

# **The effect of African Leafy Vegetables on the alleviation of micronutrient deficiencies in school children residing in the North West Province of South Africa.**

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“Three passions have governed my life:  
The longing for love, the search for knowledge,  
And unbearable pity for the suffering of mankind.  
Love brings ecstasy and relieves loneliness.  
In the union of love I have seen  
In a mystic miniature the prefiguring vision  
Of the heavens that saints and poets have imagined.  
With equal passion I have sought knowledge.  
I have wished to understand the hearts of [people].  
I have wished to know why the stars shine.  
Love and knowledge led upwards to the heavens,  
But always pity brought me back to earth;  
Cries of pain reverberated in my heart  
Of children in famine, of victims tortured  
And of old people left helpless.  
I long to alleviate the evil, but I cannot,  
And I too suffer.  
This has been my life; I found it worth living.”

**Bertrand Russell**

*Adapted from Prologue – Autobiography of Bertrand Russell, 1951*

## ABSTRACT

### Background

Food and nutrition insecurity severely compromises the quality of life in farm communities in South Africa. Although food-based approaches are more sustainable strategies to address malnutrition, most other strategies only target the alleviation of single micronutrients. Synergies between nutrients demand a wider scope; food-based strategies need to focus on improving both overall diet quality and the well-being of rural and urban populations. Agricultural biodiversity is crucial in such strategies to improve food security and health. This thesis aims to investigate the effect of African leafy vegetables (ALVs) on the alleviation of micronutrient deficiencies in school children residing in the North West Province of South Africa.

### Methods

Four focus group discussions assessed primary caregivers' (n=29) knowledge, perceptions and use of indigenous and traditional plants. Thereafter, the research focused on the leaves of *Amaranthus cruentus* (amaranth), *Cleome gynandra* (spiderplant), *Cucurbita maxima* (pumpkin) and *Vigna unguiculata* (cowpea). Sensory acceptability to children of selected ALV dishes, prepared in a traditional way (n=98) and prepared with gravy, was assessed (n=80). The nutrient composition and the bio-accessibility of iron and zinc in these ALVs were determined. A randomised controlled trial to investigate the effect of consumption of these ALVs on the iron, vitamin A and zinc status of primary school children (grade R – grade 4) followed. Children of two rural farm schools were randomly allocated per grade and school to receive either daily (five days/week) 300 gram cooked ALVs with the school meal starch (N=86) or the normal school meal (N=81) for three months.

**Results**

Caregivers were positive about using ALVs, transferring knowledge from generation to generation. Children found dishes made with ALVs, prepared in the traditional way as well as with gravy, acceptable in terms of colour, smell and taste. ALVs contributed 11.6 - 15.8 mg iron and 1.4 - 3.7 mg zinc per meal. Amaranth-and-spiderplant has the highest amount of bio-accessible iron (0.42 mg iron). All dishes contain 0.3 mg bio-accessible zinc. At baseline, intervention and control children were deficient for Hb <11.5 g/dL (16.0% and 10.5%), serum ferritin <15 µg/L (16.3% and 18.5%), serum retinol <20 µg/dL (7.0% and 2.5%) and serum zinc <65 µg/dL (75.6% and 75.3%). No significant estimated intervention effect was found.

**Conclusion**

Caregivers possessed knowledge of ALVs and were positive about their use. Based on dialyzable iron and zinc, the contribution of the ALV dishes towards dietary requirements is more substantial for iron than zinc. The randomised controlled trial showed that ALVs unable to improve serum retinol, serum ferritin or hemoglobin in mildly deficient children or those with low status zinc. Furthermore, despite the low zinc status in our population, ALV consumption did not improve serum zinc concentrations. Based on the more theoretical and indirect study results, including both caregivers' and children' positive image of ALVs, and the nutrient composition and iron and zinc bio-accessibility of the ALVs, these selected vegetables do have the potential to contribute to the micronutrient intake of school children. However, the importance of ALVs might not necessary be to serve as a strategy for micronutrient deficiency alleviation, but rather in the diversification of the diet in resource-poor settings and thereby contribute to the micronutrient intake.

**Key words**

African leafy vegetables, malnutrition, micronutrients, food and nutrition security, food based strategy, agricultural biodiversity

## OPSOMMING

### Agtergrond

Voedsel- en voedingonsekerheid is 'n ernstige bedreiging vir lewenskwaliteit in plaasgemeenskappe in Suid-Afrika. Voedselgebaseerde benaderings is meer volhoubare strategieë om wanvoeding reg te stel, maar die meeste andere strategieë het slegs die aanvulling van individuele mikrovoedingstowwe ten doel. Sinergie tussen voedingstowwe vereis meer reikwydte; voedselgebaseerde strategieë moet fokus op die verbetering van algehele dieetkwaliteit, sowel as die welsyn van landelike en stedelike gemeenskappe. Landboudiversiteit speel 'n sleutelrol in so 'n strategie om voedselsekureit en gesondheid te verbeter. Die doel van hierdie tesis was om die effek van Afrika-blaargroentes (ABG) op die verbetering van mikrovoedingstoftekorte in skoolkinders woonagtig in die Noord-Wesprovinsie van Suid-Afrika te ondersoek.

### Metodes

In vier fokusgroepbesprekings is kennis, persepsies en gebruik van inheemse en tradisionele plante onder primêre versorgers (n=29) vasgestel. Daarna is gefokus op die blare van *Amaranthus cruentus* (amarant), *Cleome gynandra* (oorpeultjie), *Cucurbita maxima* (pampoens) and *Vigna unguiculata* (akkerbone). Die sintuiglike aanvaarbaarheid vir kinders van geselekteerde ABG-disse wat tradisioneel voorberei is (n=98) en met sous voorgesit is (n=80), is vasgestel. Die voedingstofsamestelling en die biotoeganklikheid van yster en sink in hierdie ABGs is vasgestel. Daarna is 'n ewekansig gekontroleerde proefneming uitgevoer om die effek van inname van hierdie ABGs op die yster-, vitamien A- en sinkvlakke van laerskoolkinders (graad R – graad 4) te ondersoek. Kinders uit twee landelike plaasskole is ewekansig per graad en skool gekies om òf 300 gram gaar ABGs en skoolmaaltydstysel (N=86) òf die normale skoolmaaltyd (N=81) as daaglikse maaltyd te ontvang (vyf dae/week) vir drie maande.

## Resultate

Die benadering van versorgers tot (die gebruik van) ABGs was positief. Kennis daarvan is van geslag tot geslag oorgedra. Kinders het ABG-disse, tradisioneel voorberei en met sous, aanvaarbaar gevind wat kleur, reuk en smaak betref. ABG het 11.6 mg - 15.8 mg yster en 1.4 - 3.7 mg sink per maaltyd bygedra. Amarant-en-oorpeultjie het die grootste hoeveelheid biobeskikbare yster gehad (0.42 mg). Al die disse het 0.3 mg bio-toeganklike sink bevat. Op die aanvangslyn het ingryping- en kontrolekinders tekorte aan Hb <11.5 g/dL (16.0% en 10.5%), serumferritien <15 µg/L (16.3% en 18.5%), serumretinol <20 µg/dL (7.0% en 2.5%) en serumsink <65 µg/dL (75.6% en 75.3%) gehad. Geen beduidende verwagte ingrypingeffek is waargeneem nie.

## Gevolgtrekking

Versorgers was bewus van ABGs en positief oor die gebruik daarvan. Op grond van die biobeskikbare yster en sink, is die bydrae van yster in ABG-disse tot die dieetvereistes meer beduidend as dié van sink. Die ewekansig gekontroleerde proefneming toon dat ABGs nie die vlak van serumretinol, serumferritien of hemoglobien kan verhoog as daar slegs geringe tekorte is nie. Ten spyte van die lae sinkstatus van die bevolking, het inname van ABG ook nie serumsinkkonsentrasies verbeter nie. Gebaseer op die meer teoretiese en indirekte studieresultate, wat beide die versorgers en kinders se positiewe beeld van ABGs en die voedingstofsamestelling en die bio-toeganklikheid van yster en sink in hierdie ABGs insluit, het hierdie geselekteerde groentes die potensiaal om by te dra aan die mikrovoedingstoftekorte van skoolkinders. Daarteen, mag die belangrikheid van ABGs dalk nie noodsaaklik as 'n strategie vir die aanvulling van mikrovoedingstoftekorte dien nie, maar eerder in die diversifisering van die dieet in hulpbron-arme omgewings en so by te dra tot die mikrovoedingstofinname.

## Sleutelwoorde

Afrika-blaargroentes, wanvoeding, mikrovoedingstowwe, voedsel- en voedingsekuriteit, voedselgebaseerde strategie, landboudiversiteit

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## ABBREVIATIONS

AGP:  $\alpha$ 1-acid glycoprotein

ALVs: African leafy vegetables

BAZ: BMI-for-age z-score

CE: catechin equivalents

CRP: C-reactive protein

CRP: C-reactive protein

GLVs: green leafy vegetables

HAZ: height-for-age z-score

Hb: hemoglobin

ICP-OES: Inductively Coupled Plasma – Optical Emission Spectrometry

ITPs: Indigenous and traditional plants

NFCS: National Food Consumption Survey

NFCS-FB: National Food Consumption Survey-Fortification Baseline

NWU: North-West University

QFFQ: Quantitative food frequency questionnaire

RAE: retinol activity equivalent

RDA: Recommended dietary allowance

SANHANES-1: South African National Health and Nutrition Examination Survey

SAVACG: South African Vitamin A Consultative Group

SD: standard deviation

SF: serum ferritin

TfR: serum transferrin receptor

WAZ: weight-for-age z-score

WHO: World Health Organization

ZnPP: erythrocyte zinc protoporphyrin

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# 1. INTRODUCTION

## 1.1. Background and rationale

Malnutrition, hunger and inadequate food supply are universal problems, facing the majority of the world's poor and needy people and continuing to dominate the health of the poorest nations (United Nations, 2011; United Nations system & Standing committee on nutrition, 2010). Malnutrition also comprises the so-called "hidden hunger" for micronutrients. Micronutrient deficiencies of most public health significance include iron, vitamin A and zinc deficiencies (Müller *et al.*, 2005). Iron is needed in all tissues of the body for cellular respiration and many other reduction-oxidation enzyme systems and has particular functions in red blood cells, muscle and brain, including cognitive development (Stoltzfus *et al.*, 2004). Anaemia affects an estimated 1.62 billion people worldwide, especially in the developing world (McLean *et al.*, 2009). It has been estimated that nutritional iron deficiency affects 1.5-2.0 billion people globally (Lynch, 2011). Vitamin A is an essential nutrient required for maintaining immune function, eye health, vision, growth and survival in human beings (Rice *et al.*, 2004). Nearly 190 million preschool children are sub-clinically vitamin A deficient and many more school-age children, pregnant women, and others are affected. Disorders caused by vitamin A deficiency include xerophthalmia and anaemia and it contributes significantly to raised morbidity and mortality in at-risk populations by increasing the risk of common infectious diseases (WHO, 2009). In children with vitamin A deficiency, the risk of dying from diarrhoea, measles and malaria is increased by 20–24% (Black *et al.*, 2003). Zinc plays a crucial role in the immune system, physical growth and neuro-behavioural and brain development (Prasad, 2007; Gibson, 2006). Although there is no information on the global prevalence of zinc deficiency, the prevalence of inadequate zinc intake was estimated at 17.3% in 2012 (Wessels & Brown, 2012). In addition, zinc deficiency is thought to contribute substantially to the morbidity (especially diarrhoea, pneumonia and malaria) and mortality of young children globally (Caulfield & Black, 2004). Iron, vitamin A and zinc are all involved in linear growth in children, hence deficiencies in these nutrients

may result in stunting (height-for-age below minus two standard deviations from the reference median) (Rivera *et al.*, 2003). Furthermore, these deficiencies might coexist and interact.

Four national surveys to investigate nutritional status, including macro- and micronutrient deficiencies, have been conducted in South Africa. Dietary intake data collected during the National Food Consumption Survey (NFCS) in 1999 showed that children's intake of energy and several micronutrients, among which iron, vitamin A and zinc, were below two-thirds of the recommended dietary allowances (Labadarios *et al.*, 2005). Furthermore, these children (1-9 years) had low mean scores for dietary diversity and dietary variety (Steyn *et al.*, 2006). The South African Health and Nutrition Examination Survey (SANHANES-1) in 2012 showed that under-nutrition remains a national concern, especially in the rural informal areas. The prevalence of stunting in children 0-14 years of age was 15.4% (Shisana *et al.*, 2013). Compared to the 2005 NFCS-Fortification Baseline (NFCS-FB), the SANHANES-1 showed that there was an increase in stunting in the age group under five years (Shisana *et al.*, 2013; Labadarios *et al.*, 2007). In 2005 one out of three children (1-9 years) were found to be anaemic (haemoglobin < 11.5 g/dL) and one out of seven children (1-9 years) had a poor iron status (serum ferritin concentration <15 µg/L) (Labadarios *et al.*, 2007). SANHANES-1 reported a decrease in the prevalence of anaemia and iron deficiency anaemia in children younger than five years compared to NFCS-FB. However, the prevalence of iron deficiency in this age group appears to have increased (Shisana *et al.*, 2013). The national vitamin A deficiency prevalence in children younger than five years appeared to have decreased from 2005 to 2012, although the prevalence of 43.6% remained high (Shisana *et al.*, 2013; Labadarios *et al.*, 2007). In 2005 45.3% of children (1-9 years) had an inadequate zinc status (serum zinc concentration <70 µg/dL); an inadequate zinc status was more prevalent in rural and urban formal areas (Labadarios *et al.*, 2007).

Dietary inadequacy plays a key role in malnutrition and consequently in micronutrient deficiencies. In South Africa, malnutrition drives a combination of poverty-related infectious and lifestyle-related non-communicable diseases (WHO/FAO, 2003; Bourne *et al.*, 2002). The challenge is to address the

problems of the nutrition transition to avoid this double burden of disease. For this reason many advocate the use of food-based strategies to achieve optimal dietary requirements to combat micronutrient deficiencies (Faber & Wenhold, 2007; Johns & Eyzaguirre, 2006; Vorster & Kruger, 2006; Johns & Sthapit, 2004). Such strategies include supplementation, food fortification, biofortification, dietary diversification or modification, diversification of crops, introducing new crops, use of indigenous and local foods and implementation of home gardens to increase household food production.

A food-based strategy can only be a sustainable solution when its approach is holistic. A biodiversity-focused strategy is therefore relevant within a multi-pronged approach that includes improved and sustainable production technologies, changes in trade agreements and food-pricing policies, poverty reduction, education and improved health care (Johns & Sthapit, 2004). Biodiversity or biological diversity refers to the variety and variability amongst all living organisms on earth, including the number of different species, the genetic wealth within each species, the interrelationships between them, and the natural areas where they occur (Penafiel *et al.*, 2011; Collins, 2001). Because of rapid urbanisation and the degeneration of cultural heritage, knowledge on indigenous biodiversity that may traditionally be passed on from generation to generation has been lost (Vorster *et al.*, 2007). A study done by Vorster *et al.* (2005) showed that urbanisation of South Africans in the North West Province has resulted in an improvement in micronutrient intakes and status, but also increases in overweight, obesity and several risk factors for non-communicable diseases. These findings were attributed to higher consumption of fruit, vegetables, animal-derived foods and fats and oils by people living in urban areas than those living in rural areas (Vorster *et al.*, 2005). Traditional biodiversity use, instead of westernising diets, in the socio-cultural context can be a powerful tool for maintaining and enhancing health and nutritional status (Johns, 2003). Several studies have documented the link between biodiversity and nutrition (Uusiku *et al.*, 2010; Frison, 2007; Flyman & Afolayan, 2006; Hassan & Umar, 2006; Turan *et al.*, 2003; Vainio-Mattila, 2000). These studies have confirmed the importance of wild vegetables as sources of micronutrients. Nesamvuni *et al.*

(2001:51) and Faber *et al.* (2007:407) also underscored their significant contribution as source of micronutrients in South Africa. The nutrient composition of African leafy vegetables (ALVs) shows that these ALVs can potentially contribute to the reduction of micronutrient deficiencies and further improve nutrition and health status (Uusiku *et al.*, 2010).

There are enormous educational and economic gains to be achieved from improving the nutrition and health of school-age children. School feeding, both breakfast and lunch programmes, has been shown to improve school performance in both developing and industrialised countries (ACC/SCN, 2000). Against the background of prevailing malnutrition and its coexistence with micronutrient deficiencies and poverty in South Africa, this thesis includes these aspects, with the inclusion of school children from rural communities in the North West Province of South Africa in the research.

“We must learn more about plants [...], for they can help us create a world in which we do not use more than Earth can provide, in which people everywhere are healthy and well fed, and where future generations have as much opportunities to explore and enjoy the wonders of the planet as we and our ancestors have had” (Raven, 2009).

## **1.2. Aim and objectives**

The aim of this thesis was to investigate the effect of ALVs on the alleviation of micronutrient deficiencies in school children residing in the North West Province of South Africa. To achieve this, the study had the following objectives:

- To assess parents'/primary caregivers' knowledge, perceptions and use of indigenous and traditional plants (ITPs), focusing on access, preparation and preservation methods and perceptions of these plants.
- To assess sensory acceptability to children of selected ALV dishes, prepared in a traditional way and prepared with gravy.

- To investigate the nutrient composition (including iron and zinc content) and the bio-accessibility of iron and zinc in the selected ALVs.
- To investigate the effect of consumption of selected ALVs on blood haemoglobin, serum ferritin, serum transferrin receptor, zinc protoporphyrin, serum retinol, serum zinc of primary school children (grade R – grade 4).

### **1.3. Positioning of this study within larger research infrastructure**

The study described in this thesis was part of a joint project between South Africa, Kenya and Benin. This joint project was designed to fill the gaps in knowledge in these countries regarding the availability, acceptability and consumption and evidenced-based benefits of foods from local biodiversity. The South African leg of this study was coordinated from the North-West University (NWU) (Potchefstroom Campus) and was a transdisciplinary research project including the South African Agricultural Research Council, the National Institute of Occupational Health and different disciplines from the NWU (Agriculture, Nutrition, Consumer Sciences, Environmental Sciences, Agricultural Economics and Occupational Hygiene). This project aimed to increase agricultural biodiversity to improve nutritional and health status and livelihoods and to establish more sustainable production systems in the North West Province of South Africa. Several studies were conducted, among others:

- Assessment of the food plant diversity and management systems of home gardens (Lubbe *et al.*, 2010; Molebatsi *et al.*, 2010).
- Comparative study to assess the acceptance, consumption and utilisation of indigenous and traditional vegetables in adults (Matenge *et al.*, 2012; Matenge *et al.*, 2011).
- Market survey to identify demand and supply chains and the types of indigenous food sold in both rural and urban markets (Cloete & Idsardi, 2013).

- Description of factors that contribute to the availability and accessibility of a bio-diverse diet.
- An intervention study to determine the effect of indigenous and traditional vegetables on the nutritional and health status of school children.
- Analyses of the nutrient composition of these vegetables.

By promoting cultivation, consumption and knowledge on preparation of indigenous and traditional foods, new markets can be formed, which may lead to improved livelihoods and food security and more sustainable production systems. This thesis focuses only on the part of the study that aims to provide evidence-based knowledge of the use of foods from local biodiversity, such as ALVs and the possible benefits thereof for the nutritional status of school children in the North West Province.

#### **1.4 Ethical considerations**

Ethical approval was granted by the Ethics Committee of the NWU (NWU-00033-09-A1). Permission to conduct the study was granted by the Department of Education of the North West Province (Dr Kenneth Kaunda district) and the school governing bodies of the two schools. Several parent meetings, in the preferred language of the parents, were held on the school premises to explain the purpose and procedures of the (sub-)study and to answer any questions that the parents had. Potential participants were invited to participate in the (sub-)study and were asked to sign an informed consent form (illiterate people made a cross in front of a witness), agreeing that they themselves and their children would participate in the (sub-)study. Only children who obtained parental consent and gave assent for the study were included. Potential participating parents were assured of data confidentiality and that the data would be used for the sole purpose of the study. Participation was voluntary and the participants could withdraw at any time without any consequences. The intervention study was registered at [clinicaltrials.gov](https://clinicaltrials.gov) as NCT01920646.

### 1.5. Research team and contributions

The studies reported in this thesis were planned and executed by the team of researchers listed below. The research team was supported by several other persons, who are mentioned and thanked in the acknowledgments section.

<b>Researcher</b>	<b>Role in study</b>
M. van der Hoeven	PhD student and first author of three manuscripts in this thesis. Responsible for writing of this thesis, which included planning of studies, collection of data, design and planning of manuscripts, statistical analyses, interpretation of results and writing all manuscripts.
Prof. Dr C.M. Smuts	Promotor and co-author of three manuscripts. Supervised the writing of this thesis, which included supervising of planning of studies, collection of data, design and planning of manuscripts, statistical analyses, interpretation of results and writing all manuscripts.
Prof. Dr A. Kruger	Principal researcher in the main collaborative study, co-promotor and co-author of three manuscripts. Supervised the writing of this thesis, which included supervising of planning of studies, collection of data, design and planning of manuscripts, interpretation of results and writing of manuscripts.

Prof. Dr M. Faber Co-promotor and co-author of three manuscripts. Supervised the writing of this thesis, which included supervising of planning of studies, collection of data, design and planning of manuscripts, interpretation of results and writing of manuscripts.

J. Osei Co-author of two manuscripts. Contributed as Honours and Master's student to data collection, interpretation of results and writing of two manuscripts.

Prof. Dr M. Greeff Co-author of one manuscript. Contributed to the design and data collection, interpretation of results and writing of one manuscript.

The following is a statement from the co-authors confirming their individual role in each study and giving their permission that the three manuscripts may form part of this thesis:

*I declare that as a co-author I have approved the above-mentioned article(s), that my role in the study, as indicated above, is a representation of my actual contribution and that I hereby give consent that the manuscript(s) may be used for the PhD thesis of Marinka van der Hoeven.*



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Prof. Dr C.M. Smuts



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Prof. Dr A. Kruger



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Prof. Dr M. Faber



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Prof. Dr M. Greeff



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J. Osei

## **1.6. Thesis outline**

This thesis is presented in article format. The technical aspects of this document (except chapters three, four and five) follow the guidelines stipulated in the manual for postgraduate studies (2010) of the NWU. The bibliographic style used in this thesis (except for chapters three, four and five) is NWU Harvard style. Chapter two is a literature review on key components of the study to provide the necessary background. This chapter also contains a short introduction to the socio-demographic characteristics of the study population. Chapter three describes the knowledge, perceptions and use of ITPs of parents/primary caregivers and their children' sensory evaluation of selected ALVs. This manuscript has been accepted for publication by the Journal of Ethnobiology and Ethnomedicine and has been written according to the guidelines of this journal. Chapter four outlines the nutrient composition of the selected ALVs, including the bio-accessibility of iron and zinc. This manuscript has been prepared for submission to Public Health Nutrition and has been written according to the guidelines of this journal. Chapter five describes the intervention study in which the efficacy of the consumption of selected ALVs on the micronutrient status of primary school children was assessed. This manuscript also includes the dietary pattern and nutrient adequacy, focused on iron, vitamin A and zinc intake, of school children. This manuscript has been prepared for submission to the American Journal of Clinical Nutrition and has been written according to the guidelines of this journal. The final chapter discusses the main findings, conclusions and implications for policy and further research.

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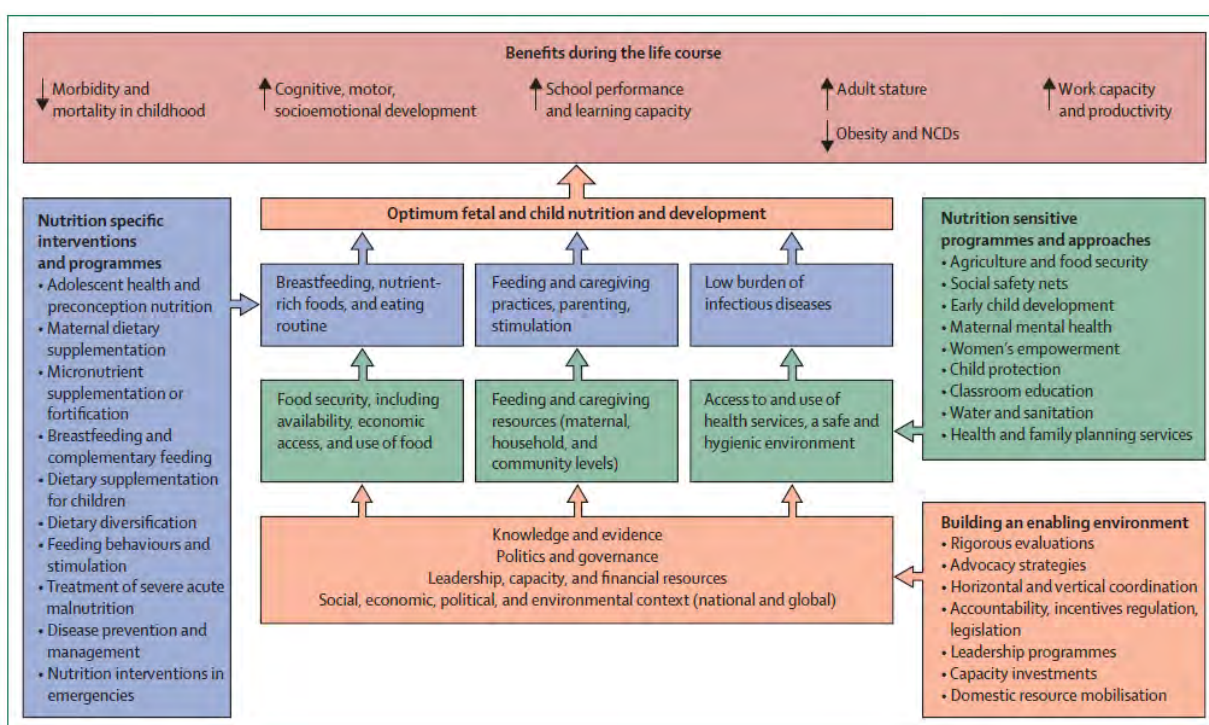
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## 2. LITERATURE REVIEW

### 2.1 Nutritional status

Nutritional status plays an important role in determining health. Nutrition influences and connects every stage of human life. Black *et al.* (2013) developed a new framework for action to achieve optimum foetal and child nutrition, growth and development (**Figure 2.1**). This framework included the dietary, behavioural, and health determinants of children's nutrition and development that are influenced by food security, feeding and caregiving resources and accessibility to health services. The wider context is formed by economic and social conditions, national and global perspectives, available knowledge and evidence, resources and governance (Black *et al.*, 2013).



**Figure 2.1** Framework for actions to achieve optimum child's nutrition and development (Black *et al.*, 2013).

The nutrition and health of school-age children in developing countries have only recently begun to receive attention after rejection of the assumption that by school age a child has survived the most

critical period and is no longer vulnerable. However, malnutrition is widespread in this age group and adversely affects school attendance, performance and learning (see **Figure 2.1**). Malnutrition has an enormous impact on both economic and social level, including increased health care cost, decreased educability and intellectual capacity and decreased adult productivity (ACC/SCN, 2000). Malnutrition is influenced by inadequate dietary intake, (frequent) infections and poor health status, inadequate care and insufficient health services and an unhealthy environment (Faber, 2010). Nutritional status can be measured by different means, such as adequacy of dietary intake, anthropometric indices and biochemical indicators.

Four national surveys to investigate nutritional status in children, including macro- and micronutrient deficiencies, have been conducted in South Africa. These include the South African Vitamin A Consultative Group (SAVACG) study (SAVACG, 1995), National Food Consumption Survey (NFCS) (Labadarios, 2000), the National Food Consumption Survey-Fortification Baseline (NFCS-FB) (Labadarios, 2007) and the South African National Health and Nutrition Examination Survey (SANHANES-1) (Shisana *et al.*, 2013). **Table 2.1** compares these four national surveys. The SAVACG, NFCS-FB and SANHANES-1 all collected biochemical data on children's vitamin A and iron status. The focus of the NFCS was on the dietary intake of children. The main results and more in-depth results will be discussed in the sections of this chapter on dietary intake (2.1.1), on anthropometric status (2.1.2) and on micronutrient deficiencies (2.1.3).

**Table 2.1** Comparison of four national surveys focusing on children's nutritional status in South Africa.

Survey	Study population	Year	Primary parameters	Main results
SAVACG study (SAVACG, 1995)	11 430 children (6-71 months)	1994	<ul style="list-style-type: none"> <li>• Haemoglobin</li> <li>• Serum ferritin</li> <li>• Serum retinol</li> <li>• Height</li> <li>• Weight</li> </ul>	<ul style="list-style-type: none"> <li>• Anaemia and poor iron status were more prevalent in urban areas. One in five children was anaemic. One in ten children was iron-depleted or iron deficient and one in 20 had iron deficiency anaemia.</li> <li>• Vitamin A deficiency was more prevalent in rural areas. One in three children had a marginal vitamin A status (serum retinol concentration below 20 µg/dL).</li> <li>• Almost one in four children was stunted and one in ten underweight.</li> </ul>
NFCS (Labadarios, 2000)	2 894 children (1-9 years)	1999	<ul style="list-style-type: none"> <li>• Dietary intake (24 h-recall and QFFQ)</li> <li>• Height</li> <li>• Weight</li> </ul>	<ul style="list-style-type: none"> <li>• Children's intakes of energy, calcium, iron, zinc, selenium, vitamins A, D, C and E, riboflavin, niacin, vitamin B6 and folic acid were below two-thirds of the recommended dietary allowance.</li> <li>• Nearly one in five children was stunted and one in ten children was underweight. Children in urban areas were least affected by nutritional disorders.</li> </ul>

Survey	Study population	Year	Primary parameters	Main results
NFCS-BF (Labadarios, 2007)	2 469 children (1-9 years)	2005	<ul style="list-style-type: none"> <li>• Haemoglobin</li> <li>• Serum ferritin</li> <li>• Serum retinol</li> <li>• Serum zinc</li> <li>• Height</li> <li>• Weight</li> </ul>	<ul style="list-style-type: none"> <li>• Almost one in three children was anaemic and one in seven children had a poor iron status.</li> <li>• Two out of three children had a poor vitamin A status.</li> <li>• 45.3% of children had an inadequate zinc status and were at risk of zinc deficiency. This was more prevalent in both urban and rural formal areas.</li> <li>• Nearly one in five children was stunted and one in ten children was underweight. One out of ten children was classified as overweight and 4% as obese.</li> </ul>

Survey	Study population	Year	Primary parameters	Main results
SANHANES-1 (Shisana <i>et al.</i> , 2013)	4 278 children (0-14 years)  Biochemistry (0-4 years):  • Haemoglobin (n=509)  • Serum ferritin (n=453)  • Serum retinol (n=436)	2011-2012	<ul style="list-style-type: none"> <li>• Haemoglobin</li> <li>• Serum ferritin</li> <li>• Serum retinol</li> <li>• Height</li> <li>• Weight</li> </ul>	<ul style="list-style-type: none"> <li>• In children under five years of age the prevalence of anaemia was 10.5%, that of iron deficiency 11% and of iron deficiency anaemia 2.1%.</li> <li>• The vitamin A deficiency was present in 43.6% of children under five years of age. Children in urban formal areas had the highest mean retinol concentration and the lowest deficiency prevalence, while the opposite was true for urban informal areas.</li> <li>• The prevalence of stunting was 15.4%, of wasting 2.9%, and of underweight 5.8%. The youngest boys and girls (0–3 years of age) had the highest prevalence of stunting (26.9% and 25.9% respectively). The prevalence of stunting was higher in rural informal areas (boys) and urban informal areas (girls). The prevalence of overweight and obesity was significantly higher in girls than boys (16.5% and 7.1% vs 11.5% and 4.7%, for girls and boys, respectively).</li> </ul>

SAVACG: South African Vitamin A Consultative Group; NFCS: National Food Consumption Survey; NFCS-FB: National Food Consumption Survey-Fortification Baseline; SANHANES-1: South African National Health and Nutrition Examination Survey; QFFQ: Food Frequency Questionnaire.

### 2.1.1 Dietary intake

Dietary intake is measured to assess the nutritional adequacy of diets and to provide information about nutrients, energy, food and eating habits (Burrows *et al.*, 2010). There are several methods to assess dietary intake, which include food balance sheets, household budget surveys, food records, dietary recalls, diet history and food frequency questionnaires (Patterson & Pietinen, 2004). A review on measuring dietary intake in children compared different assessment methods to estimate total energy intake with the golden standard (doubly labelled water). The 24-hour multiple pass recall conducted over at least a three-day period including weekdays and weekend days and interviewing both child and parent was found the most accurate method in children aged 4-11 years (Burrows, *et al.* 2010).

In South Africa, the NFCS focused on dietary intake of children aged one to nine years old. It was found that the micronutrient intake of these children was inadequate; two out of three children did not meet the recommended dietary allowance (RDA) for several micronutrients. South African children had a low iron intake; 25–35% of children did not meet 50% of the RDA for iron and 36–57% had an iron intake of less than 67% of the RDA. At least two-thirds of the RDA for vitamin A was not met by 50% of the children in all age groups and all provinces (except the Western Cape). In addition, the mean intake of zinc was inadequate, with 32–53% of children having an intake of less than half of the RDA and 50–73% of children having an intake of less than two-thirds of the RDA. Furthermore, it was found that the intake of children residing in rural areas was worse than that of children residing in urban areas (Labadarios *et al.*, 2005a). In addition, the NFCS showed that dietary diversity was very low (Steyn *et al.*, 2006). This was reflected in the most commonly consumed foods as well, which included maize, tea, sugar and bread (Steyn & Labadarios, 2002). The mean dietary diversity score for South African adults was 4.02 (3.96-4.07) which has been classified as poor (Labadarios *et al.*, 2011).

### **2.1.2 Anthropometric status**

Anthropometry measures body dimensions, which reflect cumulative exposure to diet, exercise and illness and are age- and gender specific. In order to compare different age and gender groups and to make provision for rapid changes in body size and proportions, reference standards are used (Nelson *et al.*, 2004).

The SANHANES survey showed that the mean body mass index increased with age in both boys and girls. Girls were more overweight and obese than boys at all ages. In South Africa the current prevalence of stunting in children is 15.4%, that of severe stunting 3.8%, wasting 2.9% and underweight 5.8%. The rural informal areas had the highest prevalence of undernutrition (Shisana *et al.*, 2013). In the last seven years the nutritional status of children under 10 years seems to have improved in South Africa. The prevalence of wasting and underweight has decreased, although the prevalence of stunting among children one to three years old has increased. Even though the prevalence of undernutrition has decreased on national level, in certain provinces the prevalence of undernutrition remains high, e.g. the North West, Free State and Northern Cape (Shisana *et al.*, 2013; Labadarios, 2007).

### **2.1.3 Micronutrient deficiencies**

Micronutrients are an essential part of a person's diet and include vitamins, minerals and essential fatty acids. They play a fundamental role in the prevention of disease, normal growth and development (Shergill-Bonner, 2013). This thesis will only focus on iron, vitamin A and zinc.

#### **2.1.3.1 Iron**

##### **Function in human body**

Iron is used in the human body for the binding and transport of oxygen and is involved in regulating cell growth and differentiation (Caulfield *et al.* 2006). Iron is needed in all tissues of the body for

cellular respiration and many other reduction-oxidation enzyme systems, and has particular functions in red blood cells, muscle and brain, including cognitive development (Stoltzfus *et al.*, 2004). There is no physiological mechanism for iron excretion and approximately 90% of the body's requirement is obtained from the breakdown of red blood cells (Hurrell & Egli, 2010). The body loses iron via the skin, gastrointestinal and urinary tract and through menstruation (Lynch, 2011).

### **Deficiency**

Iron deficiency arises when a sustained imbalance develops between consumed bioavailable dietary iron and utilisation of iron, increased iron requirements during pregnancy, childhood growth, helminth infections or any of the losses mentioned above (Lynch, 2011, WHO, 2011a). The major factors contributing to iron deficiency are diet-related, e.g. limited dietary diversity, caused by poverty, and the shift from animal foods to cereals, legumes and other plant diets (Lynch, 2011). In order to maintain adequate intake and absorption of iron, one would have to consume a diet containing a combination of meat, eggs, fruit and vegetables (Pettit *et al.*, 2011). Meat, red meat in particular, is the main source of heme iron, which is highly available for absorption (De Oliveira Otto *et al.*, 2012). Green leafy vegetables are recognised as good sources of iron in our diets (Gupta *et al.*, 2006). However, monotonous plant-based diets provided almost no heme iron and mainly non-heme iron. The iron absorption of the latter has been shown to be improved by ascorbic acid (Cook & Reddy, 2001) and meat (particular muscle tissue) (Geissler & Singh, 2011). Inhibitors of iron absorption include phytates, polyphenols, certain vegetable proteins (inhibitors of non-heme iron absorption), calcium (inhibitor of both heme and non-heme iron) and animal proteins (Hurrell & Egli, 2010; Faber & Wenhold, 2007).

During the first stage of iron deficiency the iron stores shrink; this is followed by the second stage of a decrease in iron transportation within the body. When the supply of this transport limits haemoglobin production, the third stage has been reached (WHO, 2011b). This severe depletion of the iron stores negatively affects the haemoglobin concentration. This in turn causes anaemia and

consequently iron-deficiency anaemia (Pettit *et al.*, 2011). Several other disorders might also be involved in the development of anaemia. These disorders include other nutritional deficiencies (particularly vitamins A and B12, and folic acid), infections (especially malaria, HIV and tuberculosis) and genetic factors (such as thalassemic syndrome) (Lynch, 2011).

The adverse effects of iron deficiency have substantial health and economic costs. Iron deficiency contributes to low work productivity in adults and these annual physical work productivity losses affect the gross domestic product (Horton & Ross, 2003). Iron deficiency in pregnancy increases maternal morbidity and mortality (Scholl 2005; Brabin *et al.*, 2001a), especially the risk of death during delivery and postpartum (Zucker *et al.*, 1994; Sarin, 1995). Furthermore, maternal anaemia during the first two trimester of pregnancy increases the risk of preterm labour (Scholl, 2005), low birth weight (Cogswell *et al.*, 2003), and infant and child mortality (Titaley *et al.*, 2010; Brabin *et al.*, 2001b), however during the third trimester it is associated with a decreased risk of preterm delivery and low birth weight (Bánhidý *et al.*, 2011). The adverse short- and long-term effects of iron deficiency on the cognitive and motor development in infants and young children remain unclear, although a recent study in South Africa showed a beneficial effect on cognition in school children with iron deficiency anaemia and poor n-3 fatty acid status (Baumgartner *et al.*, 2012). Anaemia in school children negatively affects emotional development and school performance (Zimmermann & Hurrell, 2007).

Anaemia is defined as a haemoglobin concentration lower than the normal quantity in blood. For children aged 6-11 years this is 11.5 mg/L. The main cause of anaemia is a decrease in red blood cells, often as result of blood loss (Zimmermann & Hurrell, 2007). Anaemia is often used as proxy to estimate iron deficiency. According to the World Health Organization (WHO) an anaemia prevalence of 40% or higher is classified as severe, 20–39.9% is moderate, 5–19.9% is mild and <4.9% is normal (WHO, 2011b).

### **Indicators of iron status**

One of the most frequently used indicators to detect iron deficiency is haemoglobin. However, the specificity and sensitivity of this indicator is low, especially when not adjusted for age, gender, pregnancy, ethnicity, smoking and altitude (Lynch, 2011). It is only in the third stage of iron deficiency that haemoglobin concentrations are affected (WHO, 2011b). Erythrocyte zinc protoporphyrin, a precursor of haemoglobin, can be used as a more sensitive indicator of iron deficiency. Before haemoglobin concentrations are affected, the iron in protoporphyrin is replaced by zinc and can be measured by haematofluorometry (Biesalski & Erhardt, 2007). Serum ferritin (SF) reflects the body iron stores in healthy individuals, but since it is an acute phase protein it increases independently of iron status in the presence of infection, inflammation and liver disease (Lynch, 2011; Zimmermann & Hurrell, 2007). Therefore it is necessary to include indicators of acute and chronic infection, such as C-reactive protein (CRP) or  $\alpha$ 1-acid glycoprotein (AGP) (Zimmermann & Hurrell, 2007). CRP is most commonly used, although the cut-off value is debatable (Kongsbak *et al.*, 2006). In addition, during the acute-phase response, the concentration of SF is increased for longer than that of CRP. As a result, AGP might be a better alternative marker, because it increases later during the infection than CRP and its concentrations remains high for longer (Zimmermann & Hurrell, 2007). Another indicator of iron status is serum transferrin receptor (sTfR), which can detect iron deficiency in the presence of chronic inflammation and infections. It is not substantially affected by acute-phase response, although malaria, age and ethnicity might influence sTfR (Zimmermann & Hurrell, 2007). The sTfR-to-SF ratio is a quantitative estimation of total body iron. Its advantage is that it does not rely on parameter cut-off values; it is independent of haemoglobin and the assay methods are readily automated (Lynch, 2011). Since SF increases in the presence of infection, inflammation and liver disease, the sTfR-to-SF ratio cannot be used in these conditions (Zimmermann & Hurrell, 2007).

### **Current situation in South Africa**

The most recent data with regard to the iron status of South African children (under five years of age) was collected in 2012 (Table 2.1.) by SANHANES. Because of the small sample size, data were not stratified on provincial level. It was found that the mean haemoglobin concentration was 12.2 g/dL and that the overall prevalence of anaemia (haemoglobin  $\leq$  11 g/dL) was 10.7%, that of mild anaemia 8.6% and that of moderate anaemia 2.1%. There were no gender or locality (urban – rural) differences in anaemia prevalence. The mean SF concentration was 40.7  $\mu$ g/L in children. SF concentration was negatively associated with age and was higher in rural areas. The prevalence of iron depletion (haemoglobin  $\leq$  11 g/dL and SF  $<$  12  $\mu$ g/L) was 8.1%, and that of iron deficiency anaemia (haemoglobin  $<$  11 g/dL and SF  $<$  12  $\mu$ g/L) 1.9% (Shisana *et al.*, 2013).

Compared with data from 2005 and 1994, SANHANES showed that the iron status of children under five years had improved in South Africa. The prevalence of anaemia was 21.4% in 1994 (SAVACG), 28.9% in 2005 (NFCS-FB) and 10.7% in 2012 (SANHANES). The iron deficiency prevalence remained similar to the prevalence in 2005, 8.1% (SANHANES) and 7.8% (NFCS-FB) respectively. It had however increased since 1994, when the prevalence was found to be 4.8%. The iron deficiency anaemia prevalence had decreased since 2005 from 11.3% to 1.9%. The iron deficiency anaemia prevalence was 5.0% in 1994 (SAVACG). Reasons for this improvement in iron status include among others the beneficial effects of the national food fortification programme and of the vitamin A supplementation programme in South Africa (Shisana *et al.*, 2013; Labadarios, 2007; SAVACG, 1995).

#### **2.1.3.2 Vitamin A**

##### **Function in human body**

Vitamin A is an essential fat-soluble nutrient. It plays a role in fertility and embryonic development, growth, regulating cell differentiation and proliferation, immune function and vision (Sherwin *et al.*, 2012; Nojilana *et al.*, 2007). It is derived as preformed vitamin A from animal products, such as liver, butter and egg yolks, and as provitamin A carotenoids from plant foods, such as dark-green leafy

vegetables, carrots and papaya. The conversion from provitamin A carotenoids to retinol is mainly dependent on food matrix and food processing and therefore the contribution of plant foods to the dietary needs may vary (Tang, 2010). However, the bioavailability of provitamin A carotenoids may be increased if the consumption of the plant food is accompanied by a source of fat (Kennedy *et al.*, 2003).

### **Deficiency**

Vitamin A deficiency occurs when the body's requirements are not met for a sufficient duration or level. This can be caused by increased nutritional requirements for growth, insufficient dietary intake of vitamin A food sources (with limited bioavailability) and malabsorption of vitamin A (in combination with malabsorption of fats) (Sherwin *et al.*, 2012). Malabsorption of fats is related to liver disease, protein-energy malnutrition and zinc deficiency (Mahan & Escott-Stump, 2004).

One of the first signs of vitamin A deficiency is night blindness (Mahan & Escott-Stump, 2004). This deficiency is associated with an increased risk of several infections, including diarrhoeal diseases and respiratory tract infections due to a loss of integrity of cells in the mucous membranes (Semba & Bloem, 2004). This in turn affects overall growth and development. Specific outcomes include xerophthalmia and anaemia (Sherwin *et al.*, 2012).

The WHO used the following cut-offs to define vitamin A deficiency and its level of public health significance: a prevalence of low serum retinol  $< 0.70 \mu\text{mol/L}$  of 2-9% has a mild significance, 10-19% a moderate significance and 20% or more a severe significance (WHO, 2011c).

### **Indicators of vitamin A status**

Several indicators to assess vitamin A status have been developed. These include biological, functional, histological and qualitative and quantitative biochemical indicators. Biological indicators focus on the clinical signs of deficiency and include xerophthalmia as a population-based indicator. Functional indicators include night blindness, which occurs when vitamin A stores in the eye are depleted. Histological indicators involve conjunctival impression cytology, but this has not been

widely adopted. Qualitative biomarkers include serum retinol concentrations and the carrier protein-retinol binding protein. Although serum retinol concentration is the most common indicator used, it is a static measure, which does not always respond to interventions, partly owing to retinol's homeostatic control over liver reserves (Tanumihardjo, 2011). Serum retinol is affected by acute and chronic infections; therefore it is necessary to include indicators of acute and chronic infection, such as CRP or AGP (Sherwin *et al.*, 2012). Breastmilk retinol concentrations rather reflect dietary intake than vitamin A status. Quantitative biochemical indicators include dose-response tests, isotope dilution assays and direct measurement through biopsy or autopsy samples of the liver (Tanumihardjo, 2011).

### **Current situation in South Africa**

In 2012, 43.6% of children under five years old were vitamin A deficient, which is classified by the WHO as a severe public health problem. The mean serum vitamin A was 0.72  $\mu\text{mol/L}$  and 0.79  $\mu\text{mol/L}$  (for boys and girls respectively). Children in urban informal areas had the highest prevalence of vitamin A deficiency (55.1%) and the lowest mean serum retinol (0.69  $\mu\text{mol/L}$ ). A national vitamin supplementation programme was implemented in South Africa in 2002 (see section 2.3.5.1.) (Shisana *et al.*, 2013).

Compared with data from 1995 and 2005 the current data show a decrease in vitamin A prevalence in children under five years, 43.6%, 63.6% and 33.3%, SANHANES, NFCS-BF and SAVACG respectively. Only children from rural formal areas had a decrease in serum retinol and consequently an increase in vitamin A deficiency (Shisana *et al.*, 2013; Labadarios, 2007; SAVACG, 1995).

### **2.1.3.3 Zinc**

#### **Function in human body**

Zinc plays catalytic, structural and regulatory roles in the human body (Gibson, 2012). It is involved in most metabolic pathways, in which it activates different enzymes. Zinc is required for several biochemical, immunological and clinical functions. There are no body stores for available zinc

(Gibson, 2012), except possibly during infancy (Zlotkin & Cherian, 1988). Zinc absorption is concentration-dependent and is influenced by zinc status. Individuals with low zinc status will absorb zinc with increased efficiency (FAO/WHO, 2004). When habitual dietary intake of zinc is inadequate, homeostasis will ensure that intestinal excretion of endogenous zinc is reduced and growth in children is decreased to conserve tissue zinc levels. When this imbalance is prolonged, the result is general tissue dysfunction (King, 1990). Zinc deficiency will affect the gastrointestinal system, which may result in diarrhoea, and thereby worsen malnutrition in general. Furthermore, the central nervous, immune, skeletal and reproductive systems will be affected, resulting in clinical signs such as short stature and hypogonadism (Roohani *et al.*, 2013).

### **Deficiency**

The development of zinc deficiency is strongly associated with inadequate intake of dietary zinc. This can be due to low intake of dietary zinc, poor bioavailability or a combination of both. Meat, poultry and fish are rich sources of zinc with high bioavailability (Roohani *et al.*, 2013; Gibson, 2012). Green leafy vegetables can be reasonable sources, although in small amounts (Kennedy *et al.*, 2003). Cereal and legumes are high in phytic acid and its associated phytate (Mg, Ca and K salts). Phytate limits the bioavailability of dietary zinc by the formation of insoluble complexes with zinc. In addition, phytate also forms these complexes with endogenously secreted zinc, depleting the zinc levels in the body (Gibson, 2012; Hambidge *et al.*, 2011). Furthermore, young children, adolescents, pregnant and lactating women are at risk of zinc deficiency, because of the increased requirement of zinc during growth. Malabsorption and excessive losses also contribute to the development of zinc deficiency (Gibson, 2012).

### **Indicators of zinc status**

Currently three indicators are recommended for assessing zinc status on population level: the prevalence of habitual dietary zinc intake below the estimated average requirement, the prevalence of low serum zinc concentrations in the population and the prevalence of stunting in children

younger than five years. Although a generally accepted sensitive and specific biomarker for zinc status is currently lacking, serum (or plasma) zinc is the most widely used biomarker (Moran *et al.*, 2012; Lowe *et al.*, 2009).

### **Current situation in South Africa**

The prevalence of zinc deficiency in children one to nine years old in South Africa is 45.3%, which is similar to the prevalence of 41.1% in the North West Province (Dhansay *et al.*, 2005). Troesch *et al.* (2011) also reported a high prevalence (47.4-52.5%) of zinc deficiency (serum zinc < 65 µg/dL) in school children aged 5-12 years in the Northern Cape Province of South Africa.

### **2.2.4 Micronutrient interactions**

Many micronutrients have either synergistic (e.g. ascorbic acid on non-heme absorption) or antagonistic effects (e.g. calcium on iron absorption). Zinc is required for the production of retinol and the retinol-binding protein. Although the main transport vehicle for zinc is albumin, it also competes with iron for transferrin, the main carrier for iron. Iron-rich diets can therefore limit zinc absorption, a risk factor for deficiency (Whitney & Rolfes, 2008). The efficacy of micronutrient supplements might decrease in reaction to the interaction of iron and zinc. A 2011 review article found that iron did not affect zinc absorption or the bioavailability of zinc (Yakoob *et al.*, 2011). A randomised control trial among Guatemalan women found that the addition of zinc to an iron and folic acid supplement did not modify its efficacy for iron status or improve zinc status (Nguyen *et al.*, 2012). In the presence of helminth infections, a multiple micronutrient supplement including 18 mg/d of iron had not greater effect than 60 mg/d of iron over a period of 12 weeks on haemoglobin, SF and transferrin receptors in school children (Stolzfus *et al.*, 1998).

The interaction of iron and vitamin A is complex and isotopic studies on the influence of vitamin A on iron absorption reported contradictory findings. Vitamin A can affect several stages of iron metabolism, including erythropoiesis and the release of iron from ferritin stores (Hurrell & Egli, 2010). The combined effect of an iron and vitamin A supplement was more beneficial than vitamin A

supplementation alone on the decrease in incidence of respiratory-related illnesses and the occurrences of runny nose, cough and fever among young Chinese children who lived in peri-urban communities (Chen *et al.*, 2013). In Canadian aboriginal infants, vitamin A supplementation improved haemoglobin concentrations and decreased the prevalence of anaemia, independent of iron status (Willows & Gray-Donald, 2003). Low serum retinol levels may contribute to anaemia independent of iron status in non-pregnant women (aged 15-49 years) (Jafari *et al.*, 2013).

Randomised controlled trials with multiple micronutrient supplements to address multiple nutrient deficiencies have shown modest, positive effects on infants' birth weight and children's length and weight, but not on preterm birth or perinatal mortality (Christian & Tielsch, 2012). The impact of these trials however is inadequate to demonstrate a public health benefit for policy considerations. Therefore future studies should aim to collect more meaningful data by including measures to assess the adequacy and the plausibility of the findings (Habicht & Pelto, 2012).

### **2.3 Nutrition intervention strategies to address micronutrient deficiencies**

There are several strategies to address micronutrient deficiencies. An important factor is the sustainability of these strategies. In 2010, participants at the international symposium on 'Biodiversity and Sustainable Diets: United against Hunger', formulated the following definition of sustainable diets: "Sustainable diets are those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources" (FAO, 2010). Supplementation of certain micronutrients provides the fastest, but least sustainable improvement in micronutrient status of a specific targeted population. Food fortification has a wider and more sustained impact; however the micronutrient

status will only improve gradually. Increasing dietary diversity and nutrition education is the most sustainable strategy, but this strategy takes longest to implement (Allen *et al.*, 2006).

### **2.3.1 Supplementation**

Supplementation refers to the periodic administration of pharmacological preparations of nutrients. Micronutrient supplementation should only be given to a specific target population that is unable to meet their nutritional needs through the consumption of food (Thompson, 2007).

In terms of iron, oral supplementation with ferrous iron salts (ferrous sulphate and ferrous gluconate) is preferred because of the low cost and high bioavailability. Untargeted iron supplementation might increase the risk of serious infections in endemic malaria areas (Zimmermann & Hurrell, 2007), although iron does not increase the risk of clinical malaria or death when regular malaria surveillance and treatment are provided (Ojukwu *et al.*, 2010). However, a systematic review of randomised control trials showed that iron supplementation had no harmful effect on the overall incidence of infectious diseases. It did however slightly increase the risk of developing diarrhoea (Gera & Sachdev, 2002). A systematic review of randomised clinical trials found that iron supplementation did not increase the physical growth of children (Sachdev *et al.*, 2006). A more recent review showed that iron supplementation did improve age-adjusted height among all primary-school-aged children in low- or middle-income settings and age-adjusted weight among anaemic children. Furthermore, it reduced the risk of anaemia by 50% and iron deficiency by 79% (Low *et al.*, 2013).

Several meta-analyses and review articles have evaluated the relationship between vitamin A supplementation and the risk of mortality. Reduced risk of mortality is only found in children aged between six months and five years (Sherwin *et al.* 2012). Two systematic reviews concluded that preventive vitamin A supplementation reduces diarrhoea-specific mortality (Imdad *et al.*, 2011; Imdad *et al.*, 2010). However, there was no significant effect on the specific mortality of measles,

respiratory disease and meningitis (Imdad *et al.*, 2010). Vitamin A supplementation was found to reduce the incidence of diarrhoea and measles morbidity; however there was no significant effect on the incidence of respiratory disease or hospitalisation due to diarrhoea or pneumonia (Imdad *et al.*, 2010).

Preventive zinc supplementation is associated with improvements in child health and survival (Yakoob *et al.*, 2011; Brown *et al.*, 2009) and enhanced child growth (Imdad & Bhutta, 2011). A review by Brown *et al.* (2009) indicated that zinc supplementation improved mean serum zinc and that it had no adverse effect on indicators of iron status. Zinc supplementation alone in children under five was associated with non-significant reductions in mortality due to diarrhoea, pneumonia and malaria (Yakoob *et al.*, 2011). Zinc supplementation reduced all-cause mortality in children older than 12 months by 18% and moderately increased linear growth and weight (Brown *et al.*, 2009). In order to replete the body stores, zinc supplementation might require longer duration than for example the two-week therapeutic supplementation for diarrhoea (Yakoob *et al.*, 2011).

### **2.3.2 Fortification**

Food fortification is the increase of nutrients to a higher level than they naturally occur in a food vehicle (Thompson, 2007). Effective food fortification might make supplementation unnecessary. In developed countries food fortification is a proven effective and low-cost strategy to increase micronutrient intake and reduce the consequences of micronutrient deficiencies (Dary *et al.*, 2002). There are several factors to be taken into account, before fortifying a food vehicle. These include the extent of the deficiency, the availability of other food options, dietary intake of the food vehicle, the RDAs, the production process and the associated costs (Klemm *et al.*, 2010). The best food for fortification is cereal flour (Mirmiran *et al.*, 2012).

Fortification of cereal flour with iron can be an effective approach to decrease iron deficiency (Mirmiran *et al.* 2012); however the iron compound and the amount used are crucial factors

(Troesch *et al.* 2011). In addition, inhibitors of iron absorption, such as phytate, should be taken into account (Mirmiran *et al.*, 2012). According to Hurrell *et al.* (2010), most iron fortification of wheat flour programmes are likely to be ineffective and more emphasis should be placed on the recommended iron compound and its amount (Hurrell *et al.*, 2010). A recent systematic review of randomised controlled trials evaluated 60 studies. It was found that iron fortification of food improved haemoglobin, SF, and iron nutriture. No effect was found on serum zinc concentrations, infections, physical growth and mental and motor development (Gera *et al.*, 2012). Zinc food fortification programmes are effective public health strategies to increase plasma zinc concentrations. However, overall evidence of the effectiveness of zinc food fortification is limited. A significant improvement in height velocity among very-low-birth-weight infants was found, but no impact on serum alkaline levels, serum copper levels, haemoglobin or weight gain (Das *et al.* 2013).

Multi-micronutrient food fortification was found to improve micronutrient status and to reduce the prevalence of anaemia. However, the effect on morbidity, growth and cognitive outcomes is still not clear (De-Regil *et al.*, 2013; Best *et al.*, 2011). Home fortification of foods with multiple micronutrient powders was found to be an effective intervention to reduce anaemia en iron deficiency in children (6-23 months) (De-Regil *et al.*, 2013).

### **2.3.3 Dietary diversification**

Dietary diversification/modification focuses on the improvement of the nutritional quality of the diet and thus the alleviation of multiple micronutrient deficiencies by improving the production, availability, accessibility and utilisation of foods high in bio-available micronutrients throughout the year (Faber, 2010; Gibson & Hotz, 2001; Gibson & Ferguson, 1998). Secondary data analysis of the NFCS showed that a food variety score and a dietary diversity score are both indicators of the micronutrient adequacy of a child's diet (Steyn *et al.*, 2006). Diets with a high food variety score were found to be more diverse and to be more nutritionally adequate in rural women (Ogle *et al.*

2001). Oyango *et al.* (1998) found that a higher diversity is associated with a higher intake of micronutrients and better anthropometric status in children 12-36 months old. This has been confirmed by Hatløy *et al.*, who found that food variety and dietary diversity was associated with nutritional status of children aged 6-59 months from urban communities (Hatløy *et al.*, 2000). A study, using data of 11 national demographic and health surveys, indicated that there is an association between dietary diversity and nutritional status in children 6-23 months old and that dietary diversity may be used as an indicator for diet quality and adequacy (Arimond & Ruel, 2004). Faber *et al.* (2002) reported an increase in the consumption of provitamin A-rich vegetables and also in serum retinol concentrations of 2-5 year old children in a rural village in South Africa, after implementing a home-gardening program together with nutritional education. Dietary diversification can be achieved in different ways and it is not limited to agricultural, behavioural, or preparation approaches (Gibson & Hotz, 2001). Agricultural and food policies should include home gardens and small livestock production to increase the household consumption of micronutrient-rich foods (Faber *et al.* 2011; Faber *et al.*, 2002; Tontisirin *et al.*, 2002). Dietary diversification is the optimal and most sustainable strategy to reduce micronutrient deficiencies and malnutrition. However, poverty and people's dietary preferences should be taken into account (Lynch, 2011). Policies and programmes have to include support for integrated farming systems focused on improving and maintaining household food security and should be based on a variety of foods that will meet total dietary needs. The cultivation of edible indigenous plants can also be included, although the low bioavailability of some of the required micronutrients should be enhanced by the right food combinations and appropriate food processing and preparation techniques (Tontisirin *et al.*, 2002).

### 2.3.4 Nutrition intervention strategies in South Africa

#### 2.3.4.1 Vitamin A supplementation programme

A vitamin A supplementation programme was implemented in 2002 in South Africa to comply with the recommendation of the SAVACG study. The schedule for routine high-dose vitamin A supplementation in vitamin A-deficient populations, as recommended by the International Vitamin A Consultative Group, is as follows:

- Infants 0–5 months: 150 000 IU as three doses of 50 000 IU with an interval of at least one month between doses.
- Infants 6–11 months: 100 000 IU as a single dose once
- Children 12 months and older: 200 000 IU as a single dose every four to six months (WHO, 2011c).

In 2005 the Western Cape Vitamin A Supplementation protocol stipulated a supplementary dose of 50 000 IU vitamin A to be given at six weeks of age to non-breastfed infants (Dhansay, 2007). This is not in line with the recommended three doses of the International Vitamin A Consultative Group. No data are available on the current provincial protocol or on other provincial protocols. Despite the vitamin A supplementation programme and a decrease in the prevalence of vitamin A deficiency since 2005, vitamin A deficiency remains a severe public health problem in South Africa. In 2011, the annual vitamin A supplementation coverage for children 12 -59 months was 43.4% (Health System Trust, 2011).

A study in the Philippines among 11 620 children (aged one to four years) showed that the magnitude of the effect of high dose vitamin A capsules on serum retinol is limited. In addition, the effect does not last for six months (Pedro *et al.*, 2004). It is been advocated therefore that the focus should be on preventing vitamin A deficiency by means of promoting and supporting optimal breast feeding of infants and young children and consumption of an adequate and varied diet with vitamin

A-rich foods by children and women, while at the same time implementing other health measures such as the control of infectious diseases (Shisana *et al.*, 2013). The vitamin A supplementation programme has to be followed up by other strategies, such as food fortification and nutritional education, to address vitamin A and other micronutrient deficiencies in the long term (Labadarios *et al.*, 2005b).

#### **2.3.4.2 Multiple-micronutrient fortification initiatives**

Since 2003 it has been mandatory for all maize meal and wheat flour to be fortified according to **Table 2.2** in South Africa (South Africa, 2002). Maize meal and wheat flour are fortified to provide a person of 10 years or older with the following percentages of the RDA per 200 g of raw maize meal/wheaten flour: vitamin A (31%), thiamin (25%), niacin (25%), pyridoxine (25%), folate (50%), riboflavin (17% from maize and 20% from wheat flour), iron (25% from unsifted maize meal and 50% from maize meal), and zinc (20%) (Labadarios *et al.*, 2005b).

Steyn *et al.* (2008) calculated that with the implementation of the national fortification of the two most commonly consumed staple foods the micronutrient intake of children aged one to nine years, as well as the overall micronutrient density of the diet, would improve substantially. For micronutrient status to improve, the fortificants have to be bioavailable. The WHO recommended ferrous sulphate, ferrous fumarate, ferric pyrophosphate and electrolytic iron powder as iron fortificants. The current level of electrolytic iron used as fortificant in bread might not be effective in improving iron or haemoglobin status. Furthermore, because of the high phytate content in maize meal, this might be applicable to maize meal as well (Van Stuijvenberg *et al.*, 2008). Zinc oxide and ZnSO<sub>4</sub> are the least expensive and most commonly used zinc fortificants in the food industry. Suggested levels for fortification of flour are 30-70 mg zinc/kg (Roohani *et al.*, 2013). The WHO recommended using retinyl acetate and retinyl palmitates, along with provitamin A ( $\beta$ -carotene) as a vitamin A fortificant. A study in the Philippines showed that a fortification level of 2.2  $\mu$ g retinol/g of wheat flour provided 33% of the recommended dietary intake for school children and retinol liver

stores were significantly increased in vitamin A-deficient children at the end of a 30-week efficacy trial. The impact of maize fortification on the vitamin A status of the general population is not known (WHO, 2009).

**Table 2.2** Fortificants and micronutrient requirements of wheat flour, maize meal and unsifted maize meal (per 1 kg meal) (taken from South Africa, 2002).

Fortificants	Wheat flour	Maize meal	Unsifted maize meal
Vitamin A Palmitate (min 75000 $\mu\text{gRE}^2/\text{g}$ activity) ( $\mu\text{g RE}$ )	1785	2085	2085
Thiamine mononitrate (min. 78% activity) (mg)	1.9	2.2	2.2
Riboflavin (mg)	1.8	1.7	1.7
Nicotinamide/niacinamide (mg)	23.7	25.0	25.0
Pyridoxine HCL (min. 81% activity) (mg)	2.7	3.1	3.1
Folic acid (min. 90.5% activity) (mg)	1.4	2.0	2.0
Electrolytic iron (min. 98% activity) (mg)	35.0	35.0	17.5
Zinc oxide (min. 80% activity) (mg)	15.0	15.0	15.0

### 2.3.4.3 National school feeding programme

School feeding was implemented in September 1994 in South Africa and is part of the National School Nutrition Programme. Approximately 15 000 schools participated (in the period 2002 to 2005), and an average of 5 million school children received meals during this period (Labadarios, *et al.*, 2005b). A survey commissioned by the directorate of nutrition showed that the targeting of schools was diluted, because provinces wanted to include as many schools and children as possible. This however affected the quality and quantity of the food provided as school meals and the

neediest schools were not necessarily included. In general, it resulted in poor adherence to the menu options and guidelines. Problems with the school feeding programme included the nutritional value of the school meal, including the RDA for energy, delayed feeding time, lack of running water and poor food hygiene practices (Labadarios, *et al.*, 2005b).

## **2.4 Biodiversity food-based strategy**

In the nutrition science community, biodiversity refers to food and nutrients available from wild and gathered species or varieties, often underutilised and underexploited. Food composition forms the link between biodiversity and nutrition and biodiversity food-based strategy focuses on dietary diversity (Toledo & Burlingame, 2006). There are over 80 000 plant species available to humans, of which three (maize, wheat and rice) are extensively used. Many other plant species have been neglected or forgotten (Frison *et al.*, 2006), as diets have been simplified owing to the nutrition transition. This transition describes the process of a change in dietary consumption and energy expenditure, which coincides with urbanisation, economic development, increased wealth and a shift from poverty-related infectious diseases to lifestyle-related non-communicable diseases (WHO/FAO, 2003; Bourne *et al.*, 2002). Changes in employment patterns, especially for women, lead to the replacement of time-consuming traditional prepared meals by westernised 'convenience' meals, the most popular being easy-to-prepare cereals such as rice (Frison *et al.*, 2006). In many cases the low cost of staples relative to non-staples lead to diets simultaneously adequate in energy but deficient in micronutrients. Such a diet coinciding with low energy expenditure contributes to the development of obesity and micronutrient deficiencies in the same person (Vorster *et al.*, 2011). Given the rapidity with which traditional diets and lifestyles are changing in many developing countries, it is not surprising that food insecurity, undernutrition and obesity persist in the same countries where chronic diseases are emerging as a major epidemic (Popkin, 2004; WHO/FAO, 2003). Healthy and nutritious diets for populations depend on the availability and accessibility of a

variety of plant and animal foods, in a context that promotes and supports healthy behaviour. Traditional biodiversity use, instead of westernising diets, in the socio-cultural context can be a powerful tool for maintaining and enhancing health and nutritional status (Johns, 2003). Although many plant species have been neglected or forgotten (Frison *et al.*, 2006), there are several studies that have documented the the variety of contributions which diverse plant foods can make to nutrition (Laurie & Faber, 2008; Bushamuka *et al.*, 2005; Jones *et al.*, 2005; Makhotla & Hendriks, 2004; Faber *et al.*, 2002; Schipani *et al.*, 2002; De Pee *et al.*, 1998; Schmidt & Vorster, 1995). The Convention on Biological Diversity promotes the sustainable use of biological diversity to increase diversity in diets and to tackle both under- and overnutrition (CBD, 2006). Several reviews have been conducted on the nutritional impact of agricultural interventions adopting biodiversity food based strategies. Ruel (2001) reviewed evidence on food based strategies addressing iron and vitamin A deficiencies. It focused on the production, availability and access to vitamin A and iron-rich foods, dietary intake and bioavailability of iron and vitamin A and how to increase these aspects and on plant breeding approaches. Fourteen studies in the period 1995 to 1999 have been included with a strong focus on home gardening interventions. Ruel concluded that although there is some evidence on the impact of vitamin A, evidence on the impact on nutritional status is scant (Ruel, 2001). Berti *et al.* (2004) identified 30 intervention studies in the period from 1985 to 2001, which included home gardening, livestock, mixed garden and livestock, cash cropping, and irrigation. Nutritional impact was assessed by dietary intake, biomarkers, anthropometry and morbidity. Although food production was increased in most interventions, there was not always an impact on nutrition or health status. The home garden interventions had more often a nutritional impact than the other interventions. The overall conclusion was that agricultural interventions had mixed results in terms of improving nutritional status (Berti *et al.*, 2004). Masset *et al.* (2012) reviewed the impact of agricultural interventions that aiming to improve children's nutritional status by improving income and diet of rural poor populations. This review identified 23 intervention studies, including biofortification, home gardening, aquaculture and small fisheries, dairy development and animal

source food promotion, in the period from 1990 to 2010. Outcome indicators included programme participation, income, diet composition, micronutrients intake and children's nutritional status. The interventions appeared to have a positive impact on the production and consumption of the food item promoted, although it remains unclear if this has an impact on income improvement. The impact of these interventions on micronutrients intake was inconclusive; there was no evidence of impact on iron intake and although the impact on vitamin A intake appeared positive, this should be interpreted with care due to the small number of studies available. This review confirmed the little impact on nutritional status (in this case of children), although it was rather attributed to the lack of statistical power of the intervention studies included than to the lack of efficacy of these interventions (Masset *et al.*, 2012). Including micronutrient-rich foods, such as vegetables and fruit, in people's diets, based on locally available indigenous foods, has several advantages. These indigenous vegetables are an affordable (Cloete & Idsardi, 2013) alternative to commercially available vegetables, which are often not accessible to resource-poor communities. In addition, indigenous vegetables are adapted to the local environment, which increases the chances of successful cultivation (Faber *et al.*, 2010; Vorster *et al.*, 2007; Aphané *et al.*, 2003). Food-based approaches are complex to implement and to evaluate (Greiner, 2013) and often designed with long-term planning in mind (Masset *et al.*, 2012). Although food-based approaches are more sustainable strategies to address malnutrition, most only target the alleviation of single micronutrients, mostly focused on vitamin A. Synergies between nutrients however ask for a wider scope; a food-based strategy needs to focus on improving overall diet quality as well as improving the well-being of rural and urban populations. Agricultural biodiversity plays a key role in such a strategy to improve food security and health (Faber *et al.*, 2011; Frison *et al.*, 2006). This includes the revitalisation and mobilisation of indigenous and traditional food systems with the focus on staples and non-staples, rich in micronutrients and non-nutrients (Frison *et al.*, 2006), such as indigenous and traditional vegetables.

### 2.4.1 Indigenous and traditional leafy vegetables

The terms 'indigenous' and 'traditional' have been adopted from Maundu *et al.* (2009). 'Indigenous' refers to vegetables "known to be native or to have originated in a specified geographical location". 'Traditional' refers to vegetables "brought into the region of focus (exotic or introduced species) that have been used for a sufficient length of time to be part of the local food habits, knowledge systems and customs of communities." Most indigenous vegetables will also be traditional vegetables (Maundu *et al.*, 2009). The term 'leafy vegetable' is used to describe plant species of which the leafy parts (including young, succulent stems) are used as a vegetable. In South Africa, leafy vegetables are often collectively referred to as *morogo* (Sesotho, isiPedi) or *imifino* (isiZulu, isiXhosa). This dynamic concept is subject to spatial and temporal availability and includes besides indigenous and traditional vegetables also recently introduced species such as Swiss chard (*Beta vulgaris*) (Jansen van Rensburg *et al.*, 2007). The term 'African leafy vegetable' (ALV) has been adopted from Jansen van Rensburg *et al.* (2007). It refers to "the collective of leafy vegetable species that form part of the culinary repertoire of particular contemporary African communities" (Jansen van Rensburg *et al.*, 2007). There are over 45 000 plant species in sub-Saharan Africa, of which approximately 1000 are green leafy vegetables (Shiundu & Oniang'o, 2007). Jansen van Rensburg *et al.* listed the ALVs of particular importance in South Africa: amaranth (*Amaranthus spp*), spiderplant (*Cleome Gynandra*), cowpea (*Vigna inguiculata*), pumpkins (*Cucurbita pepo*, *C. maxima* and *C. moshata*), Chinese cabbage (*Brassica rapa* subsp. *chinensis*), nightshade (*Solanum retroflexum*), melons (*Citrulus lanatus* and *Cucumis melo*) and balsam pear (*Momordica balsamina*) (Jansen van Rensburg *et al.*, 2007). Many leafy vegetables in South Africa are not cultivated, but rather collected. Several species grow naturally when soils are disturbed (Jansen van Rensburg *et al.*, 2007), but the cultivation of ALVs can also be successful in home gardens (Molebatsi *et al.*, 2010; High & Shackleton, 2000). Benefits of home gardens to the household include among others access to fresh vegetables, cost savings (and occasionally an extra income), and increase in food production. Challenges include year-round availability of water, availability of seeds and knowledge and damage cause by animals

(Oluoch *et al.*, 2009). The overall average mean harvests in one season varies per ALV and per area, ranging from a yield of 82 ton/hectare (amaranth leaves) to a yield of 16 ton/hectare (spiderplant leaves) (Oluoch *et al.*, 2009). The management of ALVs is relatively low, since they are grown under rainfed conditions in between other plants either in natural vegetation or in home gardens (Hart & Vorster, 2006). Furthermore, ALVs grow on soils of limited fertility, are relatively drought-tolerant and pest-resistant, and can be harvested within a short period of time (Faber & Wenhold, 2007). However, constraints to the successful production of ALVs included non-availability of high-quality seeds, lack of improved cultural practices and lack of effective technologies for post-harvest preservation and processing of leaves (Oluoch *et al.*, 2009).

Several studies show the importance of indigenous and traditional leafy vegetables for household food security (Faber *et al.*, 2010, Twine & Hunter, 2009; Kaschula, 2008; Jansen van Rensburg *et al.*, 2007; Vorster *et al.* 2007; Flyman & Afolayan, 2006). In South Africa ALVs are mainly consumed by poor households residing in rural areas, that rely on food collected in the wild or harvested from home gardens (Faber *et al.*, 2010, Jansen van Rensburg *et al.*, 2007; Vorster *et al.*, 2007). The Pedi proverb, “Meat is a visitor, but *morogo* a daily food”, is their daily reality (Hart & Vorster, 2006). The consumption pattern of ALVs varies among households in different countries and depends on factors such as poverty, degree of urbanisation, accessibility and availability of ALVs, gender and ethnicity (Uusiku *et al.*, 2010, Vorster *et al.*, 2007). In South Africa indigenous and traditional leafy vegetables are mostly eaten as a condiment with a starchy staple, such as maize meal porridge (Faber *et al.*, 2010; Vorster *et al.*, 2007). Ingredients of these vegetable dishes may vary and include oil, butter, groundnuts, coconut, milk, bicarbonate of soda, tomato and onion (Uusiku *et al.*, 2010).

ALVs can play a crucial role in improving the nutrient value of the starch-based diets consumed by many of the rural poor (Uusiku *et al.*, 2010). It is well known that the consumption of sufficient fruit and vegetables reduces the risk of disease (Naude, 2013; Lock *et al.*, 2004). The health-protecting properties of non-nutrient bio-active compounds in ALVs can play an essential role in the success of

the WHO's global initiative on the consumption of fruit and vegetables (Smith & Eyzaguirre, 2007). It was estimated that the theoretical-minimum-risk distribution (required adequate intake to prevent disease) for fruit and vegetable intake was 480 grams in children aged 5–14 years (Lock *et al.*, 2004). The South African paediatric food-based dietary guidelines recommend a fruit and vegetable intake of 320-480 gram per day for children aged one to seven years (Bowley *et al.*, 2007). The 1999 NFCS showed that the reported average fruit and vegetable intake in children aged one to nine years was 110-205 gram per day (Labadarios *et al.*, 2005a).

The ALVs selected for the intervention study were those that are most frequently consumed and used based on the results of previous research in urban and rural areas in the North West Province (Matenge *et al.*, 2011) and have been recognised as important ALV species in South Africa (Jansen van Rensburg *et al.*, 2007). They include the following: *Amaranthus cruentus* (amaranth), *Cleome gynandra* (spiderplant), *Cucurbita maxima* (pumpkin) and *Vigna unguiculata* (cowpea).

#### **2.4.1.1 Amaranthus cruentus**

*Amaranthus cruentus* L. (**Figure 2.2**) is part of the Amaranthaceae family. It is an erect annual herb with oblong pointed green leaves and clusters of greenish wind-pollinated flowers. It originated in Southern Mexico and Central America and can be traced back to 4000 BC (Van Wyk, 2005). Amaranth species are widely distributed in most parts of South Africa and therefore widely available as leafy vegetable (Jansen van Rensburg *et al.*, 2007; Schippers 2000). The optimal growth conditions for these C4 plants are warm conditions (day temperature above 25 °C and night temperature not below 15 °C), bright light and adequate availability of nutrients (Jansen van Rensburg *et al.*, 2007; Van Wyk, 2005). The edible parts of *Amaranthus cruentus* include the young leaves, growth points and ripe seeds (grain) (Jansen van Rensburg *et al.*, 2007; Van Wyk, 2005). An edible portion of 100 gram (wet weight) of *Amaranthus* spp. contains 327 µg RE vitamin A, 0.3 – 3.8 mg iron and 0.02 – 8.4 mg zinc (Uusiku *et al.*, 2010).



**Figure 2.2** Photographs of *Amaranthus cruentus* seed (left) and leaves (right).

#### 2.4.1.2 *Cleome gynandra*

*Cleome gynandra* L. (**Figure 2.3**) is part of the Capparaceae family. It is a herbaceous, perennial, erect plant consisting of many branches (Onyango *et al.*, 2013; Jansen van Rensburg *et al.*, 2007). *Cleome* is thought to be native to Africa (Onyango *et al.*, 2013). The leaves are compound and palmate with three to seven leaflets. Spiderplant requires full exposure to sunlight and does not grow well when (night) temperatures drop below 15 °C. Optimal growth conditions include well-drained medium-textured soils and adequate water supply, although the plant may be drought-tolerant (Jansen van Rensburg *et al.*, 2007; Schippers, 2000). The edible parts include the leaves and the growth tips (Jansen van Rensburg *et al.*, 2007). An edible portion of 100 gram (wet weight) of *Cleome* spp. contains 1200 µg RE vitamin A, 2.6 – 2.9 mg iron and 0.6 – 0.8 mg zinc (Uusiku *et al.*, 2010).



**Figure 2.3** Photographs of *Cleome gynandra* seed (left) and leaves (right).

### 2.4.1.3 *Cucurbita maxima*

*Cucurbita maxima* Lam. (**Figure 2.4**) is part of the Cucurbitaceae family. It is a trailing annual plant with rounded leaves and yellow male and female flowers (Jansen van Rensburg *et al.*, 2007; Van Wyk, 2005). Its origin might be in Peru from where it spread to Mexico and the rest of the world (Van Wyk, 2005). It is a popular leafy vegetable in South Africa and one of the few ALVs that is cultivated in the country (Jansen van Rensburg *et al.*, 2007). *Cucurbita maxima* is not frost-resistant, but is drought-tolerant and requires little water (Jansen van Rensburg *et al.*, 2007; Schippers 2000). Edible parts of this vegetable include the leaves, flowers, fruit (both young and mature) and the seed (Jansen van Rensburg *et al.*, 2007; Van Wyk, 2005). An edible portion of 100 gram (wet weight) of *Cucurbita maxima* contains 325 µg RAE vitamin A, 9.2 mg iron and 0.75 mg zinc (Van Jaarsveld *et al.*, 2013).



**Figure 2.4** Photographs of *Cucurbita maxima* seed (left) and leaves (right).

### 2.4.1.4. *Vigna unguiculata*

*Vigna unguiculata* (L) Walp (**Figure 2.5**) is part of the Leguminosae family (Jansen van Rensburg *et al.*, 2007). It is an annual bushy or climbing herb with compound leaves and pods. Cowpea is indigenous to Africa and from there it spread to Europe, Asia and the Americas (Jansen van Rensburg *et al.*, 2007; Van Wyk, 2005). *Vigna unguiculata* is drought-tolerant and resistant to many diseases (Jansen van Rensburg *et al.*, 2007). Edible parts include ripe seeds, (green) pods, young leaves and growth points (Jansen van Rensburg *et al.*, 2007; Van Wyk, 2005). An edible portion of 100 gram

(wet weight) of *Vigna unguiculata* contains 99 µg RE vitamin A, 0.3 – 3.0 mg iron and 0.23 mg zinc (Uusiku *et al.*, 2010).



**Figure 2.5** Photographs of *Vigna unguiculata* seed (left) and leaves (right).

#### 2.4.2 Nutrient composition

Several studies emphasised the importance of indigenous and traditional vegetables as sources of micronutrients (Van Jaarsveld *et al.*, 2013; Uusiku *et al.*, 2010; Faber *et al.*, 2007; Odhav *et al.*, 2007; Nesamvumi *et al.*, 2001; Steyn *et al.*, 2001; Kruger *et al.*, 1998). Van Jaarsveld *et al.* (2013) determined the nutrient content of the following eight cultivated ALVs: Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*), black nightshade (*Solanum retroflexum* Dun.), pigweed (*Amaranthus cruentus* L.), Jew's mallow (*Corchorus olitorius* L.), spider flower (*Cleome gynandra* L.), cowpea (*Vigna unguiculata* L. Walp.), pumpkin (*Cucurbita maxima* Duchesne) and tamma melon (*Citrullus lanatus* (Thunb.)). These vegetables contained substantial amounts of β-carotene and iron, but the vitamin C content was much lower than expected. Most of these vegetables contributed to to daily dietary recommendations (Van Jaarsveld *et al.*, 2013). Uusiku *et al.* (2010) reviewed the nutritional value of 22 ALVs. *Manihot esculenta*, which contains 1970 µg RE per 100 g edible portion and 311 mg/100 g of vitamin C, as well as *Chenopodium album*, with up to 6 mg/100 g iron, 18.5 mg/100 g zinc, 226 mg/100 g calcium and up to 211 mg/100 g magnesium, stood out. There were large differences in the β-carotene content of different ALVs. It varied from 99 µg RE in *Vigna unguiculata*

to 1970 µg RE in *Manihot esculenta*, both per 100 gram edible portion. Children will have to consume 300 gram of fresh ALVs to satisfy their dietary requirement for vitamin A (Uusiku *et al.*, 2010). Environmental factors, such as soil type and pH, availability of water, plant age, variety, use of fertilizers and climatic conditions influence the nutrient composition of plants, especially minerals (Uusiku *et al.*, 2010; Greenfield & Southgate, 2003). The content of dietary iron per 100 gram edible portion varied widely, from 0.2 to 12.8 mg iron for *Lesianthera africana* and *Solanum nigrum*, respectively. The zinc content in the same species was also highly variable, from 0.02 to 8.4 mg zinc for *Amaranthus* spp. and from 1.4 to 18.5 mg/100 g for *Chenopodium album*. In addition, these 22 ALVs contained dietary fibre and non-nutrient components, in many instances more than conventional vegetables, such as spinach or cabbage (Uusiku *et al.*, 2010).

However, different processing techniques and anti-nutritional factors such as phytate and vitamin C can influence the bioavailability of these micronutrients. Cooking increased the bioavailability of β-carotene and reduced the vitamin C content, as a result of leaching, but had no effect on iron and zinc bioavailability (Uusiku *et al.*, 2010). Optimisation of processing techniques can lead to better bioavailability of certain micronutrients, as shown by Amoussa-Hounkpatin *et al.* (2013). They studied the effect of the multi-step preparation of traditional sauces based on amaranth leaves, cooked palm nuts or red palm oil on the carotenoid content of the final dish. The blanching of amaranth leaves and production of palm nut juice had almost no effect on the retinol activity equivalent (RAE) values of the final product. The heating of red palm oil to 180-200 °C did affect the RAE value, but the final dish can still contribute to the reduction of vitamin A deficiency (Amoussa-Hounkpatin *et al.*, 2013). Furthermore, leafy vegetables might enhance the bioavailability of micronutrients in the staple crops they accompany, as they contain vitamin C (Aphane *et al.*, 2003). The reported nutrient composition of different ALVs shows that several of them contribute to meeting the daily micronutrient recommendations and this therefore suggests that a biodiversity food-based strategy, such as the use of ALVs, can address micronutrient deficiencies (Krawinkel 2009; Tontisirin *et al.*, 2002). There is however a need for more data on the nutritional value of

these vegetables, since the currently available data are limited and often incomplete (Uusiku *et al.*, 2010). In addition, it is important to take the bioavailability of these nutrients into account to determine if ALVs can contribute to the alleviation of micronutrient deficiencies (Flyman & Afolayan, 2006; Faber *et al.*, 2010).

### **2.4.3 Bioaccessibility and bioavailability**

Bioaccessibility refers to the amount of an ingested nutrient that is potentially available for absorption, whereas bioavailability is the amount of an ingested nutrient that is absorbed and available for physiological functions. Both absorption and availability are dependent on digestion and release from the food matrix. Bioavailability however includes absorption by the gastrointestinal tract and transport to body cells (Etcheverry *et al.*, 2012). There is great variation in the bioavailability of minerals and trace elements in different foods and food components and under different gastrointestinal conditions (Gupta *et al.*, 2006). Bioavailability may be negatively influenced by dietary fibre, calcium, phytate, oxalate, tannins and phosphates (Hurrell & Egli, 2010; Gupta *et al.*, 2006). The polyphenolic compounds present in plant foods include phenolic acids, flavonoids and their polymerisation products. They all act as inhibitors of iron absorption, but to a different extent (Gupta *et al.*, 2006). Phytate is the main factor inhibiting iron absorption in plant-based diets (Hurrell & Egli, 2010). Phytic acid forms insoluble complexes with iron and calcium that are not available for absorption under the pH conditions of the small intestine (Gupta *et al.*, 2006). Food processing, preparation methods and the addition of exogenous phytase can be used to remove or degrade phytate to varying degrees. Enhancers of iron absorption include ascorbic acid and muscle tissue (Hurrell & Egli, 2010). Zinc bioavailability in healthy individuals is determined by the individual's zinc status, the total zinc content of the diet and the availability of soluble zinc from the diet's food component. Inhibitors of zinc absorption include phytate and the potential interaction between iron and zinc (Roohani *et al.*, 2013).

Few studies provide data on iron and zinc availability from vegetables and no recent studies on zinc availability have been conducted (Lucarini *et al.*, 2000). Lucarini *et al.* (2000) studied the in vitro iron and zinc dialysability from several vegetables. The dialysability of leafy vegetables ranged from 15.0%-23.1% and 32.7%-49.3% for iron and zinc, respectively. The addition of cereal products (macaroni) to vegetables drastically lowered the dialysability of both (from 40% to 75%) confirming the inhibitory effect of phytate on iron and zinc bioavailability (Lucarini *et al.*, 2000). The iron content found by Gupta *et al.* (2006) in 13 green leafy vegetables varied widely, ranging from 0.0275 g/kg – 0.1847 g/kg. *Amaranthus tricolor* was among the vegetables with the highest iron content and the highest amount of bioaccessible iron. Because of variations in total iron content and the percentage of bioaccessibility of iron in these vegetables, the absolute quantity of bioaccessible iron was similar in the 13 samples. Oxalic acid has been found to have a moderate depressing effect on iron absorption. However, its role in modifying the bioavailability of iron is not clearly understood yet. Its availability was influenced by the presence of different inhibitory factors and could not be attributed to one single component. Oxalic acid, tannin and phytic acid accounted for 25%, 17% and 16% of inhibition of iron availability, respectively (Gupta *et al.*, 2006). The total amount of iron in green leafy vegetables was higher (by 9%) in samples cooked in iron utensils than in samples cooked in non-iron utensils, while the averages of total dietary fibre, oxalates and tannin were comparable. In addition, it was found that the bioavailability of iron increased in green leafy vegetables cooked in iron utensils (Kumari *et al.*, 2004).

The type of food matrix in which carotenoids are located is a major factor determining their bioavailability. Processing, including mechanical homogenisation or heat treatment, has the potential to enhance the bioavailability of carotenoids from vegetables (from 18% to a sixfold increase). The addition of dietary fat increases carotenoid absorption although it depends on the physicochemical characteristics of the carotenoids ingested (Van het Hof, *et al.*, 2000). A rat model showed that the leaves of *Moringa oleifera* (both fresh and dehydrated) significantly improved

serum vitamin A, liver retinol levels and body weight in vitamin A-deficient rats, although the improvements in the group replete with vitamin A acetate were more pronounced. This indicates that leaves of *Moringa oleifera* can make a valuable contribution to the alleviation of vitamin A deficiency (Nambiar & Seshadri, 2001). Graebner *et al.* (2004) also showed that carotenoids from dark-green leafy vegetables were bioavailable in a rat model. They found that the relative bioavailability of  $\beta$ -carotene was 36%, 16% and 9% for *Sonchus oleraceus*, *Amaranthus viridis* and *Xanthosoma sagittifolium* leaves, respectively. The carotenoids of these dark green leafy vegetables were absorbed, converted to retinol and stored in the liver (Graebner *et al.*, 2004). However, De Pee *et al.* (1995) did not find an improvement in vitamin A status of children who consumed an additional daily portion of dark green leafy vegetables, although a similar amount of  $\beta$ -carotene from a simpler food matrix did show improvement. In addition, in an intervention study comparing the effectiveness of orange fruit and dark green leafy vegetables in improving serum retinol and  $\beta$ -carotene, De Pee *et al.* (1998) observed that the apparent mean vitamin A activity of carotenoids in fruit and in leafy vegetables and carrots was 50% (95% CI: 21%, 100%) and 23% (95% CI: 8%, 46%) of what had been assumed, respectively. There is evidence from isotope-dilution tests that green and yellow vegetables can maintain body stores of vitamin A in children, suggesting that green-yellow vegetables can protect children from becoming vitamin A deficient during seasons when the provitamin A food source is limited (Tang *et al.*, 1999). Carotenoid bioavailability and bioconversion are influenced by different factors, such as the species of carotenoids, molecular linkage, amount of carotenoids consumed in a meal, matrix of the food, nutrient status of the host and genetic factors, among others (Graebner *et al.*, 2004). More research to quantify and improve bioavailability and bioconversion of carotenoids in dark green leafy vegetables is therefore needed (De Pee *et al.* 1998).

#### 2.4.4 Knowledge and perceptions of African leafy vegetables

Knowledge of ALVs tended to be the domain of women only, whereas knowledge of fruit and cereals seemed to be in the male domain (Vorster *et al.*, 2007). Knowledge of these plants has been passed down over generations (Faber *et al.*, 2010; Vorster *et al.*, 2007) in the community (Shava, 2005). Elderly women are regarded as the custodians of indigenous knowledge with regard to ALVs, their uses, preparation and preservation (Dweba & Mearns, 2011). Several studies have shown a decline in indigenous knowledge of traditional plants (Dweba & Mearns, 2011; Jansen van Rensburg *et al.*, 2007; Odhav *et al.*, 2007; Vorster *et al.*, 2007). Young women from a rural Eastern Cape village had a negative attitude to traditional vegetables. They indicated that they preferred modern foods and would not serve traditional vegetables at a public gathering. Furthermore, they associated these vegetables with rural communities, elderly people and poverty (Dweba & Mearns, 2011). The association of these plants with poverty is a major contributor to loss of knowledge in several communities in South Africa (Dweba & Mearns, 2011; Jansen van Rensburg *et al.*, 2007). In addition, the introduction of new and exotic vegetables and urbanisation contributed to this loss (Jansen van Rensburg *et al.*, 2007). Labels such as “backward knowledge” have been linked to traditional vegetables and associated knowledge, thus discouraging the youth from learning about them (Vorster *et al.*, 2007; Modi *et al.*, 2006). Furthermore, this decline in knowledge of the usefulness of indigenous and traditional vegetables has led to a decline in consumption (Mbhenyane *et al.*, 2005; Modi, 2003; Nesamvumi *et al.*, 2001). Reasons for the decline in this indigenous knowledge may include inadequate documentation due to the death or migration of the principal custodians of this knowledge, secrecy of the custodians of this knowledge, and globalisation by promoting universal values and beliefs while adopting western knowledge (Tabuti & Van Damme, 2012). However, several studies reported frequent use and positive perceptions of these vegetables among rural communities (Faber *et al.*, 2010; Shackleton, 2003). Faber *et al.* (2010) concluded that availability and access to nutrition-related uses of ALVs are content-specific, with inter- and intra-provincial

rural/urban differences. Different communities may have different perceptions and beliefs about the ALVs that grow in their area and this affects the consumption and use of the plants (Faber *et al.*, 2010). Not only do the ALVs species differ between communities, ethnicity also plays a role in taste and consequently preparation methods (Hart & Vorster, 2006). Vorster *et al.* (2007) found that in seven communities in South Africa, ranging from peri-urban to deep rural, amaranth and pumpkin were the most popular ALVs, whereas jute mallow and spiderplant were more often preferred in the northern areas of South Africa, where they occur naturally. Amaranth was considered better tasting than Swiss chard, but not better than spiderplant in a rural community in Limpopo. Spiderplant leaves were boiled with little water for an hour and 30 minutes and no water was discarded (Faber *et al.*, 2010). In a rural community in the Eastern Cape spiderplant leaves were regarded as bitter and it was therefore stated that the plant needed to be boiled and the cooking water needed to be discarded more than once to get rid of the bitter taste (Dweba & Mearns, 2011).

As observed by other researchers, sun drying is regarded as the most common method of preservation in South Africa (Nesamvuni *et al.*, 2001; Vorster, 2007) and other African countries (Smith & Eyzaguirre, 2007). Drying of food is practised in Africa to make the products more durable and preserve them for food-insecure periods (Vorster *et al.*, 2007). Sun drying is the principal mode of extending the shelf life of ALVs (Nesamvuni *et al.*, 2001; Vorster, 2007; Faber *et al.*, 2010). In South Africa most of the leaves are dried in direct sunlight and they are sometimes blanched before drying (Vorster *et al.*, 2007). Blanching of vegetables has proven to improve colour and carotene retention because of inactivation of enzymes; however, it causes losses of ascorbic acid (Nguni & Mwila, 2007).

#### **2.4.4.1 Sensory evaluation**

Sensory characteristics of food, such as appearance, smell, texture and taste, also play an important role in people's decision to consume a particular food (Guinard, 2001). Research on the acceptability of food is needed to determine the impact of taste and preference on the dietary intake patterns of

consumers that can be used to improve the general acceptance of ALVs (Babu, 2000). There are three reasons for sensory testing among children: to understand their sensory perceptions (basic research), to conduct a sensory evaluation with children as judges (e.g. difference testing, descriptive analysis) and consumer testing with children as consumers. It is important to take the cognitive abilities of children into account while conducting sensory evaluations. These include verbal skills, attention span and task comprehension. The limited verbal skills of children demand consideration of the phrasing of questions and the vocabulary used (Guinard, 2001). To ensure school children from a low socio-economic background are able to discriminate between verbal anchors, it is recommended that a five-point ordinal scale be used (Dalton *et al.*, 2006). The test environment, including the duration of the sensory testing, has to be adjusted to maximise the child's attention span. This requires the right balance between comfort and familiarity and distraction. Furthermore, it is recommended that the test protocol be explained to the child by using visual stimuli, such as pictures of food, before the actual tasting. Sensory testing in children in the age group between six and ten years can include hedonic scaling (with appropriated facial scales with wording), paired-comparison, a duo-trio test and intensity ranking or scaling (Guinard, 2001).

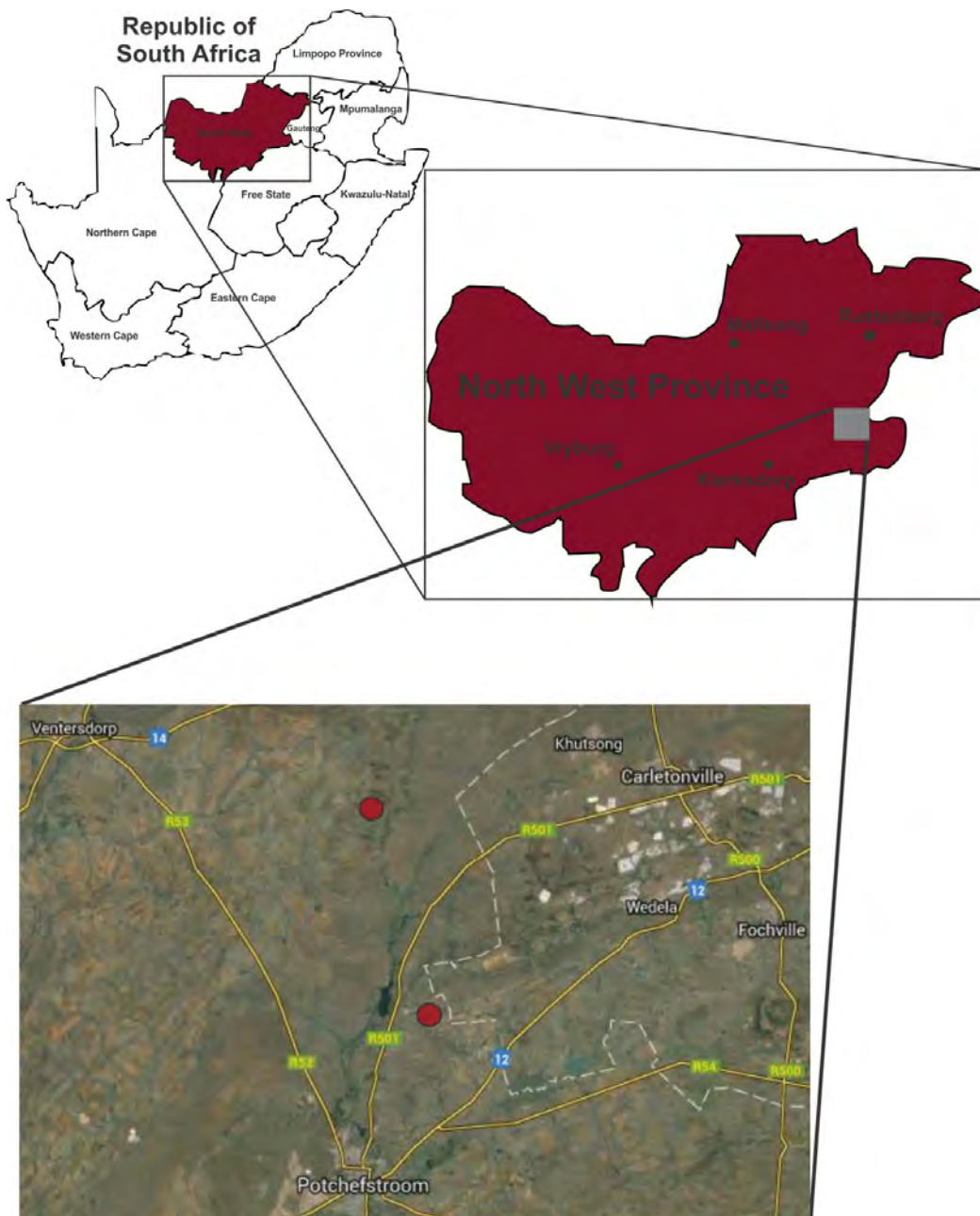
## **2.5 Farm communities in South Africa**

Farm communities in South Africa are seen as the least privileged and the most vulnerable population in terms of income, health status, education and household nutrition security (Chopra *et al.*, 2009; Kruger *et al.*, 2006). Although agriculture is an important sector in South Africa, farm workers are of the most vulnerable employees, earning the lowest wages. The South African government set minimum wages for farm workers in 2003 to improve their situation. As a consequence, sometimes farmers cut previous benefits, such as housing and food rations, which left the farm workers with less than before. Farm workers are exposed to occupational hazards in agriculture, migrancy, social discrimination and poverty. In addition, these communities are

characterised by alcohol abuse, domestic violence and chronic malnutrition (Lemke, 2005). Children from these communities indicated during focus group interviews that the alcohol use of their parents compromised their food situation (Kruger *et al.*, 2006).

### 2.5.1 Study site

The current study was conducted within the infrastructure of two primary farm schools (indicated by the maroon circles in **Figure 2.6**) in a rural area approximately 50 kilometres from Potchefstroom in the south-eastern part of the North West Province.



**Figure 2.6** Location of the selected farm primary schools (satellite image courtesy of Google Maps).

The main farming activities in this area include maize, sunflower and chicken farming. Both primary schools were located in similar farm surroundings (with only very small shops, so-called tuck shops) and were fully sponsored by the South African Department of Education and by the farm owners themselves. Both schools have implemented the National School Nutrition Programme and provide their learners with one meal per school day. For the North West Province each lunch meal consists of 40-45 gram of protein, 60 gram of starch and 60 gram of vegetables and/or fruit (South Africa, 2010/2011). One school was situated approximately 25 kilometres from an urban area with markets and grocery stores, the other school approximately 35 kilometres from such an area. Schools were chosen as entry point because they are closely linked to the community.

Dietary intake was assessed during the 1999 NFCS (Labadarios, 2000). The median iron, vitamin A and zinc intake of children four to nine years old residing in the North West Province was below the RDA (Labadarios *et al.*, 2005a) (**Table 2.3**). Furthermore, it was found that the intake of children

**Table 2.3** Iron, vitamin A and zinc intake of children four to nine years old residing in the North West Province (adapted from Labadarios, 2000).

		Iron (mg)	Vitamin A (RE)	Zinc (mg)
4-6 years (n=33)	RDA	10	500	10
	Median	6.2	383	5.4
	Q1 - Q3	4.2 - 8.6	226 - 690	3.7 - 7.6
7-9 years (n=27)	RDA	10	700	10
	Median	4.9	336	4.9
	Q1 - Q3	4.2 - 7.4	228 - 591	4.2 - 7.4

RE: Retinol equivalent; RDA: Recommended dietary allowance; Q1 - Q3: Interquartile range

residing in rural areas was worse than that of children residing in urban areas (Labadarios *et al.*, 2005a). An intervention study in the North West Province, focusing on the effects of black tea and

Rooibos on iron status, found that more than two-third of the included rural primary school children had deficient intake of iron (38%) and vitamin A (40%) (Breet *et al.*, 2005). Adults residing in rural areas in the North West Province consumed less fruit, vegetables, animal-derived foods and fats and oils than urban inhabitants of the same province (Vorster *et al.*, 2005). In the North West Province, 44.1% of the adult population has a poor dietary diversity score (lower than 4) (Labadarios *et al.*, 2011). It is likely that children's diets also lack diversity.

The 1999 NFCS reported that children living on commercial farms were severely affected in terms of stunting (Labadarios *et al.* 2005a). The prevalence of stunting among farm school children in the North West Province was 24.5% and 19.1% of them were underweight (Kruger *et al.*, 2006). SANHANES reported that the incidence of overweight in boys was lowest in the North West Province (6.4%) and that of obesity second lowest (3.3%). The prevalence of overweight and obesity among girls in the North West Province was 15.2% and 4.3% respectively. The population of the North West Province was one of the most undernourished in the country. Undernutrition among boys in the North West Province was reflected in prevalence percentages of 23.7%, 8.5% and 15.2% for stunting, wasting and underweight respectively. The trend among girls in the North West Province was similar, with percentages of 17.8%, 5.2% and 7.9% for stunting, wasting and underweight respectively (Shisana *et al.*, 2013). A regional intervention study among rural primary school children found indicated that 40% of the participants (N=150) had weight-for-age below the 10<sup>th</sup> percentile and 36% of the participants had height-for-age below the 10<sup>th</sup> percentile, indicating a malnourished population (Breet *et al.*, 2005).

Data on the micronutrient status of primary school children in farm or rural communities in the North West Province in South Africa is limited. The data of the latest national survey, SANHANES, was not stratified on provincial level due to the small sample size (Shisana *et al.*, 2013). The last national survey reporting data on anaemia and iron deficiency in the North West Province was the NFCS-FB. This study reported an anaemia (haemoglobin  $\leq$  11 g/dL) prevalence of 27% (N=37) and an

iron deficiency (haemoglobin  $\leq$  11 g/dL and SF  $<$  12  $\mu$ g/L) prevalence of 2.9% (N=35; no correction for CRP) in the North West Province (Labadarios, 2007). An intervention study in the North West Province, focusing on the effects of black tea and Rooibos on iron status, found that the mean haemoglobin concentration was 12.8 g/dL in rural primary school children and that the overall prevalence of anaemia (haemoglobin  $\leq$  12 g/dL) was 13-15% (in both groups). The mean SF concentration was 40.5-41.8  $\mu$ g/L in the same children and 11-12% had deficient SF concentrations at baseline (in both groups) (Breet *et al.*, 2005). Another study in the North West Province, using the baseline data from the BeForMi multiple micronutrient beverage intervention study conducted in 556 urban school children, found a lower prevalence of anaemia (Hb  $<$  11.5 g/dl) was 6.8%, although iron deficiency (Hb  $>$  11.5 g/dl and SF  $<$  12 lg/L) was present in 13.9% and iron deficiency anemia (Hb  $<$  11.5 g/dl and SF  $<$  12 lg/L) was present in 5.6% of the children (Ojabanjo *et al.*, 2010). Taljaard *et al.* (2013) concluded that national data is not necessary representative for regional populations and that the anaemia prevalence, based on the haemoglobin concentration in primary school children, might have improved in some regions since the NFCS-FB has been conducted.

## 2.6 Conclusion

Given the rapidity with which traditional diets and lifestyles are changing in South Africa, it is not surprising that food insecurity, micronutrient deficiencies, undernutrition and obesity persist in the areas where chronic diseases are emerging as a major epidemic. Healthy and nutritious diets for populations depend on the availability and accessibility of a variety of plant and animal foods, in a context that promotes and supports healthy behaviour. Although food-based approaches are more sustainable strategies to address malnutrition, most only target the alleviation of single micronutrients. Synergies between nutrients however demand a wider scope; a food-based strategy needs to focus on improving overall diet quality as well as improving the well-being of rural and urban populations. Farm communities in South Africa are said to be the most vulnerable population

with poor nutritional status, especially in the North West Province, which is one of the most undernourished provinces. Agricultural biodiversity plays a key role in such a strategy to improve food security and nutritional status. This includes the revitalisation and mobilisation of indigenous and traditional food systems with the focus on staples and non-staples, rich in micronutrients and non-nutrients, such as ALVs. In order to promote the biodiversity food strategy to improve nutritional status and food security, it is important to investigate the potential and success factors of such a strategy. Although there is ample evidence of the rich nutrient composition of ALVs, there is limited knowledge of the bioaccessibility and bioavailability of these nutrients, including iron, vitamin A and zinc and their effect on the micronutrient status of children. Furthermore, it is important to look at current knowledge, uses and perceptions, including sensory evaluations of ALVs, to create support for the food-based strategy.

## 2.7 References

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### 3. MANUSCRIPT 1

## **Indigenous and traditional plants: South African caregivers' knowledge, perceptions and uses and their children's sensory acceptance**

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#### **ABSTRACT**

**Background** The dietary shift from indigenous and traditional plants (ITPs) to cash crops and exotic plant food sources increases the risk of malnutrition and other nutrition-related non-communicable diseases, especially in poor rural communities. Farm communities in South Africa have been associated with poor nutritional status and extreme poverty. ITPs have been found to be affordable sources of several micronutrients. However, knowledge of and the use of these plants are declining, and little is known about the child's acceptance of dishes prepared with ITPs. This knowledge can be used to improve the general acceptance of ITPs. This study aimed to gain insight into parents' knowledge and perceptions and their use of ITPs in a farming community in the North West Province

and to assess children's acceptance of and preference for dishes made with African leafy vegetables (ALVs) and Swiss chard. **Methods** Parents (n=29) responsible for food preparation for children in grade 2 to 4 in two schools were purposively selected for four focus group discussions. A sensory evaluation assessed the children's (n=98) acceptance of, preference for and intended consumption of dishes made with leafy vegetables. The dishes were made of *Amaranthus spp.*, *Cleome gynandra*, *Cucurbita maxima*, *Vigna unguiculata* and *Beta vulgaris*. **Results** Parents mentioned 30 edible ITPs during the focus group discussions. Parents had knowledge of available ITPs and their use as food. Location, seasonal variation and rainfall affected the availability of and access to ITPs. Sun-dried ITPs were stored in sacks for later use. ITPs were perceived as healthy, affordable and delicious, hence acceptable to the parents. The children also evaluated the dishes made with ALVs as acceptable in terms of colour, smell and taste. Swiss chard was preferred, most likely because of the children's exposure to this vegetable. Children indicated that they would like to eat these leafy vegetables twice a week. **Conclusion** These results look promising for the promotion of ITPs as a strategy to reduce malnutrition in rural farm communities and for potential inclusion of these micronutrient-rich ALVs in school feeding programmes to improve the nutritional status of children.

## KEYWORDS

Indigenous plants, traditional plants, African leafy vegetables, knowledge, perception, use, sensory evaluation, parents, primary school children, South Africa

## BACKGROUND

Over millennia, indigenous and traditional plants (ITPs) have been the main source of food for many rural communities. However, colonial economies and post-independence development schemes placed greater emphasis on the production and consumption of cash crops, introduced foods that

led to the displacement of indigenous food crops and caused subsequent changes in the diet of African people (1). Their food patterns reflected an increasing intake of a limited number of domesticated plant staples, while intake of the edible wild plant species that once sustained health and nutritional status was reduced (2). It is evident that urbanisation has contributed to a decline in knowledge of the usefulness of ITPs, hence the reduction in the consumption of these foods. This dietary change, especially in poor rural communities, put people at risk of malnutrition and other nutrition-related non-communicable diseases. According to the United Nations Children's Fund conceptual framework on malnutrition, the underlying causes of malnutrition and death in children are poor household food security, inadequate maternal and child care, insufficient health services and an unhealthy environment or lack of education and information (3). The South African National Food Consumption survey of 1999 showed that a large number of children had inadequate intake of energy, vitamin A, vitamin C, thiamine, riboflavin, niacin, vitamin B6, vitamin B12, folic acid and zinc (4). It also showed that rural children were worse off than those who lived in urban areas. Lemke stated that in South Africa, with regard to socio-economic status, health status, household nutrition security and education, farm worker households tended to be most vulnerable among all groups (5). ITPs can play an important part in alleviating hunger and malnutrition. They are important sources of micronutrients, including vitamins A and C, iron and other nutrients, and are sometimes better nutritional sources than modern vegetables (6). Modernisation of South African rural communities has led to people perceiving ITPs as inferior. Faber *et al.* reported that African leafy vegetables (ALVs) were often regarded as a poor people's food in South Africa (7). Labels such as "backward knowledge" have been linked to traditional vegetables and associated knowledge, thus discouraging the youth from learning about them (8, 9).

Knowledge of the use of indigenous plants needs urgent scientific investigation and documentation before it is irreversibly lost to future generations (6). Several studies in South Africa have reported a decline in the use of ITPs (10, 11, 12). However, Shackleton reported the frequent use of wild edible herbs among rural communities (13). Faber *et al.* (7) concluded that availability and access to

nutrition-related uses of ALVs are content-specific, with inter- and intra-provincial rural/urban differences. Different communities may have different perceptions and beliefs about the ITPs that grow in their area and this affects the consumption and use of the plants. Sensory characteristics of food, such as appearance, smell, texture and taste, also play an important role in people's decision to consume a particular food. Research on the acceptability of food is needed to determine the impact of taste and preference on dietary intake patterns of consumers that can be used to improve the general acceptance of ITP foods (14).

As information collected during small studies within a specific area cannot be generalised to the entire South African population, the objectives of this contextual study were to gain insight into the parent<sup>1</sup>'s knowledge and perceptions of and their use of ITPs in a farming community in the North West Province and to assess children's acceptance of and preference for dishes made with ALVs and Swiss chard.

## **METHODOLOGY**

### **Research design**

The study was conducted in two phases. The first phase used a qualitative interpretive description approach (15) to explore and describe parents' knowledge and perceptions and their use of ITPs. Household socio-demographic characteristics were also obtained from these parents. The second phase used a quantitative cross-sectional approach in the form of sensory evaluation to assess children's acceptance of and preference for dishes made with ALVs and Swiss chard.

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<sup>1</sup> Parent or guardian, hereafter referred to as parent.

**Setting**

The North West Province of South Africa is approximately 116 320 square kilometres in area and almost all its rainfall occurs in the summer months between October and April. Average rainfall of 539 mm per annum decreases from east to west. There is a short growing season for frost-sensitive crops between October (last cold) and the end of April (first frost). Regular droughts occur in this province. Sixty percent of the province's 3.2 million inhabitants live in rural areas (16, 17). According to the North West Province State of the Environment Report in 2002, mining and agriculture, including both crop cultivation and livestock production systems, were the two most important economic sectors. In the same year, the estimated unemployment rate was 38% and approximately a third of the population was illiterate (17). According to Cloete *et al.*, 53% of the population lives in poverty and 41% is economically dependent on social funding from government (18). The current study was conducted within the infrastructure of two farm primary schools in a rural area approximately 50 kilometres from Potchefstroom in the south-eastern part of the North West Province (Figure 1). The main farming activities in this area include maize, sunflower and chicken farming. Both these primary schools were located in similar farm surroundings (with only very small shops, so-called tuck shops) and were fully sponsored by the South African Department of Education and by the farm owners themselves. One school was situated approximately 25 kilometres from an urban area with markets and grocery stores, the other school approximately 35 kilometres from such an area. Schools were chosen as entry point because they are closely linked to the community.

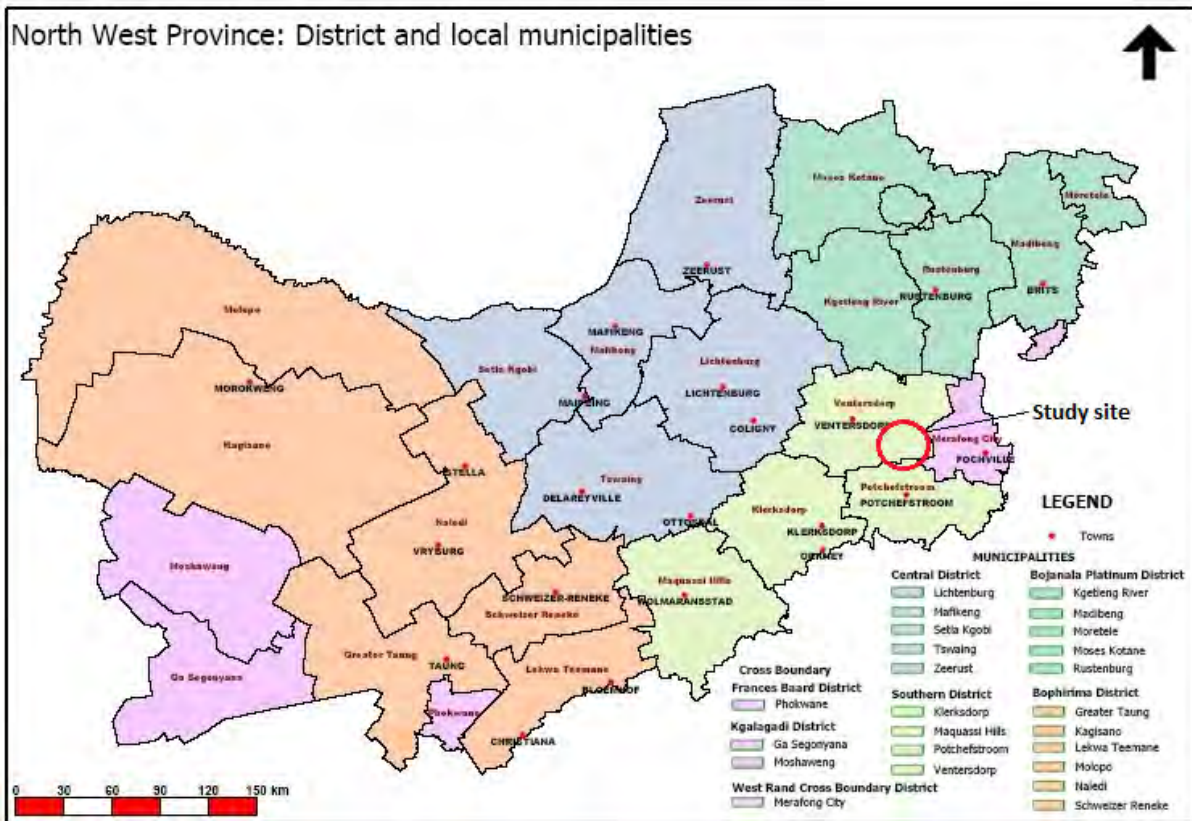


Figure 1: Map indicating the study site in the North West Province of South Africa (adapted from 17)

### Ethical considerations

Ethical approval was granted by the Ethics Committee of the North-West University (NWU-00033-09-A1). Permission to conduct the study was granted by the Department of Education of the North West Province (Dr Kenneth Kaunda district) and the school governing bodies of the two schools. Several parent meetings, in the preferred language of the parents, were held at the school premises to explain the purpose and procedures of the study, and to answer any questions that the parents had. Potential participants were invited to participate in the study and were asked to sign an informed consent form (illiterate people made a cross in front of a witness) agreeing that they themselves and their children would participate in the study. Only children who obtained parental consent and gave assent for the study were included. Potential participating parents were assured of data confidentiality and that the data would be used for the sole purpose of the study. Participation was voluntary and the participants could withdraw at any time without any consequences.

**Phase 1: Parent's knowledge, perceptions and use of indigenous and traditional plants*****Sample***

Prospective households from which to recruit a purposive voluntary sample of participants were identified through the two primary schools (19). Two focus group discussions per school were planned, with the possibility of increasing this number if data saturation was not reached. Participants were recruited through house visits. The participant had to be a parent or primary caregiver (hereafter called parent) of a child attending grade 2 to 4 at the selected primary school, responsible for procuring and preparing food in the household, living in the selected community and 18 years or older. After the parent meetings and a week before the focus group meeting, the prospective participant was verbally informed about the research again and invited to participate. If the prospective participant indicated not being available for the focus group, a different parent was asked to participate. Although eight parents were invited to participate per focus group, only one focus group consisted of eight participants. The other three focus groups had seven participants each. Repetition in knowledge and themes was noted after the third focus group discussion. The fourth focus group discussion did not provide any significant new information, therefore no more parents were selected to participate.

***Data collection***

Household socio-demographic characteristics data for the 29 parents participating in the focus group discussions was collected by means of a structured questionnaire prior to the focus group discussions.

The focus group discussions were held in a classroom at the two schools during school holidays. The school environment was chosen in order to make participants feel that they were in an environment to which they were already used. Transport was provided to get to the schools. All participants

received refreshments before and after the focus group discussions and were given a small monetary incentive for their participation. Participants were seated in a circle. This allowed them to see one another during the discussions and it also encouraged a sense of group atmosphere and bonding. All focus groups were conducted in the local language (Setswana), audio-recorded and transcribed verbatim. Each focus group was conducted by an interviewer and an assistant moderator, responsible for operating the tape, making observations and taking notes. A photo atlas from the South African Agricultural Research Council with pictures of ITPs (20) was used to assist participants to identify the ITPs and to connect the common names of the plants to their botanical names. All botanical names used in this manuscript have been verified with the International Plant Names Index and the World Checklist of Selected Plant Families (21, 22).

The focus groups followed a semi-structured format to ensure accuracy in topics covered across the different groups and still permit a certain level of flexibility within the group (23). A discussion guide with six open-ended questions was designed to collect data on the knowledge and use of ITPs. The interview guide was discussed with other experts in the field, adjusted and pilot tested (24). The following open-ended questions were included:

1. Can you tell me more about the indigenous and traditional plants in your community/surroundings?
2. You have mentioned all these plants; let us talk about where you get them.
3. These plants, how do you use them?
4. These plants, how and why do you store them?
5. Please tell me more about the beliefs about these plants?
6. Please tell me more about your feelings and your views regarding these plants?

***Concepts of knowledge and use***

For the purpose of this study, ITPs and ALVs are foods/vegetables that are either native to the region, or were introduced to it a long time ago to evolve through natural processes or farmer selection, including both wild foods/vegetables and ones traditionally cultivated by the inhabitants of a region. In describing the results of the focus groups, it was important to contextualize the term “knowledge” and “use”. Gadgil *et al.* defined knowledge as an outcome of model-making about the functioning of the natural world. They further defined indigenous knowledge as “a cumulative body of knowledge and beliefs handed down through generations by cultural transmission about survival and the relationship of beings (including humans) with one another and their environment” (25). This definition of knowledge was adapted to this study. In this context, parents were considered to be knowledgeable if they could express or give any form of relevant information related to the topic of study. The term “use” in this context referred to the purposes the ITPs served in the community and the processes that were involved in preparation to serve their purpose.

***Data analysis***

Household socio-demographic characteristics data was analysed by means of descriptive statistics using the IBM Statistical Package for Social Sciences (IBM SPSS 20.0 for Windows). A quality check was carried out on the transcribed data of the focus groups by a research assistant who was fluent in both English and Setswana, to ensure that the discussion was correctly translated without the original meaning being lost in translation. The notes taken during the focus group discussion by the assistant moderator were used to complement the rich data. The transcripts were coded and analysed with Atlas.ti 6 computer software, using the framework approach as described by Rabiee (26). The coding of the transcripts was done independently by two researchers (MvdH and JO). After

the second focus group no new codes were added. Differences between researchers were minimal and consensus was easily reached.

### ***Trustworthiness***

The principle of trustworthiness was adhered to using the approach of Guba (in 27). The pilot study and the focus groups ensured prolonged engagement (truth value) with the participants in this study. Truth value was further increased through triangulation of investigators and sources. Applicability was ensured by conducting multiple focus groups, using a detailed interview guide, encouraging participants to share their knowledge, the saturation of data and a dense description of the methodology. Transferability was obtained through purposive sampling and using direct quotations when presenting findings. The establishment of an audit trail for stepwise replication of the research was possible and a co-coder was used during data analysis, ensuring consistency and neutrality.

### **Phase 2: Child's acceptance**

#### ***Participants***

Children from grade 2 to 4 in the two primary schools (n=98; M/F: 40/58, 7-10 years) were randomly selected to participate in the sensory evaluation.

***Food sample preparation and presentation***

Four different dishes made from ALVs harvested in the study area and one dish made from store-bought Swiss chard were tested for acceptability. Swiss chard was included as reference sample. Each dish had a different vegetable content (see Table 1); however, the remaining ingredients were the same. These included tomatoes, onions, salt and vegetable oil. The ALVs selected for this study and the recipe for the dishes were based on the results of previous studies in the North West Province (28, 29). Sufficient samples of the five dishes were prepared, transported to the study site and heated in a microwave oven in a standardized manner. Each sample (numbered with three-digit random numbers) was served on a small, white plate accompanied by a small spoon.

Table 1: Leafy vegetables used in dishes tested in sensory evaluation

Leafy vegetables used in dish	Common name
ALV: <i>Amaranthus cruentus</i> L. (100%)	Amaranth
ALV: <i>Amaranthus cruentus</i> L. (80%) and <i>Cleome gynandra</i> L. (20%)	Amaranth and spiderplant
ALV: <i>Amaranthus cruentus</i> L. (80%) and <i>Cucurbita maxima</i> Lam. (20%)	Amaranth and pumpkin
ALV: <i>Amaranthus cruentus</i> L. (80%) and <i>Vigna unguiculata</i> (L.) Walp (20%)	Amaranth and cowpea
Conventional vegetable: <i>Beta vulgaris</i> L.	Swiss chard

***Procedure for sensory evaluation***

The sensory evaluation followed the procedures as described by Dalton *et al.* (30). An hour before the sensory evaluation took place, the participant ate a sandwich with margarine and polony to prevent potential hunger from influencing the rating of the different dishes. The sensory evaluation took place in an empty classroom, in sessions of 10 participants per group. A facilitator conversed with the participants in the local language (Setswana) in a friendly manner to put them at ease. The facilitator explained the procedure and the score sheet to the group. Each participant was then allocated to a trained fieldworker for a one-on-one interview in the child's preferred language. They were seated in such a way that interaction between the participants was minimised. Each child (n=98) evaluated four of the five different samples (each dish was therefore evaluated at least 77 times), randomly allocated by means of a Latin square design. One by one, the participant evaluated each sample (30 gram) for colour, smell, taste and overall acceptance. Between tasting the samples the participants were asked to take a sip of water and eat a small piece of apple to cleanse the palate in order to reduce possible overlap of flavours. The participants could indicate their opinion by pointing at the relevant smiley face representing the score or whisper their response to the fieldworker. In both instances the fieldworker recorded the appropriate score. After the evaluation of four samples, the participant was asked if he or she had a preference for one of the samples and if so, for which sample. The last question was how many times per week the participant was willing to eat this type of food (leafy vegetables). The participants received a piece of candy as incentive after completion of the sensory evaluation.

***Score sheet***

The procedure for sensory evaluation and the score sheet were standardised during a pilot study conducted in one of the schools. The 10 randomly selected children (M/F: 5/5; 7-10 years)

participating in the pilot study were excluded from participation in the sensory evaluation. Chen *et al.* (1996) and Kroll (1990), both cited by Guinard (31), recommend using hedonic scales with verbal anchors. The score sheet used a five-point ordinal scale, ranging from super good (value=5) to super bad (value=1) (see Fig. 2). The five-point ordinal scale has an equal number of positive and negative categories and is often used in consumer studies accommodating different language groups (32). To avoid potential comprehension problems the five-point ordinal scale was explained by the facilitator using visual stimuli (31) in the form of a large lollipop (super good) and cod liver oil (super bad). The score sheet also included a question on a potential preferred sample and a seven-point food action rating scale to score consumption intent relating to each sample. This was done by asking how many times per week the participant was willing to eat leafy vegetables (29). From the pilot study it was evident that the participants understood the score sheet used.

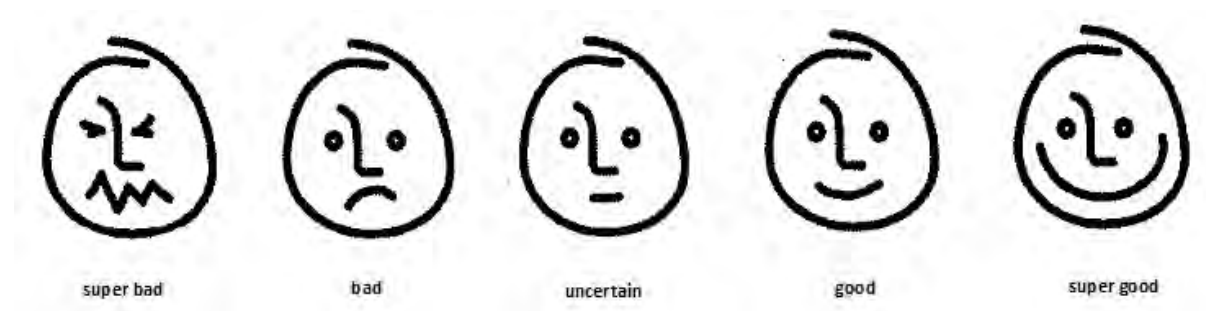


Figure 2: Five-point ordinal scale used for sensory evaluation (adapted from 30)

### **Data analysis**

Each verbal anchor used in the sensory evaluation was allocated a value ranging from super good (value=5) to super bad (value=1). Mean values for the different attributes were calculated. Data was found not to be normally distributed, therefore the Kruskal-Wallis, Mann-Whitney U test and chi-square test for independence were performed using the IBM Statistical Package for Social Sciences

(IBM SPSS 20.0 for Windows), controlling for Type 1 error across tests by using the Bonferroni approach. A p-value of <0.05 was regarded as significant.

## **RESULTS AND DISCUSSION**

### **Parent's knowledge, perceptions and use**

In total, 29 parents (median age 40.1 years, range 20.7 – 82.9 years) participated in four focus groups. Most of them (n=26) were female. This was expected, because women are usually responsible for taking care of children in the home and also are responsible for ensuring that the household has access to food. Table 2 describes the characteristics of these participants. More than half of the participants (58.6%) indicated that their husbands were the heads of the household, whereas 34% of the participants indicated that they themselves were the heads of the household. On average, two people ( $2.69 \pm 1.58$ ) in a household contributed to the income of the household. A child support grant was received by 69% of participants, whereas 19% received a pension grant. Most of participants indicated that their household did not receive any food from a feeding scheme (excluding the national school feeding programme) (89.7%) or that the household did not grow any food for its own use (72.4%). The socio-economic data indicated that these participants had a low educational background and were of low socio-economic status. This made them a vulnerable group, hence their significance for this study.

Table 2: Characteristics of focus group participants (n=29)

	<b>n</b>	<b>Percentage</b>	<b>Median</b>	<b>Range</b>
<b>Education level</b>				
- No education	5	17.2		
- Primary education	14	48.3		
- Secondary education	10	34.5		
<b>Household size</b>				
- Children (0-18 years)			3.0	1-7
- Adults (19-64 years)			2.0	0-5
- Adults (65 and older)			0.0	0-2
Number of children taking care of			3.0	1-7
<b>Work status</b>				
- Employed	17	58.6		
- Unemployed / retired	12	41.4		
<b>Total monthly household income</b>				
- Less than ZAR 1000	6	20.7		
- ZAR 1000 – ZAR 2000	12	41.4		
- ZAR 2000 – ZAR 3000	7	24.1		
- More than ZAR 3000	4	13.8		
<b>Monthly expenditures on food</b>				
- Less than ZAR 400	8	27.6		
- ZAR 400 – ZAR 800	13	44.8		
- More than ZAR 800	8	27.6		

ZAR: South African Rand; 1 USD ≈ 9.08 ZAR.

Direct quotations from participants were included in the description of the findings in order to give a richer description of the context. Results of the focus groups in general showed that the participants appeared to be knowledgeable on various edible ITPs in their surroundings in terms of their use.

### **Edible ITPs**

The themes that emerged from the data with regard to the edible ITPs included availability and access, preservation and storage, preparation and perceptions, including beliefs and feelings.

Table 3 shows the edible ITPs identified by the participants. The most commonly used edible plants were *Amaranthus spp.*, commonly known as *thepe*, and *Chenopodium album*, commonly known as *senkgampapa*. The parts of the plants that were mostly consumed were the leaves. Participants referred to the leafy vegetables as “morogo” or “wild spinach”.

Two of the identified plants, *Momordica balsamina* (Motangtang/Mistrikadika) and *Physalis peruviana* (Sepatlapatla), were found to be used for both medicinal and food purposes. According to the participants, the fruits of the *Momordica balsamina* plant were eaten, mainly as a snack, whereas the leaves were used as eye medication for children. The leaves of the *Physalis peruviana* plant were used as a condiment for starchy foods (mostly porridge made with maize meal, locally called “pap”). It was also known to cure ailments associated with pain.

Table 3: Identified edible ITPs

Local name in Setswana	Botanical name	Part consumed	FG 1	FG 2	FG 3	FG 4
Amadumbe (IsiZulu)	<i>Colocasia esculenta</i> (L.) Schott and <i>Xanthosoma sagittifolium</i> (L.) Schott	Leaves, stem	-	Y	-	-
Bobete	<i>Urtica urens</i> L.	Leaves	Y	-	-	-
Chencha-keledi	unknown	Fruit	-	Y	-	-
Kgobe-di-metsing	<i>Portulaca oleracea</i> L.	Leaves	-	-	-	Y
Leleme-la-kgomo	Looks like <i>Ricinodendron rautanenii</i> Schinz	Fruit, seeds	Y *	Y *	-	-
Lekatane	<i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai	Fruit, leaves	-	-	Y	-
Lekgomane	<i>Lagenaria siceraria</i> (Molina) Standl.	Leaves	Y	Y	-	-
Lerotho	<i>Cleome gynandra</i> L.	Leaves	Y	Y	-	-
Leshabe	<i>Sonchus asper</i> Vill. and <i>S. oleraceus</i> L.	Leaves	Y	-	-	-
Motangtang/ Mistikadika (fruit)	<i>Momordica balsamina</i> L.	Fruit	-	Y	-	-
Mmilo	<i>Vangueria infausta</i> Burch.	Fruit	-	Y	-	Y
Mmoko	unknown	Fruit	-	Y	-	-
Moetsa-wa-pere	unknown	Roots	Y *	-	Y	-
Mokofi	unknown	Seeds	-	-	-	Y
Morwetla	<i>Grewia flava</i> D.C.	Fruit	-	Y	Y	Y

Local name in Setswana	Botanical name	Part consumed	FG 1	FG 2	FG 3	FG 4
Monokotshwai	unknown	Fruit	-	-	-	Y
Motswetswejane	unknown	Fruit	-	-	Y	-
Qhela	unknown	Leaves	-	-	-	Y
Rapa/ rape	<i>Brassica rapa</i> L.	Leaves, stem	-	-	Y	-
Rotsane	unknown	Fruit	-	Y	-	-
Sebitsa	unknown	Leaves	-	-	Y	-
Sehuwe	unknown	Leaves	Y	-	Y	Y*
Sekgalo	unknown	Leaves	Y*	Y	Y	Y
Solele	<i>Portulaca oleracea</i> L.	Leaves	-	-	-	Y
Senkgane/Sekgapapane/ Imbikilicane	<i>Chenopodium album</i> L.	Leaves	Y*	Y*	Y*	Y*
Spaile	<i>Brassica carinata</i> A. Braun	Leaves	Y	Y*	-	Y*
Sepatlapatla	<i>Physalis peruviana</i> L.	Leaves, stem	Y	-	Y	-
Sthwanya	unknown	Leaves	-	-	Y	Y
Storfyn	unknown	Leaves	-	Y	-	-
Thepe	<i>Amaranthus spp: A. blitum</i> L., <i>A. graecizans</i> L., <i>A.</i> <i>cruentus</i> L., <i>A. tricolor</i> L.	Leaves, stem	Y*	Y*	Y*	Y*

The local names are given in Setswana, unless differently indicated. FG: Focus Group. (Y): the majority (75% or more) of participants within a particular focus group knew about the particular ITP mentioned and was familiar with its use. (\*): the IPT was commonly/mostly used by the participants. (-): the ITP was not used and/or known.

Participants of focus group three and four were less familiar with some of the identified ITPs. A possible reason for this might be that these participants were mostly working or living closer to the urban area than those of the other two groups. When asked during the focus group discussions about the ITPs that they used, they tended to mention more exotic, cultivated plants such as cabbage, spinach and apples. It was thus evident that participants in group three and four used fewer of the plants that grow naturally and as a result were less exposed to them. Urbanisation has been associated with loss of knowledge of ITPs and their use, even by people who live in rural areas (1, 33). Vorster *et al.* reported that access to a market had a negative influence on the use of traditional vegetables (9). People who did not have easy access to places where food is sold tended to rely more on those edible plants that grow naturally.

None of the participants had a negative perception of ITPs, which is in contrast to studies that found that especially members of the younger generation would label ITPs as inferior and as “poverty foods” (34, 9). The current study included mostly Tswana people, while Dweba and Mearns included predominantly Zulu people in their study (34) and Vorster *et al.* Pedi, Amaphondo-Xhosa, Tembu-Xhosa, Shangaan and Zulu people (9). Cultural differences probably contribute to the difference in perceptions of ITPs reported across studies. Participants in the current study perceived ITPs as advantageous, since there are no monetary costs involved in obtaining these edible plants. According to the socio-demographic data, a typical household with about eight people only had ZAR601-800 (≈USD 66-88) to spend on household food monthly, which was insufficient to sustain the whole household.

### ***Availability and accessibility***

According to the participants the edible, leafy ITPs and fruits were mostly found in the bushes, on farms and in fields and areas where water is widely available:

*“We get all the types of wild spinach from the farms and fields, like thepe and senkgane. On these farms, they grow maize and sunflowers and more. The wild spinach will grow in between these crops.”*

Participants said that the leafy ITPs, especially *thepe* and *spaille*, were available at certain times of the year and it was during those periods that the ITPs were mostly used or being preserved for when they were not available in the wild:

*“Thepe grows when it rains, but you eat it around August.”*

Participants who work on the farms may have access to these plants because they can pick them while working on the farms. The availability of these plants, however, can be compromised if they grow among cultivated crops as the farmers, who may have less knowledge about them, may see them as weeds and hence destroy them with herbicides. Vorster *et al.* reported that ITPs are still perceived as weeds by research and extension personnel who criticize farmers for not keeping them under control (9). Farmers and research and extension personnel should thus be informed of the importance of these plants.

Participants indicated that the seasonal availability of most ITPs affected the frequency of consumption. In addition, drought periods affect their availability as well. Ways of improving the availability of ITPs during off-season and drought periods include using recycled water (9) and collection of seeds of ITPs. Collection of seeds is not the current practice in rural communities, where people rely on the plants' self-sowing abilities of ITPs (35).

### ***Preservation, storage and preparation***

Participants were knowledgeable on how to access these plants during periods of low availability. One of the storage methods described was first to dry the leaves in the sun and then to store them

in sacks. During storage, other plants (e.g. beans) were used as preservatives for the plants being stored.

Only one-third of the participants (34.5%) had access to a working fridge or freezer, meaning that the majority had to rely on traditional methods of preservation and storage. Sun drying of leaves is a cheap and convenient way of preserving these plants, especially in this population, but it may lead to a great loss of essential micronutrients such as vitamins A and C (36, 37). Some participants said the leaves of the plants could be cooked before being dried and stored. According to Mnkeni *et al.*, all vegetables should be blanched in steam before drying, to deactivate the action of enzymes and also to prevent the loss of some nutrients (38). Ndawula *et al.* observed that blanching reduced the loss of  $\beta$ -carotene in cowpea leaves (36).

When describing how they used the leafy ITPs, participants said that these vegetables were mostly used as condiments to accompany starch-based dishes (especially “pap”). Participants had different opinions on which ingredients to use during preparation of the leafy ITPs in order to enhance the taste.

*“With thepe you pick it and then cook it the way you know ... if you have potatoes, you can add them. If you have onions, you can add them and also a bit of milk. You do not add water. If you have any spices, you add them, and there you will have a nice meal that you can enjoy with pap.”*

*“Then you add salt, maybe spices, and then eat it. It does not really need all that stuff, like, potatoes, tomatoes. To really enjoy thepe, you can just cook it and add onions and salt.”*

The leaves of the ITPs were often picked by hand, washed, and boiled in water. Certain types of leafy ITPs, such as spiderplant, were said to have a bitter taste, and as a result were boiled with plenty of water. Similar to the findings reported by Dweba and Mearns (34), this water was discarded two to three times in an attempt to get rid of the bitterness. Participants had mixed views and beliefs on

the effect the rinsing and draining had on leafy ITPs. Some believed that the rinsing was good, as it improved the taste, while others did not think it was good because of nutrient losses:

*“...when you cook it, if you do not want to lose the nutrients, you do not pour out the water you cook it in, and you should not cook it for too long. You can add more vegetables to it ... when you pour out the water that is when you lose all the vitamins. If you fry it until it is golden, you will lose all the nutrients and it will not be healthy for you ... you must time it properly so that you do not overcook it and lose all the nutrients.”*

*“We wash it, put it in a pot, and boil it for maybe 30 minutes ... my grandmother believed that you would drain out the vitamins.”* (The water was not drained.)

*“It tends to be very bitter, so you have to drain it ... yes, it also has vitamins.”*

Draining and discarding the water used in cooking vegetables are likely to cause the loss of water-soluble vitamins such as vitamin B complex and vitamin C (34). It is therefore important to educate this population on cooking methods that retain most of the nutritional value of the ITPs.

### ***Perceptions, beliefs and feelings***

When participants were asked about how they feel about the ITPs in their surroundings, the responses they gave were mostly positive and were related to the benefits these plants have for their wellbeing. The responses were related to the role the ITPs play in improving their health, the monetary benefits and lastly, the acceptability of these plants.

Edible ITPs were perceived as good sources of nutrients, especially vitamins, which they believed were essential in providing energy, boosting the immune system and preventing illnesses and infections. Similar to the findings of Nesamvuni *et al.* (10), participants were passionate and knowledgeable about these plants, especially wild spinach, and their importance in good nutrition.

In contrast, Vorster *et al.* found that although ITPs were perceived to be nutritious because they had been consumed by previous generations, there is little awareness of the importance of these plants (9).

Participants felt that the edible plants were crucial in their lives because they always provided them with a source of food and they did not have to spend money to acquire these plants.

*"I feel proud of them because most of the time when you do not have money, you just go out, find them and collect them. Sometimes, you may have pap but nothing to eat it with. Then you can look for wild spinach, and you go to bed with a full stomach."*

Throughout the focus group discussions participants expressed their acceptance of the taste of edible ITPs. The variety of ways in which they could be prepared with different ingredients made them very enjoyable to add as part of a meal. This is contrary to the findings of Dweba and Mearns, who reported that the lack of variety in cooking methods of traditional vegetables could make them less appealing and therefore affect consumption (34). Participants also expressed a high preference for the leafy ITPs compared to meat.

Several studies have reported the nutritional composition of ALV, indicating that these vegetables are rich in various micronutrients such as  $\beta$ -carotene, iron, calcium, magnesium, zinc and vitamin C (6, 7). Increased availability of and access to these plants may thus help to address micronutrient deficiencies. ITPs could also play a role in the diversification of diets and improving household food security in resource-poor households (34, 39). Matenge *et al.* showed that consumption of these foods could be increased through education, increased availability, marketing and gradual introduction to ITPs (29). This, combined with a positive attitude to ITPs, could benefit the promotion of ITPs. The concept of affordability should be avoided or used carefully in marketing strategies, as some ethnic groups/cultures associate these foods with poverty (7).

### Transfer of knowledge

Several methods for preservation and storage had been passed on through generations, thus indicating transfer of knowledge from one generation to another:

*“Back then, our mothers used to dry them and save them for the future.”*

In describing the cooking methods used for the various leafy ITPs, participants also indicated that there was transfer of knowledge through generations and hence the practice had not faded away completely:

*“I drain the water. Back home, my grandmother used to drain the water and then add peanuts and some oil. My grandmother believed they add[ed] flavour to the wild spinach and that peanuts were nutritious.”*

It is evident that the older generation in this population was seen as custodians of knowledge related to ITPs, and they passed their knowledge on in the hope that it would be transferred and not lost through generations. This finding was similar to the findings of a study conducted by Shava in the Eastern Cape, who found the younger generation to have extensive knowledge of ITPs in their surroundings, probably because of the close relationship they had with the elderly people in their community (40).

The focus groups were female-dominated, usually including only one male, and as a result females tended to dominate the discussions. However, the facilitator was able to insist on male participation regularly, and as a result, the males were able to describe their knowledge as well, thus avoiding bias. At times, some male participants were hesitant to make comments because they associated some aspects of the discussions with female roles, for example, cooking of food or the use of ITPs in the household. At times the older participants tended to hold back as the younger participants dominated the discussions. One elderly woman said: *“I just don’t understand ... see the younger ones are managing well on their own with the information”*. The group facilitator was able to bridge these

gaps and get the older participants more involved in the discussions as they went along. McLafferty stated that there are several ways of creating homogeneity in focus groups. In explorative studies homogeneity can also be classified according to status, class, occupation and other characteristics instead of gender and age (41). The homogeneity between the participants in the focus groups was based on their role as parents of children and the person involved in food preparation, since the main focus of this study was on knowledge and usage of ITPs. For future studies, it may be more appropriate to separate the focus group participants by age and gender.

### **Children's sensory evaluation of African leafy vegetables**

Although parents participating in the focus groups were knowledgeable about various edible ITPs and especially passionate about their importance in good nutrition, this study also sought to provide answers on children's acceptance of ALVs by means of a sensory evaluation. Knowledge of children's acceptance of the sensory attributes of ALVs is important for the potential future promotion of consumption of ITPs, including ALVs, as a strategy for reducing malnutrition (e.g. in a school nutrition programme).

The results of the sensory evaluation showed significant differences between the five dishes in the mean ratings for smell, taste and overall acceptability. When the dish made with Swiss chard was excluded from the analysis, there was no significant difference between any of the ratings of the four dishes made of ALVs (See Table 4).

Table 4: Evaluation of differences between the different dishes including and excluding the dish made with Swiss chard.

	Dishes including Swiss chard		Dishes excluding Swiss chard	
	K-W p-value <sup>1</sup>	P $\chi^2$ p-value <sup>2</sup>	K-W p-value <sup>1</sup>	P $\chi^2$ p-value <sup>2</sup>
<b>Colour</b>	0.120	0.386	0.639	0.603
<b>Smell</b>	<0.001	0.003	0.426	0.261
<b>Taste</b>	<0.001	0.002	0.632	0.931
<b>Overall</b>	<0.001	0.039	0.599	0.931

p-value of <0.05 was considered statistically significant.

<sup>1</sup> Kruskal-Wallis p-value refers to mean values. <sup>2</sup> Pearson  $\chi^2$  p-value refers to frequency.

A comparison of the responses for gender revealed no statistically significant differences between different genders' mean ratings for the five different dishes (Mann-Whitney U Test: p colour=0.631; p smell=0.268, p taste=0.518 and p overall=0.415). In the entire group, the dish made with Swiss chard was rated statistically significantly higher for smell, taste and overall acceptability than any of the dishes made with ALVs. There was no significant difference between the rating of the colour of Swiss chard and ALVs. Ratings for the dishes made with ALVs did not differ significantly in terms of colour, smell, taste and overall acceptability (see Table 5) and were thus equally acceptable regarding sensory characteristics to females and males. These four dishes combined were rated "good" or "super good" by 78.0%, 73.3%, 58.9% and 65.2% of the participants, respectively for colour, smell, taste and overall acceptability. Five of the 98 children did not have a preference for a specific dish. Of the 93 participants who did have a preference for one of the four dishes they evaluated, 75 had evaluated a dish made with Swiss chard. More than half (57.3%) of these participants preferred the sample made with Swiss chard. The median number of days per week the

participants would like to eat these leafy vegetables was 2.0 (range 1-7). There was no statistically significant difference in the number of days per week the participants would like to eat these leafy vegetables between the participants who did and did not evaluate a dish made with Swiss chard (Mann-Whitney U test:  $p=0.651$ ). The intended consumption was consistent with the positive rating of the dishes made with ALVs.

Table 5: Sensory evaluation scores (mean $\pm$ SD) for different dishes made with leafy vegetables

Dish	N	Colour	Smell	Taste	Overall
100% Amaranth	77	3.83 $\pm$ 1.13 <sup>a</sup>	3.66 $\pm$ 1.22 <sup>a</sup>	3.53 $\pm$ 1.34 <sup>a</sup>	3.82 $\pm$ 1.44 <sup>a</sup>
80% Amaranth + 20% Cowpea	80	4.05 $\pm$ 0.98 <sup>a</sup>	3.95 $\pm$ 1.02 <sup>a</sup>	3.50 $\pm$ 1.45 <sup>a</sup>	3.71 $\pm$ 1.35 <sup>a</sup>
80% Amaranth + 20% Pumpkin	79	3.85 $\pm$ 1.16 <sup>a</sup>	3.65 $\pm$ 1.25 <sup>a</sup>	3.33 $\pm$ 1.47 <sup>a</sup>	3.57 $\pm$ 1.47 <sup>a</sup>
80% Amaranth + 20% Spiderplant	78	3.83 $\pm$ 1.19 <sup>a</sup>	3.85 $\pm$ 1.17 <sup>a</sup>	3.31 $\pm$ 1.41 <sup>a</sup>	3.59 $\pm$ 1.48 <sup>a</sup>
100% Swiss chard	78	4.21 $\pm$ 0.93 <sup>a</sup>	4.33 $\pm$ 0.94 <sup>b</sup>	4.26 $\pm$ 1.19 <sup>b</sup>	4.38 $\pm$ 1.13 <sup>b</sup>

Score: five-point ordinal scale ranging from 5 (super good) to 1 (super bad). Dishes with different superscript differed statistically significant ( $p < 0.005$ ).

The dish made with Swiss chard was included as a reference dish, as it was expected that children would be exposed to this vegetable more often at home and at school via the daily cooked school meal. This was evident in the preference for the dish made with Swiss chard with regard to smell and taste. Although the dish made with Swiss chard was preferred, the four dishes made with ALVs were found to be of acceptable colour, smell and taste. These results are promising for the inclusion of ALVs in children's diet, particularly as Michicich *et al.* found a positive correlation between liking and consumption (42). Therefore, ALVs might be successfully introduced into a school nutrition programme that has a limited budget per school meal. Developing a range of recipes with different ingredients, as mentioned in the focus group discussions, will add variety to the flavours of the

dishes. ALVs have been advocated as excellent sources of several micronutrients (6, 7) and could potentially address co-existing multiple micronutrient deficiencies in individuals. Edible ITPs can add variety to the diets of people, especially those who do not have easy and regular access to markets or other fresh produce.

## **CONCLUSION**

This study indicated that there was not only a wealth of knowledge on various edible ITPs and their use in this farm community, but traditionally prepared ALV dishes were also sensorily acceptable to children. Edible ITPs were usually found growing on farms and fields among cultivated crops, and their availability and accessibility were influenced by seasonality and environmental conditions. Drying and storage methods for use during off-season periods should be optimized to minimize nutrient losses. Edible ITPs were perceived as rich sources of health-promoting nutrients and an affordable source of food and were appreciated for their taste. Knowledge about edible ITPs was transferred from one generation to another. The positive perceptions and knowledge of ITPs of the parents, and the children's acceptance of the taste of dishes made with ALVs indicate great potential for the promotion of ITPs as a strategy for improved child nutrition. It also looks promising for future use of edible ITPs in, for example, school feeding programmes. The researchers will be investigating the effect of these ALVs as part of the school meal on the nutritional status of children. Results of this study to date strongly indicate that compliance in the intervention study will be high.

## **LIST OF ABBREVIATIONS**

ALVs: African Leafy Vegetables; ITPs: Indigenous and Traditional Plants

**COMPETING INTERESTS**

The authors declare that they have no competing interests.

**AUTHORS' CONTRIBUTIONS**

MvdH: design; acquisition of data; analysis and interpretation of data; drafting the manuscript. JO: acquisition of data; analysis and interpretation of data; drafting the manuscript. MG: design; critical revision of manuscript for intellectual content. AK: critical revision of manuscript for intellectual content. MF: design; critical revision of manuscript for intellectual content. CMS: design; critical revision of manuscript for intellectual content. All authors read and approved the final manuscript.

**AUTHORS' INFORMATION**

MvdH is currently a PhD student in Nutrition at the Centre of Excellence for Nutrition (CEN) at the North-West University, South Africa. She is studying the effect of African leafy vegetables on the alleviation of micronutrient deficiencies in school children residing in the North West Province of South Africa. She is also the research co-ordinator for the South African leg of the Biodiversity study at Africa Unit for Transdisciplinary Health Research (AUTHeR) at the same university. This transdisciplinary research aims to increase agricultural biodiversity to improve nutritional and health status , as well as livelihoods and to establish more sustainable production systems in the North West Province of South Africa.

JO is currently a PhD student in Nutrition at the CEN at the North-West University, South Africa. Her PhD research is focused on iodine nutrition in mothers and their infants in the North West Province, South Africa. She has just completed her MSc in Nutrition; her research focused on the potential

contribution of African leafy vegetables to the nutritional status of school children residing in the North West Province of South Africa.

MG is a professor and senior researcher in the AUTHeR in the Faculty of Health Sciences of the North-West University, South Africa. She holds an MCur and a PhD in Psychiatric Nursing. She is an acknowledged researcher and has published extensively in national and international scientific journals and presented her research findings at many national and international conferences. Her research over the past few years has mainly focused on the quality of life of people living with HIV and AIDS and HIV stigma reduction on a community base. She is a South African nationally rated researcher and an inducted member of the International Nurses Hall of Fame.

MF is a chief specialist scientist at the Medical Research Council and an extra-ordinary professor at the University of the Western Cape as well as the University of Pretoria , South Africa. She is an acknowledged researcher and her research over the past few years has mainly focused on food-based approaches to address micronutrient malnutrition in children, particularly in rural areas. Her research also covers aspects of food security and indigenous foods.

CMS is a professor in nutrition and senior scientist in the CEN, North-West University, South Africa. He is currently the president of the Nutrition Society of South Africa. He is a well-recognised researcher and has an interest in the role of essential fatty acids in health and disease, and the interactions between iron and essential fatty acids in cognitive development. His research mostly targets vulnerable children residing in low socio-economic areas.

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## 4. MANUSCRIPT 2

### Iron and zinc content and bio-accessibility of selected green leafy vegetables

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#### Abstract

*Objective:* To assess iron and zinc content and bio-accessibility of selected green leafy vegetables (GLVs); as well as iron and zinc content, phytate:mineral molar ratios and bio-accessibility of cooked dishes made from these GLVs.

*Design:* Leaves of *Amaranthus cruentus*, *Vigna unguiculata*, *Cucurbita maxima* and *Cleome gynandra* were analysed for iron and zinc and *in vitro* biodialysability. Cooked GLV dishes were analysed for iron, zinc, calcium, phytate, total polyphenols, tannins and *in vitro* biodialysability.

*Results:* Iron content per 100 g edible portion for raw GLVs varied from 1.36 – 3.17 mg; while zinc content varied from 0.664 – 1.360 mg. The bio-accessible amount of iron ranged from 0.004 mg – 0.030 mg in GLVs cooked for five minutes; and for zinc from 0.006 mg – 0.032 mg. With a longer cooking time, more iron (except for pumpkin leaves) and zinc became bio-accessible. In the cooked GLV dishes phytate content per wet weight ranged from 2.07 – 2.99 mg/g, total polyphenols from 1.74 – 2.00 mg CE/g and tannins from 0.01 – 0.03 mg CE/100 mg. In the cooked GLV dishes, the molar ratios for phytate:iron (>1) and phytate:zinc (>15) both indicated low absorptions. The

calculated contribution of the GLV dishes (per 100g) towards dietary requirements of 4-6-year-old children is between 24% and 54% for iron, and towards 4% for zinc.

*Conclusions:* Based on dialyzable iron and zinc, the contribution of these GLVs towards dietary requirements is more substantial for iron than zinc. Whether the iron content is sufficient to affect iron status should be further investigated, using optimal preparation methods to enhance bioavailability.

## **Introduction**

Malnutrition, hunger and inadequate food supply are universal problems, facing the majority of the world's poor and needy people and continuing to dominate the health of the poorest nations <sup>(1,2)</sup>. Malnutrition also comprises the so-called "hidden hunger" for micronutrients. Micronutrient deficiencies of most public health significance include iron, vitamin A, zinc and iodine deficiencies <sup>(3)</sup>. Dietary inadequacy plays a key role in malnutrition and consequently in micronutrient deficiencies. In South Africa, malnutrition drives a combination of poverty-related infectious and lifestyle-related non-communicable diseases <sup>(4,5)</sup>. The challenge is to address the problems of the nutrition transition to avoid this double burden of disease. For this reason many advocate the use of food-based strategies to achieve optimal dietary requirements to combat micronutrient deficiencies <sup>(6-8)</sup>. Including micronutrient-rich foods, such as vegetables and fruit, in people's diets, based on the local available indigenous foods, has several advantages. These indigenous vegetables are a low-cost alternative to commercially available vegetables, which are often not accessible to resource-poor communities. In addition, indigenous vegetables are culturally acceptable and adapted to the local environment, which increases the chances of successful cultivation <sup>(9-11)</sup>. There is evidence that green leafy vegetables (GLVs) can contribute to the reduction of vitamin A deficiency <sup>(12-18)</sup>. However limited data are available on the effect of GLVs on iron and zinc status <sup>(12, 19)</sup>. Phytate is a known

inhibitor of iron and zinc bioavailability in plant-based foods by forming insoluble complexes with iron and zinc in the gastrointestinal tract<sup>(20, 21)</sup>. Furthermore, phytate can form complexes with endogenously secreted minerals, such as zinc and calcium, making them unavailable for reabsorption<sup>(22)</sup>. The suggested desirable levels for mineral absorption are phytate:iron < 1, phytate:zinc < 18, and phytate:calcium < 0.17<sup>(22)</sup>. Currently it is unclear if these phytate-to-mineral molar ratios are appropriate for children. Therefore, the aim of this study was to evaluate the potential of selected GLVs in improving the iron and zinc status of children (4-6 years) by investigating the phytate-to-mineral molar ratios and the bioaccessibility of cooked GLV dishes.

## Methods

### *Samples*

The vegetables included the leaves of amaranth (*Amaranthus cruentus* L.), cowpea (*Vigna unguiculata* (L) Walp), pumpkin (*Cucurbita maxima* Lam.) and spiderplant (*Cleome gynandra* L.). Seeds of these GLVs were supplied by the Agricultural Research Council, Roodeplaas, South Africa. These seeds were sown directly in the black, clay soil, which received irrigation and in which compost was incorporated before sowing. The GLVs were cultivated for study purposes on three hectares on a commercial farm, approximately 45 km from Potchefstroom, South Africa for two consecutive growing seasons (2010-2011 and 2011-2012). As reference, commercially bought Swiss chard (*Beta vulgaris* Linn.) was included during the first growing season. In the first growing season the GLVs were harvested before 10:00 in the morning three months after sowing. Three separate lots were collected at random from different positions across the cultivation area on the same day to ensure a representative sample. The edible and therefore harvested parts of the amaranth, cowpea and spiderplant included the young leaves and growth points, and of the pumpkin only the leaves. During the second growing season harvesting started two to three months after sowing for a period

of three weeks. The primary purpose of this second harvest was to collect material for sensory evaluation and a possible intervention trial. All harvested leaves were collected in labelled black plastic bags, pierced to avoid condensation and to allow airflow and were immediately brought to the North-West University where they were processed the same day. After harvesting, damaged leaves and non-edible parts were removed. The three lots per species were then combined into a composite sample. The samples were washed thoroughly three times under a running tap to remove soil debris, followed by washing with distilled water. The leaves were air-dried on absorbent paper at room temperature. During the first growing season, the samples were blanched and afterwards homogenised by grinding with a kitchen food processor and a stick blender. The samples were aliquoted in 50 gram portions, which were kept frozen at -20 °C until analysis. In the second growing season the leaves were washed thoroughly and dried in the same way as described for the leaves in the first growing season. The harvest from the second growing season was then used to prepare four cooked GLV dishes according to a traditional recipe (based on <sup>23</sup>). On the day of harvest, the vegetables were processed into the GLV dishes using an oil-jacketed pot (VULCAN BP 225). This recipe has been used in an intervention study to assess the potential effect of GLVs on children' nutritional status (Unpublished manuscript – chapter 5). The GLVs in the first dish consisted of 100% amaranth; the GLVs in the other three dishes consisted of 80% amaranth plus 20% spiderplant, pumpkin or cowpea respectively. The GLV content in each dish was 49%. The remaining ingredients included chopped tomato-and-onion mix (22%), vegetable oil (3%), a commercially available instant gravy powder (5%) and water (21%). After processing the samples were aliquoted in 300 gram portions, which were kept frozen at -20 °C until analysis. Before analysis the samples were thawed and homogenised by grinding with a kitchen food processor and a stick blender. Each sample of the GLV dishes was compiled of three random samples from initial, mid- and end harvest (total 9 samples) to ensure a representative sample for the whole harvest period. All samples were analysed in duplicate. The GLV were analysed in the Laboratory of Human Nutrition, ETH, Zurich and the dishes made from GLV were analysed in the Centre for Nutrition at the University of Pretoria. The

vitamin C analyses were done by Microchem Laboratories in Cape Town, which is accredited by the South African National Accreditation System.

#### *Anti-nutrients*

A modified Folin Ciocalteu method <sup>(24)</sup> was used to measure total polyphenol concentration. Tannin content was analysed with the vanillin HCl assay of Price, Van Scoyoc, and Butler <sup>(25)</sup>. Reagent blanks that corrected for the colour of the extracts from the ALVs were included. Tannin content was expressed as mg catechin equivalents (CE) per 100 g sample. Phytate content in the GLVs was measured by means of anion-exchange chromatography, as described by Frubeck *et al.* <sup>(26)</sup> The following columns and resin were used: Glass barrel Econo-columns, 0.7 x 15 cm (BioRad, Johannesburg, South Africa), Dowex 1; anion-exchange resin-AG 1 x 4, 4% cross-linkage, chloride form, 100–200 mesh (Sigma, Johannesburg, South Africa).

#### *Mineral content*

Acid digestion of the GLV samples was done according to the method of Zasoski and Burau <sup>(27)</sup>. The iron and zinc contents of the GLV samples were analysed by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) (Spectro Arcos, SPECTRO Analytical Instruments GmbH, Kleve, Germany). Standard solutions were adjusted so that the concentration of the samples fell within the linear parts of the standard curve.

### *Vitamin content*

Vitamin C content was analysed by means of a modified method described by Fellman *et al.* <sup>(28)</sup> using High-Performance Liquid Chromatography (Thermo Separation Products, Fremont, CA, USA; P400 quaternary pump; Rheodyne Model 7125 sample injector; 100  $\mu$ L sample loop) and fluorescence detection (Jasco 821-FP Intelligent Fluoremeter, Tokyo, Japan). Chromatography was performed with a LiChrosorb RP-18 5  $\mu$ m (4.6 x 250 mm) column with guard column (Phenomenex, Torrance, CA, USA); the mobile phase consisted of 50% methanol; the flow rate was 1.0 mL/min and the excitation and emission wavelengths were 350 nm and 430 nm, respectively. Vitamin A ( $\beta$ -carotene) was not analysed due to technical reasons.

### *In vitro dialysability of iron and zinc*

The samples were freeze-dried and homogenised by grinding with a kitchen food processor before being stored in an airtight container until analysis. Each sample was then analysed for the dialysability of its native iron and zinc during *in vitro* digestion. *In vitro* digestion was determined according to the dialysis method of Luten *et al.* <sup>(29)</sup>, with minor alterations. Because of potential precipitation of minerals, the tubing contents were acidified with 0.002 mL 65% nitric acid/mL dialysate when decanted, to keep minerals soluble. The mineral content of the GLVs and dialysate was analysed through ICP-OES. The availability of iron was calculated as the percentage of dialysable iron, compared to the total iron content. Enzymes used were pepsin (P-7000), pancreatin (P-1750) and bile extract (B-8631) (all Sigma). The dialysis tubing used was Spectra/Por 7 ( $\emptyset$  = 20.4 mm) with a molecular mass cut-off of 10 000 Da (Labretoria, Pretoria, South Africa).

## Results and discussion

### *Iron and zinc content of raw leaves and in vitro dialysability*

Table 1 shows the iron content and *in vitro* dialysability of iron of selected GLVs, including Swiss chard as reference vegetable. The iron content of the selected GLVs varied from 1.360 mg – 3.170 mg per 100 mg edible portion, with amaranth leaves having the highest iron content. Van Jaarsveld *et al.* <sup>(30)</sup> and Schönfeldt *et al.* <sup>(31)</sup> report higher iron content values on all GLVs (except spiderplant leaves where the content was similar to the findings of Van Jaarsveld *et al.* <sup>(30)</sup>) on raw and cooked vegetables. Especially the iron content for pumpkin leaves was reported by Van Jaarsveld *et al.* and Schönfeldt *et al.* to be much higher, 9.2 mg and 15.7 mg per 100 gram edible portion. Schönfeldt *et al.* indicated that the high iron content could have resulted from the chicken and cattle manure used as soil fertilisers <sup>(31)</sup>. Environmental factors, such as soil type and pH, availability of water, plant age, variety, use of fertilizers and climatic conditions influence the nutrient composition of plants, especially minerals <sup>(32, 33)</sup>. Another explanation for the high reported iron values in pumpkin leaves might be soil contamination rather than iron intrinsic to the leaves, since pumpkin is a trailing plant. In the current study there were GLVs with higher iron content and with lower iron content compared to Swiss chard, the conventional vegetable. The bioaccessible amount of iron ranged from 0.004 mg – 0.030 mg in GLVs cooked for five minutes in a 100°C oven. When the GLVs were cooked longer, more iron became bioaccessible within all GLVs, except for pumpkin leaves. Schönfeldt *et al.* <sup>(31)</sup> observed no change in the amount of total iron in GLVs after the cooking process for cowpea leaves, pumpkin leaves and spiderplant leaves. In amaranth leaves (*Amaranthus tricolor*) however a decrease in total iron concentration of 16.2 mg in the raw sample to 8.5 mg in the cooked sample was noticed. However, they did not report any data on bioaccessibility <sup>(31)</sup>.

Table 1. Content and *in vitro* dialysability of iron of selected GLVs after different cooking periods (edible portion – wet weight).

GLV	Vitamin C (mg/100g)	SD	Iron <sup>1</sup> (mg/100g)	SD	Short cooking - 5 min.			Long cooking - 20 min.		
					% available	SD	Bioaccessible (mg)	% available	SD	Bioaccessible (mg)
Amaranth	4.2	0.1	3.170	0.120	0.400	0.024	0.013	4.060	0.600	0.129
Cowpea	14.9	0.7	1.360	0.110	0.440	0.027	0.006	1.479	0.012	0.020
Pumpkin	3.7	0.4	1.620	0.160	0.250	0.028	0.004	0.190	0.080	0.003
Spiderplant	20.5	1.3	2.980	0.160	0.990	0.370	0.030	1.600	0.190	0.048
Swiss chard	16.1	0.9	2.710	0.170	0.490	0.034	0.013	2.340	0.290	0.063

GLV: Green leafy vegetable. <sup>1</sup> Mean iron content of raw leaves

Harvest from season 1.

Table 2 shows the zinc content and *in vitro* dialysability of zinc of selected GLVs, including Swiss chard as reference vegetable. The zinc content of the selected GLVs ranged from 0.495 – 1.360 mg per 100 gram edible portion, with spiderplant leaves containing most zinc. Van Jaarsveld *et al.* <sup>(30)</sup> and Schönfeldt *et al.* <sup>(31)</sup> both report lower zinc content values in all GLVs on raw and cooked vegetables, except for the zinc content value of pumpkin leaves. The bioaccessible amount of zinc ranged from 0.006 mg – 0.032 mg in GLVs cooked for five minutes in a 100°C oven. It was observed that with longer cooking in a 100°C oven the bioaccessible zinc increased. Schönfeldt *et al.* <sup>(31)</sup> observed no change in the amount of total zinc in GLVs after the cooking process for the leaves of amaranth, cowpea, pumpkin and spiderplant. Although Swiss chard did not have the least amount of zinc, it contains the least amount of bioaccessible zinc (0.015 mg).

Table 2. Content and *in vitro* dialysability of zinc of selected GLVs after different cooking periods (edible portion – wet weight).

GLV	Zinc <sup>1</sup> (mg/100g)	SD	Short cooking - 5 min.			Long cooking - 20 min.		
			% available	SD	Bioaccessible (mg)	% available	SD	Bioaccessible (mg)
Amaranth	0.909	0.013	2.170	0.150	0.020	11.530	0.860	0.105
Cowpea	0.664	0.030	3.570	0.250	0.024	13.760	1.710	0.091
Pumpkin	0.495	0.020	6.400	0.080	0.032	20.980	0.470	0.104
Spiderplant	1.360	0.021	2.160	0.240	0.029	10.630	0.680	0.145
Swiss chard	0.530	0.030	1.220	0.300	0.006	2.770	0.310	0.015

GLV: Green leafy vegetable. <sup>1</sup> Mean zinc content of raw leavesHarvest from season 1.

*Mineral content and in vitro dialysability of cooked GLV dishes*

Table 3 shows the iron, zinc and calcium content (dry weight) and the phytate-to-mineral molar ratios. The iron content per edible portion varied from 1.69 – 3.77 mg/100 g with the amaranth-and-pumpkin dish presenting with the highest content. The phytate:iron molar ratio ranged from 5.13 to 10.41, with the GLV dish amaranth-and-pumpkin having the lowest ratio and the dish amaranth-and-spiderplant the highest. All phytate:iron molar ratios are above 1, which is higher than the suggested desirable level of  $<1$ <sup>(22)</sup>, indicating that iron absorption could be limited. The zinc content per edible portion ranged from 0.40 – 0.44 mg/100 g, with the amaranth-and-pumpkin dish demonstrating the highest content. The phytate:zinc molar ratio ranged from 48.11 to 73.38, with the GLV amaranth-and-spiderplant dish having the lowest ratio and the amaranth dish the highest. Phytate:zinc molar ratios higher than 15 according to the World Health Organization (WHO), or 18 according to the International Zinc Nutrition Consultative Group gradually inhibit zinc absorption and have been associated with suboptimal zinc status in humans<sup>(20)</sup>. It has been estimated that the zinc bioavailability with a phytate:zinc molar ratio above 15 is low (10-15%)<sup>(34)</sup>. The calcium content per edible portion varied from 79.42 mg to 138.86 mg per 100 gram. The phytate:calcium molar ratio ranged from 0.10 to 0.16, with the GLV dish amaranth-and-pumpkin having the lowest ratio and the amaranth-and-spiderplant dish the highest. All the phytate:calcium molar ratios are under the suggested desirable level of  $< 0.17$ <sup>(22)</sup>, indicating that calcium absorption is probably not limited. Furthermore, during infancy and early childhood phytate does not limit calcium absorption, and calcium deficiency is rather a consequence of a low dietary intake than poor absorption<sup>(35)</sup>.

Table 3. Iron, zinc, and calcium content and their phytate molar ratios in GLV dishes (edible portion - wet weight).

Sample	Fe	SD	Zn	SD	Ca	SD	Ph:Fe	Ph:Zn	Ph:Ca
	mg/100 g		mg/100 g		mg/100 g				
Amaranth	2.58	0.15	0.40	0.00	136.09	15.88	9.80	73.38	0.13
Amaranth-and-cowpea	2.74	0.04	0.42	0.01	117.46	9.08	9.11	70.33	0.15
Amaranth-and-pumpkin	3.77	0.12	0.44	0.04	138.86	13.09	5.13	51.75	0.10
Amaranth-and-spiderplant	1.69	0.16	0.43	0.02	79.42	0.00	10.41	48.11	0.16

Ph:Zn: phytate:zinc molar ratio; Ph:Fe: phytate:iron molar ratio; Ph:Ca: phytate:calcium molar ratio.

Each GLV dish consisted out GLV (49%), chopped tomato-and-onion mix (22%), vegetable oil (3%), a commercially available instant gravy powder (5%) and water (21%).Harvest from season 2.

Table 4 shows inhibitors and enhancers of iron and zinc availability in GLV dishes. The phytate content per dry edible portion ranged from 2.07 – 2.99 mg/g with amaranth-and-spiderplant indicating the lowest and amaranth only the highest content. The total polyphenols content per edible portion ranged from 1.74 – 2.00 mg CE/g. The tannins varied from 0.01 – 0.03 mg CE/100 mg. These values (when converted to per dry mass) are lower compared to the tannin values reported by Gupta *et al.* <sup>(36)</sup>, whose study showed that oxalic acid, tannin, phytic acid and dietary fibre accounted for 53% for the inhibition of iron availability. Tannin and phytic acid accounted for 17% and 16% of inhibition of iron availability, respectively <sup>(36)</sup>. Soaking grains for 16 hours and germination of grains for 24 hours were shown to improve the iron bioavailability in these grains by a reduction in tannin and phytate content <sup>(37)</sup>. However, data on iron bioavailability and the soaking of leaves is limited. One study looked at soaking and cooking of green leafy vegetables, and found that 5.5-9.8% of the total available iron and 46.6-51.4% of the total available zinc were bioaccessible <sup>(38)</sup>. The GLV amaranth-and-spiderplant dish has the lowest values for the three inhibitors of iron and zinc status. This is been reflected in this dish having the highest amount of bioaccessible iron available and dialysability of zinc (table 5). The vitamin C content in the cooked dish ranged from 12.50 mg to 16.50 mg per 100 gram edible portion and may contribute to the bioavailability of iron. Furthermore, certain antinutrients in the added ingredients, such as the tomato, will influence the bioavailability of iron and zinc.

Table 4. Inhibitors and enhancer of iron and zinc bioavailability in GLV dishes (wet weight).

Sample	Phytate		Total polyphenols		Tannins		Vitamin C	
	mg/g	SD	mg CE/g	SD	mg CE /100 mg	SD	mg /100 gram	SD
Amaranth	2.99	0.24	1.77	0.06	0.03	0.02	12.50	3.82
Amaranth-and-cowpea	2.95	0.33	1.89	0.12	0.03	0.01	13.37	3.46
Amaranth-and-pumpkin	2.28	0.23	2.00	0.13	0.02	0.00	16.50	0.71
Amaranth-and-spiderplant	2.07	0.13	1.74	0.12	0.01	0.01	13.00	1.23

CE: Catechin equivalents.

Each GLV dish consisted out GLV (49%), chopped tomato-and-onion mix (22%), vegetable oil (3%), a commercially available instant gravy powder (5%) and water (21%).

Harvest from season 2.

The iron and zinc content, including their bioaccessibility, per 100 gram edible portion, in cooked GLV dishes is shown in table 5, and in single raw GLVs in tables 1 and 2. When compared to the single GLVs, the iron content of the cooked 100%-amaranth dish was less than the raw amaranth leaves, because of dilution with other ingredients in the dish. The iron content of the selected GLVs dishes (table 3) varied from 1.69 mg – 3.77 mg per 100 mg edible portion, with the GLV amaranth-and-pumpkin dish having the highest total iron content. However, looking at the bioaccessible iron (table 5), it is the GLV amaranth-and-spiderplant dish that has the highest amount of bioaccessible iron while having the least amount of total iron. The amount of dialysable iron in the four dishes ranged from 0.25 mg – 0.42 mg. Foods naturally rich in ascorbic acid, such as tomato, can be added to a GLV dish to increase the iron dialysability (up to 53%)<sup>(39, 40)</sup>. When compared to the single GLVs (table 2), the zinc content of the 100%-amaranth dish (table 3) was also less than the raw amaranth leaves, because of dilution with other ingredients in the dish. The four GLV dishes all have approximately the same total zinc amounts, ranging from 0.40 mg – 0.44 mg. The amount of bioaccessible zinc was also similar for the four dishes.

Table 5. Iron and zinc content and *in vitro* dialysability of GLV dishes per 100 gram edible portion (edible portion - wet weight).

Sample	% available iron	SD	Bioaccessible iron mg	% available zinc	SD	Bioaccessible zinc mg
Amaranth	9.70	3.60	0.25	7.00	0.40	0.03
Amaranth-and-cowpea	10.10	1.40	0.28	7.30	0.40	0.03
Amaranth-and-pumpkin	6.80	0.90	0.26	7.00	0.40	0.03
Amaranth-and-spiderplant	25.00	3.50	0.42	7.90	0.30	0.03

Each GLV dish consisted out GLV (49%), chopped tomato-and-onion mix (22%), vegetable oil (3%), a commercially available instant gravy powder (5%) and water (21%).Harvest from season 2.

The recommended daily nutrient intake for 4-6 year-old children is 6.3 mg iron (based on a diet with 10% bioavailability) and 9.6 mg zinc (based on a diet with low zinc bioavailability)<sup>(41)</sup>. Young children will consume an average portion size of 90 g boiled leaves<sup>(42)</sup>. Looking at the single GLVs cooked for 20 minutes (table 1), the contribution to iron will be between 23% (pumpkin leaves) and 45% (amaranth leaves) and to zinc between 5% (pumpkin leaves) and 13% (spiderplant leaves). The contribution of GLV dishes to iron will be between 24% (amaranth-and-spiderplant dish) and 54% (amaranth-and-pumpkin dish) and the contribution to zinc 4% (both amaranth and amaranth-and-pumpkin dishes). The phytate-iron molar ratio estimated limited bioavailability for iron based on the concentration of phytate and iron. However, the contribution of GLVs to the recommended dietary nutrient intake of iron is reasonable with regard to the amount of dialysable iron. In addition, the GLVs are rich in vitamin C, a known enhancer of iron absorption. The contribution to zinc seems insubstantial. Taking into account the amount of dialyzable zinc and the phytate-zinc molar ratio it appears that GLVs are limited sources of dietary zinc, which do not meet the recommended daily nutrient intake. However, the phytate-zinc molar ratio should be interpreted with care for children, since it is unclear if this ratio based on adults' diets is appropriate for children as well<sup>(43)</sup>. In addition, although *in vitro* dialysability is the only method thus far that has been validated against human studies to study zinc bioaccessibility<sup>(44)</sup>, it has been suggested that the Caco 2 cell uptake assay is more accurate in estimating zinc absorption<sup>(45)</sup>. Zinc absorption is also dependent on nutritional status; a zinc-deprived person will be more efficient in the absorption of zinc<sup>(20)</sup>. Although GLVs are not a primary source of iron and zinc, these vegetables can contribute to the dietary requirements of these micronutrients. Optimisation of iron and zinc bioavailability of GLVs should be explored further, especially when using GLVs as a strategy to address micronutrient deficiencies in at-risk populations which often consume monotonous plant-based diets with limited animal source foods in which the dietary iron and zinc bioavailability is low<sup>(46)</sup>. Although the inclusion of (small amounts of) animal source foods from a young age will be most efficient in addressing micronutrient deficiencies, the feasibility of this strongly depends on the availability and affordability of these foods<sup>(47)</sup>.

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**Conflict of interest**

None.

## Authorship

MvdH: design; acquisition of data; analysis and interpretation of data; drafting the manuscript. MF: design; critical revision of manuscript for intellectual content. AK: design; critical revision of manuscript for intellectual content. CMS: design; critical revision of manuscript for intellectual content. All authors read and approved the final manuscript.

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## 5. MANUSCRIPT 3

### **Consumption of African leafy vegetables failed to improve micronutrient status in school children residing in a farm community in South Africa: a randomized controlled trial**

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#### **ABSTRACT**

**Background:** Food and nutrition insecurity severely compromises quality of life in rural farm communities in South Africa. African leafy vegetables (ALVs) can potentially contribute to alleviating micronutrient malnutrition by achieving optimal dietary requirements and further improve nutritional status.

**Objective:** The aim was to investigate the effect of ALV consumption on iron, zinc and vitamin A status.

**Design:** Children (mean age 8.4 years) of two farm schools were randomly allocated to receive either a 300 gram cooked ALV dish and school meal starch (N=86) or the normal school meal (N=81) five times per/week for three months. Vegetables in the ALV dish consisted mainly of *Amaranthus cruentus* (at least 80%) and the remainder (20%) of *Cleome gynandra*, *Cucurbita maxima*, or *Vigna unguiculata*. Potential intervention effects were analyzed using ANCOVA on the endpoint measurement, adjusting for baseline values, gender, age, school and adherence. Nutrient analysis and consumer acceptance of the ALV dish were also determined.

**Results:** The ALVs contributed 11.6 - 15.8 mg iron and 1.4 - 3.7 mg zinc per meal. At baseline, prevalence of deficiencies in the intervention group were 16.0%, 16.3%, 7.0% and 75.6%, respectively for anemia (Hb <11.5 g/dL), iron deficiency (serum ferritin <15 µg/L), vitamin A deficiency (serum retinol <20 µg/dL) and zinc deficiency (serum zinc <65 µg/dL). The prevalence of deficiencies in the control group at baseline were 10.5%, 18.5%, 2.5% and 75.3%, respectively for anemia, iron deficiency, vitamin A deficiency and zinc deficiency. No significant estimated intervention effect was found. The overall acceptance rating of the ALV dishes was “good” or “super good” by 56.8% of the participants.

**Conclusions:** This randomized controlled trial showed that ALVs were unable to improve serum retinol, serum ferritin or hemoglobin if there are only mild deficiencies present. Furthermore, despite the low zinc status in our population, ALV consumption did not improve serum zinc concentrations either. However, the sensory evaluation and adherence to the ALV consumption in this intervention show potential for ALVs to diversify the diet of school children in a low-cost and sustainable way.

#### **LIST OF ABBREVIATIONS**

ALVs, African leafy vegetables; BAZ, BMI-for-age z-score; CRP, C-reactive protein; HAZ, height-for-age z-score; Hb, hemoglobin; QFFQ, quantitative food frequency questionnaire; RAE, retinol activity equivalent; RDA, Recommended dietary allowance; SD, standard deviation; SF, serum ferritin; TfR, serum transferrin receptor; WAZ, weight-for-age z-score; ZnPP, erythrocyte zinc protoporphyrin.

## BACKGROUND

The combination of poverty-related infectious diseases and lifestyle-related non-communicable diseases, both driven by malnutrition, causes significant problems for South Africa. The 2005 National Food Consumption Survey Fortification baseline (NFCS-FB-I) revealed that stunting and underweight were the most prominent nutritional disorders in children aged one to nine years (1). This was confirmed by the South African National Health and Nutrition Examination Survey (SANHANES-1) executed in 2011-2012. In children younger than five years the prevalence of stunting has increased slightly and the prevalence of underweight has decreased since 2005 (2). Moreover, the NFCS-BF-1 showed that one out of seven children was iron-deficient, 45.3% of children had inadequate zinc status, and two out of three children had poor vitamin A status (1). The NFCS of 1999 is the only national survey that reports nutrient intake data for children. It reported that the energy and essential micronutrient intake of one out of two children was less than half of what is recommended, especially in rural areas. The micronutrients that were found to be consumed at less than 67% of the recommended dietary allowances (RDAs) were iron, vitamin A, zinc and calcium. The NFCS also documented the insufficient intake of vitamin C, thiamine, riboflavin, niacin, vitamin B6, vitamin B12 and folate among a large number of children (3).

Healthy and nutritious diets for populations depend on the availability and accessibility of a variety of plant and animal foods, in a context that promotes and supports healthy behavior. Traditional biodiversity use, instead of westernizing diets, in the socio-cultural context can be a powerful tool for maintaining and enhancing health and nutritional status (4). Although many plant species have been neglected or forgotten (5), there are several studies that have documented the the variety of contributions which diverse plant foods can make to nutrition (6-13). These studies have confirmed the importance of wild vegetables as sources of micronutrients. Uusiku *et al.* (14), Faber *et al.* (10) and Nesamvuni *et al.* (15) in South Africa also underscored the significant contribution of African leafy vegetables (ALVs) as source of micronutrients, in particular iron, zinc and vitamin A ( $\beta$ -

carotene). The iron, zinc and  $\beta$ -carotene content of ALVs is known to range from 0.2–12.8 mg to 0.02–18.5 mg and 99–1970  $\mu$ g RE per 100 g edible portion respectively (5). Moreover, apart from being good plant resources of iron, zinc, and  $\beta$ -carotene, dark green ALVs supply other nutrients such as folate, ascorbic acid and phytonutrients (5, 16).

Consumption of ALVs and thereby dietary diversity can potentially contribute to the reduction of micronutrient malnutrition and further improve nutritional status and human health. However, to our knowledge, no studies have been carried out on how these vegetables can make a contribution to the nutritional status of children in farm communities in Africa, particularly in the North West Province of South Africa. The objectives of this study therefore included establishing the nutritional composition of dishes made with selected ALVs and investigating whether inclusion of these ALV dishes in school children's diets would improve their nutritional status. Hence, the effect of consumption of selected ALVs on blood hemoglobin, serum ferritin, serum transferrin receptor, zinc protoporphyrin, serum zinc and serum retinol of school children was investigated.

## **METHODOLOGY**

### **Setting**

The study was conducted in two farm schools in a malaria-free rural area approximately 50 kilometers from Potchefstroom in the North West Province, South Africa. Both these primary schools were located in similar farm surroundings (where the main farming activities include maize, sunflower and chicken) and were fully sponsored by the South African Department of Education and by the farm owners themselves. Children attending these schools receive one meal each school day (around 10:30) as part of the National School Nutrition Program in South Africa. These farm schools were selected because farm communities in South Africa are regarded as the least privileged and the

most vulnerable population in terms of income, health status, education and household nutrition security (17, 18).

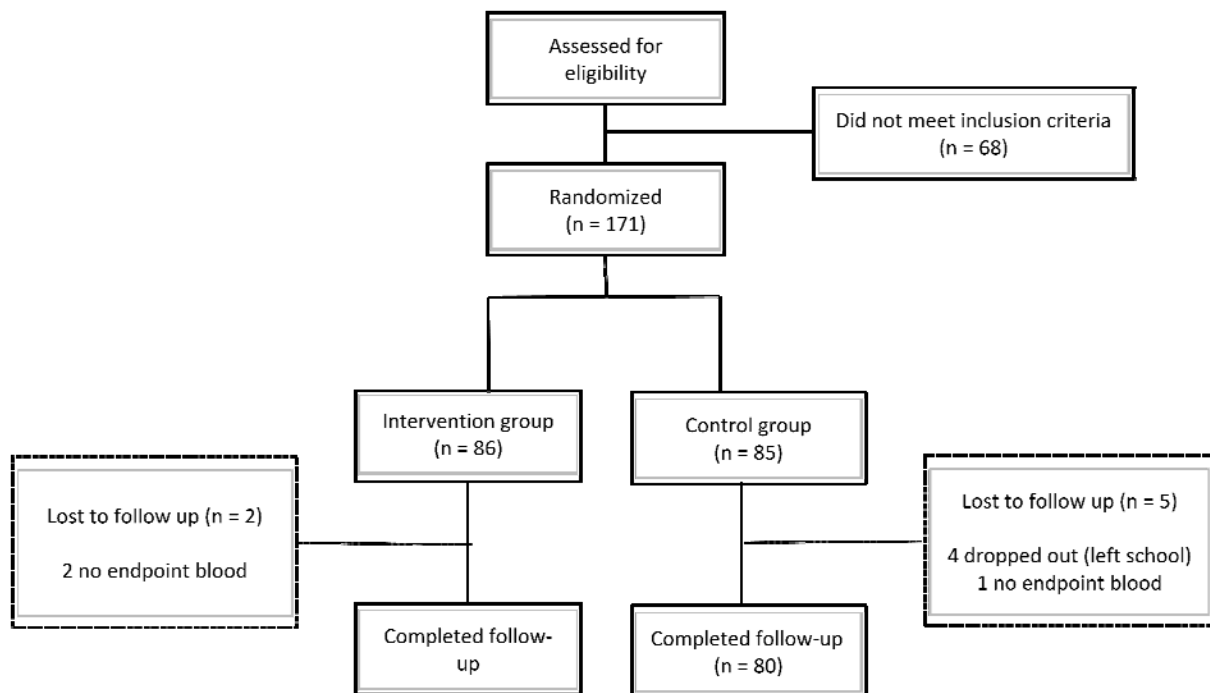
### **Participants**

The sample size requirement was calculated by using data from De Pee *et al.* (12). A sample size of 63 learners per group would be adequate to detect a 20% increase in serum ferritin and a 20% increase in serum retinol at a 5% significance level with 80% statistical power. The study was conducted from March to June 2012. All children from grade R to grade 4 (6-12 years old) were invited to participate in the study. The response rate of the study was 87.2%; parents of 171 children gave consent to participate. Participating children were dewormed at baseline with an oral dose of 400 mg Albendazole (Cipla-Medpro (Pty) Ltd). Participants were apparently healthy and had no signs and symptoms of acute illness at the time of baseline blood collection. Children with a hemoglobin concentration <8 g/dL were excluded from the study and referred for medical treatment. Children who were taking micronutrient supplements were also excluded from the study.

### **Study design**

The study used a randomized controlled design (see **Figure 1**); participants were stratified by school and by grade, and were randomly allocated to either the intervention (n= 86) or control group (n= 81). Two weeks before the start of the intervention, baseline data were collected at both schools. Each child in the intervention group received 300 grams of a cooked ALV dish with the starch of the school meal for 62 school days. This included 30 times the amaranth-and-cowpea dish, 25 times the amaranth-and-pumpkin dish, three times the amaranth-and-spiderplant dish and three times the amaranth dish. The yields of the various ALVs used were different and this resulted in a none-equally distribution. The children in the control group received the normal school meal. Meals were eaten in the class rooms under the supervision of a fieldworker who recorded adherence and absence - including the reason. Adherence was measured by how much of the meal was consumed in four

categories (plate empty, plate 75% empty, plate 50% empty or plate full). If the child ate very little (less than 25%) of the meal then this was recorded as if the child did not eat the meal.



**Figure 1:** Flow diagram of intervention study.

### Intervention product

The following ALVs were used to prepare the intervention dish: *Amaranthus cruentus* (amaranth), *Cleome gynandra* (spiderplant), *Cucurbita maxima* (pumpkin) and *Vigna unguiculata* (cowpea). These ALVs were found to be those mostly consumed and used in urban and rural areas in the North West Province (19). The vegetables were cultivated on farmland approximately 50 kilometers from Potchefstroom in the North West Province, South Africa from October 2011 till February 2012. Harvesting took place from 9 January 2012 until 2 February 2012. After harvesting, the vegetables were brought to the North-West University research kitchen and stored at +5°C. Either on the day of harvesting or the next day, the vegetables were processed into the intervention dishes using an oil-jacketed pot (VULCAN BP 225). The ALVs in the first dish consisted of 100% amaranth; the ALVs in the other three dishes consisted of 80% amaranth plus 20% spiderplant, pumpkin or cowpea. The

ALV content of each dish was 49%. The remaining ingredients included chopped tomatoes and onion mix (22%), vegetable oil (3%), a commercially available instant gravy powder (5%) and water (21%). Three different flavours of the same gravy have been used to avoid product fatigue during consumption and thereby assuring better adherence. The intervention dishes were packed per four portions of 300 gram each, labeled with the type of dish and the harvest and processing date and stored at -20°C. The recipe that was used to prepare the dishes was based on the study results by Matenge *et al.* (20) in the North West Province, which showed that this recipe was found to be the most acceptable and preferred of the traditional dishes used in the area. Prior to the intervention the ALV dishes were evaluated by children (n=80) from grade 2-4 attending the selected schools on sensory attributes in order to ensure adherence to the intervention during the entire intervention period. Swiss chard (*Beta vulgaris*) was added as reference dish and was prepared in the same way as the ALV dishes. The methods used for this sensory evaluation have been described in Van der Hoeven *et al.* (21). The intervention dish (300 gram) was served together with the starch (125 gram) which was provided by the school meal. The control meal consisted out of the same portion of starch accompanied by a serving spoon of relish, including vegetables (often cabbage) or legumes and sometimes meat or soy mince (not more than a teaspoon).

### **Nutritional composition**

The ALV dishes were analyzed for iron and zinc content. At least three composite samples (consisting of three samples each) of each ALV dish were prepared at the beginning, mid-way and end of the harvest and processing time. The amaranth-and-spiderplant dish was only prepared at the beginning of the harvest and processing time, because the yield of spiderplant was low. The composite samples were sent to Microchem Laboratories in Cape Town, which is accredited by the South African National Accreditation System. Inductively Coupled Plasma–Optical Emission Spectrometry (Optima 5300 DV, PerkinElmer, Johannesburg) (S.O.P.C No 45) was used for the analysis of iron and

zinc. The nutrient content of the ALV dishes was used to calculate their contribution to iron and zinc intake.

### **Biochemical indicators**

At baseline and at the end registered nurses drew venous blood samples (10 mL) into a 6 mL serum tube and a 4 mL EDTA-coated tube from non-fasting subjects, using a sterile Venofix infusion set (BRAUN, 23G, 0.65x20 mm) and syringes. All blood samples were transported within one to two hours to the laboratory at the Centre of Excellence for Nutrition, North-West University. Hemoglobin (Hb) and erythrocyte zinc protoporphyrin (ZnPP) were measured immediately in the laboratory, whereas for all other measurements, baseline and end samples were analyzed together immediately after the completion of the study. Hb concentration was measured on whole blood in the EDTA tubes using the Beckman Coulter (Coulter Ac.T5diff CP). After the Hb was measured, the tubes were centrifuged for 10 minutes at 3500 rpm. The plasma was collected and stored at -80°C. The remaining red blood cells (RBCs) were washed three times with 5 mL saline solution, inverted and spun off. ZnPP was measured on these washed RBCs using an Aviv ZPP Hematofluorometer (Biomedical Inc.; Model 206D). Care was taken throughout to ensure that the samples were protected from direct light. The tubes used for the zinc sample were trace element-free (tubes washed with 10–20% HNO<sub>3</sub>) to avoid contamination. For serum preparation, whole blood collected in the 6 mL serum tube was clotted in the tube, centrifuged at 3500 rpm for 10 minutes at room temperature (Universal 16R<sup>TM</sup>, Hettich), transferred into micro-tubes and stored. Serum ferritin (SF) was measured using a Ferritin Elisa kit (Ramco Laboratories Inc.). Serum transferrin receptor (TfR) was determined by using an *in vitro* enzyme immunoassay (Ramco Laboratories Inc.). Serum retinol, zinc and C-reactive protein (CRP) were measured by the Medical Research Council (MRC) in Cape Town. Serum retinol was determined by a reversed phase high-performance liquid chromatography method described by Catignani and Bieri (22) (SpectraSERIS). Serum zinc was analyzed with a flame atomic absorption spectrophotometer (Philips Pye Unicam SP9, Cambridge, United Kingdom) with a

commercial control serum (Serorm Trace Elements Serum; SERO AS, Billingstad, Norway) as a quality control. CRP was measured by an immunoturbidimetric method (Technicon method no. SM4-0183G89, Technicon RA-1000 auto analyser; Technicon Instruments, NY), using Bayer TESTpoint Serum Protein Controls (Bayer Diagnostics, Fernwald, Germany).

Anemia was defined as Hb concentration  $<11.5$  g/dL (23). Iron deficiency was defined as either an SF concentration  $<15$   $\mu$ g/L (24), ZnPP concentration  $>70$   $\mu$ mol/mol heme (25) or TfR concentration  $>8.3$  mg/L (test-kit reference value). Iron-deficiency anemia was defined as Hb  $<11.5$  g/dL and SF  $<15$   $\mu$ g/L. Inflammation was defined as a CRP concentration of  $>5$  mg/L (26) and the SF and serum retinol values of participants with inflammation were excluded from the analyses because of the confounding effects of inflammation on SF. Zinc deficiency was defined as serum zinc concentration  $<65$   $\mu$ g/dL (27). Sub-clinical vitamin A deficiency was defined as serum retinol  $<20$   $\mu$ g/dL (28).

### **Anthropometric measures**

Body weight was measured without shoes and in minimal clothing to the nearest 0.1 kg using a digital Tanita Ironman Innerscan body composition monitor scale (BC 554 – Elite Series), which was calibrated by using fixed weights. Height was measured with the Seca Leicester height measure mounted on a board. During height measurement, the subject stood upright without shoes on a flat surface against a wall with the head in the Frankfort plane. Height-for-age z-score (HAZ), weight-for-age z-score (WAZ) and BMI-for-age z-score (BAZ) were calculated using the 2007 World Health Organization (WHO) reference for children aged 5-19 years (29). Stunting was defined as HAZ  $<-2$  standard deviations (SD) of the reference population and mild stunting as  $-2$  SD  $<$  HAZ  $<$   $-1$  SD. Underweight was defined as WAZ  $<-2$  SD and mild underweight as  $-2$  SD  $<$  WAZ  $<$   $-1$  SD. WAZ was only available for children under 11 years. WAZ cannot be used as a monitoring tool for growth beyond childhood, since it is not able to distinguish between relative height and body mass (29). Overweight was defined as BAZ  $>1$  SD and obesity as BAZ  $>2$  SD.

**Dietary intake assessment**

Dietary intake was assessed by means of 24-hour dietary recall method and a Quantitative Food Frequency Questionnaire (QFFQ) among a sub-sample of the study population (n=100) after completion of the intervention study. The 24-hour recall was used to collect information on the nutrient intakes of the intervention and control groups, and included dietary intake on week – and on weekend days. The QFFQ collected data on the food items consumed in the previous month. Food portion sizes were estimated using fresh food (stiff and soft maize meal porridge and rice), plastic food models, household utensils and food packaging materials. The adult responsible for the food preparation for the child was present during the 24-hour recall and QFFQ interview with the child. These interviews were conducted in the mother tongue of the interviewees by two trained and experienced interviewers. All 24-hour recalls and QFFQ were checked for completion (e.g. preparation methods included and portion sizes) and possible errors. Data were coded and quantified manually by a registered dietician using the MRC Food Quantities Manual (30). Data were computerized and analyzed using the MRC FoodFinder3 software, which is based on the South African food composition database (31).

**Data analyses**

Statistical analyses were performed using the IBM Statistical Package for Social Sciences (IBM SPSS 20.0 for Windows). Data were checked for normal distribution and the presence of outliers ( $\pm 3$  SD from the mean). Skewed variables were transformed before analysis. Five imputations were produced for all outcome variables for missing data for the intervention study (<3%) under the assumption that values were missing at random. These multiple imputations were produced using an iterative Markov chain Monte Carlo method and a linear regression model, which included the intervention (ALV consumption), age, gender, school and adherence as independent predictors. Independent t-tests were conducted to investigate potential differences in baseline characteristics between the intervention and the control group. Estimated intervention effects were analyzed by

using ANCOVA on the endpoint measurement with baseline value, gender, age, school and adherence as individual level covariates. Baseline differences in nutritional status outcomes between the intervention and control groups between males and females, between children who were stunted and those who were not and between deficient and non-deficient children were analyzed by independent t-tests. A two-factor ANCOVA including either gender (males compared to females) or HAZ (stunted compared to not stunted) or deficiencies (deficient compared to not deficient) and ALV consumption as fixed factors was performed to investigate potential interactions of gender, stunting or deficiencies with ALV consumption on endpoint nutritional status outcomes. Appropriate subgroup analyses were performed if the gender x ALV consumption or deficiencies x ALV consumption interaction was significant. A p-value of <0.05 was regarded as significant.

#### **Ethical consideration**

Ethical approval was granted by the Ethics Committee of the North-West University (NWU-00033-09-A1). Permission was also granted by the Department of Education of the North West Province (Dr Kenneth Kaunda district) and the School governing bodies of the two schools. Meetings with the parents/guardians were held in their preferred language to explain the purpose and the procedures of the study and they were given the opportunity to ask questions or make remarks. Afterwards guardians were asked to give their written consent for their children to participate in the study. Only children who obtained parental consent and gave assent for the study were included. Participation was voluntary and participants could withdraw any time without any consequences. The intervention study was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT01920646.

## RESULTS

### Acceptance of the intervention dish

Each child (n=80) evaluated four different samples and each dish was evaluated at least 62 times.

**Table 1** shows the mean rating scores of the four ALV dishes with regard to color, smell, taste and overall acceptability. Although all dishes are rated as acceptable, Swiss chard had higher rating scores. The ALV dishes were rated “good” or “super good” by 62.3%, 57.2%, 52.2% and 56.8% of the participants, respectively for color, smell, taste and overall (not shown in table).

Table 1: Sensory evaluation scores<sup>1</sup> (mean±SD) for different dishes made with leafy vegetables.

Dish <sup>x</sup>	N	Color	Smell	Taste	Overall
100% Amaranth	68	3.3±1.3 <sup>a</sup>	3.3±1.2 <sup>a</sup>	3.26±1.35 <sup>a, b</sup>	3.40±1.35 <sup>a</sup>
80% Amaranth + 20% Cowpea	64	3.7±1.1 <sup>a, b</sup>	3.6±1.3 <sup>a</sup>	3.48±1.10 <sup>a, b</sup>	3.66±1.25 <sup>a</sup>
80% Amaranth + 20% Pumpkin	62	3.5±1.2 <sup>a</sup>	3.3±1.2 <sup>a</sup>	3.44±1.22 <sup>a, b</sup>	3.24±1.33 <sup>a</sup>
80% Amaranth + 20% Spiderplant	63	3.6±1.3 <sup>a, b</sup>	3.31±1.16 <sup>a</sup>	3.26±1.35 <sup>a, c</sup>	3.40±1.35 <sup>a</sup>
100% Swiss chard	63	4.0±1.2 <sup>b</sup>	3.98±1.16 <sup>b</sup>	4.40±0.93 <sup>d</sup>	4.27±0.95 <sup>b</sup>

<sup>1</sup> Score: Five-point ordinal scale ranging from 5 (super good) to 1 (super bad).

<sup>x</sup> Each dish consisted of 49% ALVs, tomatoes and onion mix (22%), vegetable oil (3%), a commercially available instant gravy powder (5%) and water (21%).

Dishes with different letters differed statistically significantly with regard to color, smell, taste and overall acceptability ( $p < 0.05$ ).

### Nutritional composition of the ALV dishes

**Table 2** shows the iron and zinc content of the ALV dishes and their contribution to the RDA for iron and zinc. The RDAs for children from four to eight years for iron and zinc are 10 mg/day and 5 mg/day respectively. For children 9-13 years the RDA for iron and zinc are 8 mg/day and 8 mg/day

respectively (32). One single serving of an ALV dish provided 116.4% - 157.8% of the RDA for iron and 27.6% - 73.8% of the RDA for zinc (based on RDA for children from four to eight years). The dish made with 80% amaranth and 20% pumpkin contributed most to the RDA for iron (15.8 mg per 300 gram portion) and the dish made with 100% amaranth contributed most to the RDA for zinc (3.7 mg per 300 gram portion).

Table 2: Contribution of iron and zinc content of different ALV dishes (per 300 gram serving) to RDA.

Dish <sup>x</sup>	Iron			Zinc		
	(mg)	% RDA <sup>1</sup> of iron		(mg)	% RDA of zinc	
		4-8 yrs <sup>2</sup>	9-13 yrs		4-8 yrs	9-13 yrs
100% Amaranth	11.6	116.4	145.5	3.7	73.8	46.1
80% Amaranth + 20% Cowpea	12.5	124.8	156.0	1.4	27.6	17.3
80% Amaranth + 20% Pumpkin	15.8	157.8	197.3	2.2	43.2	27.0
80% Amaranth + 20% Spiderplant	14.9	149.4	186.8	1.6	31.8	19.9

<sup>1</sup> RDA: Recommended dietary allowance. <sup>2</sup> yrs: years. <sup>x</sup> Each dish consisted of 49% ALVs, tomatoes and onion mix (22%), vegetable oil (3%), a commercially available instant gravy powder (5%) and water (21%) (starch was excluded from analysis).

### Intervention

In total 167 children participated in this study (Figure 1). Four children (all included in the control group) did not complete the study because they left the school. Baseline characteristics are shown in **Table 3**. There were no significant differences in any baseline characteristics between the intervention and the control group. Morbidity patterns were similar for the intervention and control group. Adherence was high in both the intervention (79%) and the control group (89%), taking into account absenteeism and leftovers. Adherence to the intervention meals became less toward the end, most likely a form of product fatigue. Furthermore, there was more variety in the control meals, the intervention meal always consisted out of ALVs and a starch. The meat included in the control meals were small portions (less than a teaspoon). If meat was included, it was only once or twice every two weeks. Children from the intervention group were allowed to eat the control meal

(although not the starch) as well after they finished their intervention meals. During the study duration only two children from the intervention group ate the control meal (not the starch) as well. The full portion of the intervention meal was eaten 71% of the days and the control meal was eaten in full 89% of the days. For the intervention group the mean daily ALV consumption was 236 gram.

Table 3: Baseline characteristics of study population.

	Intervention (n=86)	Control (n=81)
Age (years; mean±SD)	8.4±2.2	8.4±2.2
Male:Female ratio (%)	55:45	49:51
Stunted (HAZ <-2 SD)	12 (14.0%)	10 (12.3%)
Mildly stunted (-2 SD < HAZ < -1 SD)	24 (27.9%)	22 (27.2%)
Underweight (WAZ <sup>4</sup> <-2 SD)	4 (4.7%)	5 (6.2%)
Mildly underweight (-2 SD < WAZ < -1 SD)	26 (30.2%)	20 (24.7%)
Overweight (2 SD > BAZ >1 SD)	3 (3.5%)	2 (2.5%)
Obese (BAZ >2 SD)	0 (0.0%)	1 (1.2%)
Anemia (Hb <11.5 g/dL)	9 (10.5%)	13 (16.0%)
Iron deficiency based on SF (<15 µg/L)	14 (16.3%)	15 (18.5%)
Iron deficiency based on TfR (>8.3 mg/L)	4 (4.7%)	9 (11.1%)
Iron deficiency based on ZnPP (>70 µmol/mol heme)	2 (2.3%)	2 (2.5%)
Iron-deficiency anemia (Hb <11.5 g/dL and SF <15 µg/L)	2 (2.3%)	1 (1.2%)
C-reactive protein >5 mg/L	4 (4.7%)	4 (4.9%)
Zinc deficiency (serum zinc <65 µg/dL)	65 (75.6%)	61 (75.3%)
Sub-clinical vitamin A deficiency (serum retinol <20 µg/dL)	6 (7.0%)	2 (2.5%)

All values are given as n (%), unless indicated otherwise. HAZ: Height-for-age z score. WAZ: Weight-for-age z-score. WAZ score only available for participants < 11 years (n=141). <sup>7</sup> BAZ: BMI-for-age z score. Hb: haemoglobin. SF: serum ferritin. SF values of participants with a C-reactive protein concentration >5 mg/L were excluded. TfR: transferrin receptor. ZnPP: zinc protoporphyrin.

### Dietary intake

Analysis of the macronutrient intake showed no differences between the intervention and the control group. The median energy intake (based on QFFQ) was 7291 (5768 – 9960) kJ of children in the intervention group. The control group had a median energy intake of 6493 (5258 – 8457) kJ. The median macronutrient intake (as percentage of energy) was within the acceptable macronutrient distribution range for both groups as determined by the Institute of Medicine (33). Children in both the intervention and the control group had an adequate median intake of iron, vitamin A and zinc (**table 4**). The food items providing these micronutrients were mainly cereals and cereal products and meat. Stiff maize meal porridge and brown bread, both fortified, were among the food items contributing to more than one micronutrient and provided the largest amounts of iron, vitamin A and zinc (**table 5**). Table 5 shows that almost every child consumed both fortified stiff maize meal porridge and bread. Taking into account the median portion size of both the 24h recall and the QFFQ, the porridge provided 27-32%, 20-23% and 18-22% of the RDA for children four to eight years old for iron, vitamin A and zinc respectively. The bread was a richer source of zinc; 14-16%, 7-8% and 30-36% of the RDA for children four to eight years old for iron, vitamin A and zinc respectively.

Table 4. Median and interquartile range for daily micronutrient intake.

	EAR	24 h recall		QFFQ	
		Median	Q1 – Q3	Median	Q1 – Q3
<b>Intervention (n=49)</b>					
Iron (mg)	4.1	13.8	10.2 – 16.6	12.5	10.0 – 17.3
Vitamin A (RE µg)	500	495	367 – 827	955	450 – 1583
Zinc (mg)	4.0	7.4	6.1 – 11.2	8.6	6.7 – 10.9

<b>Control (n=51)</b>					
Iron (mg)	4.1	11.9	8.5 – 15.6	11.0	8.3 – 16.4
Vitamin A (RE µg)	500	517	238 – 817	700	374 - 1106
Zinc (mg)	4.0	8.1	5.4 – 11.2	7.4	6.0 – 10.1

EAR: Estimated average requirement for children aged 4-8 years, 24h recall: 24 h dietary recall method, QFFQ: Quantitative food frequency questionnaire, Q1 - Q3: interquartile range.

Table 5. Consumption of fortified food items rich in iron, vitamin A and zinc based on QFFQ.

	<b>Intervention (n = 49)</b>	<b>Control (n = 51)</b>
<b>Stiff maize meal porridge</b>		
Median portion per consumer (g)	229	195
No consumers	49	50
Contribution iron (mg)	3.2	2.7
Contribution vitamin A (µg RE)	92	78
Contribution zinc (mg)	1.1	0.9
<b>Bread (brown and white)</b>		
Median portion per consumer (g)	34	39
No consumers	48	51
Contribution iron (mg)	1.4	1.6
Contribution vitamin A (µg RE)	29	33
Contribution zinc (mg)	1.5	1.8

QFFQ: Quantitative food frequency questionnaire

**Intervention**

Concentrations of biochemical indicators of iron, zinc and vitamin A status at baseline and endpoint for both the intervention and control group are shown in **Table 6**. There were no significant estimated intervention effects. There were no significant gender x ALV consumption interaction effects on the biochemical outcomes either. Other deficiencies (anemia, zinc deficiency and iron deficiency based on SF) did not have a significant interaction with ALV consumption. However, the Hb and the serum zinc concentration improved significantly in the intervention group,  $p = 0.001$  and  $p = 0.022$  respectively. The same observation was evident in the control group,  $p < 0.001$  and  $p = 0.006$  respectively. There was no difference in baseline and endpoint value for serum retinol and serum ferritin. However, the prevalence of sub-clinical vitamin A deficiency decreased significantly in the group receiving ALVs from 7.0% at baseline to 1.3% at endpoint ( $p = 0.015$ ), but there was no change in prevalence in the control group. The anemia prevalence decreased from 10.5% to 1.9% in the intervention group ( $p = 0.015$ ) and decreased from 16.0% to 2.5% in the control group ( $p = 0.001$ ). The prevalence of zinc deficiency decreased as well, non-significantly from 75.6% to 68.8% in children consuming ALVs and significantly from 75.3% to 53.8% in the control group ( $p < 0.001$ ). The prevalence of iron deficiency based on SF decreased non-significantly from 16.3% to 15.0% in the intervention group, and increased non-significantly from 18.5% to 21.3% in the control group.

Table 6: Effects of ALV consumption on biochemical indicators of iron, zinc and vitamin A status<sup>1</sup>.

Indicator	Intervention (n=86)	Control (n=81)	Estimated intervention effect
Blood hemoglobin (g/dL)			
Baseline	12.80±0.98 <sup>2</sup>	12.66±1.01	-0.024 (-0.232, 0.184) <sup>3</sup>
End	13.08±0.76	13.05±0.89	
Serum ferritin (µg/L) <sup>4</sup>			
Baseline	25.97 (2.6, 75.4) <sup>5</sup>	22.90 (0.5, 66.15)	-0.016 (-0.294, 0.326)
End	25.84 (1.7, 86.6)	23.80 (0.6, 68.8)	
Serum transferrin receptor (mg/L) <sup>6</sup>			
Baseline	4.36 (1.94, 11.68)	4.41 (2.01, 13.8)	-0.015 (-0.027, 0.056)
End	4.71 (1.74, 86.6)	4.41 (2.01, 16.6)	
Zinc protoporphyrin (µmol/mol heme) <sup>6</sup>			
Baseline	35.15 (18.5, 88)	37.5 (17.00, 74.37)	-0.014 (-0.041, 0.014)
End	44.36 (22.12, 111.48)	46.85 (22.5, 106.5)	

Table 6 continued.

Indicator	Intervention (n=86)	Control (n=81)	Estimated intervention effect
C-reactive protein (mg/L) <sup>6</sup>			
Baseline	0.94 (0.10, 20.2)	0.50 (0.10, 17.46)	0.036 (-0.162, 0.232)
End	0.65 (0.10, 27.75)	0.60 (0.10-8.70)	
Serum zinc (µg/dL)			
Baseline	59.06±9.68	60.66±8.15	-0.274 (-2.959, 2.411)
End	61.61±9.49	63.01±8.73	
Serum retinol (µg/dL)			
Baseline	32.56±8.20	32.77±7.03	-0.415 (-2.108, 1.278)
End	33.59±7.85	33.58±6.55	

<sup>1</sup>Intervention effects were estimated by using ANCOVA adjusted for respectively baseline value, gender, age, school and adherence. <sup>2</sup> Mean±SD (all such values). <sup>3</sup> β (95% confidence interval) (all such values). <sup>4</sup> Data were square root transformed to perform ANCOVA. SF values of participants with a C-reactive protein concentration >5 mg/L were excluded (at endpoint: intervention = 12; control = 7). <sup>5</sup> Median (minimum, maximum) (all such values). <sup>6</sup> Data were log transformed to perform ANCOVA.

## DISCUSSION

The four ALV dishes used in this intervention were acceptable with regard to color, taste and smell for school children, as well as the same dish prepared in a traditional way without the gravy (21). Although the ALV dishes appeared to be rich in micronutrients and (mild) micronutrient deficiencies did exist in this population, the consumption of ALVs for three months did not improve the micronutrient status of children from a rural farm community in the North West Province, South Africa.

This randomized controlled trial contributes to the limited existing evidence on the effect of ALVs on the micronutrient status of children (12, 34-36). Adherence in our study was good and the portion size of the ALVs was larger than reported in the other studies. The ALVs used in our study provided a substantial amount of iron. Anemia prevalence decreased significantly in both groups and the mean hemoglobin increased significantly in both groups as well. Nawiri *et al.* (34) reported that the prevalence of anemia decreased in their intervention group, although hemoglobin concentrations did not change significantly. However, when adjusted for baseline value, gender, age, school and adherence, no effect of ALV consumption was found in the current study. Macharia-Mutie *et al.* (37) conducted a food-based intervention trial to alleviate iron deficiency anaemia, but despite the high prevalence of iron deficiency anaemia no effect of the food naturally high in iron was found. Our intervention showed no effect on zinc status, despite the fact that serum zinc was low in many children. Taking the bioavailability of zinc in plant foods into account as well we conclude that ALVs are limited zinc sources. Still, the mean serum zinc improved significantly in both groups. An explanation for this increase in both groups might be regression to the mean. Although a generally accepted sensitive and specific biomarker for zinc status is currently lacking, serum (or plasma) zinc is the most widely used biomarker (38, 39).

Our study found a significant decrease in vitamin A deficiency prevalence in the intervention group, although the sample size was small. There was no significant increase in mean serum retinol. The

results of previous studies indicated changes in mean serum retinol as well (12, 34-36, 40). ALVs seem to be more effective in improving low levels of serum retinol (34) and the mean serum retinol of the children included in our study was relatively high. Furthermore, the bioconversion to retinol is a complex process in which dietary carotenoids have to be released from the food matrix and incorporated into mixed micelle before they can be absorbed intestinally. This process is influenced by several factors including species of carotenoids, amount of carotenoids, interactions of the carotenoids, effectors of absorption, host-related factors, such as nutrient status and genetics, and food matrix. To release  $\beta$ -carotene molecules from its food matrix, Takyi (35) suggests homogenization of the sample. However, meal preparation in the other studies in which researchers did observe an improvement in serum retinol and in our study only involved boiling, steaming and cooking (12, 34, 36). Although we did not analyze the  $\beta$ -carotene levels in our vegetables due to technical reasons, Nawiri *et al.* (34) reported that both amaranth and cowpea leaves were rich in  $\beta$ -carotene.

Bioavailability of iron may be inhibited by dietary fiber, calcium, phytate, oxalate, tannins, phosphates, and polyphenolic compounds (41, 42). The high phytic acid concentration in the maize meal is regarded as the principal dietary factor that influences the absorption of native and fortification iron and zinc, especially in a plant-based diet (41, 43, 44). Although the bioavailability of non-heme iron from a plant-based diet is less than that of heme iron, the inclusion of small amounts of food of animal sources (especially muscle tissue) can improve this (41, 45). De Pee *et al.* emphasized the importance of quantifying and improving the bioavailability of dietary carotenoids (12). Several strategies have been suggested to increase the bioavailability of iron, zinc and vitamin A. In a rat pup model, the use of a low phytic acid genotype of *Zea mays* L. (maize) led to an increase in zinc absorption (46). However, the effect of the use of low phytic acid maize in humans is less conclusive, especially in the long term (47, 48). The addition of the microbial enzyme phytase could be efficacious in improving the iron and zinc absorption of maize meal porridge (41, 49), as also proved by Troesch *et al.* (50).

In soaked and cooked green leafy vegetables, 5.5-9.8% of the total available iron and 46.6-51.4% of the total available zinc were bioaccessible (51). The bioaccessibility of  $\beta$  carotene in cooked spinach was 1.5% (52). Optimization of food processing and preparation methods, including traditional recipes, might increase the bioavailability of iron (41, 45), vitamin A (53), and zinc (46, 49) and should be further explored. Future research should also focus on the potential contribution of ALVs to improve micronutrient status, including iron, vitamin A and zinc, in populations with an inadequate dietary intake.

Data on the micronutrient status of primary school children in farm or rural communities in the North West Province in South Africa is limited. The last national survey reporting data on anaemia and iron deficiency in the North West Province was the NFCS-FB. This study reported an anaemia (haemoglobin  $\leq 11$  g/dL) prevalence of 27% (N=37) and an iron deficiency (haemoglobin  $\leq 11$  g/dL and SF  $< 12$   $\mu$ g/L) prevalence of 2.9% (N=35; no correction for CRP) in the North West Province (54). An intervention study in the North West Province, focusing on the effects of black tea and Rooibos on iron status, found that the mean haemoglobin concentration was 12.8 g/dL in rural primary school children and that the overall prevalence of a low haemoglobin status (haemoglobin  $\leq 12$  g/dL) was 13-15% (in both groups). The mean SF concentration was 40.5-41.8  $\mu$ g/L in the same children and 11-12% had deficient SF concentrations at baseline (in both groups) (54). Taljaard *et al.* (56) concluded that national data is not necessary representative for regional populations and that the anaemia prevalence, based on the haemoglobin concentration in primary school children, might have improved in some regions since the NFCS-FB has been conducted. The nutritional status of our study population was also better than reported in the other studies. According to available data on micronutrient status in South Africa and the North West Province, one would have expected a study population like this one to be more deficient in iron and vitamin A and that an intervention study such as this would improve children's micronutrient status. Although the national prevalence of vitamin A deficiency in children under five years has decreased by 20%, the mean retinol value has decreased (0.73  $\mu$ mol/L to 0.67  $\mu$ mol/L) in the North West Province and consequently the

prevalence of vitamin A deficiency increased (2). Since the current study population's micronutrient status was better than expected, the sample size might not have been adequate to detect the possible impact of the consumption of the selected ALVs. However, Agte *et al.* (40) found that daily intake of 100 gram green leafy vegetables with 10 gram oil improved plasma  $\beta$ -carotene, hemoglobin, plasma vitamin C and plasma zinc in 40 young healthy adults. Their dietary intake, however, was not sufficient in terms of micronutrients (40).

School children included in our study had an adequate median intake of iron, vitamin A and zinc. Stiff maize meal porridge and brown bread provided approximately 33-50% of the RDA for iron, vitamin A and zinc (54). The mandatory fortification of maize meal and wheat flour with iron, vitamin A and zinc, among others, in South Africa was implemented in 2003 (57). It appears that the micronutrient status of children in South Africa has improved in the last years (2). Although the fortification of maize meal and wheat flour included zinc, the prevalence of zinc deficiency in this study population was higher than the provincial (41.1%) and the national (45.3%) prevalence in children one to nine years old (58). Troesch *et al.* (50) also reported a high prevalence (47.4-52.5%) of zinc deficiency (serum zinc <65  $\mu$ g/dL) in school children aged 5-12 years in South Africa. Faber *et al.* (59) reported that fortified porridge, which supplied 100% of the RDA of zinc in infants, failed to improve serum zinc concentrations. It was therefore suggested that the amount of zinc fortification was too low and required an adjustment (59). Traditionally, ALVs are mainly consumed as relish to accompany maize meal porridge, also in this study.

In addition to the nutritional contribution of ALVs, the children included in the intervention group did eat a substantial portion of ALVs five days a week for a period of three months. Vegetables and fruit are often neglected, contributing to a monotonous diet in many South African households (43, 60). The children in this study were not reluctant to eat a substantial portion of vegetables daily. The South African Youth Risk Behaviour Survey 2008 reported that 51.6% of children in grade 8-11 from the North West Province consumed cooked vegetables 4 or more times a week (preceding the

survey) and that 66.7% ate at least a cup of cooked vegetables per serving (61). It is well known that the consumption of sufficient fruit and vegetables reduces the risk of disease (62, 63). The health-protecting properties of non-nutrient bio-active compounds in ALVs can play an essential role in the success of the WHO's global initiative on the consumption of fruit and vegetables (64). It was estimated that the theoretical-minimum-risk distribution (required adequate intake to prevent disease) for fruit and vegetable intake was 480 grams in children aged 5–14 years (63). The South African paediatric food-based dietary guidelines recommend a fruit and vegetable intake of 320-480 gram per day for children aged one to seven years (65). Despite these recommendations the guideline of the Departement of Education to include vegetables and fruit in the daily school meal is not always implemented at school level due to limited access to these perishables (66). Therefore, these natural available indigenous vegetables, when wild collected in season, can play an important role in the diversification of diets in rural and farm communities.

Overall, this randomized controlled trial showed that ALVs were unable to improve serum retinol, serum ferritin or hemoglobin in a mildly deficient population. Furthermore, the zinc in ALVs is very limited available, and despite the low zinc status in our population, ALV consumption did not improve serum zinc concentrations. However, the sensory evaluation and adherence to the ALV consumption in this intervention show potential for ALVs to diversify the diet of school children.

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### **CONFLICT OF INTEREST**

None of the authors has any conflict of interest with regard to this publication.

### **AUTHORS' CONTRIBUTIONS**

MvdH: design; acquisition of data; analysis and interpretation of data; drafting the manuscript. MF: design; critical revision of manuscript for intellectual content. JO: acquisition of data; critical revision of manuscript for intellectual content. AK: design; critical revision of manuscript for intellectual content. CMS: design; critical revision of manuscript for intellectual content. All authors read and approved the final manuscript.

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## 6. CONCLUSIONS

Against the background of prevailing malnutrition and its coexistence with micronutrient deficiencies and poverty in South Africa, the aim of this thesis was to investigate the effect of African leafy vegetables (ALVs) on the alleviation of micronutrient deficiencies in school children residing in the North West Province of South Africa. Johns (2003) argued that traditional biodiversity use, instead of westernising diets, in the socio-cultural context can be a powerful tool for maintaining and enhancing health and nutritional status (Johns, 2003). Therefore this study focused on the potential of ALVs as a strategy to improve iron, vitamin A and zinc status among school children. Furthermore, this study focused on the children's and their caregivers' attitudes towards these ALVs to assess support for this strategy. This research made use of a mixed-methods approach, combining a randomized controlled trial, focus groups, sensory evaluation, nutrient composition analyses, socio-demographic semi-structured interviews, dietary intake assessment, and anthropometric measurements. It contributes therefore in a unique way to the existing evidence as most studies only include on one or two of these aspects.

The assessment of caregivers' knowledge, perceptions and use of indigenous and traditional plants (ITPs) indicated that caregivers had readily applied knowledge on and positive attitudes towards ITPs (**Objective 1 – Chapter 3**). In total 30 edible ITPs were identified and discussed. The most commonly used edible plants were *Amaranthus spp.*, commonly known as *thepe*, and *Chenopodium album*, commonly known as *senkgampapa*. The parts of the plants that were mostly consumed were the leaves. Participants referred to the leafy vegetables as "morogo" or "wild spinach". Caregivers had knowledge of available ITPs and their use as food. Location, seasonal variation and rainfall affected the availability of and access to ITPs. These vegetables were mostly used as condiments to accompany starch-based dishes (especially maize meal porridge). Participants had different opinions on which ingredients to use during preparation of the leafy ITPs in order to enhance the taste. The

variety of ways in which they could be prepared with different ingredients made them very enjoyable to add as part of a meal. Participants had mixed views and beliefs on the effect rinsing and draining had on leafy ITPs. Only one-third of the participants had access to a working fridge or freezer, meaning that the majority had to rely on traditional methods of preservation and storage. Sun drying of leaves is a cheap and convenient way of preserving these plants, especially in this population, but it may lead to a great loss of essential micronutrients such as vitamins A and C (Kiremire *et al.*, 2010; Ndawula *et al.*, 2004). None of the participants had a negative perception of ITPs, which is in contrast to studies that found that especially members of the younger generation would label ITPs as inferior and as “poverty foods” (Dweba & Mearns, 2011; Vorster *et al.*, 2007). Our finding has been confirmed by Cloete & Idsardi (2013) who found that the perception of indigenous and traditional food crops as ‘poor people’s food’ in the North West Province is unjustifiable based on consumption and expenditure patterns of their study population (Cloete & Idsardi, 2013). ITPs were perceived as healthy, affordable and delicious in the current study, hence acceptable to the caregivers. It is evident that the older generations in this population were seen as custodians of knowledge related to ITPs, and they passed their knowledge on in the hope that it would be transferred and not lost through generations.

Not only attitude, but also sensory characteristics of food, such as appearance, smell, texture and taste, play an important role in people’s decision to consume a particular food. These attributes were investigated on the following ALVs in particular: *Amaranthus cruentus* (amaranth), *Cleome gynandra* (spiderplant), *Cucurbita maxima* (pumpkin) and *Vigna unguiculata* (cowpea). The determination of the sensory acceptability to children of ALV dishes (**Objective 2**) was conducted in two phases; testing of dishes prepared in a traditional way (**Chapter 3**) and testing of the dishes used in the intervention study with added gravy (**Chapter 5**). The children evaluated the dishes made with ALVs and Swiss chard as acceptable in terms of colour, smell and taste. The results of the two sensory evaluations showed significant differences between the five dishes in the mean ratings for smell, taste and overall acceptability, with Swiss chard as most preferred vegetable in both

evaluations. When the dish made with Swiss chard was excluded from the analysis, there was no significant difference between any of the ratings of the four dishes made from ALVs in both evaluations. School children evaluated all dishes prepared in a traditional way as acceptable and indicated that they would like to eat these leafy vegetables twice a week (**Chapter 3**). It appears that the scores given to the ALV dishes prepared in a traditional way are higher than the scores for the intervention product, although the intervention product was still acceptable to the children. Three different flavours of the same gravy have been added to the intervention product to prevent product fatigue to assure adherence to the intervention, although only one flavour was used in the sensory evaluation as described in chapter 5.

The focus of the nutrient composition (including iron and zinc content) and the bio-accessibility of iron and zinc in the selected ALVs alone and in composite dishes (**Objective 3 – Chapter 4**) was on the same ALVs as used in the sensory evaluation. Cooking increased the bio-accessibility of iron and zinc. Amaranth-and-spiderplant had the highest amount of bio-accessible iron (0.42 mg/100 g). All dishes had an amount of 0.3 mg bio-accessible zinc. The phytate:iron and phytate:zinc molar ratios both indicated low iron and zinc absorption in the cooked dishes. Still, the calculated contribution of the ALV dishes (per 100g) towards dietary requirements of 4-6-year-old children was between 24% and 54% for iron, and towards 4% for zinc. Although ALVs were found not to be such good sources of iron and zinc as meat, these vegetables can contribute to the dietary requirements of these micronutrients. Based on dialysable iron and zinc, the contribution of these green leafy vegetables to dietary requirements was more substantial for iron than zinc. Due to technical reasons the  $\beta$ -carotene concentration was not assessed in the selected ALVs. However, there is evidence that the selected ALVs contain considerable amounts of  $\beta$ -carotene (Van Jaarsveld *et al.*, 2013). Based on the nutrient composition, including the bio-accessibility of iron and zinc, of the selected ALVs, it was expected that these ALVs had the ability to improve the iron, vitamin A and zinc status of deficient school children. The randomized controlled trial conducted to investigate the effect of consumption of the selected ALVs on iron, vitamin A and zinc status of primary school children (**Objective 4 –**

**Chapter 5)** however did not find evidence to support this hypothesis. The school children included in the intervention had a better nutritional status than expected, especially with regards to their iron and vitamin A status. Furthermore, despite the low zinc status in this population, ALV consumption did not improve serum zinc concentrations. Since individuals with low zinc status will absorb zinc with increased efficiency (FAO/WHO, 2004), one may wonder if serum zinc is a good indicator for zinc status in school children and if *in vitro* dialyzability is a good indicator for zinc bio-accessibility in leafy vegetables. Although a generally accepted sensitive and specific biomarker for zinc status is currently lacking, serum zinc is the most widely used biomarker (Lowe *et al.*, 2009). A recent dose-response meta-analysis concluded that if the amount of dietary zinc intake doubles, serum zinc increased with 9% in apparently healthy children aged 1-7 years (Moran *et al.*, 2012). In addition, while *in vitro* dialyzability is currently the only method to study zinc bio-accessibility that is validated against human studies, there is a need for more studies providing validation of *in vitro* methods measuring zinc bioavailability. The CaCo 2-uptake model looks promising to assess bioavailability, although further refining (e.g. eliminating the need to heat inactivate proteases) is necessary (Etcheverry *et al.*, 2012). Although the dietary intake appeared to be sufficiently adequate for all micronutrients, including iron, vitamin A and zinc, this only reflected in the low prevalence of iron and vitamin A deficiencies. Dietary intake data was based on one 24h-recall and one QFFQ and was collected after the intervention took place. A more comprehensive dietary assessment of the current study population might also give new insights. This would include at least three non-consecutive 24h recalls and one QFFQ per season to take seasonal availability into account. In addition, dietary intake data of multiple household members (including children and caregivers) might contribute to answer the question how vulnerable farm communities are in terms of nutrition security and why the nutritional status of children was not as poor as expected based on previous research (Shisana *et al.*, 2013; Labadarios, 2007; Kruger *et al.*, 2006; Lemke, 2005). A screening before the start of the intervention, including a dietary intake assessment and current micronutrient status based on blood parameters, might have been necessary. Although this would not have guaranteed that the selected

ALVs would have the expected effect on the micronutrient status of the study population, it would have ensured that the intervention had the potential to show the expected effect. The selected ALVs were not commercially available on the scale needed for this research project and therefore these ALVs had to be cultivated especially for this project while ensuring the fine balance between the agricultural reality (e.g. weather conditions and yield) and academic wishes.

Based on the more theoretical and indirect study results, including both caregivers' and children' positive image of ALVs, and the nutrient composition and iron and zinc bio-accessibility of the ALVs, these selected vegetables do have the potential to contribute to the micronutrient intake of school children. However, the importance of ALVs might not necessary be to serve as a strategy for micronutrient deficiency alleviation, but rather in the diversification of the diet in resource-poor settings and thereby contribute to the micronutrient intake.

## **6.1 Implications for policy**

Naude (2013) substantiate the food based dietary guideline "Eat plenty of vegetables and fruit every day" by recommending that school children should consume at least 400 gram of vegetables and fruit daily, which translate to five servings of 80 gram. Furthermore, these servings should include a wide variety of different coloured vegetables and fruit (Naude, 2013). Currently the National School Nutrition Programme recommends that each lunch meal consists of 40-45 gram of protein, 60 gram of starch and 60 gram of vegetables and/or fruit in the North West Province (South Africa, 2010/2011). The current study showed that school children were not reluctant to consume sustainably larger portions of leafy vegetables during one meal. It is therefore suggested that larger portions of vegetables should be included in the National School Nutrition Programme in order to meet the food based dietary guideline. With this in mind, more attention should also be given to an increased local production of vegetables allocated for the school meals, either by communities or local farmers, to ensure better access to fresh vegetables. Currently, a large proportion of schools

participating in the National School Nutrition Programme was not able to serve their learners the recommended serving of fresh vegetables every day (Faber *et al.*, 2014). Taking into account aspects such as seasonality, yield and climatic conditions, the role the cultivation of ALVs can play in the supply of fresh vegetables should be investigated from a farming and agronomic perspective.

This research underwrites the importance of Indigenous Knowledge Systems (IKS) and the positive attitude the studied community had towards its indigenous knowledge. However, Molebatsi *et al.* (2010) have recorded 525 useful plant species, of which 98 food plants, in Tswana tshimo (home gardens) in the North West Province. In the current study only 30 food plants were identified and this might be an indication that this knowledge is declining. It is important for future generation that this knowledge is conserved and protected. Integrating indigenous knowledge on useful plant species, in particular food plants, in the school curriculum could be a way to conserve this important element of culture and tradition. A school demonstration garden can showcase examples of these plants and thereby demonstrate how to grow a variety of foods which can be included to improve diets. Furthermore such a garden would be a natural setting for environmental education and awareness and horticultural programmes.

Implementation of a surveillance system for monitoring health and nutritional status at local clinics could contribute to the evidence-based decisions made on targeting populations for nutritional interventions for governmental programmes. In addition, this will increase the sample size per district and thereby province during data collection for national survey investigating nutritional status. In this way recommendations and policies can more easily and timely be adapted to the current situation without first have to conduct a large national survey.

## 6.2 Implications for future research

More research is needed on the bioavailability of iron, zinc and  $\beta$ -carotene in ALVs. The bioaccessibility data with regard to iron and zinc collected in this study cannot evaluate the bioavailability. Although the current study did not include  $\beta$ -carotene in the nutrient composition due to technical reasons, several studies have showed that dark leafy vegetables can improve serum retinol (Nawiri *et al.*, 2013; Agte *et al.*, 2006; Tang *et al.*, 1999; Takyi, 1999; De Pee *et al.*, 1998). Therefore it is proposed that CaCo2 cell uptake assay and a suckling rat pup absorption model will be used to assess the bioavailability of iron, zinc and  $\beta$ -carotene in ALVs. The assessments of iron and zinc bioavailability should be integrated (perhaps with the inclusion of calcium) because they susceptible to complex interactions (Etcheverry *et al.*, 2012). If these results look promising, a more costly stable isotope study can be conducted in human subjects (iron and zinc). Furthermore, the food processing and preparation methods, including traditional recipes, should be optimized as this might increase the bioavailability of iron, zinc and  $\beta$ -carotene. This requires an integrated approach, taking into account multiple processing and preparation methods, focusing on both the increase of micronutrient bioavailability and the decrease of antinutrients. In addition, since ALVs are mostly consumed as a relish together with other ingredients to accompany a starch dish (mostly maize meal porridge), more research should be done with regard to the combination of food-to-food fortifications (e.g. addition of tomato and oil) and the decrease of antinutrients, like phytic acid e.g. addition of phytase).

It has been suggested that African indigenous vegetables play a role in income generation and subsistence in the North West Province (Cloete & Idsardi, 2013) and throughout Africa (Oluoch *et al.*, 2009). However, there is limited information on the contribution of these vegetables, including ALVs, towards (household) food security in South Africa. In addition, more research is needed on the coping strategies and mechanisms of households with food insecurity. Our target population was vulnerable as appeared from the socio-demographic interviews, but the nutritional status (based on

both micronutrient status and anthropometry) of the children was not as poor as expected based on previous research (Shisana *et al.*, 2013; Labadarios, 2007; Kruger *et al.*, 2006; Lemke, 2005). A longitudinal cohort study might reveal working coping mechanisms which cannot be detected with a cross-sectional study design.

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Like Alice, I did not experience this adventure on my own. I had the privilege to be surrounded by fantastic people who inspired and motivated me throughout my Ph.D. study. I could not have succeeded without the invaluable support of this diverse group of people.

First of all, my promotor and co-promotors. **Prof. Dr Marius Smuts**, I would like to thank you for being an inspiring researcher and mentor, your genuine caring and concern and your faith in me. Marius, baie dankie dat jou deur altyd oopstaan. **Prof. Dr Annamarie Kruger**, thank you for your continuous support and your faith in this project. Your thoughts and inputs were always meaningful. **Prof. Dr Mieke Faber**, thank you for your advice and critical questions. You always made time to fit me into your hectic schedule. I will remember our conversations, especially the one on coping with poverty and injustice. You are an outstanding role model for me.

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My Ph.D. research was the constant factor through the most turbulent period in my life: migrating from the Netherlands to South Africa, planning the most beautiful wedding together with Martin, travelling back and forth between three continents and now, while writing the final parts of my thesis, looking into the eyes of our daughter, Aria. It will be strange, not having to think about my precious vegetables anymore. However, if there is only one thing I have learnt as a student, it is never to stop asking questions, finding answers and asking more questions. Research is never finished and you could always explore more. Lucky me!

## ABOUT THE AUTHOR

Marinka van der Hoeven was born on 6 July 1983 in Burgerveen, the Netherlands. She graduated from secondary school, Gymnasium, at Herbert Vissers College. In September 2002 she started her studies for her Bachelor of Science (Health Sciences) degree at the VU University (Amsterdam). During this part of her studies, Marinka completed an internship at the Hasan Sadikin Hospital in Bandung, Indonesia. The title of her dissertation was *'Nutrition in HIV/AIDS: Food security in HIV infected patients, Bandung, Indonesia.'* After obtaining her Bachelor's degree in 2006, Marinka started the two-year master's program in 'Public Health Research'. During her studies for this qualification she completed two international research internships. The first was at the North-West University (Potchefstroom Campus) in South Africa. The title of the thesis written from this internship was *'Determinants of health care seeking behavior in urban and rural communities in North West Province, South Africa.'* The second internship was at *Wockhardt-Harvard Medical International HIV/AIDS Education and Research Foundation (WHARF) in India. This resulted in a thesis with the following title: 'Household food security and child nutritional status in HIV/AIDS affected families in Aurangabad, India.'* After obtaining her Master's degree in 2008, Marinka moved back to Potchefstroom, South Africa to start working as research coordinator of the Biodiversity project. This multi-funded, transdisciplinary research project is aimed at increasing agricultural biodiversity and thus to improve nutritional and health status and livelihoods and establish more sustainable production systems in the North West Province of South Africa. In 2009 the preparations for Marinka's Ph.D. study started. She finished writing her Ph.D. thesis in the always inspiring town of Stellenbosch, South Africa, where she, her husband, Martin, and their daughter, Aria, are currently living.

## PUBLICATIONS

Van der Hoeven, M., Osei, J., Greeff, M., Kruger, A., Faber, M. & Smuts, C.M. 2013. Indigenous and traditional plants: South African parents' knowledge, perceptions and uses and their children's sensory acceptance. *Journal of ethnobiology and Ethnomedicine*, 9(78) - doi:10.1186/1746-4269-9-78.

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### Other publications

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### Conference presentations

Van der Hoeven, M., Osei, J., Kruger, A., Faber, M. & Smuts, C.M. The effect of African Leafy Vegetables on the micronutrient status of school children in South Africa: a randomized controlled trial. 20<sup>th</sup> International Congress on Nutrition. Joining Cultures through Nutrition. Grenada, Spain. September 2013 (Oral presentation).

Osei, J., van der Hoeven, M. & Smuts C.M. Potential contribution of African Leafy vegetables to the nutritional status of children. 20<sup>th</sup> International Congress on Nutrition. Joining Cultures through Nutrition. Grenada, Spain. September 2013 (Poster presentation).

Van der Hoeven, M., Kruger, A., Faber, M. & Smuts C.M. Potential of including African leafy vegetables in school feeding programme in rural communities. Nutrition Congress Africa 2012. Bloemfontein, South Africa. October 2012. (Oral presentation).

Osei, J., van der Hoeven, M. & Smuts C.M. Knowledge on and the use of indigenous and traditional plants in a semi-rural farm community. Nutrition Congress Africa 2012. Bloemfontein, South Africa. October 2012. (Poster presentation).

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## **ADDENDUM 1: COVER LETTERS AND CONSENT FORMS**

### **Cover letter and consent form – focus group interview**

Dear parent/guardian,

The school your child is attending has been selected to participate in a research project of the North-West University. This research project is about the effect of African leafy vegetables on nutritional status. It is been expected that by including African Leafy Vegetables in the children's daily diets their nutritional and health status will improve. Permission to undertake this research has been granted by the Department of Education of the North-West. We are also interested in learning more about what you know about indigenous and traditional plants in your community and how you use these plants. We would like to have a group interview, with different members of the community. This interview will take approximately 2 hours. The interview will be recorded on tape and video, so that we can listen carefully what you told us. Everything you say will be kept confidential and your name will not be disclosed. Should you feel any discomfort after the discussion and would like to talk to someone I will make these arrangements for you.

There are no known risks involved in this study. Participation will be voluntary. If you agree to participate, you can withdraw at any stage of the study with no consequences. All names will be separated from all answers and will be kept confidential. Information provided by you will be stored without your names to keep it completely anonymous. This information will help us to understand more about your eating habits and your knowledge on African leafy vegetables. This can help us and the schools to improve the school meal your child receives every day.

With a token of appreciation we would like to thank you for your time and participation. Please ask us if you have any questions at any time. You can contact Marinka van der Hoeven of the North-West University at 018 299 2099 or at 071 355 4090 for questions.

Yours sincerely,

Marinka van der Hoeven



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**CONSENT FORM**

I, \_\_\_\_\_ (Full name of parent/guardian), fully understand the aims of this study which were explained to me in the cover letter and at a parent meeting at the school. By ticking the first box, I give permission to use information I provide.

Yes, I will participate and give permission to use the information gained from me for research purposes only.

No, I will not participate.

Signature parent/guardian: \_\_\_\_\_

Signed at: \_\_\_\_\_ on \_\_\_\_\_ (MM/DD/YY)

Signature witness 1: \_\_\_\_\_

Signature witness 2: \_\_\_\_\_

Signed at: \_\_\_\_\_ on \_\_\_\_\_ (MM/DD/YY)

**Cover letter and consent form – intervention study**

Cover letter

Dear parent/guardian,

We are from the North-West University. The school your child is attending has been selected to participate in a research project of the North-West University. This research project is about the effect of African Leafy Vegetables on nutritional and health status. It is been expected that by including African Leafy Vegetables in the children's daily diets their nutritional and health status will improve. African Leafy Vegetables contain lots of vitamins and minerals. We would like to see if these vegetables can be used to improve nutritional and health status. This information can help us and the schools to improve the school meal your child receives every day. Permission to undertake this research has been granted by the Department of Education of the North West Province.

To see if African Leafy Vegetables have an effect on the nutritional and health status, we would like to ask your permission of including your child in our research project. Children from grade R – grade 4 will be randomly, or by chance, allocated to the intervention or the control group. The children in the intervention group will receive a portion of African Leafy Vegetables with pap or rice as school meal. For the children in the control group nothing changes, they will receive their school meal as usual. This study will be running for 12 weeks. Please remember that participation in the study is voluntary and free and you may withdraw your child from the study at any time without any consequences.

To see if the child's nutritional and health status improves (in both the intervention and the control group), we have to measure the vitamins and the minerals in the blood of your child. This will mean that a blood sample (15 mL; one tablespoon) be taken at baseline and the end of the study by a paediatric nursing sister or a medical technologist. Sterile (free of germs), disposable needles and syringes will be used once only, so there will be no chance of transfer of infection from one child to another. The technique is safe and there is only a slight prick as the needle is placed through the skin. The weight and height of your child will be measured at baseline and end of the study. The age and gender of your child will also be recorded. You will also be requested to give dietary information on your child's eating patterns. You will gain information on your child's nutritional status.

## ADDENDUM 1: COVER LETTERS AND CONSENT FORMS

All information collected about your child will be treated as confidential and only the researchers will have access to it. Your child will be given a participation number. Should anything abnormal be found, we will refer the child to the local clinic or a medical doctor for the necessary treatment. Children with iron deficiency at the end of the study will receive a once off treatment for this. You will be kept informed in this regard and are welcome to discuss with us any concerns that you may have.

Please ask us if you have any questions at any time. You can contact Prof Marius Smuts, Prof Annamarie Kruger or Marinka van der Hoeven of the North-West University at 018 299 2099 or at 082 928 4550 for questions.

Yours sincerely,

Prof Marius Smuts, Prof Annamarie Kruger and Marinka van der Hoeven



## General Principles

To the undersigned:

You are invited to take part in the research project as described in Cover letter. It is important that you read understand the following:

Participation is voluntary and no pressure however subtle may be enforced upon you to take part.

It is possible that you might not personally gain any benefit from your participation in the project, although the knowledge that is gained through the project might benefit other people or communities. In extreme cases where you might receive financial compensation for your participation it would be solely for transport and for personal maintenance such as meals. You are not allowed to be bribed to take part in the project.

You are free, to at any time, without any reason, stop participation and you will by no means be negatively affected. You may also request that your data not be used further in the study and/or that any of the biological samples be destroyed. We are however requesting that you would not withdraw without thinking it through thoroughly, seeing as it might influence the statistical reliability of the project negatively.

By giving consent to take part in this project you are also giving consent that the data generated be used by the researchers for scientific purposes as they choose, providing that it be confidential and that your name or that of your child not be linked to any data.

Medicine Control Board, Department of Health and/or any justifiably court may request access to the information to determine / insure the ethical responsibility of practices to the public.

You will be allowed to access your own data on request, unless the Ethical Committee have granted a temporary non-disclosure (In this instance the reasons will be explained in Cover letter).

A summary of the nature of the project, possible risks and factors that might cause discomfort as well as the benefits that can be expected and / or possible permanent consequences that your participation in this project might have are set out in Cover letter.

You are urged to at any stage address any questions regarding the project and procedures, to the Project leader or co-workers who will gladly answer your questions. They will discuss the project with you in full.

Signed consent is given in this case by the parent or legal guardian for participation in the project.

The project objectives are always secondary to the well-being of the participants and there will be at all times acted in your child's best interest.

No project are allowed to start unless approved by the Ethical Committee. The Project head therefore must accept any negative effects that might be experienced throughout the execution of the project, and must report it in detail and immediately to the chairperson of the Ethics Committee. In case of unforeseen serious detrimental consequences the project will be terminated immediately.



Consent form

I, the undersigned

**Full names & Surname**

Parent/guardian of

**Full names & Surname child 1**

Parent/guardian of

**Full names & Surname child 2**

Parent/guardian of

**Full names & Surname child 3**

have read and understand all the information pertaining to the project as discussed in cover letter and general principals of this informed consent as well as heard the verbal communication. I was offered the chance to discuss some issues with the project leader and hereby declare that I am taking part in the project out of free will.

2	0			-			-		
C	C	Y	Y		M	M		D	D

<b>Signature of Participant Parent/Guardian</b>	<b>Date</b>
---	-------------

Signed at

**Place**

**Witnessess**

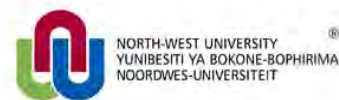
2	0			-			-		
C	C	Y	Y		M	M		D	D

<b>Signature of witness (translator, if applicable)</b>	<b>Date</b>
---	-------------

Signed at

## ADDENDUM 2: SOCIO-DEMOGRAPHIC QUESTIONNAIRE

ALV STUDY



### DEMOGRAPHIC QUESTIONNAIRE

– Interview with caregiver–

School \_\_\_\_\_

Number first child (check class list – first on class list)  
 Number second child (check class list– second on class list)  
 Number third child (check class list– third on class list)


Interviewer \_\_\_\_\_

Date of interview (dd/mm/yyyy)

D	D	M	M	Y	Y	Y	Y
---	---	---	---	---	---	---	---

#### Information on the caregiver

1. What is your date of birth? (dd/mm/yyyy)

D	D	M	M	Y	Y	Y	Y
---	---	---	---	---	---	---	---

2. What is the gender of caregiver?

1.Male       2.Female

3. How many children do you take care of (permanent – 24 hrs/day)?  children

4. What is your marital status?

<input type="text"/>	1. Single	<input type="text"/>	2. Steady partner, not living together
<input type="text"/>	3. Married	<input type="text"/>	4. Traditional marriage
<input type="text"/>	5. Living together	<input type="text"/>	6. Widowed
<input type="text"/>	7. Divorced or separated		
<input type="text"/>	8. Other, please specify:		

---

5. What is your highest formal education level?

<input type="text"/>	1. None	<input type="text"/>	2. Primary school
<input type="text"/>	3. Std 6-8/ Gr 8-10	<input type="text"/>	4. Std 9-10/Gr 11-12
<input type="text"/>	5. Further studies incomplete	<input type="text"/>	6. Diploma/Other postschool complete
<input type="text"/>	7. Degree		
<input type="text"/>	8. Other, please specify:		

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6. What type of work do you do?

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**Information on the first child**

7. What is the first child's date of birth? (dd/mm/yyyy) 

D	D	M	M	Y	Y	Y	Y
---	---	---	---	---	---	---	---

8. What is your relationship to the first child (on class list)?

<input type="text"/>	1. Father	<input type="text"/>	3. Grandpa	<input type="text"/>	5. Uncle	<input type="text"/>	7. Brother/sister	<input type="text"/>	9. Other
<input type="text"/>	2. Mother	<input type="text"/>	4. Grandma	<input type="text"/>	6. Aunt	<input type="text"/>	8. Friend		

9. Does the first child (on class list) use any medication at this moment?

<input type="text"/>	1. No	
<input type="text"/>	2. Yes, medication given by clinic, doctor or nurse	Please specify: _____
<input type="text"/>	3. Yes, self bought medication	Please specify: _____
<input type="text"/>	4. Don't know/unsure	_____

10. If yes, why was the medication used for the first child (on class list)?

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**Information on the second child**

11. What is the second child's date of birth? (dd/mm/yyyy)

D	D	M	M	Y	Y	Y	Y
---	---	---	---	---	---	---	---

12. What is your relationship to the second child (on class list)?

<input type="checkbox"/>	1. Father	<input type="checkbox"/>	3. Grandpa	<input type="checkbox"/>	5. Uncle	<input type="checkbox"/>	7. Brother/sister	<input type="checkbox"/>	9. Other
<input type="checkbox"/>	2. Mother	<input type="checkbox"/>	4. Grandma	<input type="checkbox"/>	6. Aunt	<input type="checkbox"/>	8. Friend		

13. Does the second child (on class list) use any medication at this moment?

<input type="checkbox"/>	1. No	
<input type="checkbox"/>	2. Yes, medication given by clinic, doctor or nurse	Please specify: _____
<input type="checkbox"/>	3. Yes, self bought medication	Please specify: _____
<input type="checkbox"/>	4. Don't know/unsure	_____

14. If yes, why was the medication used for the second child (on class list)?

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**Information on the third child**

15. What is the first child's date of birth? (dd/mm/yyyy)

D	D	M	M	Y	Y	Y	Y
---	---	---	---	---	---	---	---

16. What is your relationship to the first child (on class list)?

<input type="checkbox"/>	1. Father	<input type="checkbox"/>	3. Grandpa	<input type="checkbox"/>	5. Uncle	<input type="checkbox"/>	7. Brother/sister	<input type="checkbox"/>	9. Other
<input type="checkbox"/>	2. Mother	<input type="checkbox"/>	4. Grandma	<input type="checkbox"/>	6. Aunt	<input type="checkbox"/>	8. Friend		

17. Does the first child (on class list) use any medication at this moment?

	1. No	
	2. Yes, medication given by clinic, doctor or nurse	Please specify: _____
	3. Yes, self bought medication	Please specify: _____
	4. Don't know/unsure	_____

18. If yes, why was the medication used for the first child (on class list)?

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**Information on the household**

A *household* consists of a group of persons who occupy a common dwelling (or part of it) for at least four days a week and who provide themselves jointly with food and other essentials for living. In other words, they live together as a unit.

19. How many people does your household consist of?

	children (0-18 years)
	adults
	Pensioners / elderly

20. Who is the head of your household (from the caregiver's perspective)?

	1. Father		4. Wife		7. Uncle		10. Friend
	2. Mother		5. Grandpa		8. Aunt		11. Self
	3. Husband		6. Grandma		9. Sibling		12. Other

21. Who usually prepares the food in your household (from the caregiver's perspective)?

	1. Father		4. Wife		7. Uncle		10. Child
	2. Mother		5. Grandpa		8. Aunt		11. Self
	3. Husband		6. Grandma		9. Sibling		12. Other

22. Who decides on what types of food are bought for your household (from the caregiver's perspective)?

<input type="checkbox"/>	1. Father	<input type="checkbox"/>	4. Wife	<input type="checkbox"/>	7. Uncle	<input type="checkbox"/>	10. Child
<input type="checkbox"/>	2. Mother	<input type="checkbox"/>	5. Grandpa	<input type="checkbox"/>	8. Aunt	<input type="checkbox"/>	11. Self
<input type="checkbox"/>	3. Husband	<input type="checkbox"/>	6. Grandma	<input type="checkbox"/>	9. Sibling	<input type="checkbox"/>	12. Other

23. Who decides how much money is spent on food for your household (from the caregiver's perspective)?

<input type="checkbox"/>	1. Father	<input type="checkbox"/>	4. Wife	<input type="checkbox"/>	7. Uncle	<input type="checkbox"/>	10. Child
<input type="checkbox"/>	2. Mother	<input type="checkbox"/>	5. Grandpa	<input type="checkbox"/>	8. Aunt	<input type="checkbox"/>	11. Self
<input type="checkbox"/>	3. Husband	<input type="checkbox"/>	6. Grandma	<input type="checkbox"/>	9. Sibling	<input type="checkbox"/>	12. Other

24. Do one or more members of your household receive a social grant (from the government)? If yes, what type of grant do they get? (More than one answer can be given)

<input type="checkbox"/>	1. No	<input type="checkbox"/>	2. Child support grant	<input type="checkbox"/>	3. Disability grant	<input type="checkbox"/>	4. Old age pension
<input type="checkbox"/>	5. Other, please specify _____						

25. Does anybody in your household receive food from a feeding scheme?

<input type="checkbox"/>	1.No	<input type="checkbox"/>	2. Yes	<input type="checkbox"/>	3. Don't know
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26. How many people contribute to the total income in this household (including children receiving a grant, or other people receiving a grant)?

number of people

27. What is the total household income in general per month?

<input type="checkbox"/>	1. None	<input type="checkbox"/>	5. R1501-R2000	<input type="checkbox"/>	9. R3501-R4000
<input type="checkbox"/>	2. R1-R500	<input type="checkbox"/>	6. R2001-R2500	<input type="checkbox"/>	10. More than R4000
<input type="checkbox"/>	3. R501-R1000	<input type="checkbox"/>	7. R2501-R3000	<input type="checkbox"/>	11. Don't know
<input type="checkbox"/>	4. R1001-R1500	<input type="checkbox"/>	8. R3001-R3500	<input type="checkbox"/>	

28. How much money is spent on food monthly?

<input type="checkbox"/>	1. R0-R200	<input type="checkbox"/>	5. R801-R1000	<input type="checkbox"/>	9. R1601-R1800
<input type="checkbox"/>	2. R201-400	<input type="checkbox"/>	6. R1001-R1200	<input type="checkbox"/>	10. R1801-R2000
<input type="checkbox"/>	3. R401-600	<input type="checkbox"/>	7. R1201-R1400	<input type="checkbox"/>	11. More than R2000
<input type="checkbox"/>	4. R601-R800	<input type="checkbox"/>	8. R1401-R1600	<input type="checkbox"/>	12. Don't know

**Information on the house and surroundings**

29. What is your house mainly made of:

<input type="checkbox"/>	1. Brick, concrete	<input type="checkbox"/>	2. Mud	<input type="checkbox"/>	3. Tin	<input type="checkbox"/>	4. Plank, wood
<input type="checkbox"/>	5. Other, please specify: _____						

30. How many rooms does your house have? (Including kitchen or cooking area, excluding bathroom or toilet, sheds, garages, stables or any other rooms unless people live in them.)

number of rooms

31. What type of toilet does your household have?

<input type="checkbox"/>	1. Flush	<input type="checkbox"/>	2. Bucket, pot	<input type="checkbox"/>	3. Pit, vip	<input type="checkbox"/>	4. None
<input type="checkbox"/>	5. Other, please specify: _____						

32. Do you share your toilet with other households?

<input type="checkbox"/>	1. No	<input type="checkbox"/>	2. Yes
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33. Where do you get your drinking water from most of the time?

<input type="checkbox"/>	1. Own tap	<input type="checkbox"/>	2. Communal tap	<input type="checkbox"/>	3. River, dam	<input type="checkbox"/>	4. Borehole, well
<input type="checkbox"/>	5. Other, please specify: _____						

34. When cooking food, what fuel do you use most of the time?

<input type="checkbox"/>	1. Electric	<input type="checkbox"/>	2. Gas	<input type="checkbox"/>	3. Paraffin	<input type="checkbox"/>	4. Wood	<input type="checkbox"/>	5. Coal
<input type="checkbox"/>	6. Other, please specify: _____								

35. Do you or someone in your household have any of the following (please note that it needs to be in a working condition) (Tick all that apply)

<input type="checkbox"/>	1. Fridge	<input type="checkbox"/>	2. Freezer	<input type="checkbox"/>	3. Fridge-freezer combination	<input type="checkbox"/>	4. Microwave	<input type="checkbox"/>	5. Stove
<input type="checkbox"/>	6. Radio	<input type="checkbox"/>	7. TV	<input type="checkbox"/>	8. Landline (Telkom; telephone)	<input type="checkbox"/>	9. Cell phone		

**Information on home gardens**

36. Do you grow any foods for your own use?

<input type="checkbox"/>	1.No, continue with Q 37	<input type="checkbox"/>	2.Yes, continue with Q 38
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37. Why do you not grow any foods for your own use? (continue with Q 39)

1. We have no need for more food; we can afford to buy all the food we need.
2. We do not have the land available.
3. We have the land available, but there is no money for seeds/plants and supplies.
4. We do not know how to grow food.
5. Other reason, please specify:

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38. What foods do you grow for your own use? (continue with Q 39)

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39. If you were to learn a new skill to generate income for yourself and your family, what would you like to learn?

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**Thank you for your participation and your time!**

## ADDENDUM 3: 24-HOUR-RECALL

### ALV study 24-hour recall dietary intake

Today's date:    Fieldworker: .....

*year month day*

#### Information on CHILD:

Number:  Gender:  female   
male

Date of birth:    Age:  years  
year month day

School: Sizamela Buffelsvlei Grade:

#### Information on CAREGIVER:

Number:  Gender:  female   
male

Date of birth:    Age:  years  
year month day

#### What day was yesterday? (tick correct one)

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
--------	---------	-----------	----------	--------	----------	--------

#### Would you describe the food that the child ate yesterday as typical of his/her usual food intake?

Yes  1       No  2

If NO, describe the reason: .....

#### Greetings!

Thank you for giving up your time to participate in this study. Here we want to find out what the children living in this area eat and drink. This information is important to know as it will tell us if the children are eating enough and if they are healthy.

There are no right or wrong answers.

Everything you tell me is confidential. Only the number of the child and your subject number appear on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

I want to first ask you a few general questions about your child's food intake, the preparation of food and the type of food that you use in your home.

#### Instruction

Circle the subject's answer.

**1. What type of pot do you usually use to prepare food in? (may answer more than one)**

Iron pot .....	<b>1</b>	<input type="checkbox"/>
Stainless steel pot .....	<b>2</b>	<input type="checkbox"/>
Aluminium pot .....	<b>3</b>	<input type="checkbox"/>
Glass ware .....	<b>4</b>	<input type="checkbox"/>
Other (specify) .....	<b>5</b>	<input type="checkbox"/>

**2. Does the child eat maize meal porridge?**

**1**       Yes       No

If YES, what type do you have at home now?

Brand name: \_\_\_\_\_ **1**

Don't know: \_\_\_\_\_ **2**

Grind self: \_\_\_\_\_ **3**

If brand name is given, do you usually use this brand?      **1**       Yes       No       Don't know  
 (By "usually" it is meant at least 4 times a week)

Where do you get your maize meal from? (may answer more than one)

Shop .....	<b>1</b>	<input type="checkbox"/>
Employer .....	<b>2</b>	<input type="checkbox"/>
Harvest and grind self .....	<b>3</b>	<input type="checkbox"/>
Other (specify) .....	<b>4</b>	<input type="checkbox"/>
Don't know .....	<b>5</b>	<input type="checkbox"/>

**3. Does the child eat fat/margarine/is it used in preparation of food?**

**1**       Yes       No

If YES, what type do you have at home now?

Brand name: \_\_\_\_\_ **1**

Don't know: \_\_\_\_\_ **2**

If brand name is given, do you usually use this brand?      **1**       Yes       No       Don't know

**4. Do you use oil in the preparation of food?**

**1**       Yes       No

If YES, what type do you have at home now?

Brand name: \_\_\_\_\_

Don't know: \_\_\_\_\_ **2**

If brand name is given, do you usually use this brand? **1**  Yes  No  Don't know

What type of oil do you buy for deep frying? (vetkoek, samoosas, chips)

Brand name: \_\_\_\_\_

Do you use the same oil more than once? **1**  Yes  No

If yes, how many times will you use the same oil? \_\_\_\_\_

**5. What type of salt do you use?**

Give brand names \_\_\_\_\_

Do you add salt to food while it is being cooked? 

Always	Sometimes <b>2</b>	Never	Don't know
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Does the child add salt to his/her food after it has been cooked? 

Always	Sometimes	Never
--------	-----------	-------

Does he/she like salty foods eg salted peanuts, crisps, chips, fritos, biltong, dried sausage, etc 

Very much	Like it	Not at all
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**6. Does the child use any of the following:**

	Name of product	Amount per day
Vitamins/vitamins and minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Other: Specify		

I want to find out about everything your child ate or drank yesterday, including water or food you pick from the field. Please tell me everything your child ate from the time your child woke up yesterday up to the time your child went to sleep. I will also ask you where your child ate the food and how much your child ate.

To help you to describe the amount of a food your child eat, I will show you and your child pictures and examples of different amounts of the food. Please say which picture or example is the closest to the amount your child eat, or if it is smaller, between the sizes or bigger than the pictures.

Time	Place	Description of food and preparation method	Amount	Amount in gram	Code (office use)

# ADDENDUM 4: QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

ALV Study Quantitative Food Frequency Questionnaire

Today's date:    Fieldworker: .....

*year month day*

Information on CHILD:

Number:  Gender: female  male

Date of birth:    Age:  years

*year month day*

School: Sizamela  Buffelsvlei  Grade:

Information on CAREGIVER:

Number:  Gender: female  male

Date of birth:    Age:  years

*year month day*

Please think carefully about the food and drink your child has consumed during the *past week* (seven days). I will go through a list of foods and drinks with you and your child and I would like you and your child to tell me:

If your child eat the food

How the food is prepared

How much of the food your child eat at a time

How many times a day your child eat it and if your child did not eat it everyday, how many times a week your child eat it.

To help you and your child to describe the amount of a food your child eat, I will show you and your child pictures of different amounts of the food. Please say which picture is the closest to the amount your child eat, or if it is smaller, between the sizes or bigger than the pictures.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

#### FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you and your child about the type and the amount of food your child has been eating in the last few weeks. Please tell if your child eats the food, how much your child eat and how often your child eat it. We shall start with maize meal porridge.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slappap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____							
Breakfast cereals	Brand name of cereals at home now: _____							
Does your child pour milk on the porridge or cereal? <input type="checkbox"/> Yes 1 <input type="checkbox"/> No 2								
If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____								
If yes, how much milk								
Does your child put sugar on the porridge or cereal? <input type="checkbox"/> Yes 1 <input type="checkbox"/> No 2								

If yes, how much sugar							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____						3262	
	Pizza	Home made: Specify topping _____					3353 (base+c h)	
		Bought: Specify topping _____					3353 (base+c h)	
<p>You are being very helpful. Can I now ask you about meat?  <u>CHICKEN, MEAT, FISH</u>  How many times does your child eat meat (beef, mutton, pork, chicken, fish) per week?  _____</p>								
Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							

	Roasted / Grilled						2925	
	Other: _____							
Does your child eat chicken ski		Always		Sometimes		Never		
Chicken bones stew								
Chicken feet							2997	
Chicken offal								
Red meat	How does your child like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	
	Other: Specify _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	
What type of vegetables is usually put into meat stews?								

Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____							
Canned meat	Bully beef							
	Other: Specify _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____						3196	
Pilchards in tomato/chilli/burine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify							

Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							
Now we come to vegetables and fruit								
VEGETABLES AND FRUIT								
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in .....							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat .....							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							

Tomato and onion gravy	Home made with fat Type of fat .....							
	Without fat						3925	
	Canned						4192	
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat .....							
	Boiled, little sugar and fat Fat .....							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat .....							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How does your child eat mealies?							
	On cob – fat added Fat .....							
	On cob – no fat added						3725	

	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat .....							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____							
Other	_____							

vegetables, specify + preparation									
Does your child like fruit?		<input type="checkbox"/> Yes	<input type="checkbox"/> No						
Apples							3592		
Pears							3582		
Bananas							3540		
Oranges							3560		
Naartjie							3558		
Grapes							3550		
Peaches	Fresh						3565		
	Canned						3567		
Apricots	Fresh						3534		
	Canned						3535		
Mangoes							3556		
Guavas	Fresh						3551		
	Canned						3553		
Avocado							3656		
Wild fruit/berries	Specify type: _____								
Dried fruit	Types: _____								
Other fruit	_____								
If child eats canned fruit: Does your child have custard with the canned fruit?		<input type="checkbox"/> Yes	1	<input type="checkbox"/> No	2				
Custard	Home made: Milk								
	Commercial eg Ultramel						2716		
<b>BREAD AND BREAD SPREADS</b>									
Bread / Bread rolls	White						3210		
	Brown						3211		

	Whole wheat						3212	
Do you spread anything on the bread?		Always		Sometimes		Never		
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fraybentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____							
Achaar								
Other spreads	Specify: _____							
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							
<u>DRINKS</u>								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea	Tea:						3989	

or coffee	Coffee:						3989	
Milk/cup tea or coffee	What type of milk does your child use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721	
	Low fat milk powder Brand: _____						2825	
	Skimmed milk powder Brand: _____						2825	
	Milk blend Brand: _____ _____						2770	
	Whitener: type _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							
Milk as such	What type of milk does your child drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life:						2772	

	2%/low fat							
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____							
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____							
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____							

Fizzy drinks	Sweetened						3981	
Coke, fanta, etc	Diet							
Mageu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify								
SNACKS AND SWEETS								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Name: _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____							
Cakes and tarts	Type: _____							

Scones								
Rusks	Type: _____							
Savouries	Sausage rolls						2939	
	Samosas: Meat filling						3355	
	Samosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____							
Jelly							3983	
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify								
<b>SAUCES, GRAVIES AND CONDIMENTS</b>								
Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:								
<b><u>WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)</u></b>								
Wild fruit								
<b><u>MISCELLANEOUS:</u> Please mention any other foods used more than once/two times a week which we have not talked about:</b>								

INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS								
Please tell me if your child uses any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat								
Specify								

## ADDENDUM 5: INSTRUCTIONS FOR AUTHORS

### Journal of ethnobiology and ethnomedicine

Overview of manuscript sections for Research Articles

#### Abstract

The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background**, the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract. **Trial registration**, if your research reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number (e.g. **Trial registration**: Current Controlled Trials ISRCTN73824458). Please note that there should be no space between the letters and numbers of your trial registration number. We recommend manuscripts that report randomized controlled trials follow the [CONSORT extension for abstracts](#).

#### Keywords

Three to ten keywords representing the main content of the article.

#### Background

The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field. The section should end with a brief statement of what is being reported in the article.

#### Methods

The methods section should include the design of the study, the setting, the type of participants or materials involved, a clear description of all interventions and comparisons, and the type of analysis used, including a power calculation if appropriate. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses in the Methods section.

For studies involving human participants a statement detailing ethical approval and consent should be included in the methods section. For further details of the journal's editorial policies and ethical guidelines see ['About this journal'](#).

#### Results and discussion

The Results and discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The Results and discussion sections may also be broken into subsections with short, informative headings.

#### Conclusions

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

#### List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

### Competing interests

Authors are required to complete a declaration of competing interests. All competing interests that are declared will be listed at the end of published articles. Where an author gives no competing interests, the listing will read 'The author(s) declare that they have no competing interests'.

### Authors' contributions

In order to give appropriate credit to each author of a paper, the individual contributions of authors to the manuscript should be specified in this section.

### Authors' information

You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.

### Acknowledgements

Please acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for each author, and for the manuscript preparation. Authors must describe the role of the funding body, if any, in design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. Please also acknowledge anyone who contributed materials essential for the study. If a language editor has made significant revision of the manuscript, we recommend that you acknowledge the editor by name, where possible.

The role of a scientific (medical) writer must be included in the acknowledgements section, including their source(s) of funding. We suggest wording such as 'We thank Jane Doe who provided medical writing services on behalf of XYZ Pharmaceuticals Ltd.'

Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements section.

### Endnotes

Endnotes should be designated within the text using a superscript lowercase letter and all notes (along with their corresponding letter) should be included in the Endnotes section. Please format this section in a paragraph rather than a list.

### References

All references, including URLs, must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. Each reference must have an individual reference number. Please avoid excessive referencing. If automatic numbering systems are used, the reference numbers must be finalized and the bibliography must be fully formatted before submission.

Examples of the *Journal of Ethnobiology and Ethnomedicine* reference style are shown below. Please ensure that the reference style is followed precisely; if the references are not in the correct style they may have to be retyped and carefully proofread.

Examples of the *Journal of Ethnobiology and Ethnomedicine* reference style

#### *Article within a journal*

Koonin EV, Altschul SF, Bork P: **BRCA1 protein products: functional motifs**. *Nat Genet* 1996, **13**:266-267.

*Article within a journal supplement*

Orengo CA, Bray JE, Hubbard T, LoConte L, Sillitoe I: **Analysis and assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction.** *Proteins* 1999, **43**(Suppl 3):149-170.

*In press article*

Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

*Published abstract*

Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42**:s250.

*Article within conference proceedings*

Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore*. Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

*Book chapter, or article within a book*

Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2*. 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

*Whole issue of journal*

Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10**:1-72.

*Whole conference proceedings*

Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore*. Stoneham: Butterworth-Heinemann; 1996.

*Complete book*

Margulis L: *Origin of Eukaryotic Cells*. New Haven: Yale University Press; 1970.

*Monograph or book in a series*

Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues*. Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology*, vol 1.]

*Book with institutional author*

Advisory Committee on Genetic Modification: *Annual Report*. London; 1999.

*PhD thesis*

Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis*. Stanford University, Computer Science Department; 1995.

*Link / URL*

**The Mouse Tumor Biology Database** [<http://tumor.informatics.jax.org/mtbwi/index.do>]

*Link / URL with author(s)*

Corpas M: **The Crowdfunding Genome Project: a personal genomics community with open source values** [<http://blogs.biomedcentral.com/bmcblog/2012/07/16/the-crowdfunding-genome-project-a-personal-genomics-community-with-open-source-values/>]

*Dataset with persistent identifier*

Zheng, L-Y; Guo, X-S; He, B; Sun, L-J; Peng, Y; Dong, S-S; Liu, T-F; Jiang, S; Ramachandran, S; Liu, C-M; Jing, H-C (2011): **Genome data from sweet and grain sorghum (*Sorghum bicolor*).** *GigaScience*. <http://dx.doi.org/10.5524/100012>.

*Clinical trial registration record with persistent identifier*

Mendelow, AD (2006): **Surgical Trial in Lobar Intracerebral Haemorrhage.** *Current Controlled Trials*. <http://dx.doi.org/10.1186/ISRCTN22153967>

Preparing tables

Each table should be numbered and cited in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title (above the table) that summarizes the whole table; it should be no longer than 15 words. Detailed legends may then follow, but they should be concise. Tables should always be cited in text in consecutive numerical order.

Smaller tables considered to be integral to the manuscript can be pasted into the end of the document text file, in A4 portrait or landscape format. These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review; this will not always be the case if columns are generated by simply using tabs to separate text. Columns and rows of data should be made visibly distinct by ensuring that the borders of each cell display as black lines. Commas should not be used to indicate numerical values. Color and shading may not be used; parts of the table can be highlighted using symbols or bold text, the meaning of which should be explained in a table legend. Tables should not be embedded as figures or spreadsheet files.

Larger datasets or tables too wide for a landscape page can be uploaded separately as additional files. Additional files will not be displayed in the final, laid-out PDF of the article, but a link will be provided to the files as supplied by the author.

## Public health nutrition

Directions to Contributors **Public Health Nutrition** (*Revised October 2013*)

Papers submitted for publication should be written in English and be as concise as possible. If English is not the first language of the authors then the paper should be checked by an English speaker. *Public Health Nutrition* operates an on-line submission and reviewing system (eJournalPress). Authors should submit to the following address: <http://phn.msubmit.net/> Receipt of papers will be acknowledged immediately.

**Authors are asked to supply three or four key words or phrases on the title page of the typescript.**

The title page should also include a statement reporting any conflicts of interest, all sources of funding, the contribution of each author to the manuscript and any ethical information as detailed above.

**The title page should be submitted online as a separate cover letter. This enables double-blind reviewing.**

(b) *Abstract*: each paper must open with a structured abstract of **not more than 250 words**. The abstract should consist of the following headings: Objective, Design, Setting, Subjects, Results, Conclusions. All the headings should be used, and there should be a separate paragraph for each one. The abstract should be intelligible without reference to text or figures.

(c) *Introduction*: it is not necessary to introduce a paper with a full account of the relevant literature, but the introduction should indicate briefly the nature of the question asked and the reasons for asking it.

(d) *Experimental methods*: methods should appear after the introduction. A paper describing any research including human subjects must include the following statement: "This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the [name of the ethics committee removed for blinding]. Written [or Verbal] informed consent was obtained from all subjects/patients. [Where verbal consent was obtained this must be followed by a statement such as: Verbal consent was witnessed and formally recorded]." The name of the ethics committee should be included in the acknowledgements section (see (g) below), for information. This will be re-inserted into the manuscript if accepted for publication.

(e) *Results*: these should be given as concisely as possible, using figures or tables as appropriate.

(f) *Discussion*: while it is generally desirable that the presentation of the results and the discussion of their significance should be presented separately, there may be occasions when combining these sections may be beneficial. Authors may also find that additional or alternative sections such as 'conclusions' may be useful.

(g) *Acknowledgments*: these should include information on source of funding, declaration of any conflicts of interest and a brief statement of the contribution(s) of each author, as specified above. The name of the approving ethics committee should also be included in this section. **The author will be asked to provide this information during the submission process and should also include it at the bottom of the Title Page. This enables double-blind reviewing.**

(h) *References*: these should be given in the text using the Vancouver system. They should be numbered consecutively in the order in which they first appear in the text using superscript Arabic numerals in parentheses, e.g. 'The conceptual difficulty of this approach has recently been highlighted<sup>(1,2-4)</sup>'. If a reference is cited more than once the same number should be used each time. References cited only in tables and figure legends and not in the text should be numbered in sequence from the last number used in the text and in the order of mention of the individual tables and figures in the text. At the end of the paper, on a page(s) separate from the text, references should be listed in numerical order. When an article has more than three authors only the names of the first three authors should be given followed by 'et al.' The issue number should be omitted if there is continuous pagination throughout a volume. Names and initials of authors of unpublished work should be given in the text as 'unpublished results' and not included in the References. Titles of journals should appear in their abbreviated form using the NCBI LinkOut page <http://www.ncbi.nlm.nih.gov/projects/linkout/journals/jourlists.fcgi?typeid=1&type=journals&operation=Show> References to books and monographs should include the town of publication and the number of the edition to which reference is made. Thus:

1. Setchell KD, Faughnan MS, Avades T *et al.* (2003) Comparing the pharmacokinetics of daidzein and genistein with the use of <sup>13</sup>C- labeled tracers in premenopausal women. *Am J Clin Nutr* **77**, 411–419.
2. Barker DJ, Winter PD, Osmond C *et al.* (1989) Weight in infancy and death from ischaemic heart disease. *Lancet* **ii**, 577–580.
3. Forchielli ML & Walker WA (2005) The role of gut-associated lymphoid tissues and mucosal defence. *Br J Nutr* **93**, Suppl. 1, S41–S48.
4. Bradbury J, Thomason JM, Jepson NJA *et al.* (2003) A nutrition education intervention to increase the fruit and vegetable intake of denture wearers. *Proc Nutr Soc* **62**, 86A.
5. Frühbeck G, Gómez-Ambrosi J, Muruzabal FJ *et al.* (2001) The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab* **280**, E827–E847.
6. Han KK, Soares JM Jr, Haidar MA *et al.* (2002) Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. *Obst Gynecol* **99**, 389–394.
7. Uhl M, Kassie F, Rabot S *et al.* (2004) Effect of common Brassica vegetables (Brussels sprouts and red cabbage) on the development of preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in liver and colon of Fischer 344 rats. *J Chromatogr* **802B**, 225–230.
8. Hall WL, Vafeiadou K, Hallund J *et al.* (2005) Soy isoflavone enriched foods and inflammatory biomarkers of cardiovascular risk in postmenopausal women: interactions with genotype and eouol production. *Am J Clin Nutr* (In the Press).
9. Skurk T, Herder C, Kraft I *et al.* (2004) Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* (Epublication ahead of print version).
10. Skurk T, Herder C, Kraft I *et al.* (2005) Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* **146**, 1006–1011; Epublication 2 December 2004.
11. Bradbury J (2002) Dietary intervention in edentulous patients. PhD Thesis, University of Newcastle.
12. Ailhaud G & Hauner H (2004) Development of white adipose tissue. In *Handbook of Obesity. Etiology and Pathophysiology*, 2<sup>nd</sup> ed., pp. 481–514 [GA Bray and C Bouchard, editors]. New York: Marcel Dekker.

13. Bruinsma J (editor) (2003) *World Agriculture towards 2015/2030: An FAO Perspective*. London: Earthscan Publications.
14. Griinari JM & Bauman DE (1999) Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In *Advances in Conjugated Linoleic Acid Research*, vol. 1, pp. 180–200 [MP Yurawecz, MM Mossoba, JKG Kramer, MW Pariza and GJ Nelson, editors]. Champaign, IL: AOCS Press.
15. Henderson L, Gregory J, Irving K *et al.* (2004) *National Diet and Nutrition Survey: Adults Aged 19 to 64 Years*. vol. 2: *Energy, Protein, Fat and Carbohydrate Intake*. London: The Stationery Office.
16. International Agency for Research on Cancer (2004) *Cruciferous Vegetables, Isothiocyanates and Indoles*. *IARC Handbooks of Cancer Prevention* no. 9 [H Vainio and F Bianchini, editors]. Lyon, France: IARC Press.
17. Linder MC (1996) Copper. In *Present Knowledge in Nutrition*, 7th ed., pp. 307–319 [EE Zeigler and LJ Filer Jr, editors]. Washington, DC: ILSI Press.
18. World Health Organization (2003) *Diet, Nutrition and the Prevention of Chronic Diseases. Joint WHO/FAO Expert Consultation. WHO Technical Report Series* no. 916. Geneva: WHO.
19. Keiding L (1997) *Astma, Allergi og Anden Overfølsomhed i Danmark – Og Udviklingen 1987–1991 (Asthma, Allergy and Other Hypersensitivities in Denmark, 1987–1991)*. Copenhagen, Denmark: Dansk Institut for Klinisk Epidemiologi.

References to material available on websites should include the full Internet address, and the date of the version cited. Thus:

20. Department of Health (1997) Committee on Toxicity of Chemicals in Food Consumer Products and the Environment. Statement on vitamin B<sub>6</sub> (pyridoxine) toxicity. <http://www.open.gov.uk/doh/hef/B6.htm>
21. Kramer MS & Kakuma R (2002) *The Optimal Duration of Exclusive Breastfeeding: A Systematic Review*. Rome: WHO; available at [http://www.who.int/nut/documents/optimal\\_duration\\_of\\_exc\\_bfeeding\\_review\\_eng.pdf](http://www.who.int/nut/documents/optimal_duration_of_exc_bfeeding_review_eng.pdf)
22. Hooper L, Thompson RL, Harrison RA *et al.* (2004) Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database of Systematic Reviews*, issue 4, CD003177. <http://www.mrw.interscience.wiley.com/cochrane/clsysrev/articles/CD003177/frame.html>
23. Nationmaster (2005) HIV AIDS – Adult prevalence rate. [http://www.nationmaster.com/graph-T/hea\\_hiv\\_aid\\_adu\\_pre\\_rat](http://www.nationmaster.com/graph-T/hea_hiv_aid_adu_pre_rat) (accessed June 2005).

**Tables.** Tables should carry headings describing their content and should be comprehensible without reference to the text. Tables should not be subdivided by ruled lines. The dimensions of the values, e.g. mg/kg, should be given at the top of each column. Separate columns should be used for measures of variance (SD, SE etc.), the  $\pm$  sign should not be used. The number of decimal places used should be standardized; for whole numbers 1.0, 2.0 etc. should be used. Shortened forms of the words weight (wt) height (ht) and experiment (Expt) may be used to save space in tables, but only Expt (when referring to a specified experiment, e.g. Expt 1) is acceptable in the heading.

Footnotes are given in the following order: (1) abbreviations, (2) superscript letters, (3) symbols. Abbreviations are given in the format: RS, resistant starch. Abbreviations appear in the footnote in the order that they appear in the table (reading from left to right across the table, then down each column). Abbreviations in tables must be defined in footnotes. Symbols for footnotes should be used in the sequence: \*†‡§||¶, then \*\* etc. (omit \* or †, or both, from the sequence if they are used to indicate levels of significance).

For indicating statistical significance, superscript letters or symbols may be used. Superscript letters are useful where comparisons are within a row or column and the level of significance is uniform, e.g. <sup>a,b,c</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ). Symbols are useful for indicating significant differences between rows or columns, especially where different levels of significance

are found, e.g. 'Mean values were significantly different from those of the control group: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ '. The symbols used for  $P$  values in the tables must be consistent. Tables should be placed at the end of the text. Each table will be positioned near the point in the text at which it is first introduced unless instructed otherwise.

Please refer to a recent copy of the journal for examples of tables.

## American journal of clinical nutrition

### Abstract

A properly constructed and informative abstract is helpful for the initial editorial review of the submitted manuscript. Original research articles must include a structured abstract that contains no more than 300 words, is written in **complete sentences**, and includes the following headings:

**Background:** Provide 1 or 2 sentences that explain the context of the study.

**Objective:** State the precise objective, the specific hypothesis to be tested, or both.

**Design:** Describe the study design, including the use of cells, animal models, or human subjects. Identify the control group. Identify specific methods and procedures. Describe interventions, if used.

**Results:** Report the most important findings, including results of statistical analyses.

**Conclusions:** Summarize in 1 or 2 sentences the primary outcomes of the study, including their potential clinical importance, if relevant (avoid generalizations).

Review articles, special articles, and reports should include an unstructured abstract (no more than 300 words) that states the purpose of the article and emphasizes the major concepts and conclusions. Any abbreviations used in the abstract should be defined in the abstract at first mention.

### Introduction

Clearly state the purpose of the article. Summarize the rationale and background for the study or observation, giving only strictly pertinent references. Do not include methods, data, results, or conclusions from the work being reported. The Introduction should be limited to 1.5 manuscript pages.

### Subjects (or Materials) and Methods

Describe clearly your selection of the experimental and control subjects and provide eligibility and exclusion criteria and details of randomization. Describe the methods for, and success of, any masking (blinding) of observations. Report any complications of experimental treatments. Identify the methods, apparatus (manufacturer's name in parentheses), and procedures in sufficient detail to allow other researchers to reproduce the results. Define all group designations parenthetically at first mention [for example, "control (CON) and high-fat (HF) groups"] and include definitions for these abbreviations in the abbreviation footnote on the title page. Do not use trademark names, such as Teflon, as generic terms. Give references for established methods, including statistical methods; provide references and brief descriptions of methods that have been published but are not well known; and describe new or substantially modified methods, giving reasons for using them and evaluating their limitations. Identify precisely all drugs and chemicals used, including generic names, dosages, and routes of administration. If trade names for drugs and chemicals are included, give the manufacturer's name and location.

**Ethics.** When reporting experiments on human subjects, indicate that the procedures followed were in accordance with the ethical standards of the responsible institutional or regional committee on human experimentation or in accordance with the Helsinki Declaration of 1975 as revised in 1983. Do not use patients' names, initials, or hospital identification numbers. When reporting experiments on animals, indicate approval by the institution's animal welfare committee and state whether the National Research Council's guide for the care and use of laboratory animals was followed.

*Statistics.* Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (eg, CIs, SDs, or SEs), even for differences that were not significant. Report the numbers of observations. Specify any general-use computer programs used, including the version number and the manufacturer's name and location. Include general descriptions of statistical methods in the Subjects (or Materials) and Methods section and specific descriptions in each table and figure legend. Indicate whether variables were transformed for analysis. Provide details about what hypotheses were tested, what statistical tests were used, and what the outcome and explanatory variables were (where appropriate). Indicate the level of significance used in tests if different from the conventional 2-sided 5% alpha error and whether or what type of adjustment is made for multiple comparisons.

When data are summarized in the Results section, specify the statistical methods used to analyze them. Avoid nontechnical uses of technical statistical terms, such as *random* (which implies a randomizing device), *normal*, *significant*, *correlation*, *sample*, and *parameter*. Define statistical terms, abbreviations, and symbols not listed under "Abbreviations for statistical terms." If there are 3 or more abbreviations used in the text, prepare an abbreviation footnote. The footnote should be associated with the first abbreviated term in the text and should be an alphabetized listing of all author-defined abbreviations and their definitions. Detailed statistical analyses, mathematical derivations, and the like may sometimes be suitably presented as one or more appendixes.

### *Results*

Present your results in a logical sequence in the text, tables, and figures. Do not present specifics of data more than once and do not duplicate data from tables or figures in the text; emphasize or summarize only important observations. Do not present data from individual subjects except for very compelling reasons. Report losses to observation (such as dropouts from a clinical trial). Use boldface for the first mention of each table or figure.

### *Discussion*

The Discussion should not exceed 4 typewritten pages except in unusual circumstances as approved by the Editor. Emphasize concisely the novel and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results. Include the implications of the findings and their limitations and relate the observations to other relevant studies. Link conclusions with the goals of the study and avoid unqualified statements and conclusions that are not completely supported by the data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses and recommendations when warranted by the results and label them clearly as such.

### **Acknowledgments**

Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name and for providing to the Editor a copy of the permission, if requested. Authors must disclose any financial or personal relationships with the company or organization sponsoring the research at the time the research was done. Such relationships may include employment, sharing in a patent, serving on an advisory board or speakers' panel, or owning shares in the company. If an author or authors have no potential conflicts of interest, please state this. The source of support for the research reported in the paper should be listed on the title page, not as an acknowledgement. Each author is required to list his or her contribution to the work.

### **References**

Number references consecutively in the order in which they are first mentioned in the text. Identify references by Arabic numerals in parentheses. References cited in tables or in legends to figures should be numbered according to the first citation of the table or figure in the text. Appendixes should have a separate reference section.

### *Journals*

1. Journal article with DOI: If an article has a DOI number ("digital object identifier" number unique to the publication), it may be included at the end of the reference.

- Hamer M, Steptoe A. Prospective study of physical fitness, adiposity, and inflammatory markers in healthy middle-aged men and women. *Am J Clin Nutr* 2009;89:85–89. doi:10.3945/ajcn.2008.26779
2. Standard journal article: list all authors when 10 or fewer; when >10, list only the first 10 and add "et al." Abbreviate journal titles according to *Index Medicus* style, which is used in MEDLINE citations. Jeffery RW, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *Am J Clin Nutr* 2003;78:684–9.
  3. Corporate author  
National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143–421.

*Books and other monographs*

4. Personal authors  
Shils M, Shike M, Olson J, Ross AC. *Modern nutrition in health and disease*. 9th ed. Baltimore: Lippincott Williams & Wilkins, 1998.
5. Committee report or corporate author  
National Research Council. *Recommended dietary allowances*. 10th ed. Washington, DC: National Academy Press, 1989.  
Food and Nutrition Board, Institute of Medicine. *Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids*. Washington, DC: National Academy Press, 2000.
6. Chapter in book  
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**Tables**

Tables must be included in the text file, and each should appear one per page. Double-spacing of tables is preferred but not required. Number tables consecutively with Arabic numerals (do not use 1A, 1B, etc) and supply a brief descriptive title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading or table title. Each table should contain enough detail (including statistics) that the table is intelligible without reference to the text. All nonstandard abbreviations, including group designations, used in a table or table title should be defined in a footnote to the table title, and the abbreviations should be listed in alphabetic order. If the footnote to the table title contains multiple items, the definitions of the abbreviations should be the last item. If a table contains only one abbreviated term in the body of the table, then a separate footnote placed after that abbreviation should be used to define that term. Commonly used approved abbreviations (see Units and Abbreviations) may be used without explanation. Additionally, explanations are not needed for ANOVA, BMI, F (females), and M (males). For footnotes, use superscript Arabic numerals. For reporting results of statistical analyses, superscript letters can be used if explaining the results in the usual manner would be too complicated (see a recent issue of the *AJCN* for examples). The first appearance in a horizontal row determines the order of the footnotes. Identify statistical

measures of variation, such as SD and SE. **Omit internal horizontal and vertical rules.** Cite each table in the text in consecutive order. Use boldface for the first mention of each table. If you use data from another published source, acknowledge the source fully. Number references in tables according to the location of the first citation of each table in the text.