et al. 2011)

Within a pH range of pH 1–7, tannins mainly form mixtures of mono- and bis-type complexes with iron, but may also form tris-complexes (Friman et al. 2004) (Figure 2.7-3).

Siegenberg et al. (1991) found a significant inhibitory effect of tannic acid on iron absorption. Meals infused with a solution of 3 mg iron as $FeSO_4 \bullet 7H_2O$ were served with varying amounts of tannic acid. It was found that 12 mg tannic acid reduced iron absorption by one third, and 50 mg reduced absorption by 70%. Brune et al. (1989) found that inhibition of iron absorption by tannic acid was strongly dose-related. An amount of 5 mg tannic acid added to a test meal inhibited iron absorption by 20%, 25 mg by 67% and 100 mg by 88%. Gallic acid inhibited iron absorption to the same extent as tannic acid, per mole galloyl groups, whereas no inhibition was found when catechin was added to the test meal.

Unlike iron, very few studies have documented the relationship between phenolic compounds in foods and zinc bioavailability. Greger and Lyle (1988) found a reduction in zinc absorption from tea in rats.

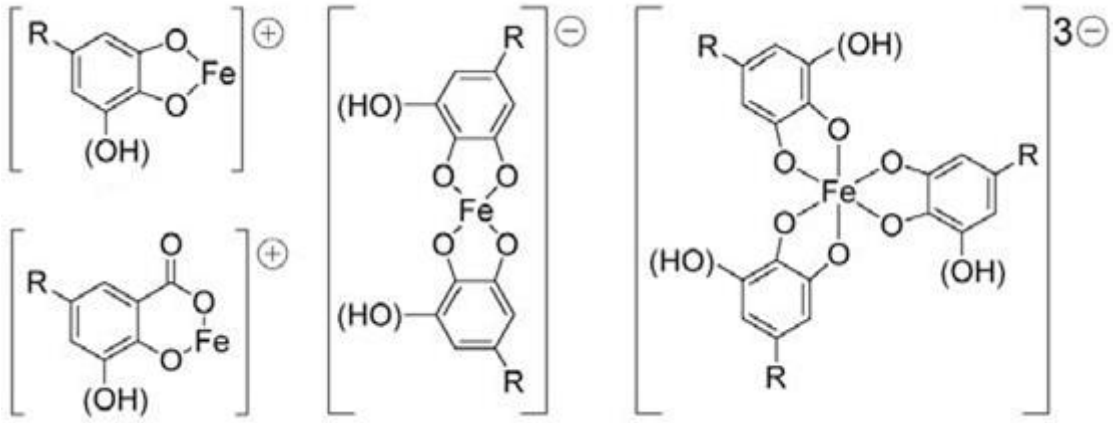

Figure 2.7-3: Tannin-iron complexes, tannin-iron mono-, bis-, and tris- complexes. R = residual part of the tannin (Friman et al. 2004)

2.7.4 Phytate

Unrefined cereal and legume grains contain high levels of phytate, also referred to as phytic acid (myo-inositol hexaphosphate) (Figure 2.7-4A), which is a potent inhibitor of iron and zinc absorption in both adults and children (Gibson 2006). Both iron and zinc deficiencies have been reported as a consequence of high phytate intakes (Reale et al. 2007). Phytate forms strong, insoluble Fe-phytate or Zn-phytate complexes (Figure 2.7-4B) in the intestinal tract (Lönnerdal 2000), rendering the minerals unavailable for absorption and reabsorption.

The first step in mineral membrane transport for absorption requires that the mineral remain in the ionic state; however, in the ionic state the mineral is very unstable and highly susceptible to sequestering by phytate (Lopez et al. 2002). Phytate is able to reduce the bioavailability of some minerals such as zinc, iron, calcium, copper and magnesium as the phosphate groups can form strong and insoluble complexes with their divalent cations (Lönnerdal 2000). When precipitation by phytate occurs the metal is not in ionic state and the carrier proteins, located on the intestinal cell membrane, cannot bind themselves to the metal ion for membrane transport (Lopez et al. 2002).

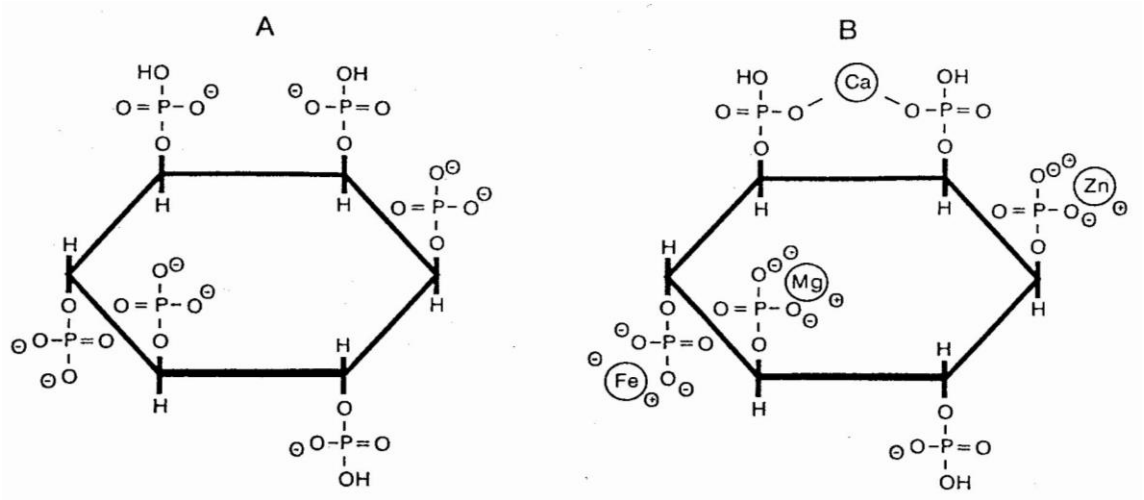

Figure 2.7-4: Structure of phytate (A) and mineral-phytate complexes (B) (Schinckel).

Zinc appears to be the element whose bioavailability is most reduced by phytate (Walter et al. 1998). Phytate has a dose-dependent response on zinc absorption, and the phytate:zinc molar ratio of a diet can be used to predict the proportion of bioavailable dietary zinc. Diets with molar ratios of more than 15:1 have been associated with biochemical zinc deficiency in humans (Gibson 2006). Phytate:zinc molar ratios greater than 20:1 are associated with clinical evidence of zinc deficiency, whereas ratios of 10:1 or less indicate adequate zinc bioavailability (Antony and Chandra 1998).

Mixtures of different phosphorylated forms of inositol phosphate are found in plant foods, of which the major form is usually hexaphosphate (IP-6), but penta- (IP-5), tetra- (IP-4), and triphosphates (IP-3) are also present (Lönnerdal 2000). In a rat pup model, it has been found that only IP-6 and IP-5 inhibit zinc absorption, IP-4 and IP-3 had no significant effect (Lönnerdal 2000). Zinc absorption in women was measured by extrinsically labelling phytate containing meals with ^{65}Zn and measurement of whole body retention (Sandström and

Sandberg 1992). White bread (22 μmol zinc) containing no detectable phytate had 43.3% zinc absorption, white bread containing 400 μmol inositol phosphates of forms IP-6, IP-5, and IP-4 had 14.3%, 18.1% and 41.5% zinc absorption, respectively. These results indicated that only IP-6 and IP-5 also inhibit zinc absorption in humans.

Degradation of phytate by processing techniques such as sprouting (malting) and fermentation can enhance the bioavailability of bound metals (Lopez et al. 2002). It should be noted; however, that phosphates from phytate hydrolysis can still react with mineral ions to form insoluble precipitates. Phosphates precipitate in alkaline condition, such as found in the intestine, trapping minerals within the insoluble compounds and thus, rendering the metals unavailable for absorption.

2.7.5 Dietary fibre

The level of total dietary fibre varies greatly between cereal grains. Values of total dietary fibre content for rye and maize have been reported as approximately 15%, db, and sorghum at 11%, db, (Charalampopoulos et al. 2002). Elsewhere 18%, and 21%, db, total dietary fibre content have been reported for rye and sorghum, respectively (Ragaee et al. 2006).

Péneau et al. (2008) using fibre-rich fruit and vegetable juices found that fibre only had an effect on non-haem iron absorption in groups whose non-haem iron absorption was high due to low iron stores. However, fibre had no effect on non-haem iron absorption in groups with high iron stores. In fact, conflicting results have been found concerning the effect of fibre on iron bioavailability. Studies have found weak associations (Cowin et al. 2001) or no associations (Thane et al. 2000) between serum ferritin and fibre intake in children. No effect of fibre on serum ferritin was found in woman (Cade et al. 2005), and conflicting results were found in elderly individuals (Fleming et al. 2002).

Concerning zinc absorption, Turnlund et al. (1984) conducted studies with alpha-cellulose as an isolated fibre component, and found no significant inhibitory effect on zinc absorption. Cook et al. (1983) found that only bran had a statistically significant inhibition on iron bioavailability from wheat muffins, prepared with added bran, cellulose, or pectin. They demonstrated that inhibition of iron absorption is not a universal property of all fibre sources. Moreover, the modest effect of maximally altering the natural fibre levels of a meal, suggests that fibre is not a major determinant of food iron bioavailability in humans. Rather it is the

high phytate levels of some forms of fibre which inhibits non-haem iron absorption (Cook et al. 1997).

Furthermore, high fibre containing foods with reduced phytate levels showed increased mineral absorption, to a degree similar to that of low fibre containing foods (Navert and Sandström 1985). Hence, Lönnerdal (2000) concluded that fibre on its own has little or no effect on mineral absorption.

2.7.6 Oil / fat

Few studies have been made on the effect of oils on iron bioavailability, and those on zinc bioavailability are even scarcer. Johnson et al. (1987) studied haem and non-haem iron absorption by altering the type and level of fat in diets fed to rats. They found that both haem and non-haem iron absorption was higher in diets containing high (30%) dietary fat, rather than low (5%) fat, regardless of the type of fat. However, rats fed coconut oil showed significantly greater haemoglobin (Hb), ΔHb and liver iron stores, compared to safflower oil (Johnson et al. 1987). The effect of a high fat diet on non-haem iron only showed increased liver iron, but not Hb or ΔHb as compared to a low fat diet; however, with haem iron, all three these indices increased significantly with high fat.

It has been shown that high fat diets fed to rats favoured non-haem iron absorption and iron absorption was greater from diets containing more saturated fats (Bowering et al. 1977). It has been proposed that the effect of fatty acids on iron absorption may be either exerted directly on iron absorption or indirectly through alteration of the fatty acid composition of the intestinal brush border membrane (Pabón and Lönnerdal 2001). Dietary fat composition has been shown to have an effect on the membrane composition of different types of cells (Pabón and Lönnerdal 2001) and individual fatty acids have been shown to affect iron absorption by brush border vesicles (Chang and Chen 1992).

Pabón and Lönnerdal (2001) fed rat pups with human milk, defatted human milk (DHM), DHM + 4% coconut oil, maize oil, soy oil, or olive oil to study the iron bioavailability as affected by oil. They found that pups dosed with a diet high in saturated fat (DHM + coconut oil), had higher iron absorption than those dosed with human milk or DHM + soybean oil. They thus, found a positive effect of fatty acid saturation on iron absorption. These findings support the hypothesis that the effects of fatty acids on iron absorption are mediated through changes in the fatty acid composition of the brush border membrane, which in turn modifies

the absorption capacity of the membrane. This would suggest that fatty acid saturation should also have a positive effect on zinc and other mineral absorptions.

2.7.7 Other minerals

Calcium

Calcium is known to have an absorption depressing effect on iron, as demonstrated in single-meal, as well as short-term diet intervention studies (Hallberg et al. 1991). The inhibitory effect of calcium on iron absorption has been found to be dose related. Hallberg et al. (1991) found that doses of 300–600 mg calcium added to wheat rolls, reduced iron absorption in human subjects by 50–60%. A calcium dose of 165 mg added to hamburgers, also showed a significant reduction in haem iron absorption. It has been found that the effect of calcium is related to the mucosal transfer of iron. Barton et al. (1983) studied the site of calcium effect on iron absorption in rats. It was found that animals given iron/calcium solution had 78 intravillous deposits per 1000 microvilli, whereas mid duodenal microvilli exposed to iron alone had 395 intravillous stain deposits per 1000 microvilli. Thus, calcium affects iron absorption at the mucosal site of iron transfer.

Calcium in itself appears unlikely to have a negative effect on zinc absorption (Lönnerdal 2000). When calcium was added to cow's milk formula to a level of 1300 mg/L, there was no significant difference in zinc absorption as compared to a formula with 500 mg/L calcium (Lönnerdal et al. 1984). Spencer et al. (1984) administered varying levels of calcium, up to 2000 mg, to adult men and found no significant change in urinary or faecal zinc excretions or zinc balance. Similarly, Dawson-Hughes et al. (1986) administered 500 mg elemental calcium with a test meal to postmenopausal women and found no significant difference in zinc retention; however, iron retention was significantly reduced. They concluded that iron absorption, but not zinc, may be significantly reduced when calcium supplements are taken with meals.

Yan et al. (1996) studied the effect of long-term supplementation with 1000 mg calcium, taken between meals, on indices of iron and zinc in women in Gambia. They found no deleterious effects on plasma ferritin or zinc concentrations in women who were at risk of iron and zinc deficiency.

However, the calcium levels of the diet may adversely affect iron and zinc bioavailability from phytate-containing meals (Lönnerdal 2000) as calcium has the tendency to co-precipitate with phytate and zinc, forming insoluble Ca_4Zn_2-phytate complexes, thus, rendering the mineral unavailable for absorption (Sandström 2001). Calcium-bound phytate has a higher affinity for zinc than phytate alone, thereby reducing the reabsorption of endogenous zinc, as well as decreasing the bioavailability of dietary zinc. The [Ca]:([phytate]/[zinc]) ratio formula has been devised as a predictor of zinc bioavailability (Fordyce et al. 1987).

Iron and zinc

The potential negative impact of iron on zinc absorption and status has caused concern. Many iron fortification and supplementation programmes have been implemented to improve iron nutrition. However, such programmes could possibly further exacerbate poor zinc status due to the negative impact of iron provision on zinc absorption and status (Lönnerdal 2000). It has been found that high doses of inorganic iron can reduce zinc bioavailability, as measured by changes in plasma zinc in a fasted state (Solomons and Jacob 1981). Human adults were administered 25 mg zinc (as $ZnSO_4$) in water solution, with 25, 50 or 75 mg of iron added. Plasma zinc absorption was significantly reduced when 25 mg iron was added, and the effect was magnified when 50 or 75 mg was added. However, Lönnerdal (2000) only found a significant reduction in zinc absorption in the fasting state when iron was added to zinc in a dose in solution at 25:1 molar ration, but not at 2.5:1 ratio. It was suggested that the iron and zinc interaction is much less pronounced when zinc intake is close to a physiological level. Lönnerdal (2000) also found that when high iron doses were given as part of a meal, no inhibitory effect on zinc absorption was found, even when the ratio between iron and zinc was 25:1. Davidsson et al. (1995) studied the effect of iron fortification of bread (65 mg/kg), weaning cereal (500 mg/kg) and infant formula (12 mg/L) in human adults and also demonstrated that iron has no significant negative effect on zinc absorption when given as fortification in food matrixes. This was also confirmed by Fairweather-Tait et al. (1995) WHO studied the effect of iron fortification of a weaning food on zinc absorption in infants. Lönnerdal (2000), thus, suggested that the effect of iron on zinc is exerted only at very high ratios of iron to zinc, as can only be achieved by supplementation, and only in water solution in fasting state.

2.8 Bioaccessibility of calcium and phosphorus

Bioaccessibility of calcium and phosphorus is important as these minerals are required by the human body to provide skeletal rigidity and are also involved in most metabolic processes (WHO/FAO 2004). The maintenance of the concentration of ionised calcium in extracellular fluid of the body is vital as many neuromuscular and other cellular functions are dependent on this. Several intracellular signalling pathways, such as hormonal effects on target organs, are also dependent on the calcium concentration in extracellular fluid (WHO/FAO 2004). Concerning phosphorus, it is the second most abundant element in the human body, of which approximately 85% is found in bones and teeth (NHMRC 2006). The remainder of phosphorus is distributed through soft tissues where it forms part of many important compounds, acts as an acid buffer in urine, protects blood systematic acid/base balance, activates catalytic proteins, and acts as a temporary energy store and transport mechanism.

2.9 Plant foodstuff candidates available in Africa which could increase iron and zinc bioavailabilities and provitamin A levels of pearl millet porridge

2.9.1 Moringa leaves

The moringa tree (*Moringa oleifera* Lam.*)* is considered as one of the most useful trees, as almost all the parts of the moringa leaves tree can be used for food and animal feed (Moyo et al. 2011). Leaves from the moringa leaves tree are rich in nutrients (Table 2.9.1), with a high protein content (33.5%, db). It is also rich in calcium (4.03%, db) potassium (1.66%, db), iron (54.1 mg/100 g, db), zinc (3.4 mg/100 g, db) and selenium (40.1 mg/100 g, db) (Moyo et al. 2011). Moringa leaves contain 17 fatty acids, of which alpha-linolenic acid had the highest value (49.25%, db). Vitamin E (85 mg/100 g, db) and beta-carotene (20.4 mg/100 g, db) are also present in the leaves.

2.9.2 Hibiscus calyces

The calyces of hibiscus (*Hibiscus subdariffa* L.*)* are commonly used to make tea, jellies, jams and other beverages and also for medicinal purposes (Borrás-Linares et al. 2015). The calyces

are rich in acid and pectin and also contain protein, fat, antioxidants, and minerals such as calcium, iron, zinc, and phosphorus (Table 2.9.1).

2.9.3 Baobab fruit pulp

Baobab (*Adansonia digitata* L.*)* trees are indigenous to sub-Saharan Africa and are tolerant to high temperatures and drought (Osman 2004). They are used mainly for their fruit and leaves. The fruit pulp is mainly used in beverages and food preparation, it is an excellent source of carbohydrates (85.0%, db), but low in protein and fat (9.2% and 0.3%, db, respectively) (Table 2.9.1). The fruit pulp is high in potassium, sodium, calcium and magnesium content (1384, 31, 329, and 100 mg/100 g, respectively), but low in iron, zinc and copper (10.4, 2.0, and 1.8 mg/100 g, db, respectively).

2.9.4 Mango-carrot premix (plus sunflower oil), as a provitamin A source

Mango (*Mangifera indica* L.) is a highly important commercial crop in tropical regions (Rocha Ribeiro et al. 2007). Mangoes are a good source (Table 2.9.1) of ascorbic acid, carotenoids and phenolic compounds. Mango has high carotene content, with beta-carotene being the most abundant carotenoid. Bhaskarachary (1995) reported 13.0 mg/100 g, db, total carotene with 10.1 mg/100 g, db, being beta-carotene.

Carrots (*Daucus carota* L.) are one of the most consumed plants in the world and is a major single source of provitamin A (Table 2.9.1), providing 14–17% of total vitamin A consumption (Nicolle et al. 2004). Alpha- and beta-carotene are the main pigments of orange carrots and levels of 14.3 and 34.4 mg/100 g, db, respectively, have been reported.

Sunflower oil may increase the bioavailability of provitamin A through the incorporation of provitamin A into micelles (van Het Hof et al. 2000). Formation of micelles is dependent on the presence of fat in the intestine which makes the ingestion of dietary fat along with provitamin A crucial.

Table 2.9.1: Literature values for mineral content, enhancers, and inhibitors in the micronutrient-rich plant foodstuffs, as compared to pearl millet

Foodstuff:	**Moringa leaves**	**Hibiscus calyces**	**Baobab fruit pulp**	**Mango**	**Carrot**	**Whole pearl millet**
Iron (mg/100g db)	24.42 ± 13.63[a] (4.10–49.00) 3,9,10,15,19,42,14	23.2 ± 15.32 (3.22–37.80) 1,2,4,5,14,16	8.05 ± 8.76 (1.00–29.20) 6,8,12,21,24	1.00* 40	3.56 ± 1.63 (2.04–6.58) 35,36,37,38,39,40	6.00 ± 2.40 (3.00–9.60) 41
Zinc (mg/100g db)	2.82 ± 0.46 (2.18–3.24) 3,9,15,14	6.11 ± 4.39 (1.17–12.22) 1,2,4,5,16	4.78 ± 10.18 (0.47–31.80) 6,12,21,24	0.54* 40	2.06 ± 0.46 (1.43–2.73) 35,36,37,38,39,40	3.70 ± 0.67 (2.40–4.83) 41
Calcium (mg/100g db)	2014 ± 1012 (346–3650) 3,9,15,19,42,14	574 ± 697 (3–1602) 3,9,10,15,19	535 ± 586 (3–2160) 6,8,12,21,24	66* 40	225 ± 44 (190–301) 35,36,37,38,39,40	8 ± 3 (4–14) 41
Ascorbic acid (mg/100 g, db)	856 ± 426 (48–1667) 9,22,23,42	57 ± 46 (12–140) 1,2,4,14,16	332 ± 166 (142–572) 6,8,18,24	107 ± 108 (7–418) 29,1	59 ± 49 (0–167) 35,36,37,38,39,40	Not reported 23 ± 1 (21–23) 26, 27
Oxalic acid (mg/100 g, db)	1067 ± 938 (404–1730) 19	289 ± 250 (6–480) 2	5 ± 7 (0–10) 6,25	Not reported	Not reported	
Phytate (mg/100 g, db)	1360 ± 1279 (255–3100) 9,13,20,14	715 ± 1238 (0–2144) 2,16,17	690* 25	49.17 ± 78.66 (3.5–140) 30,31,32	6 ± 6 (0–14) 32	1136 ± 207 (830–1395) 41,43
Total phenolics (mg/100 g, db GAE or TAE)	10616 ± 13967 (2020–45810) 13,15,20,22,23,42	5759 ± 2388 (2400–10000) 10	3527 ± 12 (3500–3536) 11	202 ± 144 (54–617) 1	392 ± 470 (60–1690) 33,34	495 ± 219 (147.8–790) 26,27,43,44

Foodstuff:	Moringa leaves	Hibiscus calyces	Baobab fruit pulp	Mango	Carrot	Whole pearl millet
Condensed tannins (mg CE/100 g, db)	1.67 ± 2.05 (0.22–3.12) [9,13,15,20]	2.43 ± 2.68 (0.00–5.30) [2,16,17]	0.74 ± 1.28 (0.01–2.22) [6,25]	0* [32]	6.50 ± 9.19 (0.00–13.00) [32]	None detected
B-carotene (mg/100 g, db)	33.98 ± 19.3 (10.01–61.9) [42,14]	Not reported	Not reported	54.62 ± 43.88 (14.53–184.06) [1]	64.4 ± 34 (3.3–91.7) [35,36,37,38,39,40]	None detected

[a]Values are reported as mean ± standard deviation (minimum–maximum)

*Only one value found in literature

[1](Manthey and Penelope 2009), [2](Adanlawo and Ajibade 2006), [3](Anjorin et al. 2010), [4](Lestienne et al. 2005), [5](Carvajal-Zarrabal et al., 2012), [6](Chadare et al., 2008), [7](Christian and Jackson, 2009), [8](Gebauer et al. 2002), [9](Gidamis et al., 2003), [10](Borrás-Linares et al. 2015), [11](Lamien-Meda et al., 2008), [12](Magaia et al., 2013), [13](Makkar and Becker, 1996), [14](Leone et al. 2015), [15](Moyo et al., 2011), [16](Nnam and Onyeke, 2003), [17](Ojokoh, 2006), [18](Parkouda et al., 2012), [19](Price, 2007), [20](Richter et al., 2003), [21](Sena et al., 1998), [22](Siddhuraju and Becker, 2003), [23](Sreelatha and Padma, 2009), [24](Stadlmayr et al., 2013), [25](Umaru et al., 2007), [26](Ravindran 1991), [27](Ragaee et al. 2006), [28](Hess et al. 2005), [29](Bajaj and Kaur 1981), [30](Ferguson et al. 1988), [31](Nitithan et al. 2004), [32](Hallberg and Hulthén 2000), [33](Nicolle et al. 2004), [34](Stratil et al. 2006), [35](Frontela et al. 2008), [36](Dost and Tokul 2006), [37](Puupponen-Pimiä et al. 2003), [38](Zhou and Yu 2006), [39](Patras et al. 2009), [40](USDA Nutrient Database 2015), [41](Gwamba 2016), [42](Yang et al. 2006), [43](Saharan 2015), [44](Jukanti et al. 2016)

2.10 Conclusions

The most critical and widespread micronutrient deficiencies are iron, zinc and vitamin A. Suboptimal iron, zinc and vitamin A status may be caused by an inadequate dietary intake of the nutrients and/or low bioavailability. It is of utmost importance that economically feasible approaches to alleviate these deficiencies be addressed as even mild to moderate micronutrient malnutrition, although often not apparently visible in those affected by it, has detrimental and lifelong consequences for health. Most populations in sub-Saharan Africa are dependent on cereals as their major source of nutrition, however, cereals have poor bioavailable non-haem iron and zinc due to inhibitory factors such as phytate, phenolic compounds and tannins. Cereal-based staple foods could possibly be improved by combining locally available plant foodstuffs which complement each other in such a way as to provide improved levels and bioavailabilities of iron, zinc and provitamin A. Food-to-food fortification of cereal-based foods with micronutrient-rich plant foodstuffs should be investigated as this may increase the levels of iron, zinc and provitamin A in the cereal-based foods while simultaneously reducing the inhibitory effects of the antinutrients found in cereals. This may be one of the best ways to provide nutritious diets to the sustainability of populations in sub-Sahran Africa and may lead to improved iron, zinc and vitamin A statuses. Micronutrient-rich foodstuffs, such as moringa leaves, hibiscus calyces, mango fruit pulp, carrots and sunflower oil could possibly be used as natural fortificants to increase the iron, zinc and provitamin A bioavailabilities from cereal-based foods.

Chapter 3: Hypotheses and objectives

3.1 Hypotheses

3.1.1 Hypothesis 1

The addition of mango-carrot premix (plus sunflower oil) (to aid micellisation) (van Het Hof et al. 2000), as a provitamin A source, to pearl millet porridge will increase the iron and zinc bioaccessibilities. It has been found that the addition of beta-carotene to cereal-based meals (sorghum, rice, wheat, maize), increased iron and zinc bioaccessibilities (Gautam et al. 2010) and iron absorption in adults (García-Casal et al. 1998). Provitamin A carotenoids such as beta-carotene, lycopene, and lutein have an enhancing effect on nonhaem iron absorption as soluble complexes with iron in the intestinal tract are formed (García-Casal 2006).

3.1.2 Hypothesis 2

The addition of micronutrient-rich plant foodstuffs to pearl millet porridge will improve iron and zinc bioaccessibilities in increasing order moringa leaves < baobab fruit pulp < hibiscus calyces. This is because moringa leaves, although rich in iron and zinc, contains high levels of calcium, total phenolics and phytate (Negesse et al. 2009). With the exception of tannins, baobab fruit pulp has the lowest levels of iron and zinc bioavailability inhibitors, and has the highest levels of organic acids, but is very low in iron and zinc (Magaia et al. 2013). Hibiscus calyces are rich in iron and zinc, and contains less calcium, total phenolics and phytate than moringa leaves, and also contains organic acids (Ojokoh 2006).

Calcium has an absorption depressing effect on iron, but seemingly not on zinc (Hallberg et al. 1991). Phytate is a potent inhibitor of iron and zinc absorption (Gibson 2006) as it forms strong, insoluble iron-phytate or zinc-phytate complexes in the intestinal tract (Lönnerdal 2000), rendering the minerals unavailable for absorption. Phenolics chelates metal (Khokhar and Apenten 2003) and are thus able to interact with iron and zinc, to form insoluble complexes, unavailable for absorption (Siegenberg et al. 1991). Tannins can form complexes with iron and zinc, which render the minerals unavailable for absorption (Khokhar and Apenten 2003).

Some organic acids, such as citric and acetic acids, are able to form soluble ligands with iron and zinc in the gastrointestinal tract, which easily break down in the small intestine, thus, releasing bioavailable iron and zinc for absorption (Pabón and Lönnerdal 1993). Some organic acids, such as malic, citric and ascorbic acids, prevent the formation of insoluble mineral-phytate complexes, thus, overcoming phytate inhibition of iron and zinc bioavailabilities (Siegenberg et al. 1991).

3.2 Objectives

3.2.1 Objective 1

To evaluate the effect of adding a mango-carrot premix (plus sunflower oil), as a provitamin A source, to a pearl millet porridge, on the bioaccessibility of iron and zinc as measured by the *in vitro* dialysability assay. The purpose of this study is to combat vitamin A, iron and zinc deficiencies in malnourished African populations by using plant foodstuffs as fortificants, through a food-to-food fortified cereal-based porridge that delivers provitamin A and bioavailable forms of iron and zinc.

3.2.2 Objective 2

To evaluate the effects of adding varying concentrations and combinations of micronutrient-rich plant foodstuffs (moringa leaves, hibiscus calyces, and baobab fruit pulp) on the iron and zinc bioaccessibilities (*in vitro* dialysability assay) of a pearl millet porridge. The purpose of this study is to combat iron and zinc deficiencies in malnourished African populations by using plant foodstuffs as fortificants, through a food-to-food fortified cereal-based porridge that delivers bioavailable forms of iron and zinc.

Chapter 4: The effect of addition of micronutrient-rich plant foodstuffs on the iron and zinc contents and bioaccessibilities of pearl millet porridge

4.1 Abstract

Sub-Saharan Africa has alarmingly high rates of micronutrient malnutrition, partly due to monotonous cereal-based diets which are high in iron and zinc bioavailability inhibitors. The effect of micronutrient-rich plant foodstuffs (moringa leaves, hibiscus calyces, and baobab fruit pulp) on the mineral bioaccessibilities of pearl millet porridge was evaluated. The plant foodstuffs were analysed for mineral (Ca, Fe, Zn, P), and antinutrient contents. The effects of adding 30 g/100 g pearl millet, db, of mango-carrot premix (plus 5 g/100 g pearl millet, db, sunflower oil) as a provitamin A source, plus 5 or 15 g/100 g pearl millet, db, of moringa leaves, hibiscus calyces or baobab fruit pulp to whole pearl millet porridges on iron and zinc bioaccessibilities (*in vitro* dialysability assay) were determined.

Addition of the mango-carrot premix (plus sunflower oil), as a provitamin A source, to pearl millet porridge increased the bioaccessible iron and zinc. This is because provitamin A carotenoids have an enhancing effect on nonhaem iron and zinc absorption as the carotenoids are able to form soluble complexes with these minerals in the intestinal tract.

The addition of hibiscus calyces and baobab fruit pulp to pearl millet porridges increased the iron and zinc bioaccessibilities, and even more so at increased levels of the foodstuffs. Baobab fruit pulp caused the greatest increases in iron and zinc bioaccessibilities. These increases were probably due to high levels of organic acids found in hibiscus calyces and baobab fruit pulp as most organic acids are able to form soluble complexes with iron and zinc which renders the minerals bioaccessible. The addition of moringa leaves to pearl millet porridge increased, and in some cases, decreased the iron and zinc bioaccessibilities. Moringa leaves, although it had the highest iron content of all the plant foodstuffs, had the highest levels of calcium and total phenolics as well as a substantial level of phytate. With such high levels of iron and zinc bioaccessibility inhibitors, the addition of moringa leaves at 15 g/100 g pearl millet, db, probably negatively affected the bioaccessibility of the iron and zinc contained in the pearl millet itself.

Dietary diversification by means of complementing pearl millet porridges with baobab fruit pulp, possibly hibiscus calyces, but not moringa leaves, increases the iron and zinc bioaccessibilities, as an indication of bioavailability, thus possibly leading to improved iron and zinc statuses of malnourished populations in sub-Saharan Africa.

4.2 Introduction

Sub-Saharan Africa has the highest hidden Hunger rates in the world, with 18 of the 20 countries with the highest micronutrient malnutrition scores in the world (Muthayya et al. 2013). Women of reproductive age, young children and the elderly are most susceptible to micronutrient malnutrition (Bain et al. 2013). Some of the most critical and widespread micronutrient deficiencies are of iron, zinc and vitamin A (WHO/FAO 2006). Micronutrient malnutrition leads to impaired mental and physical development, reduced work productivity, increased risk for perinatal complications and morbidity from infectious disease and mortality (Muthayya et al. 2013). Of greatest concern is the detrimental consequences of micronutrient malnutrition extending across generations (Bailey et al. 2015).

Monotonous plant-based diets, which are predominantly cereal-based, form the staple diet of a great proportion of the African population (WHO 2014). Cereal foods are low in iron and zinc bioavailability enhancers and high in inhibitors. Thus, although cereals may contain adequate amounts of these minerals to meet daily requirements, the bioavailabilities are low (Zimmermann and Hurrell 2007). Often less than 5% of iron, and less than 10% of zinc is bioavailable for absorption (WHO/FAO 2006).

Pearl millet grain is a major food staple for millions of people in sub-Saharan Africa and accounts for approximately 10% of the total cereal production in Africa (FAOSTAT 2017). In some countries of sub-Saharan Africa, millets, and in particular pearl millet, can form more than 50% of the total cereal production. Millet foods are consumed in sub-Saharan Africa as thick or soft porridges or gruels (Svanberg et al. 1993). Such porridges serve as meals for the whole family and usually the first complementary foods introduced to infants as weaning foods in most of Africa, from as early as three months (Solomons 1999).

Phenolics, condensed tannins, phytate, calcium and organic acids are modifiers of iron and zinc bioavailabilities which are often present in cereals and other plant-based foods. Certain types of food phenolics, such as phenolic acids and flavonoids, strongly inhibit non-haem iron absorption (Hurrell et al. 1999) and possibly zinc absorption (Greger and Lyle 1988). Condensed tannins are known to negatively affect iron absorption (Khokhar and Apenten 2003). However, few studies have documented their effect on zinc bioavailability (Afsana et al. 2004). Phytate is a very strong inhibitor of both iron and zinc absorption and is a contributory factor to iron and zinc deficiencies (Reale et al. 2007). Calcium on its own has

an absorption depressing effect on iron, but seems to have no negative effect on zinc absorption (Lönnerdal 2000). Calcium has a tendency to co-precipitate with phytate and zinc which renders the mineral unavailable for absorption, even more so than just phytate (Sandström 2001).

Combining locally available plant foodstuffs, high in iron and zinc and bioavailability enhancers, and low in bioavailability inhibitors, with cereal-based porridges has been advocated as a means of increasing iron and zinc contents as well as bioavailabilities (Onofiok and Nnanyelugo 1998). Pearl millet porridge could serve as a vehicle to which plant foodstuffs can be added in order to improve micronutrient levels and bioavailability. Moringa leaves, hibiscus calyces and baobab fruit pulp were selected as micronutrient-rich plant foodstuffs based on their production and consumption in Western Africa and their high iron and/or zinc contents. A mango-carrot premix (49% carrot and 51% mango) (plus sunflower oil), as a provitamin A source, was used to address underlying vitamin A deficiency. The food-to-food fortified cereal-based porridges would be served as a complete meal, addressing the three most critical micronutrient deficiencies (iron, zinc and vitamin A), thus the base porridge would consist of the cereal plus provitamin A source.

Dietary interventions to address vitamin A deficiency have been relatively established; however, research on how iron and zinc malnutrition can be addressed through dietary diversification and inclusion of specific foods to a cereal-based diet is still lacking. The purpose of this study was to evaluate the impact of micronutrient-rich plant foodstuffs on the mineral nutritive value of pearl millet porridge.

4.3 Materials and methods

Characterisation of the plant foodstuffs included determination of the mineral (iron, zinc, calcium and phosphorus), tannin, total phenolic, and phytate contents (Figure 4.3-1). This was followed by preparing various porridge formulations, which were then analysed for iron, zinc, calcium and phosphorus bioaccessibilities by the *in vitro* dialysability assay.

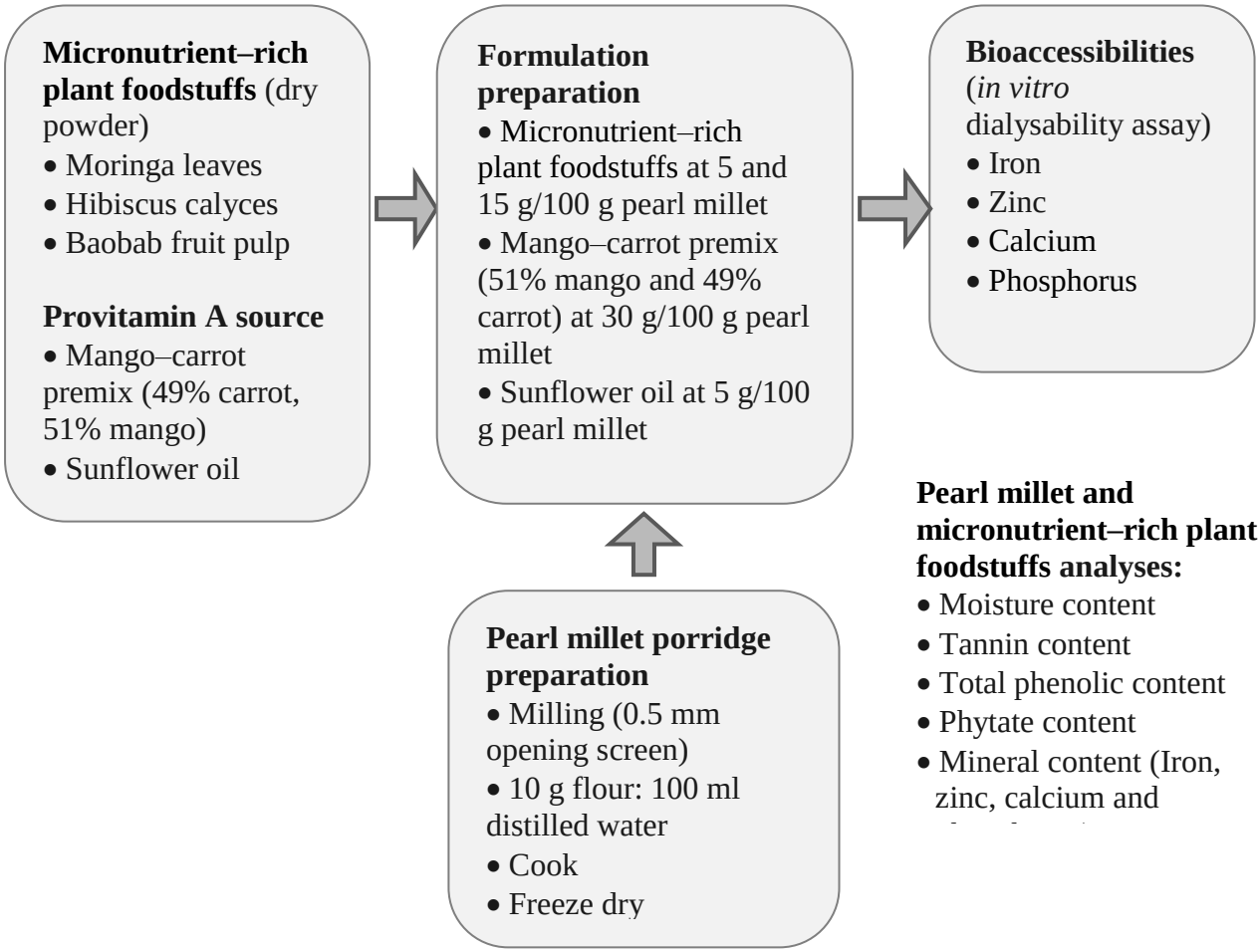

Figure 4.3-1: Experimental design–Plant Foodstuffs characterisation and porridge preparation with various amounts and combinations of micronutrient-rich plant foodstuffs (5 and 15 g/100 g pearl millet, db) and mango-carrot premix (30 g/100 g pearl millet, db) (plus sunflower oil) (5 g/100 g pearl millet, db)

4.3.1 Materials

Whole pearl millet, variety Kuphanjala-2, was obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Bulawayo, Zimbabwe. Commercially available dried moringa leaves, hibiscus calyces, and baobab fruit pulp were obtained as

micronutrient-rich plant foodstuffs. The plant foodstuffs were obtained from two suppliers: Maria Production (Dakar, Senegal) and Free Work Service (Dakar, Senegal). Four samples of a mango-carrot premix, comprising freeze-dried mango (51%) and carrot (49%), was supplied by Purdue University.

4.3.2 Porridge ingredient preparation

Whole millet grains were rubbed between gloved hands to remove glumes. The grain was further cleaned by sieving to remove foreign matter. Clean grain was quickly rinsed with distilled water, to remove dust contamination, and allowed to air dry at 24˚C for 24 hours. The grain was milled using a laboratory hammer mill (Falling Number 3100, Perten, Huddinge, Sweden) fitted with a 0.5 mm opening screen. All flour samples were stored at 10°C prior to porridge preparation and analyses.

The dry moringa leaves, hibiscus calyces and baobab fruit pulp was milled with an air cooled knife-type laboratory mill (A11 basic analytical mill, IKA®, Staufen, Germany) and passed through a 0.5 mm opening stainless steel screen. The resulting powders were stored at 10°C in double sealed, airtight plastic bags. The mango-carrot premixes were stored at -20°C in vacuum sealed plastic bags and wrapped in foil.

4.3.3 Pearl millet porridge preparation

Distilled water was added to pearl millet flour in a ratio of 1:10, flour: water (w/w). The mixture was heated to 100˚C and maintained with constant stirring for 15 min. The mixture was left to cool at ambient temperature, after which it was placed in plastic containers and frozen to -20˚C and freeze dried. Freeze dried porridge flour was crushed to a particle size that passed through a 0.5 mm opening screen before further analyses. The pre-cooked porridge flour was stored at 10°C in double sealed, airtight plastic bags.

4.3.4 Moisture

Moisture content was determined by an air oven, one stage, drying procedure, method 44-15A (AACC International, 2000). This method is based on gravimetric determination of

moisture contents, by calculating loss in moisture as the percentage of the original weight of the sample.

4.3.5 Mineral contents

Mineral contents (iron, zinc, calcium and phosphorus) were quantified using approved methods of the AOAC International (2000). Samples of accurate weight (0.500 g) were digested using a combination of 65% nitric acid and 70% perchloric acid (5:2, v/v) at 2400˚C (AOAC method 935.13). Once digested, the samples were diluted to 50 mL in a volumetric flask, with deionised water. A GBC 905 atomic absorption spectrometer (Braeside, Australia) was used to quantify iron and zinc, according to AOAC method 999.10.

Calcium content was determined according to AOAC method 935.13. A solution of 1% lanthanum chloride heptahydrate and anhydrous nitric acid was used to inhibit the interaction of other elements with calcium (Giron, 1973). A Perkin-Elmer, 5100 atomic absorption spectrometer (Walluf, Germany) was used.

Phosphorus content was measured colorimetrically (400 nm) according to AOAC method 965.17. The reaction of ammonium molybdate tetrahydrate and ammonium vanadate with phosphorus in the sample develops a yellow colour which is measured against a phosphorus standard.

4.3.6 Total phenolic contents

A modified Folin-Ciocalteu method, as described by Waterman and Mole (1994), was used to measure total phenolic contents. Extractions were prepared by using acidified methanol (1% HCl in methanol). Absorbance of a reagent blank was included in the calculation as the modification. This method is based on the reduction of the Folin-Ciocalteu phenol reagent (phosphomolybdic-phosphotungstic acid), in the presence of phenolic compounds, to a blue colour in alkaline solution. Maximum absorption of the blue molybdotungstophosphate pigments are at alkaline pH (Cicco et al. 2009). Total phenolic content was reported as mg catechin equivalents per gram (mg CE/g) sample, dry weight basis.

4.3.7 Condensed tannin contents

A modified Vanillin HCl method was used to measure condensed tannins, catechin equivalent, as described by Maxson and Rooney (1972). Extractions were prepared by using acidified methanol (1% HCl in methanol). Absorbance of a reagent blank was included in the calculation as the modification. The method is based on the reaction of vanillin with condensed tannins, which results in the formation of coloured complexes (Schofield et al. 2001). Colour blanks were prepared by reacting sample extract with 4% concentrated HCl in methanol (v/v) instead of vanillin reagent.

4.3.8 Phytate contents

Phytate content was determined by indirect quantitative analysis of phytate through anion exchange chromatography, as described by Frühbeck et al. (1995). Glass barrel Econo-columns, 0.7 x 5 cm and Dowex 1-anion-exchange resin-AG 1 x 4 (4% Cross-linkage, chloride form, 100–200 mesh) was used for purification. Samples were treated with Wade reagent after which absorbance was measured. Sodium phytate solutions were used to produce a standard curve.

4.3.9 Porridge formulations

The formulations of the pearl millet porridge consisted of up to 15 g/100 g pearl millet, db, of micronutrient-rich plant foodstuffs (moringa leaves, hibiscus calyces, and/or baobab fruit pulp), plus 30 g/100 g pearl millet, db, of mango-carrot premix (plus 5 g/100 g pearl millet, db, of sunflower oil), as a provitamin A source. Based on this, porridge formulations (Table 4.3.1) were formulated with varying concentrations (5 and 15 g/100 g pearl millet, db) and combinations of the micronutrient-rich plant foodstuffs, with or without the provitamin A source. Deionised water was used as filler for formulations which contained less than 15 g/100 g pearl millet, db, of micronutrient-rich plant foodstuff, in order to keep the provitamin A and pearl millet contents constant. Thus, in the experimental design, the micronutrient-rich plant foodstuffs were added as additional nutrient sources to the pearl millet, and did not act as a replacement or to reduce the cereal nutrient contributions.

Table 4.3.1: Porridge formulations with dried moringa leaves (M), hibiscus calyces (H) and baobab fruit pulp (B) alone and in combination with freeze dried pearl millet (PM) porridge plus provitamin A source

Formulation	**Composition** (g/100 g, db)						
	Dried moringa leaves	**Dried hibiscus calyces**	**Dried baobab fruit pulp**	**Precooked millet flour**	**Dried Mango-carrot premix**	**Sunflower oil**	**Deionised water**
PM + provit A source	0	0	0	50	30	5	15
PM alone	0	0	0	50	0	0	50
PM + provit A source + 5M + 5H + 5B	5	5	5	50	30	5	0
PM + 5M + 5H + 5B	5	5	5	50	0	0	35
PM + provit A source + 5M	5	0	0	50	30	5	10
PM + provit A source + 15M	15	0	0	50	30	5	0
PM + provit A source + 5H	0	5	0	50	30	5	10
PM + provit A source + 15H	0	15	0	50	30	5	0
PM + provit A source + 5B	0	0	5	50	30	5	10
PM + provit A source + 15B	0	0	15	50	30	5	0
PM + provit A source + 5M + 5H	5	5	0	50	30	5	5
PM + provit A source + 5M + 5B	5	0	5	50	30	5	5
PM + provit A source + 5H + 5B	0	5	5	50	30	5	5

4.3.10 Phytate:mineral molar ratios of porridge formulations

The mineral and phytate levels of each porridge formulation were calculated from the mineral and phytate levels of each ingredient. Phytate:mineral molar ratios were also calculated for each of the formulations, to predict mineral bioavailability.

4.3.11 Mineral bioaccessibility by the *in vitro* dialysability assay

The porridge formulations (Table 4.3.1) were subject to *in vitro* digestion to simulate human gastric and intestinal digestion. Mineral bioaccessibilities (iron, zinc, calcium and phosphorus) were determined according to the dialysis method of Miller et al. (1981). Digestive enzymes and bile salts used were pepsin (P-7000), pancreatin (P-1750), and bile extract (B-8631) (Sigma-Aldrich, Johannesburg, South Africa). Dialysis tubing used was Spectra/Por 7 (Ø = 20.4 mm) with a molecular weight cut-off (MWCO) of 10 kDa (G.I.C. Scientific, Johannesburg, South Africa).

Gastric stage

Triplicates of each formulation (6 g) were mixed with 40 g deionised water in a 250 ml Erlenmeyer flask. The pH was adjusted to pH 2.0 with 6 M HCl. After 10 minutes equilibration time, the pH was checked and, if necessary, readjusted to 2.0. Freshly prepared pepsin solution (16 g pepsin in 100 ml 0.1 M HCl) was added (1.8 g) and the mixture made up to 60 g with deionised water. Samples were incubated in a shaking water bath (150 rpm, circular arm movement of 2 cm) at 37˚C for 120 min. The gastric digests were cooled to 0˚C while titratable acidity was measured.

Titratable acidity

A homogeneous aliquot of the gastric digest (12 g) was heated to 20˚C and mixed with 3 g freshly prepared pancreatic mixture (4 g pancreatin, 25 g bile extract, mixed in 1 litre 0.1 M $NaHCO_3$). The pH was adjusted to pH 7.5 with 0.5 M NaOH, and if necessary, readjusted after an equilibration period of 30 minutes. Titratable acidity was defined as the amount of 0.5 M NaOH required to attain a pH of pH 7.5, when titrated against 12 g of the gastric digest plus 3 g pancreatic mixture.

Intestinal stage

Homogenised gastric digest aliquots (12 g) were weighed into 250 ml wide-necked Erlenmeyer flasks, in triplicate, and placed in a water bath at 37˚C for 5 minutes. Segments of dialysis tubing (15 cm from clamp to clamp) containing 15 g $NaHCO_3$ solution were then added, $NaHCO_3$ concentration being equivalent in moles NaOH titre. The segments of dialysis tubing were immediately added into the Erlenmeyer flasks, which were closed with parafilm to prevent CO_2 loss. Flasks were in the water bath for 30 minutes, after which 3 g of the pancreatic mixture was added. The digests were incubated in the shaking water bath for two hours at 37˚C. The dialysis bags were rinsed with deionised water, and the levels of each bag transferred into 15 ml centrifuge tubes. Dialysates were acidified with 0.45 ml HNO_3 (65%) to keep minerals in suspension. Tubes were made up to 15 ml with deionised water and frozen till analysis.

Dialysate mineral analysis

The mineral contents (iron, zinc, calcium, and phosphorus) of the dialysates were determined in duplicate by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) according to method 200.7 of the United States Environmental Protection Agency (U.S. EPA. 1994). The dialysate mineral analyses were carried out at Central Analytical Facilities (CAF), Stellenbosch University. Characteristic atomic-line emission spectra were measured by optical spectrometry and a background correction technique was used to compensate for variable background contribution to the determination of the analytes.

Bioaccessible minerals were expressed as the percentage of the mineral in the dialysate relative to the total mineral contents in the digests.

$$\%\ \text{bioaccessible mineral} = \frac{\text{Dialysable mineral (µg)}}{\text{total mineral (µg)}} \times 100$$

The amount of bioaccessible mineral was calculated based on the mineral levels of the porridges and % bioaccessible mineral.

4.3.12 Statistical analysis

Values are presented per 100 g of dry matter. Bioaccessibility values were calculated per 100 g, as consumed and data are presented as means ± 1 standard deviation (SD). The data analysed using a one-way analysis of variance (ANOVA) with XLSTAT software, using Fisher's least significant difference (LSD) test to separate means, with confidence level for significant differences at $p \leq 0.05$. Outliers of bioaccessibility results were identified using Grubbs test for outliers with XLSTAT software and not included in mean calculations.

4.4 Results and discussion

4.4.1 Mineral contents

The iron levels of moringa leaves were significantly ($p \leq 0.05$); however, not substantially, different between suppliers (Table 4.4.1). The iron levels of hibiscus calyces from Free Work Service was approximately double ($p \leq 0.05$) that from Maria Production. Also, the iron levels of baobab fruit pulp from Maria Production was approximately ten times ($p \leq 0.05$) that from Free Work Service. Variation in nutrient levels of plants from the same species can be due to genetic variation within species, variation in soil nutrients, and due to post-harvesting storage and treatments (Vreugdenhil et al. 2005). Iron is the second most abundant mineral on Earth (Reilly 2008), thus soil particulate inclusion during the crop's growth, would lead to increased iron content. The mean iron levels of moringa leaves (58.4 mg/100 g, db) and hibiscus calyces (47.1 mg/100 g, db) were the highest of the plant foodstuffs, and were the only plant foodstuffs with higher ($p \leq 0.05$) iron contents than pearl millet (7.5 mg/100 g, db). Moringa leaves had a rather higher iron content than the maximum reported in literature (49.0 mg/100 g, db) (Moyo et al. 2011).

None of the zinc levels of the plant foodstuffs differed significantly ($p > 0.05$) between suppliers (Table 4.4.1). The zinc levels of all the plant foodstuffs, apart from hibiscus calyces (2.70 mg/100 g, db), were lower than ($p \leq 0.05$) the zinc levels of the pearl millet sample (2.95 mg/100 g, db). The zinc levels of all the plant foodstuffs were within ranges reported in literature (Table 2.9.1).

Table 4.4.1: Iron (Fe), zinc (Zn), calcium (Ca), and phosphorus (P) contents (mg/100 g, db) of dried moringa leaves, hibiscus calyces, baobab fruit pulp, mango-carrot premix, and pearl millet

	Mineral contents (mg/100 g, db[2] ± SD)			
Plant foodstuffs	**Fe**	**Zn**	**Ca**	**P**
Moringa leaves *(MP[3]; FWS[4])*	$58.4 \pm 4.9^{A\,1}$ *(61.9 ± 3.1^{a}; 54.9 ± 1.1^{b})*	2.28 ± 0.15^{B} *(2.17 ± 0.15^{d}; 2.38 ± 0.00^{cd})*	2115 ± 56^{A} *(2075 ± 77^{b}; 2154 ± 43^{a})*	284 ± 1^{B} *(285 ± 1^{b}; 283 ± 1^{b})*
Hibiscus calyces *(MP; FWS)*	47.1 ± 19.0^{A} (33.7 ± 0.4^{c}; 60.6 ± 0.0^{a})	2.70 ± 0.14^{A} *(2.60 ± 0.16^{bc}; 2.80 ± 0.24^{ab})*	970 ± 22^{B} *(985 ± 33^{c}; 955 ± 24^{c})*	163 ± 43^{C} *(132 ± 0^{e}; 193 ± 2^{c})*
Baobab fruit pulp *(MP; FWS)*	13.8 ± 16.1^{B} *(25.2 ± 0.8^{d}; 2.4 ± 0.2^{g})*	1.24 ± 0.12^{C} *(1.15 ± 0.08^{ef}; 1.33 ± 0.07^{e})*	273 ± 27^{C} *(292 ± 8^{d}; 254 ± 6^{d})*	53 ± 3^{E} *(55 ± 1^{f}; 51 ± 1^{f})*
Mango-carrot premix[5]	4.3 ± 0.3^{B}	1.23 ± 0.02^{C}	140 ± 1^{D}	138 ± 2^{D}
Pearl millet (Whole grain, raw)	7.5 ± 0.1^{B}	2.95 ± 0.08^{A}	7 ± 1^{E}	416 ± 10^{A}

[1]Mineral contents values are the mean ± 1 standard deviation of samples analysed in duplicate (n=2). Values followed by different letter superscripts differ significantly according to Fisher's LSD test ($p \leq 0.05$). Lower case letters indicate differences between suppliers of each plant foodstuff and upper case letters indicate differences between means of each foodstuff.
[2]db: dry weight basis
[3]MP: Maria Production
[4]FWS: Free Work Service
[5]Mango-carrot premix composed of freeze-dried mango (51%) and carrot (49%)

The calcium levels of all the plant foodstuffs, apart from moringa leaves, did not differ significantly ($p > 0.05$) between suppliers (Table 4.4.1). Compared to the pearl millet (7 mg/100 g, db), the calcium levels of the plant foodstuffs were approximately 300, 150, 40 and 20 times greater for moringa leaves, hibiscus calyces, baobab fruit pulp, and mango-carrot premixes, respectively. The calcium levels of pearl millet were lower than reported in literature. Ragaee et al. (2006) found 51 mg/100 g, db, and Léder (2004) found 28 mg/100 g, db. Calcium values for the other plant foodstuffs were within the ranges previously reported (Table 2.9.1).

Phosphorus levels of only hibiscus calyces, differed ($p \leq 0.05$) between suppliers (Table 4.4.1). Phosphorus levels of all the plant foodstuffs were below that in pearl millet (416 mg/100 g, db).

4.4.2 Antinutrients

The total phenolic levels of only hibiscus calyces differed ($p \leq 0.05$) between suppliers; however, not substantially (Table 4.4.2). The total phenolics in the moringa leaves, hibiscus calyces, and baobab fruit pulp fruit were approximately ten times higher than the total phenolics in the pearl millet. The total phenolic levels of all the plant foodstuffs were within ranges reported in literature (Table 2.9.1). Yang and Chang (2006) found the total phenolic contents in mature moringa leaves to be 3063 mg/100 g, db, which is somewhat lower than the 4655 mg/100 g, db found here. Borrás-Linares et al. (2015) reported the total phenolic levels of a collection of 25 hibiscus varieties with varying calyx colour intensities, from green-yellow to deep red. For the deep red varieties, as used in this study, they reported total phenolic contents to range from 3000 to 10000 mg GAE/100 g, db.

Even though there were no phenolics detected in the mango-carrot premix, literature values reported for raw carrots range from 60 to 1690 mg of gallic acid equivalents/100 g, db, (Table 2.9.1) and can vary greatly between different cultivars (Nicolle et al. 2004). Puupponen-Pimiä et al. (2003) reported a reduction in total phenolic levels of carrots after blanching, freezing and frozen storage. No total phenolic levels of mango on dry basis could be found in literature; however, total phenolic contents, fw, varied greatly, from 19 to over 300 mg/100 g, fw (Reddy et al. 2010). Hung and Duy (2012) investigated the effect of freeze-drying and heat-drying (55˚C) on the total phenolic levels of various deep-coloured vegetables, including carrots. They found that heat-drying significantly reduces the total phenolic contents; heat-dried beetroot had approximately 13 times less total phenolic contents than freeze-dried beetroot. It could be that the levels of phenolics in the mango-carrot premix powder had been drastically reduced as a result of drying.

Condensed tannins were only detected in baobab fruit pulp (2286 mg/100 g, db), and did not differ significantly ($p > 0.05$) between the suppliers (Table 4.4.2). The levels of condensed tannins in baobab fruit pulp was very similar to the 2220 mg/100 g, db, reported by Umaru et al. (2007).

Table 4.4.2: Total phenolics, tannin, and phytate levels of dried moringa leaves, hibiscus calyces, baobab fruit pulp, mango-carrot premix, and pearl millet on dry weight basis

Plant foodstuffs	**Total phenolic contents[1]**	**Condensed tannin contents[1]**	**Phytate contents[2]**
	(mg CE[4]/100 g ± SD)	(mg CE/100 g ± SD)	(mg/100 g ± SD)
Moringa leaves *(MP[7]; FWS[8])*	$4655 \pm 49^{A\,3}$ *(4689 ± 43[a]; 4620 ± 175[a])*	nd *(nd; nd)*	829 ± 23^{BC} *(844 ± 45[d]; 812 ± 28[d])*
Hibiscus calyces *(MP; FWS)*	3451 ± 289^{B} *(3247 ± 31[c]; 3655 ± 53[b])*	nd *(nd; nd)*	4833 ± 799^{A} *(5398 ± 360[a]; 4268 ± 150[b])*
Baobab fruit pulp *(MP; FWS)*	3738 ± 3^{B} *(3740 ± 166[b]; 3736 ± 75[b])*	2286 ± 328^{A} *(2054 ± 106[a]; 2518 ± 101[a])*	321 ± 26^{C} *(339 ± 15[e]; 302 ± 7[e])*
Mango-carrot premix	nd[6]	nd	290 ± 85^{C}
Pearl millet (whole grain, raw)	353 ± 13^{C}	nd	1360 ± 30^{B}

[1]Total phenolic and condensed tannin contents values are the mean ± 1 standard deviation of at least two samples analysed in triplicate (n=6).

[2]Phytate contents values are the mean ± 1 standard deviation of at least two samples analysed in quadruplicate (n=8)

[3]Means followed by different letter superscripts differ significantly according to Fisher's LSD test ($p \leq 0.05$). Lower case letters indicate differences between suppliers of each plant foodstuff and upper case letters indicate differences between means of each foodstuff.

[4]CE: Catechin equivalents

[5]db: dry weight basis

[6]nd: not detected

[7]MP: Maria Production

[8]FWS: Free Work Service

The phytate levels of only hibiscus calyces differed ($p \leq 0.05$) between the suppliers (Table 4.4.2). Hibiscus calyces were the only plant foodstuff to contain more ($p \leq 0.05$) phytate (4833 mg/100 g, db) than pearl millet (1360 mg/100 g, db). In fact, the phytate levels of hibiscus calyces was more than twice higher than the maximum value reported in literature (2144 mg/100 g, db) (Ojokoh 2006).

4.4.3 Mineral bioaccessibility as measured by the *in vitro* dialysability assay

Iron bioaccessibility

With the addition of the micronutrient-rich plant foodstuffs to the pearl millet plus provitamin A source porridge, the percentage bioaccessible iron increased ($p \leq 0.05$) by a minimum of 32% and a maximum of 129%. With regard to the amount of bioaccessible iron, increases ($p \leq 0.05$) from 33 to 241% were observed (Table 4.4.3). However, with the addition of 15 g/100 g pearl millet, db, of moringa leaves to pearl millet porridge, the percentage bioaccessible iron was reduced ($p \leq 0.05$) by 52%.

The porridge consisting of only pearl millet had very low percentage bioaccessible iron (1.30%) (Table 4.4.3), and because it also had the lowest iron contents (7.5 mg/100 g, db) (Table 4.4.1) of all the plant foodstuffs analysed, it had the lowest ($p \leq 0.05$) amount of bioaccessible iron (0.044 mg/100 g, db).

Addition of the mango-carrot premix at 30 g/100 g pearl millet, db, (plus sunflower oil at 5 g/100 g pearl millet, db), as a provitamin A source, to the pearl millet porridge alone (Table 4.4.3) increased ($p \leq 0.05$) the percentage bioaccessible iron by 37% and the amount of bioaccessible iron by 84%. When the provitamin A source was added to the pearl millet plus 5 g/100 g pearl millet, db, of each micronutrient-rich plant foodstuff (moringa leaves, hibiscus calyces and baobab fruit pulp) porridge, the percentage bioaccessible iron increased ($p \leq 0.05$) by 47% and the amount of bioaccessible iron increased ($p \leq 0.05$) by 67%.

It has been reported that provitamin A carotenoids (beta-carotene, lycopene, lutein, and zeaxanthin), have an enhancing effect on nonhaem iron absorption in both *in vitro* and human absorption studies (García-Casal 2006). These carotenoids were found to form a soluble complex with iron in the intestinal tract. Gautam et al. (2010) found that the addition of carrot as a source of beta-carotene to the cereals sorghum and rice, had positive effects on iron bioaccessibility, as measured by the dialysability assay. Carrot added at 25 and 50 g (roughly providing 2 and 4 mg beta-carotene) per 100 g of cereal, fw, resulted in 37 to 68% increased iron bioaccessibility. The positive effect of beta-carotene on iron bioaccessibility was confirmed by including pure beta-carotene (2 and 4 mg/100 g cereal, fw). Beta-carotene added at the 2 and 4 mg level resulted in increases from 22 to 71% and up to 102%, respectively in iron bioaccessibility.

Table 4.4.3: Effects of adding 5 and 15 g/100 g pearl millet plus provitamin A source, db, of dried moringa leaves (M), hibiscus calyces (H) and baobab fruit pulp (B) alone and in combination to freeze dried pearl millet (PM) porridge on iron and zinc bioaccessibilities

Formulation	**Percentage bioaccessible iron** [% ± SD (percentage difference)]	**Amount of bioaccessible iron** [mg/100 g porridge, db ± SD (percentage difference)]	**Percentage bioaccessible zinc** [% ± SD (percentage difference)]	**Amount of bioaccessible zinc** [mg/100 g porridge, db ± SD (percentage difference)]
PM + provit A source	1.79 ± 0.22$^{f\,1}$	0.081 ± 0.010^{g}	10.8 ± 2.7^{gh}	0.178 ± 0.045^{ef}
PM alone	1.30 ± 0.34^{g} (-27%)^{2}	0.044 ± 0.011^{h} (-46%)	10.5 ± 1.6^{h} (-3%)	0.139 ± 0.021^{f} (-22%)
PM + provit A source + 5M + 5H + 5B	2.15 ± 0.27^{de} (20%)	0.213 ± 0.027^{b} (163%)	14.1 ± 1.1^{ef} (30%)	0.271 ± 0.022^{d} (53%)
PM + 5M + 5H + 5B	1.46 ± 0.17^{g} (-19%)	0.128 ± 0.015^{ef} (58%)	13.0 ± 2.1^{fg} (20%)	0.208 ± 0.034^{e} (17%)
PM + provit A source + 5M	2.50 ± 0.40^{c} (40%)	0.180 ± 0.029^{c} (123%)	20.1 ± 3.9^{c} (87%)	0.353 ± 0.069^{c} (99%)
PM + provit A source + 15M	0.86 ± 0.12^{h} (-52%)	0.108 ± 0.015^{f} (33%)	10.0 ± 1.5^{h} (-8%)	0.196 ± 0.029^{e} (10%)
PM + provit A source + 5H	2.37 ± 0.40^{cd} (32%)	0.156 ± 0.026^{d} (93%)	15.8 ± 0.7^{de} (47%)	0.280 ± 0.013^{d} (57%)
PM + provit A source + 15H	2.57 ± 0.24^{c} (44%)	0.276 ± 0.026^{a} (241%)	16.8 ± 1.2^{d} (55%)	0.336 ± 0.024^{c} (89%)
PM + provit A source + 5B	3.28 ± 0.25^{b} (83%)	0.168 ± 0.013^{cd} (109%)	24.4 ± 3.9^{b} (126%)	0.416 ± 0.067^{b} (134%)
PM + provit A source + 15B	4.10 ± 0.65^{a} (129%)	0.263 ± 0.042^{a} (225%)	27.5 ± 2.8^{a} (154%)	0.500 ± 0.050^{a} (181%)

Formulation	Percentage bioaccessible iron [% ± SD (percentage difference)]	Amount of bioaccessible iron [mg/100 g porridge, db ± SD (percentage difference)]	Percentage bioaccessible zinc [% ± SD (percentage difference)]	Amount of bioaccessible zinc [mg/100 g porridge, db ± SD (percentage difference)]
PM + provit A source + 5M + 5H	1.98 ± 0.29^{ef} (11%)	0.184 ± 0.027^{c} (127%)	15.6 ± 1.5^{de} (45%)	0.293 ± 0.028^{d} (65%)
PM + provit A source + 5M + 5B	1.90 ± 0.33^{ef} (6%)	0.149 ± 0.026^{de} (84%)	15.3 ± 2.1^{de} (42%)	0.278 ± 0.038^{d} (56%)
PM + provit A source + 5H + 5B	2.98 ± 0.48^{b} (66%)	0.215 ± 0.035^{b} (166%)	22.7 ± 3.5^{b} (111%)	0.415 ± 0.063^{b} (133%)

[1]Values are reported as mean ± 1 standard deviation, values followed by different letter superscripts differ ($p \leq 0.05$). Each formulation was, in duplicate, subjected to three gastric stages, which in turn were subjected to three intestinal stages; each intestinal stage was measured for mineral content in duplicate (n = 36). Outliers were removed using Grubb's test for outliers.

[2]Numbers in brackets are the percentage difference in bioaccessibility compared to pearl millet plus provitamin A source

Moringa leaves addition at 5 g/100 g pearl millet, db, to pearl millet plus provitamin A source porridge had a seemingly positive effect on iron bioaccessibility, however, when moringa was added at 15 g/100 g pearl millet, db, it had a negative effect on iron bioaccessibility. When compared to the pearl millet + provitamin A source porridge, the addition of moringa leaves at 15 g/100 g pearl millet, db, the percentage bioaccessible iron was reduced by 52% and the amount of bioaccessible iron only increased by 33%, as opposed to increases of 40% and 123%, respectively, with its addition at 5 g/100 g pearl millet, db. The positive effect on the iron bioaccessibility with the addition of moringa leaves at 5 g/100 g pearl millet, db, was probably because of the high iron content of moringa leaves. The negative effect of the addition of moringa leaves at 15 g/100 g pearl millet, db, on bioaccessible iron was possibly due to the high levels of antinutrients it contains which could have affected the iron contained in the pearl millet itself. While moringa contained less than 0.1% tannins, it contained approximately 5% total phenolics, the highest total phenolic levels of all the plant foodstuffs and more than ten times that of pearl millet (Table 4.4.2). Moringa also contained substantial levels of phytate (829 mg/100 g, db) (Table 4.4.1) and the highest level of calcium (2115 mg/100 g, db) (Table 4.4.2), all which reduce iron bioaccessibility.

The percentage and the amount of bioaccessible iron was reduced ($p \leq 0.05$) by 66% and 40%, respectively, with the addition of moringa leaves at 15 g/100 g pearl millet, db, as compared to its addition at 5 g/100 g pearl millet, db. For most of the porridge formulations, addition of moringa leaves, plus hibiscus calyces, plus/or baobab fruit pulp (each at 5 g/100 g pearl millet, db) reduced ($p \leq 0.05$) the percentage bioaccessible iron by at least 17%, with a maximum reduction of 42%, as compared to hibiscus calyces plus/or baobab fruit pulp addition alone (at 5 g/100 g pearl millet, db).

Sreelatha and Padma (2009) found 4581 mg/100 g, db, total phenolics in mature moringa leaves, similar to what was found in this study (Table 4.4.2). All porridge formulations with moringa leaves thus, contained between 400 and 880 mg/100 g, db, total phenolics, such levels would probably have had an inhibitory effect on iron bioaccessibility (Khokhar and Apenten 2003). Moringa leaves have also been reported to contain appreciable levels of saponins (8000 mg/100 g, db) (Ferreira et al. 2008), which form insoluble saponin-mineral complexes with iron, zinc and calcium, thus, inhibiting the bioaccessibility of these minerals (Milgate and Roberts 1995).

Phenolics, such as those in moringa leaves, hibiscus calyces, baobab fruit pulp, and pearl millet have metal-chelating properties (Khokhar and Apenten 2003), resulting in the formation of insoluble polymerised complexes with iron and zinc (Siegenberg et al. 1991). The galloyl and catechol groups on phenolics can form insoluble complexes with iron and possibly zinc, which render the minerals unavailable for absorption (Towo et al. 2006).

The addition of hibiscus calyces to the pearl millet plus provitamin A source porridge (Table 4.4.3) increased ($p \leq 0.05$) the percentage bioaccessible iron by a minimum of 32% and a maximum of 44%. The amount of bioaccessible iron was increased by a minimum of 93% and a maximum of 241%. The highest amount of bioaccessible iron was found with the addition of hibiscus calyces (0.276 mg/100 g, db) at 15 g/100 g pearl millet, db.

Hibiscus calyces were the plant foodstuff with the second highest iron contents (47.1 mg/100 g, db) (Table 4.4.1). The phytate (4833 mg/100 g, db) and total phenolic (3451 mg/100 g, db) levels of hibiscus calyces were respectively, four and ten times higher than that of pearl millet (Table 4.4.2). Porridge formulations containing hibiscus calyces, thus, had the highest phytate contents (between 930 and 1440 mg/100 g, db) and among the highest total phenolic contents (between 350 and 770 mg/100 g, db) of all the formulations. However, when compared to the pearl millet plus provitamin A source porridge, formulations with hibiscus calyces had up to 1.7 times the percentage bioaccessible iron and up to 3.4 times the amount of bioaccessible iron (Table 4.4.3).

As described, phytate is a potent inhibitor of iron and zinc absorption in both adults and children (Gibson 2006). Even though hibiscus calyces had the highest phytate contents, its addition to the pearl millet plus provitamin A source porridge resulted in increased iron bioaccessibility. This was likely due to the organic acids in hibiscus calyces. Hibiscus calyces are rich in organic acids, with citric acid, hydroxycitric acid, hibiscus acid, malic acid and tartaric acid being the major organic acids, and oxalic and ascorbic acids being minor compounds (Da-Costa-Rocha et al. 2014). Most of these organic acids have the ability to chelate iron, through binding with carboxyl and hydroxyl groups, and thus, increase the iron solubility and bioaccessibility (Lönnerdal 2000).

The addition of baobab fruit pulp increased ($p \leq 0.05$) the iron bioaccessibility in all formulations. The iron bioaccessibility increased by up to 225% (Table 4.4.3). Adding a higher amount of baobab fruit pulp resulted in a larger increase in the percentage and the

amount of bioaccessible iron, probably also due to the high levels of organic acids. Tembo et al. (2017) analysed fresh baobab fruit pulp for organic acids and found very high concentrations of ascorbic, citric, and malic acids (466, 3300, and 2360 mg/100 g, respectively), as well as tartaric acid (174 mg/100 g, db). Such organic acids are enhancers of iron bioavailability (Lönnerdal 2000). Salovaara et al. (2002) studied the effect of nine organic acids on the absorption of ferric (Fe^{3+}) iron (10 μmol/L) in the human epithelial cell line Caco-2. They found that tartaric acid (4 mmol/L) increased ferric iron (Fe^{3+}) absorption 43-fold. Malic, succinic, fumaric, citric, and oxalic acids also enhanced ferric iron (Fe^{3+}) absorption and ascorbic acid (80 μmol/L) resulted in a 70-fold increase in ferric iron (Fe^{3+}) absorption.

The addition of 15 g/100 g pearl millet, db, of baobab fruit pulp yielded the highest percentage bioaccessible iron (4.10%) of all the porridge formulations. Increased iron bioaccessibility was found, despite the substantial levels of antinutrients in baobab fruit pulp. Baobab fruit pulp contained less than 1% phytate but had considerable amounts of tannins and phenolics (>2% and >3.5%, respectively) (Table 4.4.2). Formulations with baobab fruit pulp thus, had the highest tannin contents (between 117 and 343 mg CE/100 g, db) and among the highest total phenolic contents (between 363 and 769 mg/100 g, db) of all the formulations.

Condensed tannins (proanthocyanidins) are high molecular weight phenolics, consisting of polymerized flavan-3-ol and/or flavan-3,4-diol units (Devi et al. 2014). Tannins are biologically active and adversely affects iron bioavailability as it is a potent chelator of iron ions, forming complexes with ferrous iron (Fe^{2+}) which renders the iron unavailable for absorption (Khokhar and Apenten 2003). The phenolic hydroxyl groups are the sites for chelation with metal ions. The presence of organic acids in baobab fruit pulp may have overcome the inhibitory effects of the phytates and tannins (Cercamondi et al. 2014a), and possibly accounts for the positive effects from the addition of baobab fruit pulp, in this study. Despite the low iron levels, and high tannin and phenolics levels of baobab fruit pulp, as compared to pearl millet, its addition to pearl millet porridges increased the iron nutritive value.

Zinc bioaccessibility

With the addition of the micronutrient-rich plant foodstuffs to the pearl millet plus provitamin A source porridge, the percentage bioaccessible zinc increased ($p \leq 0.05$) by a minimum of 30% and a maximum of 154%. The amount of bioaccessible zinc increased ($p \leq 0.05$) by a minimum of 53% and a maximum of 181% (Table 4.4.3).

The porridge consisting of only pearl millet had low percentage bioaccessible zinc (10.5%) and the lowest ($p \leq 0.05$) amount of bioaccessible zinc (0.139 mg/100 g, db) (Table 4.4.3). Zinc contents, as well as the percentage and the amount of bioaccessible zinc of the pearl millet porridge were similar to the cereals analysed by Hemalatha et al. (2007).

With the addition of the mango-carrot premix at 30 g/100 g pearl millet, db, (plus sunflower oil, 5 g/100 g pearl millet, db), as a provitamin A source, plus 5 g/100 g pearl millet, db, of each micronutrient-rich plant foodstuff (moringa leaves, hibiscus calyces and baobab fruit pulp), the amount of bioaccessible zinc increased ($p \leq 0.05$) by 31%, as compared to pearl millet alone.

As described, provitamin A carotenoids has an enhancing effect on iron bioaccessibility. The study by Gautam et al. (2010) also found that the addition of carrot to the cereals studied, had a positive effect on zinc bioaccessibility. Carrot added at 25 and 50 g (roughly providing 2 and 4 mg beta-carotene) per 100 g of cereal, fw, resulted in 16 to 94% increased zinc bioaccessibility. With the inclusion of pure beta-carotene, zinc bioaccessibility was increased by 16 to 56%, with 2 mg beta-carotene /100 g cereal, fw, and increased by up to 118% with 4 mg beta-carotene /100 g cereal, fw.

Moringa leaves addition at 5 g/100 g pearl millet, db, to pearl millet plus provitamin A source porridge had a seemingly positive effect on zinc bioaccessibility, however, when moringa was added at increased levels (15 g/100 g pearl millet, db), it reduced the zinc bioaccessibility. When compared to the pearl millet + provitamin A source porridge, the addition of moringa leaves at 15 g/100 g pearl millet, db, the percentage bioaccessible zinc was reduced by 8% and the amount of bioaccessible zinc only increased by 10%, as opposed to increases of 87% and 99%, respectively, with its addition at 5 g/100 g pearl millet, db. The positive effect on the zinc bioaccessibility with the addition of moringa leaves at 5 g/100 g pearl millet, db, was probably because of the additional zinc that was added to the porridge, from the moringa leaves. The negative effect of the addition of moringa leaves at 15 g/100 g

pearl millet, db, on bioaccessible zinc, as with iron bioaccessibility, was possibly due to the high levels of antinutrients found in the moringa leaves.

The percentage and the amount of bioaccessible zinc was reduced ($p \leq 0.05$) by 51% and 45%, respectively, with the addition of moringa leaves at 15 g/100 g pearl millet, db, as compared to its addition at 5 g/100 g pearl millet, db. For most of the porridge formulations, addition of moringa leaves, plus hibiscus calyces, plus/or baobab fruit pulp (each at 5 g/100 g pearl millet, db) reduced ($p \leq 0.05$) the percentage bioaccessible zinc by 37 to 38% and reduced ($p \leq 0.05$) the amount of bioaccessible zinc by 33 to 35%, as compared to hibiscus calyces plus/or baobab fruit pulp addition alone (at 5 g/100 g pearl millet, db). Only when moringa was added together with hibiscus calyces alone (each at 5 g/100 g pearl millet, db), there was no significant ($p > 0.05$) change in the percentage or the amount of bioaccessible zinc, as compared to hibiscus calyces addition alone (at 5 g/100 g pearl millet, db).

Although moringa leaves had lower phytate contents than hibiscus calyces, moringa leaves had more than double the calcium contents. Calcium has the tendency to co-precipitate with phytate and zinc forming insoluble Ca_4Zn_2-phytate complexes (Kumar et al. 2010). Calcium-bound phytate has a higher affinity for zinc than phytate alone, thereby reducing the reabsorption of endogenous zinc, as well as decreasing dietary zinc bioavailability.

The addition of hibiscus calyces to the pearl millet plus provitamin A source porridge (Table 4.4.3) increased ($p \leq 0.05$) the percentage bioaccessible zinc by a minimum of 7% and a maximum of 55%. The amount of bioaccessible zinc was increased by a minimum of 57% and a maximum of 89%. Apart from pearl millet, hibiscus calyces were the plant foodstuff with the highest zinc contents (2.70 mg/100 g, db) (Table 4.4.1). Compared to the pearl millet plus provitamin A source porridge, formulations with hibiscus calyces had up to 2.1 times the percentage bioaccessible zinc and up to 2.3 times the amount of bioaccessible zinc (Table 4.4.3).

As with iron, the increased zinc bioaccessibility with the addition of hibiscus calyces to the pearl millet plus provitamin A source porridge was also likely due to its organic acids. Most organic acids have positive effects on zinc bioaccessibility as soluble complexes with zinc are formed in the intestinal tract (Lönnerdal 2000). These complexes easily break down in the small intestine, thus, releasing mineral cations, bioaccessible for absorption. It is thought that

the organic acids prevent the formation of insoluble zinc-phytate complexes, although the magnitude of this effect has not been extensively studied (Gibson 2006).

The addition of baobab fruit pulp increased ($p \leq 0.05$) the zinc bioaccessibility in all formulations, zinc bioaccessibility increased by up to 181% (Table 4.4.3). Adding a higher amount of baobab fruit pulp resulted in a larger increase in the percentage and the amount of bioaccessible zinc, and was probably also due to the high levels of organic acids. The addition of 15 g/100 g pearl millet, db, of baobab fruit pulp yielded the highest percentage bioaccessible zinc (27.5%), as well as the highest amount of bioaccessible zinc (0.500 mg/100 g, db) of all the porridge formulations. As with iron, increases in zinc bioaccessibility were found, despite the substantial levels of antinutrients in baobab fruit pulp and can also be explained by the high levels of organic acids, such as ascorbic, citric, malic and tartaric acids (Tembo et al. 2017), found in baobab fruit pulp. The presence of these organic acids may have overcome the inhibitory effects of phytates and tannins on zinc bioaccessibility (Cercamondi et al. 2014a). Despite baobab fruit pulp having low zinc levels, and high tannin and phenolics levels, its addition to the pearl millet plus provitamin A source porridges increased the zinc bioaccessibilities.

Phytate to mineral molar ratios as predictions of iron and zinc bioavailabilities

Phytate to mineral molar ratios can be used as predictions of mineral bioavailability. The phytate:iron, phytate:zinc, and phytate×calcium:zinc molar ratios for each porridge formulation was thus calculated (Table 4.4.4). Phytate:mineral molar ratios ranged from 5.8:1 to 23.6:1 for iron, from 31.4:1 to 54.2:1 for zinc, and from 3:1 to 293:1 for calcium-bound phytate:zinc molar ratios. These molar ratios indicate inadequate iron and zinc bioavailabilities for all the formulations. As previously discussed, iron and zinc bioaccessibilities were mostly increased with fortification of the micronutrient-rich plant foodstuffs. The addition of the provitamin A source reduced the phytate:iron and phytate:zinc molar ratios; but, increased the phytate×calcium:zinc molar ratios. Baobab fruit pulp added at 15 g/100 g pearl millet, db, resulted in the lowest phytate:zinc molar ratio of 31.4:1 and also had the highest increase in bioaccessible zinc, thus indicating some correlation between phytate:zinc molar ratio and the dialysability assay. The low phytate:zinc molar ratio of baobab fruit pulp added at 15 g/100 g pearl millet, db, may in part explain why its addition resulted in the highest bioaccessible zinc (Table 4.4.3). However, moringa leaves added at 15 g/100 g pearl millet, db, gave the lowest phytate:iron molar ratio of 5.8:1 but had the lowest

percentage bioaccessible iron and the lowest increase in amount of bioaccessible iron. This indicates that the phytate:iron molar ratios of this study did not correlate with the bioaccessibility results found.

Table 4.4.4: Calculated mineral (iron, zinc, calcium) and phytate contents (mg/100 g, db) and calculated phytate:mineral molar ratios of each formulation

Formulation	**Phytate:mineral molar ratios**[1]		
	phytate:iron	**phytate:zinc**	**phytate×calcium:zinc**
PM + provit A source	17.1	31.8	36
PM alone	23.6	33.4	3
PM + provit A source + 5M + 5H + 5B	9.0	39.6	211
PM + 5M + 5H + 5B	9.5	42.3	181
PM + provit A source + 5M	9.9	32.0	121
PM + provit A source + 15M	5.8	32.4	293
PM + provit A source + 5H	13.7	40.1	94
PM + provit A source + 15H	11.5	54.2	259
PM + provit A source + 5B	14.6	31.7	47
PM + provit A source + 15B	11.5	31.4	68
PM + provit A source + 5M + 5H	9.6	39.9	199
PM + provit A source + 5M + 5B	9.1	31.9	131
PM + provit A source + 5H + 5B	12.5	39.8	107

[1]Phytate and mineral contents for each formulation are based on the mean phytate and mineral contents from the different suppliers for each plant foodstuff (moringa leaves [M], hibiscus calyces [H], baobab fruit pulp [B], pearl millet porridge [PM], and mango-carrot premix (plus sunflower oil) [as provitamin A source]) (Table 4.4.1 and Table 4.4.2). Phytate:mineral molar ratios were calculated based on mineral contents and molecular weights of each element. Composition of formulations are shown in Table 4.3.1

It has been proposed that the molar ratios of phytate:iron should be below the critical level of 1:1 and preferably below 0.3:1 in order to achieve significant increases in iron bioavailability (Gibson et al. 2010). Concerning zinc, phytate has a dose-dependent effect on its absorption (Tripathi and Platel 2010), and the phytate:zinc molar ratio of a diet can be used to predict the proportion of dietary zinc bioavailability. Diets with molar ratios of phytate:zinc of more than

15:1 have been associated with biochemical zinc deficiency in humans (Gibson 2006). All the formulations had phytate:zinc molar ratios above 30, despite the increased zinc bioaccessibilities. Phytate:zinc molar ratios greater than 20:1 are associated with clinical evidence of zinc deficiency, whereas 10:1 or less indicate adequately bioavailable zinc from the diet (Antony and Chandra 1998). Molar ratios of phytate×calcium:zinc are also used as an indicator of zinc bioavailability, ratios above 200:1 are associated with inadequate zinc bioavailability (Bindra et al. 1986). There seems to be some contradiction in the above mentioned ranges as some formulations had very low phytate×calcium:zinc ratios (i.e. 3, 36 and 47:1) however, still had phytate:zinc ratios above 30. It is suggested that phytate:mineral molar ratios should not be used in isolation to predict bioavailability as other antinutrients and mineral bioavailability enhancers also play a role and should also be considered.

Calcium bioaccessibility

Of the micronutrient-rich plant foodstuffs, added to the pearl millet plus provitamin A source porridge, only baobab fruit pulp at 15 g/100 g pearl millet, db, resulted in a significant increase ($p \leq 0.05$) in the percentage bioaccessible calcium, it increased by 202% (Table 4.4.5). Concerning the amount of bioaccessible calcium, moringa leaves, hibiscus calyces and baobab fruit pulp all resulted in significant increases ($p \leq 0.05$). Addition of hibiscus calyces at 15 g/100 g pearl millet, db, resulted in the highest increase (by 750%) in amount of bioaccessible calcium.

The percentage bioaccessible calcium was highest (50.15%) (Table 4.4.5) in the porridge consisting of only pearl millet, however, this porridge had the lowest amount of bioaccessible calcium (1.8 mg/100 g, db). Pearl millet had very low calcium contents (7 mg/100 g, db) (Table 4.4.1), thus, even with high percentage bioaccessibility, the amount of bioavailable calcium was still rather low.

With the addition of the mango-carrot premix (plus sunflower oil), as a provitamin A source, plus 5 g/100 g pearl millet, db, of each micronutrient-rich plant foodstuff (moringa leaves, hibiscus calyces and baobab fruit pulp), an increase ($p \leq 0.05$) in amount of bioaccessible calcium was found (Table 4.4.5). When the provitamin A source was added to the porridge containing only pearl millet, a reduction ($p \leq 0.05$) in the percentage bioaccessible calcium was found.

Table 4.4.5: Effects of adding 5 and 15 g/100 g pearl millet plus provitamin A source, db, of dried moringa leaves (M), hibiscus calyces (H) and baobab fruit pulp (B) alone and in combination to freeze dried pearl millet (PM) porridge on calcium and phosphorus bioaccessibilities

Formulation	**Percentage bioaccessible calcium** [% ± SD (percentage difference)]	**Amount of bioaccessible calcium** [mg/100 g porridge, db ± SD (percentage difference)]	**Percentage bioaccessible phosphorus** [% ± SD (percentage difference)]	**Amount of bioaccessible phosphorus** [mg/100 g porridge, db ± SD (percentage difference)]
PM + provit A source	6.26 ± 2.24^{cd} [1]	2.8 ± 1.0^{fg}	14.0 ± 5.1^{f}	34.9 ± 12.8^{f}
PM alone	50.15 ± 16.69^{a} (701%)[2]	1.8 ± 0.6^{g} (-36%)	13.3 ± 3.4^{f} (-5%)	27.7 ± 7.0^{f} (-21%)
PM + provit A source + 5M + 5H + 5B	7.23 ± 1.97^{cd} (15%)	15.4 ± 4.2^{c} (443%)	23.7 ± 3.1^{d} (70%)	65.1 ± 8.6^{cd} (87%)
PM + 5M + 5H + 5B	3.32 ± 1.16^{d} (-47%)	5.7 ± 2.0^{ef} (104%)	14.7 ± 4.8^{f} (5%)	34.2 ± 11.3^{f} (-2%)
PM + provit A source + 5M	9.53 ± 2.01^{c} (52%)	14.4 ± 3.0^{cd} (408%)	25.8 ± 3.3^{bcd} (84%)	68.0 ± 8.6^{bcd} (95%)
PM + provit A source + 15M	5.61 ± 0.70^{cd} (-10%)	20.3 ± 2.5^{b} (617%)	19.4 ± 1.9^{e} (38%)	56.6 ± 5.5^{e} (62%)
PM + provit A source + 5H	6.78 ± 0.91^{cd} (8%)	6.4 ± 0.9^{e} (124%)	29.7 ± 2.2^{a} (112%)	76.4 ± 5.6^{a} (119%)
PM + provit A source + 15H	12.63 ± 0.31^{bc} (102%)	24.1 ± 0.6^{a} (750%)	28.6 ± 1.7^{ab} (104%)	78.2 ± 4.6^{a} (124%)
PM + provit A source + 5B	10.93 ± 3.87^{c} (75%)	6.4 ± 2.3^{e} (127%)	29.6 ± 3.2^{a} (111%)	74.5 ± 8.2^{ab} (113%)
PM + provit A source + 15B	18.90 ± 2.98^{b} (202%)	16.3 ± 2.6^{c} (475%)	27.8 ± 2.0^{abc} (99%)	71.6 ± 5.3^{abc} (105%)
PM + provit A source + 5M + 5H	11.12 ± 2.63^{c} (78%)	22.2 ± 5.3^{ab} (683%)	26.0 ± 1.2^{bcd} (86%)	70.6 ± 3.3^{abc} (102%)

Formulation	Percentage bioaccessible calcium [% ± SD (percentage difference)]	Amount of bioaccessible calcium [mg/100 g porridge, db ± SD (percentage difference)]	Percentage bioaccessible phosphorus [% ± SD (percentage difference)]	Amount of bioaccessible phosphorus [mg/100 g porridge, db ± SD (percentage difference)]
PM + provit A source + 5M + 5B	7.08 ± 0.56^{cd} (13%)	11.7 ± 0.9^{d} (311%)	23.6 ± 2.0^{d} (68%)	62.7 ± 5.3^{de} (80%)
PM + provit A source + 5H + 5B	10.78 ± 0.90^{c} (72%)	11.6 ± 1.0^{d} (309%)	25.1 ± 2.8^{cd} (79%)	65.2 ± 7.2^{cd} (87%)

[1]Values are reported as mean ± 1 standard deviation, values followed by different letter superscripts differ ($p \leq 0.05$). Each formulation was subjected to three gastric stages, which in turn were subjected to three intestinal stages (n = 27). Outliers were removed using Grubb's test for outliers.

[2]Numbers in brackets are the percentage difference in bioaccessibility compared to pearl millet plus provitamin A source

Phosphorus Bioaccessibility

The addition of the micronutrient-rich plant foodstuffs to the pearl millet plus provitamin A source porridge resulted in an increase ($p \leq 0.05$) in the percentage and the amount of bioaccessible phosphorus for all the formulations (Table 4.4.5). Porridge formulations with hibiscus calyces or baobab fruit pulp addition (5 or 15 g/100 g pearl millet, db) to the pearl millet plus provitamin A source porridge resulted in the greatest increases ($p \leq 0.05$) in bioaccessible phosphorus. The percentage bioaccessible phosphorus was increased by a maximum of 112% and the amount of bioaccessible phosphorus was increased by a maximum of 124%, with the addition of hibiscus calyces, as compared to the pearl millet plus provitamin A source porridge. However, when 5 g/100 g pearl millet, db, of hibiscus calyces were added together with 5 g/100 g pearl millet, db, baobab fruit pulp, the percentage and the amount of bioaccessible phosphorus only increased ($p \leq 0.05$) by 79% and by 87%, respectively. Moringa leaves addition at 15 g/100 g pearl millet, db, resulted in the smallest increase ($p \leq 0.05$) in the percentage (by 38%) and amount (by 62%) of bioaccessible phosphorus.

4.5 Conclusions

Food-to-food fortification of pearl millet porridge with baobab fruit pulp has the greatest positive effect on the iron and zinc bioaccessibilities. When baobab is added at 15 g/100 g pearl millet, db, percentage bioaccessible iron is increased by 129%, the percentage bioaccessible zinc is increased by 154% and the amount of bioaccessible zinc is increased by 181%, as compared to the pearl millet plus provitamin A source porridge. Fortification with hibiscus calyces also increases the iron and zinc bioaccessibilities. Hibiscus calyces added at 15 g/100 g pearl millet, db, increases the amount of bioaccessible iron the most, by 241%, as compared to the pearl millet plus provitamin A source porridge. These increases are probably due to the high levels of organic acids in baobab fruit pulp and hibiscus calyces. Most organic acids are able to form soluble complexes with iron and zinc which renders the minerals bioaccessible for absorption in the gastrointestinal tract.

Fortification with moringa leaves to pearl millet porridge increases as well as decreases the iron and zinc bioaccessibilities. When moringa is added at 15 g/100 g pearl millet, db, a reduction of 52% in the percentage iron bioaccessibility and a reduction of 8% in the percentage zinc bioaccessibility, as compared to peal millet plus provitamin A porridge, is observed. This is because moringa leaves, even though it has the highest iron content (58.4 mg/100 g, db) of all the plant foodstuffs, has the highest levels of calcium and total phenolics as well as a substantial level of phytate. With such high levels of iron and zinc bioaccessibility inhibitors, fortification with moringa leaves, probably negatively affects the bioaccessibility of the iron and zinc contained in the pearl millet itself.

Addition of the mango-carrot premix (plus sunflower oil), as a provitamin A source, to pearl millet porridge results in increased bioaccessible iron and zinc. This is because provitamin A carotenoids have an enhancing effect on nonhaem iron and zinc absorption as the carotenoids are able to form soluble complexes with these minerals in the intestinal tract.

Dietary diversification by means of fortifying pearl millet porridges with baobab fruit pulp, possibly hibiscus calyces, but not moringa leaves, could increase the iron and zinc bioavailabilities of the porridge. This may possibly lead to improved iron and zinc statuses of populations in sub-Saharan Africa with micronutrient deficiencies.

Chapter 5: General discussion

This chapter is divided into three sections. The first part critically evaluates the experimental design and important methods as applied in this study and also lists additional *in vitro* and *in vivo* methods that can be applied. The second section evaluates the applicability of major findings concerning the effect of porridge composition on the contribution to iron and zinc recommended dietary allowances (RDA) and absolute (physiological) requirements (AR). The third section discusses how porridges can further be improved and suggests additional research which can be done in the future.

5.1 Methodological considerations

5.1.1 Porridge preparation and formulation

The preparation of the pearl millet porridge included cooking it with water in a pot, to mimic in home cooking of porridge. The porridge was then freeze dried as a means to preserve the cooked porridge. Freeze drying, although not economically feasible to produce porridge flour for communities in sub-Saharan Africa, was used for the purpose of this study as it is the least damaging to nutrients. Extrusion cooking of the pearl millet would be a better approach as it could be used to produce economically feasible instant porridge flour. However, only small amounts of cooked pearl millet porridge flour was needed for the purposes of this study, hence, stove cooking and freeze drying was used.

The micronutrient-rich plant foodstuffs (moringa leaves, hibiscus calyces, baobab fruit pulp), used in this study, were chosen as they are commercially available in Senegal and could possibly be used to produce food-to-food fortified pearl millet porridges for people living in that region of sub-Saharan Africa. These plant foodstuffs were added to the pearl millet at levels of 5 and 15 g/100 g pearl millet, db. The addition of the plant foodstuffs at 5 g/100 g pearl millet, db, was chosen as a realistic food-to-food fortification amount with respect to cost, as well as sensorial properties of the porridge. The addition of the plant foodstuffs at 15 g/100 g pearl millet, db, was used mainly to determine an observable effect, such high levels of food-to-food fortification with these plant foodstuffs, in practice, would be uneconomical and would also be unfavourable to the sensory properties of the porridge.

The mango-carrot premix (plus sunflower oil) as a provitamin A source was included in the study to address underlying vitamin A deficiency. It was also used to determine if plant foodstuffs, rich in provitamin A, could increase the iron and zinc bioaccessibilities from pearl millet porridge.

5.1.2 Calcium and phosphorus contents and bioavailabilities

The calcium content of the foodstuffs were determined as calcium is known to reduce iron bioaccessibility, and when bound to phytate, can reduce the bioaccessibility of zinc, as discussed previously. With regard to phosphorus contents, its levels in the foodstuffs are related to phytate, which is a potent inhibitor of iron and zinc absorption (Gibson 2006). Each phytate molecule has 6 phosphate groups, containing phosphorus, attached to its inositol ring. Free phosphates, as those from phytate hydrolysis, can also react with mineral ions to form insoluble precipitates which renders the minerals unavailable for absorption (Lopez et al. 2002). Although it was not the focus of this project, the calcium and phosphorus bioaccessibilities were also determined as these are important minerals for growth and development (FAO/WHO 2001).

5.1.3 The Folin-Ciocalteu method to determine total phenolic contents

The Folin-Ciocalteu method, used to determine the total phenolic contents, is a non-specific assay as other oxidation substances can interfere with the assay in an inhibitory, additive or synergistic way (Magalhães et al. 2010). Inhibitory effects are not likely to occur as reactions from oxidants competing with the Folin-Ciocalteu reagent should have been completed in advance. The Folin-Ciocalteu reagent is added before the alkali, thus, avoiding the inhibitory effect of air oxidation. Some nonphenolic compounds such as organic acids, reducing sugars, ferrous sulphate, and sodium sulphite, can directly reduce the Folin-Ciocalteu reagent, resulting in an additive effect. A clean-up procedure using solid phase extraction have been proposed as a means to separate phenolic compounds and eliminate interfering oxidising substances (Magalhães et al. 2010). A synergistic effect is produced by the regeneration of oxidised phenols to allow further oxidation by the Folin-Ciocalteu reagent (Magalhães et al. 2010). It is not clear what the rate of regeneration is and whether the time limit applied in the methodology would nullify the synergistic effect. It has been found that ascorbic acid at concentrations higher than 1.0 mg/L had considerable interference with the Folin-Ciocalteu

assay, as an additive effect, thus, correction based on ascorbic acid contents should be applied (Magalhães et al. 2010). Moringa leaves, hibiscus calyces, and baobab fruit pulp contain ascorbic acid (Table 2.9.1), thus, the total phenolic contents determined might have been overestimated.

Alternative, more specific methods, such as analysing methanolic extracts of plant samples by liquid chromatography-mass spectrometry can provide profiling of specific phenolic compounds (Leone et al. 2015). Qualitative HPLC profiling may also be used (Puupponen-Pimiä et al. 2003).

Iron bioaccessibility may have been overestimated. Some phenolic compounds, such as those found in tea or spinach (Brown et al. 1990), can bind iron in soluble complexes (Moran et al. 1997). Because these compounds are soluble, they readily diffuse through the dialysis membrane and thus, are measured as bioaccessible iron (Luten et al. 1996). However, the iron bound to soluble phenolic compounds is not bioavailable for absorption in the gastrointestinal tract.

5.1.4 Provitamin A

The increases in iron and zinc bioaccessibilities from the addition of the provitamin A source were in agreement with research by Gautam, Platel and Srinivasan (2010). However, in this present study it is not possible to clarify whether the improved bioaccessibilities were due to the provitamin A or other iron and zinc bioaccessibilities enhancers present in the provitamin A source. To confirm that the positive effect of the provitamin A source (mango-carrot premix plus sunflower oil) on the bioaccessible iron and zinc was due to the provitamin A, the provitamin A content would need to be measured. The same amount of provitamin A as detected in the mango-carrot premix should then be added to the pearl millet porridge and iron and zinc bioaccessibilities measured.

5.1.5 The *in vitro* dialysability assay as a measure of bioaccessible minerals

The *in vitro* dialysability assay as a measure of bioaccessibility is limited as it cannot assess the rate of absorption, absorption, or transport kinetics. Furthermore, it cannot measure nutrient or food competition at the site of absorption (Etcheverry et al. 2012). A major drawback of the *in vitro* dialysability assay is that a significant amount of the iron diffused

into the dialysis bag immediately becomes insoluble due to the higher pH of the dialysate, this amount of insoluble iron may have a significant effect on the results (Van Campen and Glahn 1999). The dialysability assay has, however, been validated against human absorption studies and found to agree to some extent (Etcheverry et al. 2012). Dialysable iron values from a study done by Luten et al. (1996) were similar to some extent to non-haem iron absorption values from a human subject study, in ranking and magnitude. The dialysability assay should rank meals in the same order and direction of the response to inhibitors and enhancers as absorption studies.

The *in vitro* dialysability assay has significant variation in the parameters between the individual models described in literature. This makes it challenging to compare results between different research-groups and to deduce general findings (Minekus et al. 2014). To aid the production of more comparable data in future studies, Minekus et al. (2014) proposed a "general standardised and practical static digestion method based on physiologically relevant conditions". A frameset of parameters were suggested and included the specific digestion fluids, the oral, gastric and intestinal phase as well as suggestions of sampling during digestion. Justifications for the recommendations of the digestion parameters were included.

An extension to the dialysis method developed by Miller et al. (1981) is a continuous-flow dialysis system which is performed by means of a hollow-fibre system (Wolters et al. 1993). The continuous-flow dialysis system takes the removal of dialysable components into account and thus, leads to a better estimate of *in vivo* bioavailability (Etcheverry et al. 2012). Sophisticated gut models have also been developed which simulate the human digestive system. The Netherlands Organization (TNO) for Applied Scientific Research has developed a computer controlled gastrointestinal model (TIM) which simulates conditions in the human stomach, duodenum, jejunum and ileum (Minekus et al. 1995). This gastrointestinal model has a much greater *in vivo* predictive value than typical *in vitro* dissolution assays (TNO 2013).

5.1.6 Estimation of bioavailability

A human colon adenocarcinoma cell line, Caco-2 cells, have demonstrated numerous morphological and biochemical characteristics of enterocytes (Au and Reddy 2000). Caco-2 cells differentiate into polarised monolayers with a well-developed brush border and

associated enzymes. These cells can be used to assess bioaccessibility through the determination of nutrient uptake, transport, or both (Etcheverry et al. 2012). Iron uptake can be estimated by the Caco-2 human epithelial cell line via ferritin formation, via atomic absorption spectroscopy or via radio-isotopic forms of the mineral. The latter two are also used for zinc and calcium uptake studies.

The *in vitro* Caco-2 absorption model has been suggested as the recommended bioaccessibility method for iron and zinc (Etcheverry et al. 2012). This assay can provide more information than bioaccessibility studies alone, such as the impact of food or nutrient components on the absorption rate and efficiency, as well as possible competition at the absorption site (Glahn et al. 2002). The Caco-2 absorption model has been validated against human absorption results, with a significant correlation found (Au and Reddy 2000). The Caco-2 cell model is thus a useful assay to assess human iron absorption and is also feasible to study iron and zinc bioavailability from various food combinations.

5.2 Application of research findings

5.2.1 Contribution to recommended dietary allowances (RDA)

Recommended dietary allowances (RDA) of iron and zinc for 2–5-year-old children, vary according to the types of diets followed (National Institute of Health 2016). Mixed western-type diets contain meat with haem iron which is the highest bioavailable form of iron (≈18%) and zinc (≈45%), whereas strict vegetarian diets have the lowest bioavailable iron (≈5%) and zinc (≈15%). Vegetarian diets are mostly followed by poor populations in developing countries where access to a variety of foods is limited. The iron RDA for vegetarian diets is 1.8 times higher than for mixed diets and is associated with moderate iron bioavailability (≈10%). People following vegetarian diets have 50% greater zinc requirements than those following mixed western diets and is based on dietary phytate:zinc molar ratios greater than 15. RDA of iron and zinc is 11.6 mg/day and 8.3 mg/day, respectively for 2–3-year-old children, and 12.6 mg/day and 9.6 mg/day, respectively for 4–5-year-old children, following a diet with low iron and zinc bioavailabilities (WHO/FAO 2004). As a large proportion of the populations in sub-Saharan Africa are reliant on vegetarian diets, the contribution one portion of each porridge formulation could have on the RDA's of iron and zinc for 2–5-year-old children, following a vegetarian diet, was calculated (Table 5.2.1). RDA's for children in this

age group were chosen as they are at greatest risk of mineral deficiencies (WHO 2014). Calculations were based on porridge-type portion sizes of 100 g, fw, for 2–3 years and 120 g, fw, for 4-5 years (derived from Mahan and Raymond 2016). The calculations were based on a porridge consisting of 30% solids. The amount of porridge required to meet 25% of iron and zinc RDAs for 2–5-year-old children was calculated for each of the formulations (Table 4.3.1). A porridge providing 25% of iron and zinc RDAs would be sufficient as the porridge would not be the only source of iron and zinc in the diet. If two portions were consumed during a day, 50% of iron and zinc RDAs for 2–5-year-old children would be met.

With the addition of the provitamin A source to pearl millet, the contribution to iron and zinc RDAs increased by 34 and 25%, respectively, as compared to pearl millet alone. For the various porridge formulations, the highest percentage contribution one portion of porridge on the iron RDA for 2–5-year-old children could make, was 35.7 and 39.5%, respectively, for 2–3 and 4–5-year-old children (Table 5.2.1). This was with moringa leaves addition at 15 g/100 g pearl millet, db, as moringa leaves had the highest levels of iron of all the plant foodstuffs. However, in Chapter 4, it was found that porridge fortified with this level of moringa leaves had the lowest bioaccessible iron, even though its contribution to the iron RDA was the highest. Concerning zinc, the highest percentage contribution of one portion of porridge could make on the zinc RDA for 2–5-year-old children was 5.8 and 6.4%, respectively, for 2–3 and 4–5-year-old children (Table 5.2.1) and was achieved with hibiscus calyces added at 15 g/100 g pearl millet, db. Hibiscus calyces were the foodstuff with the highest levels of zinc, thus it had the greatest percentage contribution to zinc RDA. Even though food-to-food fortification of pearl millet porridge with the suggested micronutrient-rich plant foodstuffs increases the zinc content of the porridges, the contribution to RDA is still very low and might not be sufficient to improve zinc statuses of children.

RDA only takes the content of nutrients into account and not the bioaccessibility of these nutrients (National Institute of Health 2016). Even though RDA's take into account the effects of the type of diet followed on the nutrient bioavailability, it is difficult to make true comparisons between the different plant foodstuffs as they affect nutrient bioavailabilities differently. Thus to be able to make better comparisons between the effects of the different plant foodstuffs on iron and zinc bioavailabilities from pearl millet porridge, the iron and zinc contents, as well as their bioaccessibilities have to be taken into account (WHO/UNICEF/UNU 2001). The contribution, various pearl millet porridge formulations could make to the absolute requirements for iron and zinc should rather be estimated.

Table 5.2.1: Effects of adding 5 and 15 g/100 g pearl millet plus provitamin A source, db, of dried moringa leaves (M), hibiscus calyces (H) and baobab fruit pulp (B) alone and in combination to freeze dried pearl millet (PM) porridge on the percentage contribution of one portion (30% solids) to the recommended dietary allowances (RDA) for iron and zinc for 2–5-year-old children, and the amount of porridge required to meet 25% of their RDA

Formulations	**% Contribution to RDA**				**Grams of porridge required to meet 25% of RDA**			
	2–3 years (Portion of 100 g)		**4–5 years** (Portion of 120 g)		**2–3 years**		**4–5 years**	
	Iron	**Zinc**	**Iron**	**Zinc**	**Iron**	**Zinc**	**Iron**	**Zinc**
PM + provit A source	13.1	4.8	14.4	5.3	191	524	173	475
PM alone	9.7	3.8	10.7	4.2	257	655	233	593
PM + provit A source + 5M + 5H + 5B	28.5	5.6	31.5	6.2	88	449	79	406
PM + 5M + 5H + 5B	25.1	4.6	27.8	5.1	99	541	90	490
PM + provit A source + 5M	20.6	5.1	22.8	5.6	121	494	110	447
PM + provit A source + 15M	35.7	5.7	39.5	6.2	70	442	63	400
PM + provit A source + 5H	19.2	5.1	21.2	5.7	131	488	118	442
PM + provit A source + 15H	31.3	5.8	34.6	6.4	80	430	72	389
PM + provit A source + 5B	14.8	4.9	16.4	5.4	168	507	152	459
PM + provit A source + 15B	18.4	5.2	20.3	5.8	136	476	123	431

Formulations	% Contribution to RDA				Grams of porridge required to meet 25% of RDA			
	2–3 years (Portion of 100 g)		4–5 years (Portion of 120 g)		2–3 years		4–5 years	
	Iron	Zinc	Iron	Zinc	Iron	Zinc	Iron	Zinc
PM + provit A source + 5M + 5H	26.7	5.4	29.5	6.0	94	462	85	418
PM + provit A source + 5M + 5B	22.4	5.2	24.7	5.8	112	479	101	433
PM + provit A source + 5H + 5B	20.9	5.3	23.1	5.8	119	474	108	429

5.2.2 Contribution to iron and zinc absolute (physiological) requirements (AR)

The contribution various pearl millet porridge formulations could make to the AR for iron and zinc, relative to the pearl millet plus provitamin A source porridge was calculated for 2–5-year-old children (Figure 5.2-1). The AR for a mineral is equal to the sum of the daily basal losses of the mineral (via faeces, urine, skin and its appendages, milk, menstrual blood, and semen) plus the amounts of the mineral needed for growth (WHO/UNICEF/UNU 2001). For 2–5-year-old children, the absolute iron requirement is 0.5 mg/day (WHO/UNICEF/UNU 2001) and the absolute zinc requirements is 1.09 mg/day (FAO/WHO 2001). The absolute bioaccessible mineral (per portion) was calculated as the amount of bioaccessible mineral multiplied by portion weight, db.

$$\text{Percentage contribution of porridge formulation to AR} = \frac{\text{Absolute bioaccessible mineral (per portion)}}{\text{AR}} \times 100$$

$$\text{Relative contribution of porridge formulation to AR} = \frac{\text{Percentage contribution to AR of formulation}}{\text{Percentage contribution to AR of pearl millet + provitamin A source}}$$

Baobab fruit pulp addition had the highest relative contribution to the AR for both iron and zinc (Figure 5.2-1). Its addition at 5 g/100 g pearl millet, db, contributed 1.9 and 1.6 times, respectively more to the AR for iron and zinc, than the pearl millet plus provitamin A source porridge. Likewise, its addition at 15 g/100 g pearl millet, db, contributed 3.3 and 2.9 times, respectively more to the AR for iron and zinc, than the pearl millet plus provitamin A source porridge. Hibiscus calyces added at 15 g/100 g pearl millet, db, had the highest relative contribution to the AR for iron, however, baobab fruit pulp was the best overall food-to-food fortificant among the plant foodstuffs as it had the greatest positive effect on both iron and zinc bioaccessibilities.

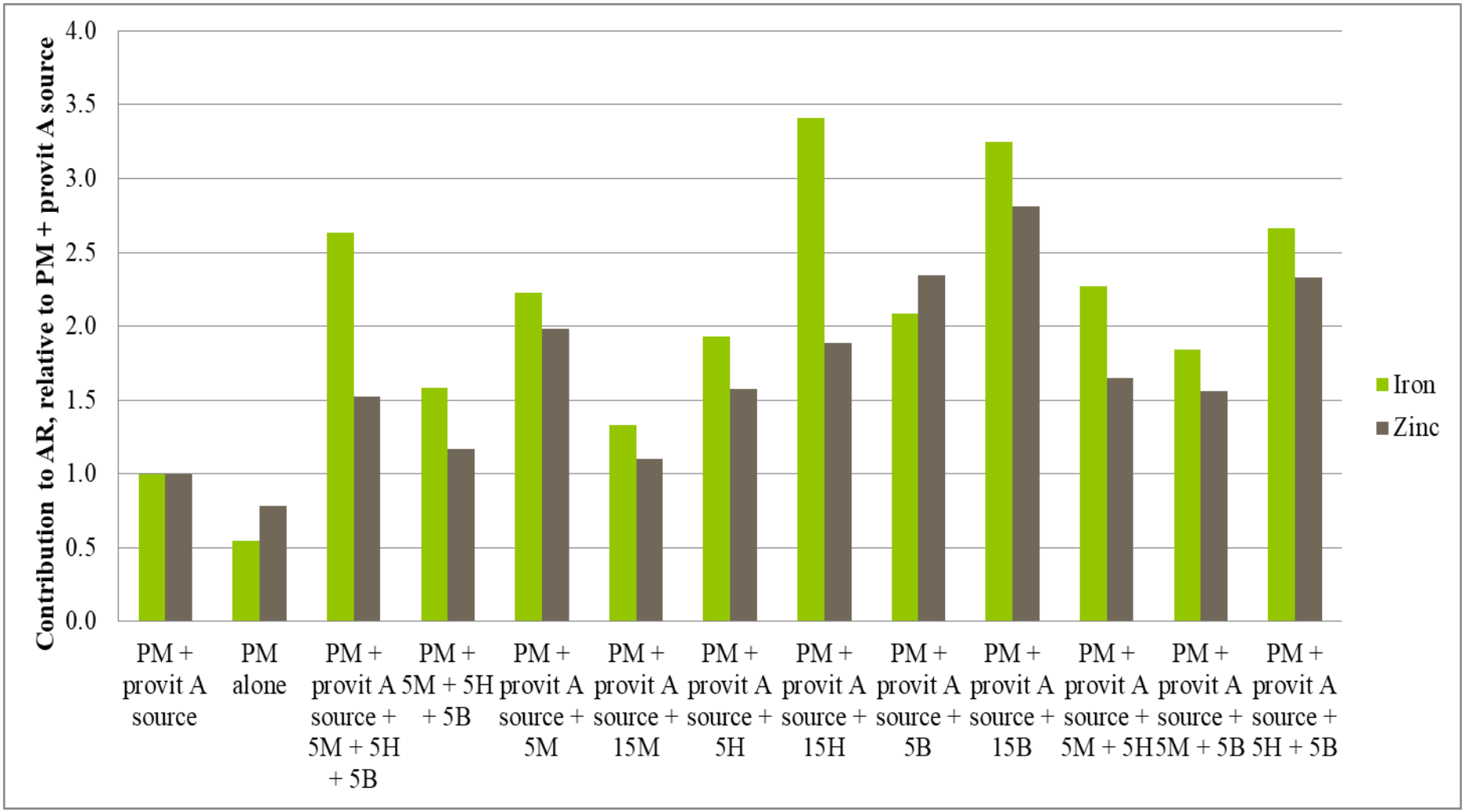

Figure 5.2-1: Effects of adding 5 and 15 g/100 g pearl millet plus provitamin A source, db, of dried moringa leaves (M), hibiscus calyces (H) and baobab fruit pulp (B) alone and in combination to freeze dried pearl millet (PM) porridge on the relative contribution to iron and zinc absolute (physiological) requirements (AR) of 2–5-year-old children, as compared to pearl millet plus provitamin A source porridge

Even though the maximum percentage contribution to zinc RDA was 5.8 and 6.4% for 2–3 and 4–5-year-old children, respectively (Table 5.2.1), and indicated insufficient zinc content to improve zinc statuses of children, the contribution to zinc AR was 2.9 times that of pearl millet plus provitamin A source and 6.8 times that of pearl millet alone. This strongly indicates that zinc statuses of children consuming such food-to-food fortified porridges would increase; however the extent of improved statuses cannot be predicted.

5.3 Future research

Iron and zinc bioavailability enhancers are also present in the micronutrient-rich plant foodstuffs. Determination of the iron and zinc bioavailability enhancers should be carried out to better explain the effects of the foodstuffs on the iron and zinc bioaccessibilities. Determination of bioavailability enhancers, such as organic acids can be measured by liquid chromatography (Nisperos-Carriedo et al. 1992).

Processing techniques of the moringa leaves, hibiscus calyces and baobab fruit pulp should be considered. It may be that heat processing was used during the drying of the moringa leaves, if that was the case, many of the organic acids which enhance iron and zinc bioavailabilities would have been destroyed (Asami et al. 2003). Other processing techniques which further improve mineral bioavailabilities should be explored; such processes may involve extrusion cooking, or acidification of the porridge by means of fermentation or artificial acidification (Motarjemi and Nout 1996). Extrusion cooking, a combined thermo-mechanical treatment, of the pearl millet and co-extrusion of the pearl millet plus the micronutrient-rich plant foodstuffs should be investigated. Research has indicated that extrusion cooking is a quicker and more consistent way to increase nutrient bioavailability, as compared to traditional processing methods (Nikmaram et al. 2017). Extrusion cooking causes thermal and chemical breakdown of antinutritional factors, such as mineral bioavailability inhibitors, and simultaneously could alter the physical, chemical and nutritional nature of nutrients in a desirable manner.

The *in vitro* Caco-2 absorption model is a closer predictor of bioavailability than the dialysability assay alone, and should be considered for future studies. Evidence for the enhancing effect of baobab fruit pulp and hibiscus calyces on iron and zinc bioavailability also needs to be confirmed in *in vivo* studies (stable isotope absorption) (Johnson 1982).

Chapter 6: Conclusions and Recommendations

Fortification of pearl millet with a mango-carrot premix (plus sunflower oil) improves the iron and zinc bioaccessibilities as mango and carrots contain very low levels of iron and zinc bioavailability inhibitors, and are rich in organic acids and provitamin A, which can act as iron and zinc bioavailability enhancers.

Additional fortification of the pearl millet plus provitamin A source porridge with the micronutrient-rich plant foodstuffs (moringa leaves, hibiscus calyces or baobab fruit pulp) further improves the iron and zinc bioaccessibilities. Baobab fruit pulp addition results in the highest iron and zinc bioaccessibilities as it contains the lowest levels of calcium and phytate, of the micronutrient-rich plant foodstuffs. Even though baobab fruit pulp contains low levels of iron and zinc, and substantial levels of tannins, it is very rich in organic acids which can act as potent iron and zinc bioavailability enhancers. Additional fortification of the pearl millet plus provitamin A source porridge with baobab fruit pulp could contribute >200% and >180% more to the iron and zinc absolute requirements (defined as the sum of the daily basal losses of the mineral plus the amounts of the mineral needed for growth), respectively, for 2-5-year-old children.

The addition of hibiscus calyces to the pearl millet plus provitamin A source porridge also considerably improves iron and zinc bioaccessibilities as it contains substantial levels of iron, zinc, and organic acids, even though it has substantial levels of phytate. The addition of moringa leaves generally results in the lowest increases and, in some cases, even reduces the iron and zinc bioaccessibilities, even though it has the highest level of iron of all the plant foodstuffs. Dried moringa leaves have the highest levels of calcium and total phenolics, and substantial levels of phytate, as well as possible low levels of organic acids, all which contribute to the low iron and zinc bioaccessibilities.

The addition of baobab fruit pulp or hibiscus calyces (with or without the mango-carrot premix) to the pearl millet plus provitamin A source porridge considerably improves the iron and zinc bioaccessibilities. Thus, the iron and zinc status of people consuming a cereal-based diet may be improved by the inclusion of baobab fruit pulp or hibiscus calyces, as natural food fortificants, to cereal-based porridges. Dietary diversification, with mango, carrots, hibiscus calyces and baobab fruit pulp, as locally available plant-based foods, to produce cereal-based porridges is one of the best ways to provide nutritious diets to the sustainability

of a population. Furthermore, locally grown and processed food can contribute substantially to economic growth and food security in both urban and rural communities in sub-Saharan Africa. Production of a porridge also has the potential to be marketed in distant markets, as it is a microbiologically stable product.

It is recommended that future studies should include phenolic profiling and organic acid profiling to further explain the iron and zinc bioaccessibility results. The results of this study cannot be applied to humans yet as further studies have to be done to confirm the dialysability findings. Thus, further *in vitro* studies (Caco-2 absorption model) as well as *in vivo* studies (stable isotope absorption) should be carried out.

Chapter 7: References

AACC International (American Association of Cereal Chemists International). 2000. Approved Methods of the American Association of Cereal Chemists: Method 10-50D. 10th ed. St. Paul: AACC International.

Adanlawo IG, Ajibade VA. 2006. Nutritive value of the two varieties of Roselle (Hibiscus sabdariffa) calyces soaked with wood ash. Pakistan J Nutr. 5(6): 555–557.

Afify AEMMR, El-Beltagi HS, Abd El-Salam SM, Omran AA. 2012. Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pac J Trop Biomed. 2(3): 203–209.

Afsana K, Shiga K, Ishizuka S, Hara H. 2004. Reducing effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. Biosci Biotechnol Biochem. 68(3): 584–592.

Anjorin TS, Ikokoh P, Okolo S. 2010. Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int J Agric Biol. 12(3): 431–434.

Antony U, Chandra TS. 1998. Antinutrient reduction and enhancement in protein, starch, and mineral availability in fermented flour of finger millet (*Eleusine coracana*). J Agric Food Chem. 46(97): 2578–2582.

Asami DK, Hong YJ, Barrett DM, Mitchell AE. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. J Agric Food Chem. 51(5): 1237–1241.

Au AP, Reddy MB. 2000. Caco-2 cells can be used to assess human iron bioavailability from a semipurified meal. J Nutr. 130(5): 1329–1334.

Bailey RL, West KP, Black RE. 2015. The epidemiology of global micronutrient deficiencies. Ann Nutr Metab. 66(suppl 2): 22–33.

Bain LE, Awah PK, Geraldine N, Kindong NP, Sigal Y, Bernard N, Tanjeko AT. 2013. Malnutrition in sub-Saharan Africa: Burden, causes and prospects. Pan Afr Med J. 15(1): 1–9.

Bajaj KL, Kaur G. 1981. Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. Analyst. 106(1258): 117–120.

Barton JC, Conrad ME, Parmley RT. 1983. Calcium inhibition of inorganic iron absorption in rats. Gastroenterology. 84(1): 90–101.

Beiseigel JM, Hunt JR, Glahn RP, Welch RM, Menkir A, Maziya-dixon BB. 2007. Iron

bioavailability from maize and beans : a comparison of human and caco-2 cell measurements. Am J Clin Nutr. 86: 388–396.

Bender DA. 1999. Benders dictionary of nutrition and food technology. 8th ed. Cambridge: Woodhead Publishing Limited.

Best C, Neufingerl N, van Geel L, van den Briel T, Osendarp S. 2010. The nutritional status of school-aged children: Why should we care? Food Nutr Bull. 31(3): 400–417.

Bhaskarachary K. 1995. Carotene content of some common and less familiar foods of plant origin. Food Chem. 54(2): 189–193.

Bindra GS, Gibson RS, Thompson LU. 1986. [Phytate][calcium]/[zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. Nutr Res. 6(5): 475–483.

Borrás-Linares I, Fernández-Arroyo S, Arráez-Roman D, Palmeros-Suárez PA, Del Val-Díaz R, Andrade-Gonzáles I, Fernández-Gutiérrez A, Gómez-Leyva JF, Segura-Carretero A. 2015. Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (Hibiscus sabdariffa). Ind Crops Prod. 69(1): 385–394.

Bowering J, Masch GA, Lewis AR. 1977. Enhancement of iron absorption in iron depleted rats by increasing dietary fat. J Nutr. 107(9): 1687–1693.

Brown RC, Klein A, Simmons WK, Hurrell RF. 1990. The influence of Jamaican herb teas and other polyphenol-containing beverages on iron absorption in the rat. Nutr Res. 10(3): 343–353.

Brune M, Hallberg L, Skanberg ANNB. 1991. Determination of iron binding phenolic groups in foods. J Food Sci. 56(1): 128–131.

Brune M, Rossander L, Hallberg L. 1989. Iron absorption and phenolic compounds: importance of different phenolic structures. Eur J Clin Nutr. 43(8): 547–557.

Cade JE, Moreton JA, Hara BO, Greenwood DC, Moor J, Burley VJ, O'Hara B, Greenwood DC, Moor J, Burley VJ. 2005. Diet and genetic factors associated with iron status in middle-aged women. Am J Clin Nutr. 82(1): 813–820.

Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Özkan H. 2004. Triticum dicoccoides : An important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Sci Plant Nutr. 50(7): 1047–1054.

Carvajal-Zarrabal O, Barradas-Dermitz, DM, Orta-Flores Z, Hayward-Jones PM, Nolasco-Hipólito C, Aguilar-Uscanga, MG, Miranda-Medina A, Bujang KB. 2012. *Hibiscus sabdariffa L*, roselle calyx, from ethnobotany to pharmacology. J Exp Pharmacol. 4(1):

25–39.

Cercamondi CI, Egli IM, Zeder C, Hurrell RF. 2014a. Sodium iron EDTA and ascorbic acid, but not polyphenol oxidase treatment, counteract the strong inhibitory effect of polyphenols from brown sorghum on the absorption of fortification iron in young women. Br J Nutr. 111(3): 481–489.

Cercamondi CI, Icard-Vernière C, Egli IM, Vernay M, Hama F, Brouwer ID, Zeder C, Berger J, Hurrell RF, Mouquet-Rivier C. 2014b. A higher proportion of iron-rich leafy vegetables in a typical Burkinabe maize meal does not increase the amount of iron absorbed in young women. J Nutr. 144(9): 1394–1400.

Chadare FJ, Linnemann AR, Hounhouigan JD, Nout MJR, Van Boekel MAJS. 2008. Baobab food products: a review on their composition and nutritional value. Crit Rev Food Sci Nutr. 49(3): 254–274.

Chang S, Chen M. 1992. Effects of dietary fat on lipid composition and iron uptake of intestinal brush-border membrane of rats. J Nutr Biochem. 3(3): 135–139.

Charalampopoulos D, Wang R, Pandiella SS, Webb C. 2002. Application of cereals and cereal components in functional foods: A review. Int J Food Microbiol. 79(1): 131–141.

Christian KR, Jackson JC. 2009. Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (Hibiscus sabdariffa) during maturity. J Food Compos Anal. 22(7): 663–667.

Cicco N, Lanorte MT, Paraggio M, Viggiano M, Lattanzio V. 2009. A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts. Microchem J. 91(1): 107–110.

Cook JD, Noble NL, Morck TA, Lynch SR, Petersburg SJ. 1983. Effect of fibre on nonheme iron absorption. Gastroenterology. 85(1): 1354–1358.

Cook JD, Reddy MB, Burri J, Juillerat MA, Hurrell RF. 1997. The influence of different infant cereal grains on iron absorption. Am J Clin Nutr. 65(1): 964–969.

Cook JD, Watson SS, Simpson KM, Lipschitz D, Skikne BS. 1984. The effect of high ascorbic acid supplementation on body iron stores. Blood. 64(3): 721–726.

Cowin I, Emond A, Emmett P. 2001. Association between composition of the diet and haemoglobin and ferritin levels in 18-month-old children. Eur J Clin Nutr. 55(4): 278–286.

Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. 2014. Hibiscus sabdariffa L. - A phytochemical and pharmacological review. Food Chem. 165(1): 424–443.

Davidsson L, Almgren A, Sandström B, Hurrell RF. 1995. Zinc absorption in adult humans: the effect of iron fortification. Br J Nutr. 74(3): 417–425.

Dawson-Hughes B, Seligson FH, Hughes VA. 1986. Effects of calcium-carbonate and hydroxyapatite on zinc and iron retention in postmenopausal women. Am J Clin Nutr. 44(1): 83–88.

Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. 2014. Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fibre: A review. J Food Sci Technol. 51(6): 1021–1040.

Domellöf M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, Hojsak I, Mihatsch W, Molgaard C, Shamir R. 2014. Iron requirements of infants and toddlers. J Pediatr Gastroenterol Nutr. 58(1): 119–129.

Dost K, Tokul O. 2006. Determination of phytic acid in wheat and wheat products by reverse phase high performance liquid chromatography. Anal Chim Acta. 558(1): 22–27.

Ejigui J, Savoie L, Marin J, Desrosiers T. 2005. Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and antinutritional factors of yellow maize (Zea mays). J Biol Sci. 5(5): 590–596.

Etcheverry P, Grusak MA, Fleige LE. 2012. Application of *in vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B 6, B 12, D, and E. Front Physiol. 3(317): 1–22.

Fairweather-Tait SJ, Wharf SG, Fox TE. 1995. Zinc absorption in infants fed iron-fortified weaning food. Am J Clin Nutr. 62(1): 785–789.

FAO/WHO. 2001. Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation. Bangkok: FAO/WHO

FAOSTAT. 2017. FAOSTAT [Internet]. [cited 2015 Jul 29]. Available from: http: //www.fao.org/faostat/en/#home

Febles CI, Arias A, Hardisson A, Rodríguez-Alvarez C, Sierra A. 2002. Phytic acid level in wheat flours. J Cereal Sci. 36(1): 19–23.

Ferguson EL, Gibson RS, Thompson LU, Ounpuu S, Berry M. 1988. Phytate, zinc, and calcium contents of 30 East African foods and their calculated phytate: Zn, Ca: phytate, and [Ca][phytate]/[Zn] molar ratios. J Food Compos Anal. 1(4): 316–325.

Ferreira PPM, Farias DF, Oliveira JTDA, Carvalho ADF. 2008. *Moringa oleifera* : bioactive compounds and nutritional potential *Moringa oleifera* : compostos bioativos e potencialidade nutricional. Rev Nutr. 21(4): 431–437.

Fischer Walker CL, Ezzati M, Black RE. 2009. Global and regional child mortality and

burden of disease attributable to zinc deficiency. Eur J Clin Nutr. 63(5): 591–597.

Fleming DJ, Tucker KL, Jacques PF, Dallal GE, Wilson PWF, Wood RJ. 2002. Dietary factors associated with the risk of high iron stores in the elderly Framingham Heart Study cohort. Am J Clin Nutr. 76(6): 1375–1384.

Fordyce EJ, Forbes RM, Robbins KR, Erdman JW. 1987. Phytate × calcium/zinc molar ratios: are they predictive of zinc bioavailability? J Food Sci. 52(2): 440–444.

Friman L, Hoglund H, Hogberg HE, Agnemo R. 2004. Tannin-iron impregnated thermomechanical pulp. Part I: Effects of extractions and heat on brightness. Nord Pulp Pap Res J. 19(2): 229–236.

Frontela C, García-Alonso FJ, Ros G, Martínez C. 2008. Phytic acid and inositol phosphates in raw flours and infant cereals: The effect of processing. J Food Compos Anal. 21(4): 343–350.

Frühbeck G, Alonso R, Marzo F, Santidrián S. 1995. A modified method for the indirect quantitative analysis of phytate in foodstuffs. Anal Biochem. 225(2): 206–212.

García-Casal MN, Layrisse M, Solano L, Baron MA, Arguello F, Llovera D, Ramırez J, Leets I, Tropper E. 1998. Vitamin A and B-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. J Nutr. 128(1): 646–650.

García-Casal MN. 2006. Carotenoids increase iron absorption from cereal-based food in the human. Nutr Res. 26(1): 340–344.

Gautam S, Platel K, Srinivasan K. 2010. Influence of B-carotene rich vegetables on the bioaccessibility of zinc and iron from food grains. Food Chem. 122(3): 668–672.

Gebauer J, El-Siddig K, Ebert G. 2002. Baobab (*Adansonia digitata* L.): a Review on a multipurpose tree with promising future in the Sudan. Gartenbauwissenschaft. 67(4): 155–160.

Gibson RS, Bailey KB, Gibbs M, Ferguson EL. 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. Food Nutr Bull. 31(Suppl 2): S134–S146.

Gibson RS, Ferguson EL, Lehrfeld J. 1998. Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. Eur J Clin Nutr. 52(1): 764–770.

Gibson RS, Hotz C. 2001. Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. Br J Nutr. 85(Suppl 2): S159–S166.

Gibson RS. 2006. Zinc: the missing link in combating micronutrient malnutrition in

developing countries. Proc Nutr Soc. 65(1): 51–60.

Gidamis AB, Panga JT, Sarwatt S V, Chove BE, Shayo NB. 2003. Nutrient and antinutrient contents in raw and cooked young leaves and immature pods of *Moringa oleifera* , Lam. Ecol Food Nutr. 42(6): 399–411.

Gillooly M, Bothwell TH, Torrance JD, Macphail AP, Derman DP, Bezwoda WR, Mills W, Charlton RW, Mayet F. 1983. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br J Nutr. 49(3): 331–342.

Glahn RP, Wortley GM, South PK, Miller DDD. 2002. Inhibition of iron uptake by phytic acid, tannic acid, and ZnCl2: Studies using an *in vitro* digestion/Caco-2 cell model. J Agric Food Chem. 50(2): 390–395.

Glew RH, Vanderjagt DJ, Lockett C, Grivetti LE, Smith GC, Pastuszyn A, Millson M. 1997. Amino acid, fatty acid, and mineral composition of 24 indigenous plants of Burkina Faso. J Food Compos Anal. 10(3): 205–217.

Greger JL, Lyle BJ. 1988. Iron, copper and zinc metabolism of rats fed various levels and types of tea. J Nutr. 118(1): 52–60.

Greiner T. 2013. Vitamin A: Moving the food-based approach forward. Seoul: FAO and WHO.

Gwamba J. 2016. Pearl millet: Influence of mineral biofortification and simple processing technologies on minerals and antinutrients. Pretoria: University of Pretoria.

Hallberg L, Brune M, Erlandsson M, Sandberg A, Rossander-Hulthen L. 1991. Calcium: Effect of different amounts on nonheme-iron and heme-iron arsorption in humans. J Nutr Educ. 53(1): 112–119.

Hallberg L, Hulthén L. 2000. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. Am J Clin Nutr. 71(1): 1147–1160.

Hambidge KM, Huffer JW, Raboy V, Grunwald GK, Westcott JL, Sian L, Miller LV, Dorsch JA, Krebs NF. 2004. Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids. Am J Clin Nutr. 79(6): 1053–1059.

Heaney R. 2001. Bioavailability of nutrients and other bioactive components from dietary supplements. J Nutr. 131(4): 1376–1382.

Hemalatha S, Platel K, Srinivasan K. 2007. Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. Food Chem. 102(4): 1328–1336.

Hess SY, Thurnham DI, Hurrell RF. 2005. Influence of provitamin A carotenoids on iron, zinc, and vitamin A status. Washington DC: HarvestPlus

Hidalgo A, Brandolini A. 2014. Nutritional properties of einkorn wheat (Triticum monococcum L.). J Sci Food Agric. 94(4): 601–612.

Hounsome N, Hounsome B, Tomos D, Edwards-Jones G. 2008. Plant metabolites and nutritional quality of vegetables. J Food Sci. 73(4): R48–R65.

Hung PV, Duy TL. 2012. Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. Int Food Res J. 19(1): 327–332.

Hurrell RF, Egli I. 2007. Optimizing the bioavailability of iron compounds for food fortification. In: Kraemer K, Zimmermann MB, editors. Nutritional Anemia. Basel: Sight and Life Press; p. 77.

Hurrell RF, Lynch S, Bothwell T, Cori H, Glahn R, Hertrampf E, Kratky Z, Miller D, Rodenstein M, Streekstra H, et al. 2004. Enhancing the absorption of fortification iron: A Sustain Task Force report. Int J Vitam Nutr Res. 74(6): 387–401.

Hurrell RF, Reddy MB, Cook JD. 1999. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. Br J Nutr. 81(4): 289–295.

Hurrell RF. 2002. How to ensure adequate iron absorption from iron-fortified food. Nutr Rev. 60(7): S7–S15.

Icard-Vernière C, Olive F, Picq C, Mouquet-Rivier C. 2015. Contribution of leafy vegetable sauces to dietary iron, zinc, vitamin a and energy requirements in children and their mothers in Burkina Faso. Plant Foods Hum Nutr. 70(1): 63–70.

IZiNCG. 2004. Assessment of the risk of zinc deficiency in populations and options for its control. Brown KH, Rivera JA, Bhutta ZA, Gibson RS, King JC, Lönnerdal B, Ruel MT, Sandström B, Wasantwisut E, Hotz C, et al, editors. Toronto, Canada: The United Nations University.

Johnson PE, Lukaski HC, Bowman TD. 1987. Effects of level and saturation of fat and iron level and type in the diet on iron absorption and utilization by the rat. J Nutr. 117(3): 501–507.

Johnson PE. 1982. A mass spectrometric method for use of stable isotopes as tracers in studies of iron, zinc, and copper absorption in human subjects. J Nutr. 112(1): 1214–1424.

Jukanti AK, Gowda CLL, Rai KN, Manga VK, Bhatt RK. 2016. Crops that feed the world 11. Pearl Millet (*Pennisetum glaucum* L.): an important source of food security, nutrition and health in the arid and semi-arid tropics. Food Secur. 8(2): 307–329.

Khokhar S, Apenten RKO. 2003. Iron binding characteristics of phenolic compounds : some

tentative structure – activity relations. Food Chem. 81(1): 133–140.

Klopfenstein CF, Hoseney RC. 1995. Nutritional properties of sorghum and the millets. In: Dendy DAV, editor. Sorghum and millets: chemistry and technology. St. Paul: American Association of Cereal Chemists; p. 125–168.

Krebs NF. 2000. Overview of zinc absorption and excretion in the human gastrointestinal tract. J Nutr. 130(5): 1374S–1377S.

Kumar V, Sinha AK, Makkar HPS, Becker K. 2010. Dietary roles of phytate and phytase in human nutrition: A review. Food Chem. 120(4): 945–959.

Lamien-Meda A, Lamien CE, Compaoré MMY, Meda RNT, Kiendrebeogo M, Zeba B, Millogo JF, Nacoulma OG. 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules. 13(3): 581–594.

Léder I. 2004. Sorghum and millets. In: Fuleky G. Cultivated plants, primarily as food sources. Encyclopedia of life support systems. Paris: EOLSS Publishers Company Limited; p. 66-83

Leone A, Fiorillo G, Criscuoli F, Ravasenghi S, Santagostini L, Fico G, Spadafranca A, Battezzati A, Schiraldi A, Pozzi F. 2015. Nutritional characterization and phenolic profiling of *Moringa oleifera* leaves grown in Chad, Sahrawi refugee camps, and Haiti. Int J Mol Sci. 16(8): 18923–18937.

Lestienne I, Icard-Vernière C, Mouquet C, Picq C, Trèche S. 2005. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. Food Chem. 89(3): 421–425.

Lönnerdal B, Cederblad A, Davidsson L, Sandström B. 1984. The effect of individual components of soy formula and cows' milk formula on zinc bioavailability. Am J Clin Nutr. 40(5): 1064–1070.

Lönnerdal B. 2000. Zinc and health : current status and future directions. Dietary factors influencing zinc absorption. J Nutr. 130: S1378-1383.

Lopez HW, Leenhardt F, Coudray C, Remesy C. 2002. Minerals and phytic acid interactions: is it a real problem for human nutrition? Int J Food Sci Technol. 37(1): 727–739.

Lopez-Martinez LX, Oliart-Ros RM, Valerio-Alfaro G, Lee CH, Parkin KL, Garcia HS. 2009. Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. LWT - Food Sci Technol. 42(6): 1187–1192.

Low JW, Arimond M, Osman N, Cunguara B, Zano F, Tschirley D. 2007. A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. J Nutr. 137(5): 1320–1327.

Luten J, Crews H, Flynn A, Van Dael P, Kastenmayer P, Hurrell RF, Deelstra H, Shen LH, Fairweather-Tait S, Hickson K. 1996. Interlaboratory trial on the determination of the *in vitro* iron dialysability from food. J Sci Food Agric. 72(4): 415–424.

Magaia T, Uamusse A, Sjöholm I, Skog K. 2013. Dietary fibre, organic acids and minerals in selected wild edible fruits of Mozambique. Springerplus. 2(1): 88.

Magalhães LM, Santos F, Segundo MA, Reis S, Lima JLFC. 2010. Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity. Talanta. 83(2): 441–447.

Mahan LK, Raymond JL. 2016. Krause's food & the nutrition care process. 14th ed. Missouri: Elsevier; [Accessed 2017 October 29]. https://books.google.co.za/books?id=DXIwDAAAQBAJ&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false.

Makkar HPS, Becker K. 1996. Nutrional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Anim Feed Sci Technol. 63(1): 211–228.

Manthey JA, Penelope PV. 2009. Influences of harvest date and location on the levels of β-carotene, ascorbic acid, total phenols, the *in vitro* antioxidant capacity, and phenolic profiles of five commercial varieties of mango (*Mangifera indica* L.). J Agric Food Chem. 57(22): 10825–10830.

Mason JB, Greiner T, Shrimpton R, Sanders D, Yukich J. 2015. Vitamin A policies need rethinking. Int J Epidemiol. 44: 283–292.

Mason JB, Lotfi M, Dalmiya N, Sethuraman K, Deitchler M. 2001. The micronutrient report. Current progress and trends in the control of vitamin A, iodine and iron deficiencies. Ottawa: The Micronutrient Initiative.

Matuschek E, Towo E, Svanberg U. 2001. Oxidation of polyphenols in phytate-reduced high-tannin cereals: Effect on different phenolic groups and on *in vitro* accessible iron. J Agric Food Chem. 49(11): 5630–5638.

Maxson ED, Rooney LW. 1972. Evaluation of methods for tannin analysis in sorghum grain. Cereal Chem. 49(6): 719–729.

McKevith B. 2004. Nutritional aspects of cereals. Br Nutr Found Nutr Bull. 29(2): 111–142.

Mendoza C, Viteri FE, Lönnerdal B, Young KA, Raboy V, Brown KH. 1998. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. Am J Clin Nutr. 68(5): 1123–1127.

Milgate J, Roberts DCK. 1995. The nutritional & biological significance of saponins. Nutr Res. 15(8): 1223–1249.

Miller DD, Schricker BR, Rasmussen RR, Van Campen D. 1981. An *in vitro* method for estimation of iron availability from meals. Am J Clin Nutr. 34(10): 2248–2256.

Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, Carrière F, Boutrou R, Corredig M, Dupont D, and Dufour C. 2014. A standardised static in vitro digestion method suitable for food–an international consensus. Food and function. 5(6): 1113-1124

Minekus M, Marteau P, Havenaar R. 1995. Multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. ATLA Altern Lab Anim. 23(1): 197-209

Moran JF, Klucas R V, Grayer RJ, Abian J, Becana M. 1997. Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: Prooxidant and antioxidant properties. Free Radic Biol Med. 22(5): 861–870.

Motarjemi Y, Nout MJR. 1996. Food fermentation: a safety and nutritional assessment. Bull World Health Organ. 74(6): 553–559.

Moyo B, Masika P, Hugo A, Muchenje V. 2011. Nutritional characterization of moringa (*Moringa oleifera* Lam.) leaves. African J Biotechnol. 10(60): 12925–12933.

Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. 2013. The global hidden hunger indices and maps: an advocacy tool for action. PLoS One. 8(6): 1–12.

Muzhingi T, Gadaga TH, Siwela AH, Grusak MA, Russell RM, Tang G. 2011. Yellow maize with high β-carotene is an effective source of vitamin A in healthy Zimbabwean men. Am J Clin Nutr. 94(2): 510–519.

National Institute of Health. 2016. Office of dietary supplements - dietary supplement fact sheet: Iron [Internet]. [cited 2016 Mar 17]. Available from: https: //ods.od.nih.gov/factsheets/Iron-HealthProfessional/

Navert B, Sandström B. 1985. Reduction of the phytate content. Br J Nutr. 53(1): 47–53.

Negesse T, Makkar HPS, Becker K. 2009. Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an *in vitro* gas method. Anim Feed Sci Technol. 154(3): 204–217.

NHMRC National Health and Medical Research Council. 2005. Phosphorus. Nutrient reference values for Australia and New Zealand, including recommended dietary intakes. [Accessed September 2005]. www.nhmrc.gov.au

Nicolle C, Simon G, Rock E, Amouroux P, Remesy C. 2004. Genetic variability influences carotenoid, vitamin, phenolic, and mineral content in white, yellow, purple, orange, and dark-orange carrot cultivars. J Am Soc Hortic Sci. 129(4): 523–529.

Nikmaram N, Leong SY, Koubaa M, Zhu Z, Barba FJ, Greiner R, Oey I, Roohinejad S. 2017.

Effect of extrusion on the anti-nutritional factors of food products: An overview. Food Control. 79(1): 62–73.

Nisperos-Carriedo MO, Buslig BS, Shaw PE. 1992. Simultaneous detection of dehydroascorbic, ascorbic, and some organic acids in fruits and vegetables by HPLC. J Agric Food Chem. 40(7): 1127–1130.

Nitithan S, Komindr S, Nichachotsalid A. 2004. Phytate and fibre content in Thai fruits commonly consumed by diabetic patients. J Med Assoc Thail. 87(12): 1444–1446.

Nnam N, Onyeke N. 2003. Chemical composition of two varieties of sorrel (Hibiscus sabdariffa L.), calyces and the drinks made from them. Plant Foods Hum Nutr. 58(1): 1–7.

Odhav B, Beekrum S, Akula U, Baijnath H. 2007. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. J Food Compos Anal. 20(5): 430–435.

Ojokoh AO. 2006. Roselle (Hibiscus sabdariffa) calyx diet and histopathological changes in liver of albino rats. Pakistan J Nutr. 5(2): 110–113.

Olney DK, Rawat R, Ruel MT. 2012. Identifying potential programs and platforms to deliver multiple micronutrient interventions. J Nutr. 142(Suppl. 1): 178S–185S.

Oniang'o RK, Mutuku JM, Malaba SJ. 2003. Contemporary African food habits and their nutritional and health implications. Asia Pac J Clin Nutr. 12(3): 331–336.

Onofiok NO, Nnanyelugo DO. 1998. Weaning foods in West Africa: Nutritional problems and possible solutions. Food Nutr Bull. 19(1): 27–33.

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Peña RJ. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J Cereal Sci. 46(3): 293–307.

Osman MA. 2004. Chemical and nutrient analysis of baobab (*Adansonia digitata*) fruit and seed protein solubility. Plant Foods Hum Nutr. 59(1): 29–33.

Pabón ML, Lönnerdal B. 1993. Effect of citrate on zinc bioavailability from milk, milk fractions and infant formulas. Nutr Res. 13(1): 103–111.

Pabón ML, Lönnerdal B. 2001. Effects of type of fat in the diet on iron bioavailability assessed in suckling and weanling rats. J Trace Elem Med Biol. 15(1): 18–23.

Parker RS. 1996. Absorption, metabolism, and transport of carotenoids. Faseb J. 10(5): 542–551.

Parkouda C, Sanou H, Tougiani A, Korbo A, Nielsen DS, Tano-Debrah K, Ræbild A, Diawara B, Jensen JS. 2012. Variability of Baobab (*Adansonia digitata* L.) fruits'

physical characteristics and nutrient content in the West African Sahel. Agrofor Syst. 85(3): 455–463.

Patras A, Brunton N, Da Pieve S, Butler F, Downey G. 2009. Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purees. Innov Food Sci Emerg Technol. 10(1): 16–22.

Péneau S, Dauchet L, Vergnaud AC, Estaquio C, Kesse-Guyot E, Bertrais S, Latino-Martel P, Hercberg S, Galan P. 2008. Relationship between iron status and dietary fruit and vegetables based on their vitamin C and fibre content. Am J Clin Nutr. 87(5): 1298–1305.

Poutanen K, Flander L, Katina K. 2009. Sourdough and cereal fermentation in a nutritional perspective. Food Microbiol. 26(7): 693–699.

Price ML. 2007. The Moringa tree. ECHO Tech Note.: 1–19.

Puupponen-Pimiä R, Häkkinen ST, Aarni M, Suortti T, Lampi AM, Eurola M, Piironen V, Nuutila AM, Oksman-Caldentey KM. 2003. Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. J Sci Food Agric. 83(14): 1389–1402.

Radhakrishnan MR, Sivaprasad J. 1980. Tannin content of sorghum varieties and their role in iron bioavailability. J Agric Food Chem. 28(1): 55–57.

Ragaee S, Abdel-Aal ESM, Noaman M. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. Food Chem. 98(1): 32–38.

Ravindran G. 1991. Studies on millets: Proximate composition, mineral composition, and phytate and oxalate contents. Food Chem. 39(1): 99–107.

Reale A, Konietzny U, Coppola R, Sorrentino E, Greiner R. 2007. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. J Agric Food Chem. 55(8): 2993–2997.

Reddy CVK, Sreeramulu D, Raghunath M. 2010. Antioxidant activity of fresh and dry fruits commonly consumed in India. Food Res Int. 43(1): 285–288.

Reilly C. 2002. Metal contamination of food: Its significance for food quality and human health. 3rd Ed. Oxford: Blackwell Science

Richter N, Siddhuraju P, Becker K. 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (Oreochromis niloticus L.). Aquaculture. 217(1): 599–611.

Rocha Ribeiro SM, Queiroz JH, Lopes Ribeiro de Queiroz ME, Campos FM, Pinheiro Sant'ana HM. 2007. Antioxidant in mango (*Mangifera indica* L.) pulp. Plant Foods Hum

Nutr. 62(1): 13–17.

Saharan M. 2015. Development and nutritional evaluation of pearl millet (*Pennisetum glaucum*) based convenience foods. [place unknown]: Chaudhary Charan Singh Haryana Agricultural University.

Salovaara S, Sandberg A-S, Andlid T. 2002. Organic acids influence iron uptake in the human epithelial cell line Caco-2. J Agric Food Chem. 50(21): 6233–6238.

Sandström B, Cederblad A. 1987. Effect of ascorbic acid on the absorption of zinc and calcium in man. Int J Vitam Nutr Res. 57(1): 87–90.

Sandström B, Sandberg A. 1992. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. J Trace Elem Electrolytes Health Dis. 6(2): 99–103.

Sandström B. 2001. Micronutrient interactions: effects on absorption and bioavailability. Br J Nutr. 85(Suppl 2): S181–S185.

Schofield P, Mbugua DM, Pell AN. 2001. Analysis of condensed tannins: A review. Anim Feed Sci Technol. 91(1): 21–40.

Sena LP, Vanderjagt DJ, Rivera C, Tsin ATC, Muhamadu I, Mahamadou O, Millson M, Pastuszyn A, Glew RH. 1998. Analysis of nutritional components of eight famine foods of the Republic of Niger. Plant Foods Hum Nutr. 52(1): 17–30.

Shahidi F, Chandrasekara A. 2013. Millet grain phenolics and their role in disease risk reduction and health promotion: A review. J Funct Foods. 5(2): 570–581.

Sharma DC, Mathur R. 1995. Correction of anemia and iron deficiency in vegetarians by administration of ascorbic acid. Indian J Physiol Pharmacol. 39(4): 403–406.

Shashi BK, Sharan S, Hittalamani S, Shankar AG, Nagarathna TK. 2007. Micronutrient composition, antinutritional factors and bioaccessibility of iron in different finger millet (*Eleusine coracana*) genotypes. Karnataka J Agric Sci. 20(3): 583–585.

Siddhuraju P, Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem. 51(8): 2144–2155.

Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG, Schmidt U, Tal A, Mayet F. 1991. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am J Clin Nutr. 53(1): 537–541.

Solomons NW, Jacob RA, Pineda O, Viteri FE. 1979. Studies on the bioavailability of zinc in man. III Effects of ascorbic acid on zinc absorption. Am J Clin Nutr. 32(1): 2495–2499.

Solomons NW, Jacob RA. 1981. Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. Am J Clin Nutr. 34(1): 475–482.

Solomons NW. 1999. Dietary sources of zinc and factors affecting its bioavailability. In: Brown KH, editor. Spec Issue Recent Interv Trials with Zinc Implic Programs Res Defic. 22nd ed. [place unknown]: Food and Nutrition Bulletin; p. 138–154.

Spencer H, Kramer L, Norris C, Osis D. 1984. Effect of calcium and phosphorus metabolism in man. Am J Clin Nutr. 40(1): 1213–1218.

Sreelatha S, Padma PR. 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum Nutr. 64(4): 303–311.

Stadlmayr B, Charrondière UR, Eisenwagen S, Jamnadass R, Kehlenbeck K. 2013. Review: Nutrient composition of selected indigenous fruits from sub-Saharan Africa. J Sci Food Agric. 93(1): 2627–2636.

Stekel A, Olivares M, Pizarro F, Chadud P, Lopez I, Amar M. 1986. Absorption of fortification iron from milk formulas in infants. Am J Clin Nutr. 43(1): 917–922.

Stratil P, Klejdus B, Kubáň V. 2006. Determination of total content of phenolic compounds and their antioxidant activity in vegetables - Evaluation of spectrophotometric methods. J Agric Food Chem. 54(3): 607–616.

Suma PF, Urooj A. 2014. Influence of germination on bioaccessible iron and calcium in pearl millet (Pennisetum typhoideum). J Food Sci Technol. 51(5): 976–81.

Svanberg U, Lorri W, Sandberg A. 1993. Lactic fermentation of non-tannin and high-tannin cereals: effects on *in vitro* estimation of iron availability and phytate hydrolysis. J Food Sci. 58(2): 408–412.

Tembo DT, Holmes MJ, Marshall LJ. 2017. Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (*Adansonia digitata*) pulp from Malawi. J Food Compos Anal. 58(1): 40–51.

Teucher B, Olivares M, Cori H. 2004. Enhancers of iron absorption: Ascorbic acid and other organic acids. Int J Vitam Nutr Res. 74(6): 403–419.

Thane CW, Walmsley CM, Bates CJ, Prentice A, Cole TJ. 2000. Risk factors for poor iron status in British toddlers: further analysis of data from the National Diet and Nutrition Survey of children aged 1.5-4.5 years. Public Health Nutr. 3(4): 433–440.

Thompson B. 2007. Food-based approaches for combating iron deficiency. In: Kraemer K, Zimmermann MB, editors. Nutritional Anemia. Switzerland: Sight and Life Press; p. 337–358.

TNO. 2013. TIM Gastrointestinal Models. A quick, cost-efficient and reliable approach for pharmaceutical research. The Hague: TNO

Towo E, Matuschek E, Svanberg U. 2006. Fermentation and enzyme treatment of tannin

sorghum gruels: Effects on phenolic compounds, phytate and *in vitro* accessible iron. Food Chem. 94(3): 369–376.

Tripathi B, Chetana, Platel K. 2010. Fortification of sorghum (Sorghum vulgare) and pearl millet (*Pennisetum glaucum*) flour with zinc. J Trace Elem Med Biol. 24(4): 257–262.

Tripathi B, Platel K. 2010. Finger millet (Eleucine coracana) flour as a vehicle for fortification with zinc. J Trace Elem Med Biol. 24(1): 46–51.

Tsuruta Y, Nagao K, Kai S, Tsuge K, Yoshimura T, Koganemaru K, Yanagita T. 2011. Polyphenolic extract of lotus root (edible rhizome of Nelumbo nucifera) alleviates hepatic steatosis in obese diabetic db/db mice. Lipids Health Dis. 10(1): 202-209.

Turnlund JR, King JC, Keyes WR, Gong B, Michel MC. 1984. A stable isotope study of zinc absorption in young men: effects of phytate and alpha-cellulose. Am J Clin Nutr. 40(5): 1071–1077.

U.S. EPA. 1994. Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry, Revision 4.4. [place unknown]: U.S. Environmental Protection Agency.

Umaru HA, Adamu R, Dahiru D, Nadro MS. 2007. Levels of antinutritional factors in some wild edible fruits of Northern Nigeria. African J Biotechnol. 6(16): 1935–1938.

USDA Nutrient Database. 2015. USDA National Nutrient Database for Standard Reference [Internet]. [cited 2015 Sep 1]. Available from: https: //ndb.nal.usda.gov/

Van Campen DR, Glahn RP. 1999. Micronutrient bioavailability techniques: Accuracy, problems and limitations. F Crop Res. 60(1): 93–113.

van Het Hof KH, West CE, Weststrate J a, Hautvast JG. 2000. Dietary factors that affect the bioavailability of carotenoids. J Nutr. 130(3): 503–506.

von Drygalski A, Adamson JW. 2012. Iron Metabolism in Man. J Parenter Enter Nutr. 37(5): 599-606

Vreugdenhil D, Aarts MGM, Koornneef M. 2005. Exploring natural genetic variation to improve plant nutrient content. In: Broadley MR, White PJ, editors. Plant Nutr Genomics. Cornwall, UK: Blackwell Publishing and CRC Press; p. 201–219.

Walter A, Rimbach G, Most E, Pallauf J. 1998. Effect of citric acid supplements to a maize-soya diet on the *in vitro* availability of minerals, trace elements, and heavy metals. Zentralbl Veterinarmed A. 45(9): 517–524.

Waterman PG, Mole S. 1994. Analysis of phenolic plant metabolites. Oxford, UK: Blackwell Scientific Publications.

Wessells KR, Brown KH. 2012. Estimating the global prevalence of zinc deficiency: Results

based on zinc availability in national food supplies and the prevalence of stunting. PLoS One. 7(11): e50568.

WHO. 2008. Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. de Benoist B, McLean E, Egli I, Cogswell M, editors. Geneva: WHO Press.

WHO. 2009. Global prevalence of vitamin A deficiency in populations at risk 1995-2005. WHO global database on vitamin A deficiency. Geneva: WHO Press.

WHO. 2014. The health of the people: What works - The African regional health report of 2014. Luxembourg.

WHO. 2015. The global prevalence of anemia in 2011. Geneva, Switzerland: WHO Press.

WHO/FAO. 2004. Vitamin and mineral requirements in human nutrition. 2nd ed. [place unknown]: World Health Organization and Food and Agriculture Organization of the United Nations.

WHO/FAO. 2006. Guidelines on food fortification with micronutrients. Allen LH, de Benoist B, Dary O, Hurrell RF, editors. Geneva, Switzerland: WHO Press.

WHO/UNICEF/UNU. 2001. Iron deficiency anaemia: assessment, prevention and control, a guide for program managers. Geneva: WHO Press.

Wolters MG, Schreuder HA, van den Heuvel G, van Lonkhuijsen HJ, Hermus RJ, Voragen AG. 1993. A continuous *in vitro* method for estimation of the bioavailability of minerals and trace elements in foods: application to breads varying in phytic acid content. Br J Nutr. 69(1): 849–861.

Yan L, Prentice A, Dibba B, Jarjou LM, Stirling DM, Fairweather-Tait SJ. 1996. The effect of long-term calcium supplementation on indices of iron, zinc and magnesium status in lactating Gambian women. Br J Nutr. 76(6): 821–831.

Yang R, Chang L, Hsu J, Weng BBC, Palada MC, Chadha ML, Levasseur V. 2006. Nutritional and functional properties of Moringa leaves–from germplasm, to plant, to food, to health. Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Paris: Moringanews, CDE, CTA, GFU.

Zhou K, Yu L. 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT - Food Sci Technol. 39(10): 1155–1162.

Žilić S, Serpen A, Akillioğlu G, Gökmen V, Vančetović J. 2012. Phenolic compounds, carotenoids, anthocyanins, and antioxidant capacity of colored maize (Zea mays L.) kernels. J Agric Food Chem. 60(5): 1224–1231.

Zimmermann MB, Hurrell RF. 2007. Nutritional iron deficiency. Lancet. 370: 511–520.

APPENDIX A: PUBLICATIONS PRESENTED BASED ON THIS RESEARCH

Van der Merwe, R., Taylor, J.R.N., and Kruger, J. Micronutrient-rich plant foodstuffs has the potential to increase iron and zinc nutritive values from a cereal-based porridge. Poster presentation at the 3rd International Congress on Hidden Hunger, Stuttgart, Germany, 20-22 March 2017.

Van der Merwe, R., Taylor, J.R.N., and Kruger, J. Food-to-food fortification of pearl millet instant porridge to increase iron and zinc nutritive values. Poster presentation at SAAFoST 22nd Biennial International Congress & Exhibition, Cape Town, South Africa, 3-6 Septermber 2017

Van der Merwe, R., Taylor, J.R.N., and Kruger, J. Food-to-food fortification of pearl millet instant porridge to increase iron and zinc nutritive values. Poster presentation at IUNS 21st International Congress of Nutrition, Buenos Aires, Argentina, 15-20 October 2017.

Mineral-rich plant foods has the potential to increase iron and zinc nutritive values from an instant cereal-based porridge

Reneè van der Merwe, John RN Taylor and Johanita Kruger

Department of Food Science, University of Pretoria, Private bag X20, Hatfield 0028, South Africa
Renee.vdmerwe89@gmail.com, john.taylor@up.ac.za, johanita.kruger@up.ac.za

INTRODUCTION

Sub-Saharan Africa has the highest hidden hunger rates in the world, 18 of the 20 countries with the highest micronutrient malnutrition scores are in this region[1]. Some of the most critical and widespread micronutrient deficiencies are of iron, zinc and vitamin A[2]. Homogenous plant-based diets of low nutritional quality, as well as frequent infections, are likely contributory factors[1]. Cereal-based foods are low in iron and zinc availability enhancers and high in inhibitors, resulting in low bioavailability[3]. Often less than 5% of iron, and less than 10% of zinc is available for absorption[5].

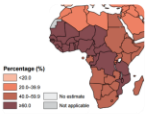

Figure 1: Prevalence of anaemia in infants and children aged 6–59 months in Africa, 2011[4].

AIM

To establish the effect of various iron and zinc rich plant floods on the mineral bioaccessibility of a cereal-based instant porridge. Various concentrations and combinations of the plant foods was used to evaluate synergistic effects.

EXPERIMENTAL

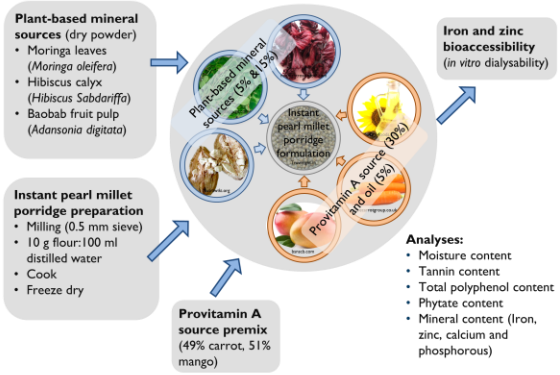

Figure 2: Sample characterisation analyses of pearl millet, moringa, hibiscus, baobab, and provitamin A source; porridge formulation with various amounts and combinations (5% &15%) of plant-based mineral sources, pearl millet (50%), provitamin A source (30%) and oil (5%) to study the effect on iron and zinc bioaccessibilities.

RESULTS AND DISCUSSION

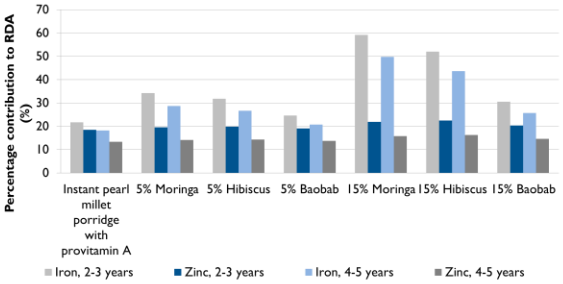

Figure 3: The effects of adding various amounts (5 & 15%) of moringa, hibiscus and baobab to pearl millet-based instant porridges on the percentage contribution to iron and zinc recommended dietary allowance (RDA) for children aged 2-5 years.
*Porridge would consist of 30% instant porridge flour and 70% water
*Portion sizes are 100 g and 120 g for children aged 2-3 and 4-5 years[6], respectively

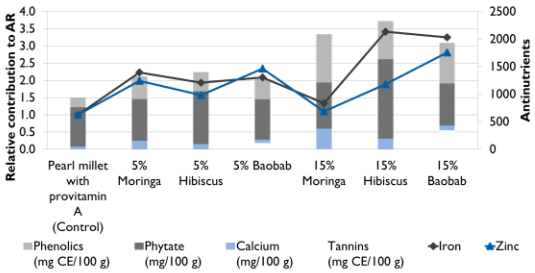

Figure 4: The effect of adding various amounts (5 & 15%) of moringa, hibiscus and baobab to pearl millet-based instant porridges on condensed tannins, phytate, total phenolics and calcium contents and the relative contribution to iron and zinc absolute (physiological) requirements (AR) for children aged 2-5 years.

- Percentage contribution towards the RDA for children aged 2-3 years to iron was 19% higher and zinc was 39% higher than children aged 4-5 years.
- Increased addition of hibiscus and baobab increased iron and zinc AR
- Moringa (15%) contributed the most to the iron and zinc RDA (Figure 3), however due to high antinutrient content (Figure 4), moringa addition (5%) roughly doubled the iron and zinc AR, whereas moringa (15%) showed no improvement to AR of 2-5 year olds, as compared to pearl millet.
- Despite high tannin content, baobab (15%) contributed the most towards iron and zinc AR for children aged 2-5 years (Figure 4), possibly due to high citric and ascorbic acid contents[7,8], which is an enhancer of zinc bioavailability.
- Hibiscus (15%) had the highest relative contribution to iron AR for children aged 2-5 years (Figure 4). Hibiscus has high ascorbic acid content[9] which is a potent enhancer of iron bioavailability, even in the presence of phytate.

CONCLUSIONS

Baobab and hibiscus shows potential to be used as natural fortificants in instant cereal-based porridges to increase iron and zinc nutritive values.

REFERENCES

1) Muthayya et al. (2013) PLoS ONE, 8 (6)
2) Allen, L.H., et al. (2009) J. Nutr., 139 (5), pp.1022–1030.
3) Zimmermann & Hurrell (2007) The Lancet, 370, pp. 511-520
4) WHO, (2015) The global anaemia prevalence in 2011.
5) WHO, (2006) Guidelines on food fortification with micronutrients.
6) Vilakati et al. (2015) LWT-Food sci. technol., 63 (2), pp.1191–1198.
7) Namratha & Sahithi (2015) Int. J. Adv. Herb. Med., 1, pp. 20-26
8) Shahat (2006). Pharm. Biol., 44(6), pp. 445-450
9) Abou-Arab et al. (2011). J Am Sci., 7(7), pp. 445-456

ACKNOWLEDGEMENTS

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Figure A-1: Micronutrient-rich plant foodstuffs has the potential to increase iron and zinc nutritive values from an cereal-based porridge

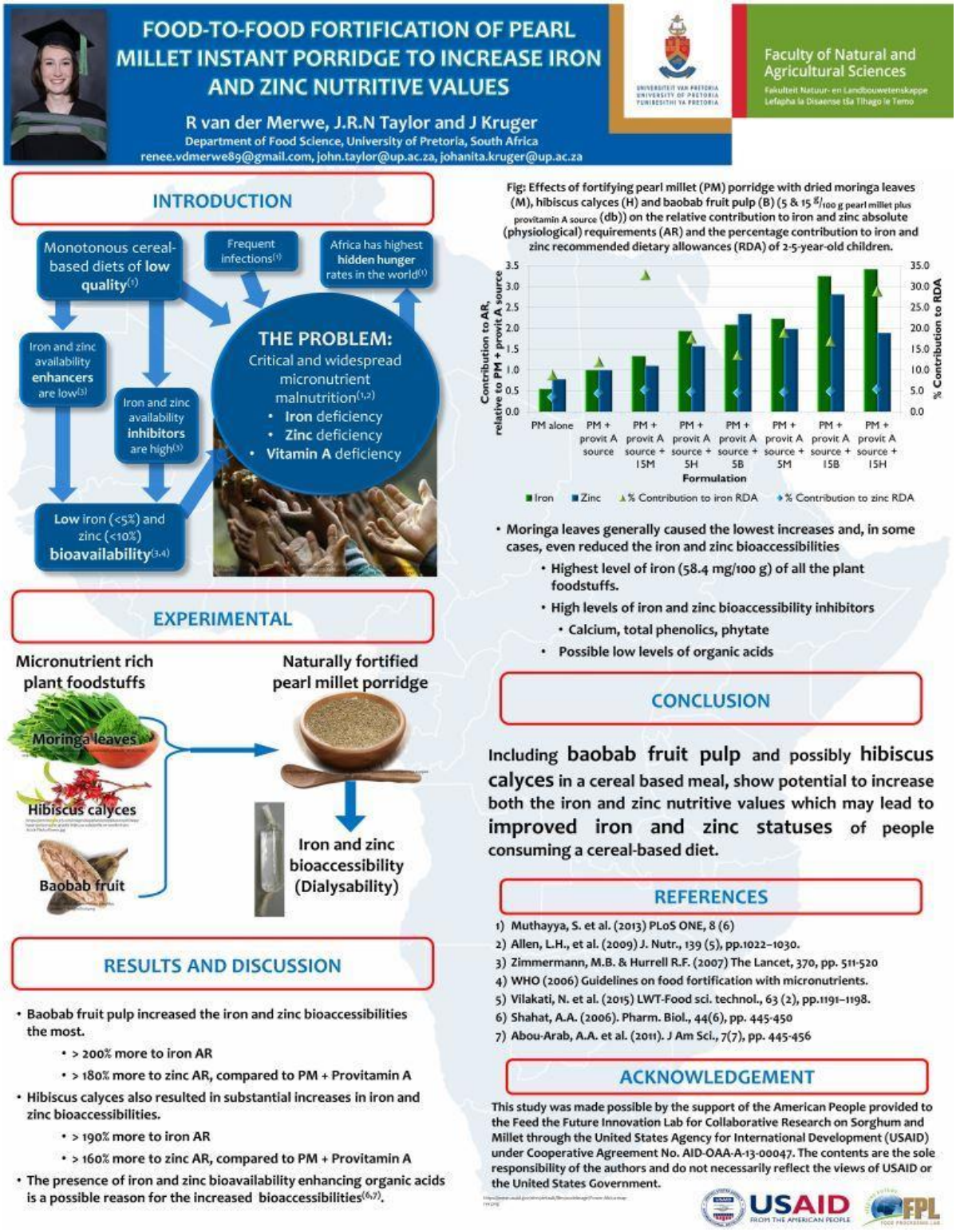

Figure A-2: Food-to-food fortification of pearl millet instant porridge to increase iron and zinc nutritive values.