

Development and validation of portion size food photographs to determine maize intake of young children in rural Eastern Cape Province

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Preface

I greatly appreciate the continuous support, guidance and encouragement from my supervisors Dr. Martani Lombard and Dr. Averalda Van Graan, who despite their tight work schedules were always available and provided constructive criticism throughout my study. Without their patience and guidance, this study would not have been possible at all.

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I am grateful to Nolo for preparing the traditional isiXhosa meals.

The National Research Fund, for providing the funding of this project.

Me Marike Cocrane, for providing statistical advice.

To my late partner this one is for you. Thank you so much, for your unconditional love and support

ABSTRACT

Objective: The objective of the study was to develop and validate a portion size food photograph series to more accurately determine maize intake of infants aged 6 - 24 months living in deep rural areas in the Eastern Cape (EC) province of South Africa.

Design: This was a community based, cross sectional, observational study.

Participants: Mothers/caregivers aged 18 years and older taking care of infants and young children (6 - 24 months).

Outcome measure: This study developed a food photograph series to improve portion size estimation of maize dishes consumed by infants and young children. The food photograph series were developed to be used alongside the validated quantitative food frequency questionnaire (QFFQ). The food photograph series consisted of photos representing portions ranging from teaspoons, tablespoons to large serving ladles. Participants were first shown the food photograph series and asked to identify the portion size most frequently given to the infant / child. Then the fieldworker recorded the portion. As part of validation participants were asked to dish up the amount of food usually consumed by the infant or child and the fieldworker recorded the dished up portion size.

Results: The data distribution was tested with the Shapiro-Wilk's test and found normal. Thus all statistical tests were conducted on parametric data. The paired t-test showed a significant difference ($p < 0.05$) between the photograph portion sizes and the dished up portion sizes for two dishes (soft porridge and crumbly *pap*). Agreement at group level was good for all dishes except soft porridge and crumbly *pap* when the t-test was conducted.

The percentage difference was acceptable for only three dishes (soft porridge, maize meal and pumpkin and crumbly *pap*). However, agreement at group level in terms of percentage difference was acceptable for soft porridge, maize meal and pumpkin and crumbly *pap* and not for the other dishes. Thus, even though the t-test indicated poor agreement for soft porridge and crumbly *pap*, it is acceptable when using the percentage difference.

Strength of association was measured with Pearson correlation coefficients. Results indicated that the association was acceptable, if not strong for only stiff *pap* and samp and beans. For all other dishes this was weak.

Lastly, the Bland-Altman analyses indicated good agreement at individual level for all dishes, although this was mostly due to the wide limits of agreements. Bias was present for all dishes with the exception of maize and pumpkin.

When looking at the overall validity of the dishes it is clear that the food photograph series is valid for three dishes, maize meal and pumpkin, stiff *pap* and samp and beans.

Conclusion: The accuracy of portion size estimates is critical in the assessment of food consumption patterns. The food photograph series is valid for three dishes (maize meal and pumpkin, stiff *pap* and samp and beans).

Key terms: infant maize dietary intake, infant and young child feeding, food photograph series

OPSOMMING

Doelwit: Die doel van die studie was om 'n porsie grote voedsel foto reeks te ontwikkel en valideer om die mielie inname van babas en jong kinders tussen die ouderdom 0 – 24 maande, wat in diep landelike gebiede in die Oos Kaap van Suid Afrika woon, meer akuraat te bepaal.

Ontwerp: Hierdie was 'n gemeenskap gebasseerde, observasie studie.

Deelnemers: Moeders / versorgers bo die ouderdom van 18 jaar wat babas en jong kinders (0 – 24 maande) versorg was ingesluit.

Uitkomst: Hierdie studie het 'n porsie grote voedsel foto reeks ontwikkel met die doel om porsie grote skatting te verbeter. Dit is gefokus op die mielie inname van babas en jong kinders. Die foto reeks is ontwikkel om saam met 'n kwantitatiewe voedsel frekwensie vraelys gebruik te word. Die foto reeks bestaan uit fotos wat 'n reeks porsies verteenwoordig wat strek van teeleples, eeteleples en opskeplepels. Deelnemers was die porsie grote foto reeks gewys en gevra om die mees algemene porsie wat aan die baba of jong kind gegee word, uit te wys. Hierna het die veldwerker die deelnemers gevra om die regte porsie op te skep. Hierdie twee porsies was dan met mekaar vergelyk.

Resultate: Verspreiding was getoets met die Shapiro-Wilk toets en was normal versprei. All statistiese toetse was gedoen op parametrise data. Die t-toets het 'n aansienlike verskil ($p < 0.05$) uitgewys tussen die foto reeks en die opgeskepte porsie vir twee geregte (sagte pap en krummel pap). Daar was 'n goeie ooreenstemming vir die groep vir alle ander geregte.

Die persentasie verskil was aanvaarbaar for slegs drie geregte (sagte pap, mielie meel en pampoen en krummel pap). Hierdie is 'n verdure assesering van ooreenstemming op groep vlak. Dus, al het die t-toets swak ooreenstemming aangedui vir sagte pap en krummel pap is hierdie geregte steeds aanvaarbaar as die persentasie verskil gebruik word.

Die sterkte van assosiasie was gemeet met Pearson korrelasie. Resultate het aangedui dat die assosiasie aanvaarbaar (maar nie sterk nie) is vir slegs stywe pap en stamp mielies en bone. Die assosiasie was swak vir alle ander geregte.

Die Bland-Altman analyses het goeie ooreenstemming aangedui vir alle geregte maar dit was meestal as gevolg van die wye limiete van ooreenstemming.

As daar gekyk word na die algehele validasie van die geregte is dit duidelik dat die voedsel foto reeks aanvaarbaar is vir drie geregte, mielie meel en pampoens, stywe pap en stamp mielies en bone.

Gevolgtrekking: Die akkuraatheid van porsie grote bepaling is krities in die bepaling van dieet inname patrone. Die voedsel foto reeks se validering is aanvaarbaar vir drie geregte (mielie meel en pampoens, stywe pap en stamp mielies en bone).

Kern terme: mikotoksiene, baba en jong kind voeding, voedsel foto reeks

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LIST OF ABBREVIATIONS

AF	Aflatoxin
BMI	Body mass index
CI	Confidence interval
DOH	Department of Health
DON	Deoxynivalenol
EC	Eastern Cape
EPIC	European Prospective Investigation into Cancer and Nutrition
FAO	Food and Agriculture Organisation
FG	Femtogram
FSA	Food Standards Agency
FPS	Food photograph series
FB	Fumonisin B
H/A	Height for age
HREC	Health Research Ethics Committee
HSRC	Human Science Research Council
IARC	International Agency for Research on Cancer
IYC	Infant and young child
IYCF	Infant and young child feeding
IQ range	Inter quartile range
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KG	Kilogram
LOA	Level of agreement

MRC	Medical Research Council
NCND	National Center for Nutrition and Dietetics
NFCS	National Food Consumption Survey
NWU	North-West University
OTA	Ochratoxin A
PPB	Parts-per-billion, 10^{-9}
PSEAs	Portion size estimation aids
PMTDI	Provisional maximum tolerable daily intake
PROMECC	Program for Mycotoxins and Experimental Carcinogens
PSMA	Portion size measurement aid
QFFQ	Quantitative food frequency questionnaire
R	Mean values of the reference measure
SANHANES-1	South African National Health and Nutrition Examination Survey
SAVACG	South African Vitamin A Consultative Group
SD	Standard deviation
UK	United Kingdom
UNICEF	United Nations Children's Fund
W/A	Weight for age
W/H	Weight for height
WHO	World Health Organisation
ZEA	Zearalenone

CHAPTER 1 INTRODUCTION

1.1 INTRODUCTION AND PROBLEM IDENTIFICATION

Infant and young child feeding (IYCF) (especially in the first two years of life) is vital to improve child survival rates, increase healthy growth and cognitive development as well as to reduce the risk of chronic diseases later in life (WHO, 2014b). Infants are particularly vulnerable to under-nutrition since relative to their body size; they have high nutritional requirements with a low capacity and can thus only eat small amounts of food at a time (Gibson *et al.*, 1998). For this reason, complementary foods (given from six months of age) should be nutrient-dense. Unfortunately these food items and dishes are often inadequate in developing countries where maize meal is often utilised as a complementary food (Faber, 2004). The World Health Organisation (WHO) has confirmed that the optimal feeding of infants and children under five years of age has become a critical public health issue (WHO, 2007). Chronic under-nutrition, which includes stunting, foetal growth restrictions and vitamin A and zinc deficiencies, together with low breastfeeding rates is associated with approximately 45% of deaths in the world of under five-year-olds (WHO, 2014b).

Concurrently, the prevalence of overweight children aged 1 - 9 years was 10% nationally and the prevalence of obese children 4% according to Kruger *et al.* (2007). The prevalence of underweight (according to the national food consumption survey - 2005) in the Eastern Cape (EC) was 7.8% (aged 1 – 9 years).

Stunting is defined as a condition where infants and young children are too short for their age (UNICEF, 2009). Stunting in early years is further associated with inadequate development and poor cognitive development leading to sub-optimal educational achievements (WHO, 2004). Poverty is one of the primary underlying causes of stunting as it leads to a lack of sufficient food.

The prevalence of stunting is the highest among young boys and girls (0 – 3 years) across South Africa (Shisana *et al.*, 2012). In the EC province, the prevalence of stunting was 15.6% for girls and 21.6% for boys (Shisana *et al.*, 2012).

According to the South African National Health and Nutrition Examination Survey (SANHANES-1) the two periods of greatest vulnerability to stunting are during intrauterine development, and during the transition from reliance on breast milk to the addition of complementary foods to the diet (Shisana *et al.*, 2012). Since the lack of adequate and sufficient complementary feeding plays such a large role in the development of stunting,

attention should be paid to the energy and nutrient density of complementary foods and the frequency of feeding (Black *et al.*, 2008).

The former Transkei region of the EC is a deep rural area characterised by a high prevalence of poverty and underdevelopment. Subsistence farming is the primary source of food and income at household level and maize consumption is an integral part of the culturally distinct dietary patterns and ethnic tradition (Lombard *et al.*, 2013, Lombard *et al.*, 2014). A preliminary survey (data not published) conducted in the area amongst mothers and primary caregivers of infants, indicated that home-grown maize and thus soft maize porridge is a primary complementary food. It has furthermore been well-documented that the home-grown maize in these rural areas are extremely high in mycotoxins (Burger *et al.*, 2010).

Mycotoxins are low-molecular-weight metabolites that are produced by fungi (Miller, 1995) that grow on the maize. Two examples of mycotoxins found in maize are, aflatoxin (AF) and fumonisin B (FB) are associated with infant and young child (IYC) growth, stunting and under-nutrition especially weight-for-age, height-for-age and weight-for-height z-scores (Gong *et al.*, 2002; Kimanya *et al.*, 2010).

The current study was part of a larger longitudinal, case control study (PhilaSana) where infant and young child dietary habits are being determined to obtain valuable information regarding mycotoxin exposure and its relationship to infant and young child growth. The study is currently conducted in the EC Amatole district. However, to accurately measure mycotoxin exposure it is essential to have accurate data on maize consumption and thus precise dietary recall assessment methods are imperative.

According to Gibson (2005), the errors associated with quantifying the portion of food consumed contribute largely to the measurement errors in most dietary assessment methods. Although it is not always possible to exclude all forms of error, it is important to understand the size and the direction of the error introduced by the dietary assessment method. One of the primary errors occurring in the measurement of food consumption is the assessment of portion sizes (Nelson *et al.*, 1996). Because of this, various aids to assist with dietary intake questionnaires have been developed for the quantitative estimation of dietary data collection. These aids include food models, food pictures, household measures and standard portion sizes (Chambers *et al.*, 2000). Food photographs were used to determine errors in conceptualization during portion size and nutrient content estimation by Nelson *et al.*, (1996). However, the effectiveness of photographs for portion size estimates requires that individuals are able to (i) remember the amounts eaten, (ii) have the ability to mentally

see the amount of food eaten in relation the food photograph presented; and (iii) directly link the food photographs to the actual food portion sizes consumed in the household (Nelson and Haraldsdóttir, 1998b). Robson & Livingstone (2000) stated that the ability of adults, to estimate the portion size of food eaten appears to be affected by the estimation skills, the quantification aid used, the consistency of the participant's perceptions and the type of food consumed.

The study population consist of semi-literate women with infants and young children living in the EC as subsistence rural farmers with a very cultural-specific diet that is significantly different from a standard Western diet. The food photograph series is thus necessary to understand the eating pattern and accurately estimate the portion size of maize intake of infants and young children. However this needs to be developed and validated before use.

1.2 AIMS AND OBJECTIVES

The following aim and objects have been identified for the study:

1.2.1 AIM

The aim of the study was to develop and validate a portion size food photograph series to more accurately determine maize intake of infants aged 6 - 24 months living in deep rural areas in the EC province of South Africa.

1.2.2 OBJECTIVES

The following objectives supported the study:

- Identification of maize based complimentary foods;
- Identification of age-appropriate portion sizes of complimentary foods specifically in the EC;
- Development of age-appropriate portion size food photograph series;
- Validation of the newly developed age-specific portion size food photograph series.

1.3 OUTLINE OF THE STUDY

The study was conducted in different phases, which included the following:

1.3.1 DEVELOPMENT OF INFANT FOOD PORTION SIZE PHOTOGRAPHS

A cultural specific Quantitative food frequency questionnaire (QFFQ) which was previously completed was used to identify the food items reportedly consumed by infants and young children in the EC. Preliminary data (unpublished) on infant and young child feeding practices has been collected during 2013 as part of the *Philasana* project was used for in this study. Data was collected from 100 infants and mothers/caregivers from two deep rural areas in the EC, Amatole district (Mazeppa Bay and Qolora by Sea).

Maize dishes consumed in the EC and mentioned in the 100 QFFQ were chosen, which were soft porridge (maize meal porridge with a soft consistency), crumbly *pap* (maize meal with a dry and crumble consistency), stiff *pap* (maize meal porridge with a thick consistency), maize meal and *imifino* (maize meal porridge with a thick consistency cooked with wild spinach-like plants), maize meal and pumpkin (maize meal porridge with a thick consistency cooked with pumpkin), maize meal and spinach (maize meal porridge with a thick consistency cooked with spinach), maize meal and beans (maize meal porridge with a thick consistency cooked with sugar beans), samp and beans (broken dried maize kernels cooked with sugar beans) and soup (watery soup made with whole maize kernels and sugar beans). Maize meal is prepared in three different ways in the EC – as soft porridge, stiff *pap* and crumbly *pap* (*umphokoqo*). Soft porridge is consumed with fermented milk or fresh milk for breakfast. Crumbly *pap* (*umphokoqo*) and Stiff *pap* which is maize meal with a thick consistency and are consumed for lunch and supper. Stiff *pap* also forms the basis of most combined dishes. Combined dishes that are consumed include: maize meal and *imifino* (spinach & cabbage), maize meal and spinach, maize meal and pumpkin (*Umqa*), maize meal and dried sugar beans, *samp* and dried sugar beans, soup (kernels and dried sugar beans), mealie rice and *imifino* (crushed maize kernels cooked with wild spinach-like plants), mealie rice and spinach (crushed maize kernels cooked with spinach) and mealie rice and pumpkin (crushed maize kernels cooked with pumpkin).

1.3.1.1 Identification of portion sizes

To accommodate the consumption of different portion sizes during infant and young child feeding, the food photograph series consisted of portions depicted on a teaspoon, tablespoon and a large serving ladle spoon, which were found to be the usual utensils used at home. The teaspoon, tablespoon and large serving ladle spoon each depicted maize-containing local food items. Since residences of Bizana and Centane combined maize with vegetables (Lombard *et al.*, 2014) and the ratio of maize meal to vegetables varies according to availability, the food photograph series (FPS) developed included four different

ratios for each combination dish. The combination dishes had four ratio photographs each, these include maize meal and *imifino* (1:2:2, 1:1:1, 1:5:5 & 2:1:1), maize meal and spinach (1:2,1:1,2:1 & 1:5), maize meal and pumpkin (1:2,1:3,3:1 & 2:1), maize meal and beans (1:2, 2:1, 3:1 & 5:1), samp and beans (1:2, 2:1, 3:1 & 5:1), soup -whole mealie kernels and beans (1:2, 1:1, 2:1 & 1:3), mealie rice and *imifino* (1:2:2, 1:3:3, 3:1:1 & 2:1:1), mealie rice and spinach (1:2, 1:3, 3:1 & 2:1) and mealie rice and pumpkin (1:2, 3:1, 1:3, & 2:1).

1.3.1.2 Development of photographs

A female born and raised in a rural area in the EC Province prepared the isiXhosa maize-containing dishes which were reported in the QFFQ in 2014. These dishes were prepared according to isiXhosa recipes determined previously (Lombard *et al.*, 2013). The raw ingredients of recipes were weighed and step-by-step preparation and cooking methods recorded. Final photographs were taken using a black background to emphasise the mostly white foods. The plate used was blue and the most common dishing-up utensil, a tablespoon was used as a scale to illustrate dimension.

The preparation and photo shoot were done over three days. As the female born and raised in the EC was cooking, as soon as the dish was ready it was taken to the makeshift studio to be photographed. The dishes had to cool down first before the photographer could take the photograph. The following procedure was followed - most of the maize meal containing dishes were prepared on day one. Mealie rice dishes were prepared and photographed on day two. The dried sugar beans and kernels were soaked overnight on the second day. The third day which was the last day, all dried sugar beans dishes were prepared and photographed. The average angle of viewing when a person is seated at a table, which is 42° above the horizon, was used for all photographs (Nelson *et al.* 1994). The dish preparations and the photo shoot took place at the North-West University and an established food photographer from the Western Cape Province was used.

1.3.1.3 Validation

The validation part of the study was conducted in two deep rural areas in the Amathole district municipality (from 10 different villages) of the EC. The study population included isiXhosa speaking mothers and caregivers aged 18 years and older with infants and young children age 6 - 24 months. The study population excluded mothers/caregivers aged 18 years and older not taking care of children 6 - 24 months and women or caregivers who do not know how to prepare complementary traditional isiXhosa food.

Due to the widely spread geographical area and the lack of infrastructure such as roads and telephones, the sampling was based on a voluntary, snowball sample.

Once everybody was clear (especially the senior males in the villages as they belong to the tribal council) about the aims and objectives of the study, as well as the inclusion and exclusion criteria and study procedure, a local area (usually the school, shop or outside the clinic) was identified where willing volunteers fitting the inclusion criteria convened the following day. This was the first data collection day. Volunteers (mothers and caregivers) were asked for individual written informed consent and data collection commenced.

Step 1: Portion size identification

The mother/caregiver were first shown the FPS. She was asked to identify the portion size most frequently given to the infant/child. This portion size was recorded by the fieldworker.

Step 2: Actual dishing up from pre-prepared dishes

Volunteers were asked to prepare dishes corresponding to the FPS used at Step 1. The research team provided the needed ingredients. Mothers and caregivers of IYC in the area were then asked to actually dish up the normal portion size they would give to the infant or young child in their household.

For the three days, each day two different stations were set up at one of the volunteers' houses. At the first station, participants would sign the consent form, and then fill in the socio-economic questionnaire and lastly would be shown a set of infant photographs of the different maize dishes. Participants had an opportunity to identify portion size that would usually be consumed by the infant and children. Participants would inform the fieldworker if a teaspoon or tablespoon or ladle was given to the infant or child and the number of the serving spoons given. The fieldworker would record the portion sizes identified for each dish. Seven dishes were validated: soft porridge, maize meal & *imifino*, maize meal & spinach, maize meal and pumpkin, stiff *pap*, *samp* & beans and crumbly *pap*.

At the second station, participants were asked to dish up the amount of food usually consumed by the infant or child. If a participant did not give a food item for a specific reason or the dish was not yet age appropriate the participant did not dish up the dish. The interviewer first weighed the empty plate using a digital, precision balance scale. The mother/caregiver dished up the usual portion size of individual food items consumed by the infant or young child. The empty plate's weight was then subtracted to obtain the actual portion size consumed.

1.4 ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the North-West University at the Potchefstroom Campus (NWU-00089-15-S1) (Addendum 1). Goodwill permission was further obtained from the relevant chiefs, headmen and traditional leaders from each village before the onset of the study. Each participant received a detailed, easy-to-understand consent form which was in isiXhosa (participants' first language), provided they could read. The field worker explained the details of the study to the participants who could not read but still gave all the participants a consent form (Addendum 2).

1.4.1 REIMBURSEMENT

Participants each received a well-wrapped and sealed gift as a sign of appreciation. All participants were informed about the gift of appreciation beforehand. No participant received any financial incentive, due to the high degree of poverty in these areas.

1.4.2 RISK AND BENEFITS

The questionnaires were completed by trained fieldworkers and research assistants. Questionnaires were completed while mothers were waiting to participate in the validation phase. As anticipated, the waiting period was long and mothers and children were offered refreshment. Mothers and caregivers did manage to breastfeed, feed and even change children's nappies while waiting to participate in the validation process as the study took place at the chief or headmen's house.

1.5 LAYOUT OF THE THESIS

Chapter 2 of the thesis provides relevant literature about infant and young children malnutrition rates in South Africa and the EC Province. It further provides information on breastfeeding, complimentary feeding, maize meal consumption and mycotoxin exposure in the EC. Lastly the literature review provides the latest information on dietary intake assessment methods, portion size estimation methods and statistical analysis used in validation of food photographs. Chapter 3 includes a layout of the different phases and the methodology used in each phase. Chapter 4 focuses on the study results. Chapter 5 provides a detailed discussion on the results while Chapter 6 provides conclusions and recommendations. Chapter 7 and 8 includes the reference list and relevant addenda.

1.6 RESEARCH TEAM

The research team consists of the following:

MSc student: Mr A Rasekhala Private practicing dietitian working in previously disadvantaged communities of Soweto and Vosloorus involved in nutrition education and behavior change.

Supervisor: Dr Martani Lombard conducted her PhD on developing and validating an adult QFFQ for people living in this area. Dr Lombard also has extensive experience in infant and young child feeding in rural and peri-urban areas.

Co-Supervisors: Dr Averalda Van Graan (Medical Research Council) has significant experience in infant and young child feeding practices as a hospital dietitian.

Statistician: Miss Marike Cockeran statistics expert for Philasana.

CHAPTER 2 LITERATURE REVIEW ON DEVELOPMENT AND VALIDATION OF PORTION SIZE FOOD PHOTOGRAPHS

The aim of this chapter is to present a review of the literature on the steps in the development of dietary assessment tools and methods. The chapter further discusses the different dietary assessment methods as well as their strengths and limitations. This is followed by a discussion on portion size estimation and the development and validation of portion size photographs.

2.1 INTRODUCTION

Infant and young child feeding (IYCF) is the most important area to improve promotion of healthy growth and development, as well as child survival (WHO, 2014b). The first two years of a child's life are important as optimal nutrition during this period reduces the risk of chronic disease, lowers mortality and morbidity, and encourages better development in the child overall (WHO, 2014b). Infants are particularly vulnerable to under-nutrition since relative to their body size; they have high nutritional requirements with limited capacity to consume foods. They can thus only consume small amounts of food at a single time (Gibson *et al.*, 1998). For this reason, complementary foods are required to be nutrient-dense and are often inadequate in developing countries where maize meal is utilised frequently as a complementary food (Faber, 2004). The World Health Organisation (WHO) has confirmed that the optimal feeding of infants and children under five years has become a critical public health issue (WHO, 2007).

2.2 INFANT AND YOUNG CHILD MALNUTRITION

Access to proper nutrition has implications for educational achievement, cognition, mental health, productivity, stress, adult obesity, household expenditure and food allocation as well as economic growth (WHO, 2004). Food insecurity remains a major public health issue in South Africa (where 35% of households are considered food insecure) with the HIV/AIDS epidemic having a significant impact on households (De Waal & Whiteside, 2003, HSRC, 2004, Kimani-Murage *et al.*, 2010).

Infants and young children have a right to access adequate nutrition and support for optimal feeding practices (WHO, 2003). Optimum nutrition during infancy and childhood is critical for optimal child health, growth and development, and inappropriate IYC feeding practices contribute to under-nutrition related conditions such as chronic (stunting) and acute (wasting) under-nutrition (WHO, 2014a).

Under-nutrition is a leading cause of global childhood morbidity and mortality (Faber & Benade, 2007). Infants and children under five years of age suffer the highest risk of disability and death as a result of under-nutrition, placing this group as the most vulnerable population (WHO, 2014a). Under-nutrition, which includes wasting, stunting, foetal growth restrictions and micronutrient (especially vitamin A and zinc) deficiencies, together with low breastfeeding rates is associated with 45% of under-five year death rates (WHO, 2014b). However, there is increasing evidence to show that childhood over-nutrition has also become an important contributor to adult diabetes and non-communicable diseases later in life (WHO, 2014b).

Over the years the nutritional status of South African children has changed especially in the EC (Table 2.1). The South African Vitamin A Consultative Group (SAVACG) survey (1994) (children under six years) found that approximately 29% of children were stunted in the EC compared to 23% nationally. The prevalence of wasting was 3% for both the province and the country. In 1996, 11% were underweight in the province and 11% nationally respectively (Labadarios *et al.*, 1996). The National Food Consumption Survey (NFCS) (1999) reflected similar results showing 21% of children (aged 1 - 9 years) were stunted in the province compared to 22% nationally. Wasting was 2% compared to 4% nationally, and underweight was 7% compared to 10% nationally (Labadarios *et al.*, 1999). The prevalence of underweight (according to the national food consumption survey - 2005) in the EC was 7.8% (aged 1 – 9 years).

Stunting (when a child is short for his/her age) is caused by chronic under-nutrition (UNICEF, 2009). Stunting is associated with inadequate growth and poor cognitive development leading to sub-optimal educational achievements (WHO, 2004). The underlying causes of stunting include poverty which leads to lack of sufficient food and lack of equity. According to the South African National Health and Nutrition Examination Survey (SANHANES-1) the two periods of greatest vulnerability to stunting are during intrauterine development, and during the transition from reliance on breastmilk to the addition of complementary foods (Shisana *et al.*, 2012). In the EC, prevalence of stunting was 15.6% for girls and 21.6% for boys (Table 2.1) (Shisana *et al.*, 2012). Under-nutrition has stayed roughly constant in South Africa since the early 1990s. Despite our relatively high per capita income, we have rates of child stunting comparable to low-income countries in its region, and higher rates of stunting than lower-income countries in other regions. In addition; children's nutritional status varies considerably among the nine provinces and possibly within each province. The lack of adequate and sufficient complementary feeding is a further determinant of stunting and

attention should be paid to the energy and nutrient density of complementary foods and the frequency of feeding (Black *et al.*, 2008).

Table 2.1. Anthropometric status of children in South Africa and the Eastern Cape Province, 1994, 1999, 2005 and 2012 (Adapted from Department of Health 2013 – 2017 Roadmap for Nutrition in South Africa)

Indicator	Survey year	Eastern Cape (%)	National (%)
Stunting (% H/A < - 2 SD) Moderate to high public health significance according to WHO standards for stunting > 20%	1994	29	23
	1999	21	22
	2005	18	18
	2012	21.6 (boys) 15.6 (girls)	26.9 (boys) 25.9(girls)
Wasting (%W/H < -2 SDs) Moderate to high public health significance according to WHO standards for wasting > 5%.	1994	3	3
	1999	2	4
	2005	4.1	4.5
Underweight (%W/A < -2 SDs) Moderate to high public health significance according to WHO standards for underweight > 10%	1994	11	9
	1999	7	10
	2005	7.8	9.3
Overweight (%W/H > +2 SDs)	1999	8	6
	2005	6.1	4.8

Shaded areas indicate prevalence at a level of high public health significance according WHO standards (stunting > 20%, for underweight > 10% and for wasting > 5%). Classification for overweight in children has not been established).

H/A = height for age, W/H = weight for height, W/A = weight for age, SD = standard deviation

2.3 COMPLIMENTARY FEEDING

Breastmilk alone cannot meet the requirements of the infant after six months (Agostoni *et al.* 2008). The UNICEF (2007) further recommended that mothers continue to breastfeed for two years or more where possible, gradually adding nutritionally adequate, age-appropriate and safe complementary feeding to the diet from six months of birth (UNICEF, 2007). The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition also recommends that complementary foods not be introduced before 17 weeks due to immature gastrointestinal and renal systems (Agostoni *et al.* 2008). There is no compelling evidence to encourage the delayed introduction of potentially allergenic foods (for example fish and eggs) however the committee highlights the importance of complementary foods to provide the majority of the infant's iron requirements (Agostoni *et al.* 2008). The WHO (2002) has recommended that complementary food contain sufficient quantities of fish or eggs, poultry and meat, as well as vitamin A-rich vegetables and fruits every day, and fortified complementary foods and micronutrient supplements are recommended to guarantee adequate nutrient intake (WHO, 2002). Only around 20% of protein requirements need to be met by complementary foods since complementary feeding is the process of introducing solids in association with breast milk (WHO, 1999).

Complementary foods are required to be nutrient-dense and are often inadequate in developing countries where maize meal is frequently utilised (Faber, 2004). Unrefined maize and wheat cereals contain phytic acid in the germ which inhibits iron, zinc and calcium absorption. Refined cereals therefore allow micronutrients to be more bioavailable and the addition of ascorbic acid or vitamin C rich plant foods to the meal will enhance absorption and is therefore encouraged (Gibson *et al.* 1998). Animal and fish protein are known to enhance the absorption of iron and zinc and the addition of even amounts as little as 10g has been shown to be beneficial (Krebs *et al.* 2006; Engelmann *et al.* 1998).

Faber (2004) conducted a survey of the nutrient composition of complementary foods consumed by 475, 6 - 12 month old South African infants in a rural area in KwaZulu-Natal (Faber, 2004). Overall, infants who consumed commercially prepared complementary foods had higher intakes of calcium, iron, zinc and vitamin A among other nutrients when compared to infants consuming home-prepared complementary meals. Despite this observation, the nutrient composition of the complementary diet for all the infants was found to be inadequate, especially with regard to iron, zinc and calcium intake (Faber, 2004). Appropriate measures should be taken to improve the nutrient density of home-prepared meals as well as commercially purchased complementary foods (Faber, 2004). An effective approach is the implementation of fortification strategies for complementary foods. The

densities of iron and vitamin B6 in complementary foods are often inadequate without fortification (Brown & Lutter, 2000; Dewey & Brown, 2003). The foods served to infants 6 – 12 months of age should ideally be culturally acceptable and resemble the family pot (Van der Merwe *et al.*, 2007). Faber & Benade (2007) reported that soft maize meal porridge enriched with margarine and sugar was the most popular solid food given to the infants in their study. Even though maize meal is fortified, with vitamin A, thiamine (vitamin B1), riboflavin (vitamin B2), niacin, folic acid, pyridoxine (vitamin B6), iron, and zinc these micronutrients are unlikely to have a positive impact on the nutritional status of infants as a result of the small amount consumed (Davidsson 1996; Gibson & Ferguson 1996).

Van der Merwe *et al.* (2007) encouraged the introduction of protein and iron-rich foods such as finely mashed or minced meat, chicken, boneless fish and liver as well as the yolk of soft boiled eggs for infants at 7 – 8 months (Van der Merwe *et al.* 2007). From nine months, finger foods are encouraged to help with the motor development of the infant; grated cheese, small pieces of soft fruit and vegetables and finely cut soft meat or chicken should be provided (van der Merwe *et al.* 2007). From 12 months of age, family food can be given to children and only mashed or chopped if necessary (Department of Health 2011b). It is important to provide vitamin A from 6 months of age (carrots, butternut, pumpkin, pawpaw and mango) and vitamin C-rich (tomato, guavas, citrus fruit) foods daily to meet the infants' requirements of these key micronutrients (Glinsmann *et al.* 1996, Lucas, 1999).

2.4 MAIZE CONSUMPTION AND MYCOTOXIN EXPOSURE IN RURAL EASTERN CAPE PROVINCE

Rural subsistence farmers residing in the EC use maize meal as a staple foods obtained from homegrown maize meal that is milled at the local miller, stamped, or grounded at home (Lombard *et al.*, 2013). The maize meal consumed by these populations is therefore by default not fortified. Infants from these subsistence communities may thus be consuming less micronutrients than their urban counterparts. The maize is however often consumed with vegetables, with the ratio of maize to vegetables varying according to seasonal availability of vegetables (Lombard *et al.*, 2014).

2.4.1 Maize consumption in the rural Eastern Cape Province

The majority of the maize crop is harvested at maturity, and the dried kernels are milled, stamped or grounded at home to make different dishes and drinks such as thin boiled porridge, or fermented maize beer, thick porridges, and weaning gruel (Lombard *et al.*, 2013). Samp and beans (dried whole maize kernels combined with beans) is also frequently consumed in the EC. Soft porridge is consumed with milk or sugar for breakfast while stiff

pap (maize meal with a thicker consistency) and crumbly *pap* are consumed for lunch and dinner with meat or spinach. Stiff *pap* also forms the basis of most combined dishes. Most households in the EC combine maize meal and *imifino*, or maize meal and vegetables such as pumpkin, cabbage or spinach to make traditional dishes (Beyers *et al.*, 1979, Lombard *et al.*, 2014). The traditional dishes' consumption is dependent on the availability of vegetables (mostly seasonal), and the availability influence the ratio of maize meal to vegetables (Lombard *et al.*, 2014). The maize meal consumed by these populations is therefore by default not fortified, with vitamin A, thiamine (vitamin B1), riboflavin (vitamin B2), niacin, folic acid, pyridoxine (vitamin B6), iron, and zinc and thus infants from these subsistence communities may be consuming less micronutrients than their urban counterparts. According to Nel & Steyn (2002) maize and maize-based products are consumed by between 67% and 83% of the population, and the average cooked maize consumption is estimated between 475 and 690 g/person/day.

Maize is often given as complimentary food and it is often given before the weaning age which is 6 months (WHO, 1999). This is especially the case in subsistence households such as those in the EC. Maize-based complementary foods often contain considerable levels of fumonisins (FB) (Shephard *et al.*, 1996). FBs have relatively high prevalence in home grown maize in tropical and subtropical countries (Miller, 1995).

Mycotoxins are secondary metabolites produced by fungi that naturally contaminate agricultural food products either during improper storage, in the field, or during food processing (Miller, 1995). Bennett & Klich (2003) stated that although there are approximately 300 –400 mycotoxins, with the four known to influence human health being zearalenone (ZEA), aflatoxin (AF), deoxynivalenol (DON) and fumonisin (FB).

2.4.2 Types of mycotoxins

Although there are hundreds of mycotoxins, five are recognized as the principal fungi that influence human health: fumonisins (FB), aflatoxins (AF), zearalenone (ZEA), deoxynivalenol (DON), and ochratoxin A (OTA) (Smith *et al.*, 2012). The most abundant mycotoxins in South Africa are FB, ZEA and DON (Smith *et al.*, 2012).

*Fumonisin*s are mycotoxins produced by *Fusarium proliferatum* and *Fusarium verticillioides* in maize (Shephard *et al.*, 2007). Fumonisin (followed by aflatoxins) are the main mycotoxins in maize worldwide. Research conducted by Marasas *et al.* (1988) found that maize from households in certain areas in South Africa had significantly higher levels of the mycotoxin fumonisin moniliforme. Zearalenone originates from *Fusarium graminearum* and

mostly infects sorghum, barley, wheat and maize (Goyarts *et al.*, 2007). According to Hepworth *et al.* (2012) DON contaminates barley, maize and wheat, and its exposure is predicted to be frequent as it is stable during processing.

Consumption of foods contaminated by mycotoxins has been linked to various adverse health outcomes in human populations including infant and young children (Shephard, 2008). For these reasons, health authorities in some countries regulate mycotoxin levels for human food (FAO, 2004). Under the South African national regulations (Act No. 54 of 1972, as amended by Government Notice No. R. 1145 of 8 October 2004), the only two mycotoxins considered are:

- Aflatoxin in all foodstuffs, but specifically peanuts and dairy milk. The legal maximum limit for aflatoxin B1 is 5 Fg/kg or 5 ppb (parts per billion), with a total aflatoxin limit not exceeding 10 Fg/kg or 10 ppb. In milk the maximum limit of aflatoxin M is 0.05 Fg/L or 0.05 ppb.
- Patulin in apple juice and apple juice-based commodities is set at a maximum legal limit of 50 Fg/L or 50 ppb.

It is therefore recommended that the South Africa government needs to broaden its food safety regulations relating to mycotoxin exposure (Rheeder *et al.*, 2009). Humans can be exposed on a daily basis to mixtures of these mycotoxins through consumption of foods contaminated with several mycotoxins or consumption of different foods contaminated by a single mycotoxin (Rheeder *et al.*, 2009).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established, for each mycotoxin, a provisional maximum tolerable daily intake (PMTDI), which includes:

- $\mu\text{g}/\text{kg}$ body weight / week for ochratoxin A (OTA) (JECFA, 2002);
- $1 \mu\text{g}/\text{kg}$ body weight / day for deoxynivalenol (DON) (JECFA, 2001);
- $0.5 \mu\text{g}/\text{kg}$ body weight / day for zearalenone (ZEA) (JECFA, 2000);
- $2 \mu\text{g}/\text{kg}$ body weight / day for fumonisins (FB) (JECFA, 2001, 2012).

A study conducted by Shephard *et al.* (2007) researching the exposure assessment of *Fusarium Verticillioides* at Centane and Bizana (deep rural areas in the EC) found that children (1 - 9 years and 10 – 17 years of age) had a high risk of exposure to *Fusarium Verticillioides* attributed to high maize consumption of homegrown maize (Table 2.2).

Table 2.2. Estimated *Fusarium Verticillioides* intake in South Africa: above maximum tolerable daily intake 2 µg/kg/day (Shepard *et al.* 2007)

	Age Group	<i>Fusaria spp</i> Intake (µg/kg/day)	
	Years	Mean	Range
Bizana	1-9	6.6	(1.0 - 18.8)
	10-17	4.0	(0.9 - 9.6)
Centane	1-9	14.1	(2.7 - 35.9)
	10-17	8.3	(2.0 - 17.1)

Aflatoxin (AF) and fumonisin B (FB) is associated with infant and young child (IYC) growth, stunting and under-nutrition especially weight-for-age, height-for-age and weight-for-height z-scores (Gong *et al.*, 2002; Kimanya *et al.*, 2010). In Tanzania, Kimaya *et al.* (2010) reported that children with FB exposure ranging between 20 – 3 201 µg/kg were 1.3 cm shorter and 328 g lighter than those exposed at lower levels. It is therefore imperative to quantify the mycotoxin exposure for children 6 – 24 months in order to find effective reduction strategies. The most effective way to accurately quantify mycotoxin exposure is to determine the dietary intake through a dietary recall method (Lombard *et al.*, 2013). From previous nutrition and exposure research conducted in the area, an accurate, age-appropriate food portion sizes photograph series, depicting a range of infant and young child portion sizes of maize dishes, will improve portion size estimation and thus dietary intake assessment. This will ultimately improve the accuracy of assessments of mycotoxin exposure and the related risks to IYCF (Lombard *et al.*, 2014). The study conducted by Gong *et al.*, (2003) determined the impact of weaning status on FB exposure and have shown that serum AF was significant and that exposure was at least two-fold higher amongst those not breastfed compared to those partially or exclusively breastfed.

2.5 DIETARY ASSESSMENT

Dietary intake assessments are conducted to get information on individual's food habits, nutrient intake, dietary patterns, and sources of nutrients (Willett, 1998). According to Gibson (2005) there are two categories of dietary intake assessment methods; quantitative and qualitative methods. Quantitative methods consist of dietary recalls and food records and measure the amount of individual foods consumed in a day. Qualitative methods on the

other hand consist of patterns of food used during a longer period of time and looks at the frequency and time to assess habitual food intake of specific food items (Gibson, 2005).

Regardless of the assessment method, Gibson (2005) reports that the success of dietary assessment depends on the ability of participants to conduct accurate portion size estimations, interviewer skills and the participant's ability to accurately recall what foods and how much of the foods have been consumed (Gibson, 2005).

Furthermore, Willet (1998) stated that the inaccuracy of dietary information assessed by various dietary assessment methods is the biggest challenge in nutrition epidemiological studies. According to Willet (1998) some dietary assessment methods need several recalls, which are human resource demanding, expensive, time consuming, and may also lead to high recall bias. This makes it inappropriate in community based or population based studies. Developing alternative dietary assessment methods is an essential component of population based studies and might reduce participants' fatigue (Subar, *et al.*, 2001). Fowles *et al.*, (2007) stated that in assessing dietary intake, various commonly used techniques have been recognized, all of which require an efficient and reliable portion size measurement aid (PSMA) to accurately estimate quantities of food consumed. The techniques include, amongst others, the multiple 24-hour recalls, quantitative food frequency questionnaires (QFFQ) and weighed food records (Fowles *et al.*, 2007).

2.5.1 24-hour Recall

The most widely used method to assess dietary intake of individuals is the 24-hour dietary intake recall since it is economical, quick and can be used for both illiterate and literate participants. Strengths and limitations are reported in Table 2.3, (Steyn *et al.*, 2011; Gibson & Ferguson, 2008; Gibson & Huddle, 1998). The interview is usually conducted telephonically or face to face. According to Wrieden *et al.*, (2003) and Gibson (2005) there are three steps that participants need to follow when conducting a 24-hour recall; (a) provide dishes consumed and list of food items during the past 24-hours; (b) provide recipes and cooking methods; and (c) provide portion sizes of each and every food item consumed. A 24-hour dietary intake recall used as a sole method in rural populations have been shown to result in systematic negative bias that can lead to significant underestimation of nutrient intake and daily average energy intake compared with weighed record method (Alemayehu *et al.*, 2011). It is thus recommended that a minimum of four 24-hour recalls per participant must be completed and that these are not consecutive and at least one must include a weekend day (Alemayehu *et al.*, 2011).

Table 2.3. Strengths and limitations of 24-Hour Recall (Steyn *et al.*, 2011; Gibson & Ferguson, 2008; Gibson & Huddle, 1998; Gibson, 2005; Alemayehu *et al.*, 2011).

Strengths	Limitations
Economical	Requires highly trained interviewers
Quick	Coding process must be standardised and pretested to prevent errors
Can be used for both illiterate and literate participants	Low reliability of the data
Culturally sensitive dietary assessment method	Systematic negative bias

2.5.2 Quantitative Food Frequency Questionnaire

Food frequency questionnaires (FFQ) assess dietary intake by determining how often a person consumes a limited number of foods (Kohlmeier & Bellach, 1995). Quantitative food frequency questionnaire (QFFQ) gives a respondent an idea of a portion size and requests that the frequency of intake is provided in terms of this given amount (Willett, 1998). According to Willett (1998), the FFQ is relatively easy to use, can better reflect long-term dietary intake and is inexpensive. However, the QFFQ is the best available method for conducting large epidemiological studies on diet and disease relationships as it assesses habitual dietary intakes (Nelson *et al.*, 1996).

The important principle of the QFFQ is long-term / habitual dietary intake (daily, weekly, monthly or yearly). Because of this, the use of the QFFQ is beneficial as it can provide more representative information on habitual intake than a few days' records or recalls (Flegal, 1999). Unfortunately the QFFQ is based on memory and may thus include a certain amount of bias, strengths and limitations are reported in Table 2.4 (Gibson 2005).

Table 2.4. Strength and limitations of quantitative food frequency questionnaire (Willett, 1998; Gibson, 2005)

Strengths	Limitations
Relatively inexpensive for a large sample size	Recall depends on memory
An indication of usual dietary intake may be obtained	Development and validation tedious Period of recall imprecise
Design can be based on large population data	Limited data in terms of food descriptions
Low responded burden	Recall of past diet maybe biased by current diet
Procedure does not alter habitual dietary intake	Long list tends to overestimate and short lists tends to underestimate intake
Suitable for epidemiology studies	
Can be machine readable if coded	Responded burden is governed by number and complexity of item list
Trained interviewers not needed	
Can be self-administered	No information on meal pattern throughout the day

2.5.3 Weighed diet record

According to Black *et al.*, (1991) the weighed diet record is considered the only fully quantified dietary assessment method. Participants are expected to weigh food before and after eating, while leftovers are weighed and recorded. The dished up amount must be subtracted from the leftovers to estimate the total food intake. Wrieden *et al.*, (2003) stated that household measurements are easier than scales to estimate portion sizes.

2.6. PORTION SIZE ESTIMATION

One of the primary errors occurring in the measurement of food consumption is the assessment of portion sizes (Nelson *et al.*, 1996). Because of this, various aids to assist with dietary intake questionnaires have been developed for the quantitative estimation of dietary data collection. These aids include food models, food pictures, household measures and standard portion sizes (Chambers *et al.*, 2000).

According to Gibson (2005), the errors associated with quantifying the portion of food consumed contributes largely to the measurement errors in most dietary assessment methods. Research done by Seligson (2003) and Young & Nestle (2002 and 1995) has

demonstrated that most people have difficulties in determining what would constitute a portion size correctly.

Portion size measurement aids are also sometimes referred to as portion size estimation aids (PSEAs).

2.6.1 Portion size estimation tools

The United Kingdom Food Standards Agency describes the photographic food atlas as a picture album of different portion sizes of commonly consumed foods and of cups, spoons, cans and plates of varying sizes (Food Safety Bulletin, 1997). The use of food photographs depicting standardized portion sizes of various foods actually consumed by a population improves accuracy of food quantification (Nelson *et al.*, 1994; Nelson *et al.*, 1996). According to Nelson and Haraldsdottir (1998a) a photographic food atlas is defined as a single volume of a photograph series bound together. Nelson and Haraldsdottir (1998) defined a portion as the amount of food that one chooses to eat at a sitting and the fact that the selected portion of food may differ from the standard that is usually smaller or larger. A photographic food atlas is a useful tool to facilitate dietary recall, educate the community regarding portion sizes estimations and has been used by dietitians as a source to provide rich qualitative data. The photographic food atlas is further an excellent research tool used to quantify food portion size (Marjan, 1995, Turconi *et al.*, 2005, Ovaskainen *et al.*, 2008).

Robson & Livingstone (2000) stated that the ability of adults to estimate portion size of food eaten appears to be affected by the estimation skills, the quantification aid used, the consistency of the participant's perceptions and the type of food consumed. Food photographs were used to determine errors in conceptualization during portion size estimation by Nelson *et al.*, (1996). The study had 136 female and male participants with an age range between 18 and 90 years. The researchers had eight photographs for each food item and the photographs had portion sizes that ranged in equal increments from the 5th to the 95th percentile. The visual analogue scale was used by participants to estimate portion sizes. The researchers realised a generalized underestimation of larger portion sizes and overestimation of small portions sizes. A large variation between estimation of portion sizes from photographs was observed. Overestimation was also associated with older participants more than with the younger participants. Those with higher body mass index (BMI) (≥ 30 kg/m²) underestimated energy and fat content of foods, whilst those with lower BMI (≤ 25 kg/m²) overestimated these nutrients. The researchers concluded that gender, BMI and age are possible essential confounders in accurate portion size estimation of food when food photographs are used (Nelson *et al.*, 1996).

Some common household measures, such as glasses, plates, bowls, cups, and measuring spoons, are frequently used to quantify portion sizes. Other models with easily recognizable shapes such as a tennis ball, deck of cards or golf ball (Weber *et al.*, 1997) have also been used to represent common household measures to demonstrate portion sizes. The PSMA was used in estimating dietary intake of 103 participants. According to Ovaskainen *et al.*, 2008; Steyn *et al.*, 2006, three-dimensional models, household measures, abstract and generic shapes, food photographs, and volume measures and utensils, drawings of foods, and plastic food replicas are other portion size measurement tools that have been used to improve portion size recall. Hence, two dimensional food photograph series, drawings of food, utensils, household measures and plastic food replicas are other portion size measurement tools that are used to improve portion size recall (Ovaskainen *et al.*, 2008). In the United States of America commonly consumed foods were presented in different serving shapes and forms to enable participants to easily recognize commonly consumed food items as well as to help with the accuracy of the dietary assessments. Different serving shapes included a block-shaped piece of cheese versus the flat cheese slice, and a slice of bread versus a roll (Hess, 1997).

A study conducted by Chambers *et al.*, (2000) using four different food aid measurement tools to estimate food portions concluded that life sized pictures presented the highest accuracy when used by participants to assess food portion size as compared to the other aids. The nutritional value of the commonly eaten foods can be estimated using the photographic food atlas, the quantity of the food items and seasonal eating behaviours (Robson & Livingstone, 2000).

2.6.2 Developing portion size photographs

Nelson & Haraldsdóttir, (1998b) stated that data based on the target population's eating habits will also provide information on specific portion sizes of food items consumed. Therefore, consultation with the study population is crucial, in order to obtain reliable information of different foods consumed and their portion sizes. Furthermore Nelson & Haraldsdóttir, (1998b) stated that an advisory group, comprising of the representatives of the study population must participate in collecting the dietary data, and be involved in developing the food photograph series. Lastly when the portion size food photograph series has been developed, it is important to return to the population to determine the practical use of the tool (Nelson & Haraldsdóttir, 1998b).

The accuracy of portion size estimation has been defined as the limitation of all self-reporting dietary intake assessment methods used (Gibson, 2005). Elwood and Bird (Elwood & Bird,

1983) are the researchers who originally described the method of diet evaluation using food photographs. They (Elwood & Bird, 1983) conducted a prospective study in which 25 participants were asked to write down their food intake. Each of the participants was given a high-speed, high-quality camera to take pre-meal and post-meal photographs, of all foods and beverages consumed within their homes. Slides of the food photographs were put beside pictures of pre-weighed and pre-measured standard meals that consisted of food and drinks. The researchers compared pre-meal and post-meal photos to finally determine estimated weights of consumed foods. Elwood and Bird (1983) concluded that the method used was a cost effective way to conduct a dietary intake assessment.

Chambers *et al.* (2000) reported that the more a food model looks like the actual food; the more participants are likely to use them to recall portion size of food because of their appeal and ease in usage. Lombard *et al.*, (2013) indicated that for a mostly maize consuming population, the colour of the plate (a white plate has little contrast with the mostly white maize dishes), type of plate (it is easier to determine depth of portion in a plate than in a bowl), background (a dark background is better for white maize dishes) and scale (knife and fork are rarely used in the area) influences portion size estimation.

The following factors must be consideration when developing portion size photographs:

- **Size of photograph**

According to Nelson *et al.*, (1994) the appearance of the photograph size does not influence accuracy, although the minimum acceptable size is 75 x 100 mm, but food on photographs must be life sized (Chambers *et al.*, 2000).

- **Background of the photograph**

The background of the photograph seems to have an effect according to Nelson *et al.*, (1994) as he stated that quality black-and-white photographs could provide the same results as colour photographs; just because colour photographs tend to hold the attention of people longer as the photographs are attractive.

- **Colour of the plate**

According to Lombard *et al.*, (2014) the colour of the plate influences the photograph outcomes: there is little contrast between the white maize dishes and the white plate while the yellow or brown plate and green plate influenced the colour of dishes containing pumpkin and spinach respectively.

- **Type of plate used**

According to Lombard *et al.*, (2014) plates are better to use than bowls especially when determining the depth of the portion size.

Nelson and Haraldsdóttir (1998a) identified 10 different photographic atlases (published between 1985 and 1997) from seven countries in an effort to research practical guidelines on how to develop photographic atlases of food portion sizes. The photographic atlases had a varied number of photos ranging between 15 and 245 as shown by Table 2.5. Other countries such as Malaysia (Marjan, 1995), South Africa (Venter *et al.*, 2000) and Italy (Turconi *et al.*, 2005) also developed photographic food atlases.

2.6.2.1 Malaysia

Marjan (1995) developed a photographic food atlas in Malaysia. The photographic food atlas was used in the assessment of the dietary intake and helped to improve the accuracy of the 24-hour recall dietary method. Common Malaysian food items such as fruit and vegetables, cereals, legumes, nuts, milk, fish and meat groups were identified as well as 250 readymade foods were included. Food items were bought or prepared according to local serving sizes from the identified foods and these were photographed. All ingredients and edible portion sizes were weighed and recorded. A computerized version of the Nutrient Composition of Malaysian Foods, the Demeter software was used to calculate the nutrient content of the foods. The bounded photographic atlas consisted of photographs of serving sizes, weighted edible portions, nutrient content of foods and large size food portions.

2.6.2.2 South Africa

South African researchers Venter *et al.*, (2000) developed a photographic food atlas of commonly consumed foods to be used in portion size estimation during a study to evaluate the health profile of 169 African volunteers from clinics in the North West province. The researchers collected data from participants on commonly consumed foods, recipes, methods and portion sizes. The researchers then prepared the photographic food atlas with 3 - 4 different portion sizes. The photographs were enlarged and bounded into a book and validated. To validate the photographic food atlas, participants had to estimate 2 959 portions of pre-weighed foods by matching them to correct portions in the photographic food atlas. The researchers indicated that 68% of food portions were accurately estimated within 10% of actual weight.

Table 2.5. Photographic Food Atlas of Food Portion Sizes by country based on reference (Nelson & Haraldsdottir., 1998b)

Country	Year	Title	Number of photos in the tool kit	Colour	Number of portions	Presentation	Instructions included
Finland	1985	Annoskuvakirja	126	Yes	3	Increasing size	Yes
Poland	1991	AlbumPorcji Produktow I Potraw	135	Yes	3	Increasing size	No
France	1994	PortionAlimentaires	245	Yes	3	Increasing size	Yes
EPIC	1995	EPIC-SOFT Portion Picture Book for Estimation of Portion Size	140	Yes	4 to 6	Increasing size	Yes
Russia	1995	Albomportsiy Productov I Bljud	63	Yes	3	Increasing size / varied	No
Portugal	1996	Manual de Quantificacao d'Alimentos	110	Yes	3	Varied	Yes
Portugal	1996	Modelos fotografico para Inquiritos Alimentares	58	Yes	4	Increasing size	No
Portugal	1996	Registro Fotografico para Inqueritos Dieteticos	71	Yes	3	Decreasing size	Yes
Sweden	1997	Swedish Photographic Atlas of Food Portion Sizes	15	Yes	5	Increasing size	Yes
UK	1997	Food Portion Sizes: A Photographic Atlas	98	Yes	8	Increasing size	Yes

EPIC = European Prospective Investigation into Cancer and Nutrition

UK = United Kingdom

In another study South African researchers Lombard *et al.*, (2013) developed a food photograph series to improve portion size estimation of maize dishes in rural areas of the EC. Two sets of photographs were developed to be used alongside the validated maize-specific food frequency questionnaire. The photographs were designed to assess portion size intakes and to facilitate estimation of maize amounts in various combined dishes using data from 24-hour recalls with 159 participants, dishing-up sessions with 35 participants, and focus group discussions with 56 participants. Women between the ages of 18–55 years were recruited to participate in the study. The food photograph series comprised of small, medium and large portion size photographs. A total of 21 maize dishes and three ratio photographs of nine combined maize-based dishes were used in the portion size estimation. The researchers reported that the food photograph series improved portion size estimation.

2.6.2.3 United Kingdom

The Department of Nutrition and Dietetics at King's College London in collaboration with the United Kingdom Nutrition Epidemiology Group developed the photographic food atlas of food portion sizes. The photographic food atlas was made up of colour photographs of 78 foods commonly consumed by British adults. The portion sizes ranged from very small to very large portion sizes. The photographic food atlas also included the user's guide which provided background information on its development, instructions on food atlas use, data on the weights of the foods in the photographs and a questionnaire intended for use with the photographs, and lastly it included the software to assist with food consumption calculations (Food Safety Bulletin, 1997).

2.6.2.4 United States of America

The American photographic food atlas, was researched and developed through the partnership of the research and development panel of Portion Photos of Popular Foods (Hess, 1997), the National Center for Nutrition and Dietetics (NCND) established by the American Dietetic Association, representatives from the Center for Nutrition Education at the University of Wisconsin-Stout, the Diabetes Research and Training Center, and the Food and Nutrition board, National Academy of Sciences. The photographic food atlas was designed for use in the public health and clinical community research setting (Hess, 1997). The atlas contains 128 laminated pages of life-size colour photos of 109 commonly consumed foods in the United States of America. Three portion size photographs of each food item were captured on an appropriate plate or bowl on each page. Portion sizes in the

photographic food atlas were based on the 1997 nutrition facts label, diabetic exchanges, and the United States of America Department of Agriculture Food Guide Pyramid.

2.6.3 Validation of Portion Size Photographs

Validation provides information on how the participants perceive the portion sizes depicted on the photographs (Lucas *et al.*, 1995). However, the effectiveness of photographs for portion size validation requires that individuals are able to (i) remember the amounts eaten, (ii) have the ability to mentally see the amount of food eaten in relation the food photograph presented; and (iii) directly link the food photographs to the actual food portion sizes consumed in the household (Nelson *et al.*, 1994). Nelson & Haraldsdóttir, (1998a) stated that this type of validation will only be used if the portion size photographs are used along with 24-hour recalls. By knowing the abovementioned components that contribute to estimation errors, improvements can be made to the tool (Nelson & Haraldsdóttir, 1998a).

Lucas *et al.* (1995) added that the type of food items, the containers and preparation methods also influence validation of food portion size photographs. While Nelson *et al.* (1994) indicated that various factors including the colour, order of presentation and the size of the photographs have an impact on validation results. Elwood and Bird conducted a validity test on data collected from records kept by 16 participants (Bird & Elwood, 1983). The records included pre-weighed and post-weighed foods consumed for a period of four days as well as photographs of all pre-weighed and post-weighed meals. Correlation coefficients calculated, ranged from 0.84 to 0.97 when data from the weighed food record and the photographic record were compared. This indicated a strong relationship between food photographs and weight of actual foods. However the Bird and Elwood photographic method had various limitations including high participant burden, a high cost of equipment, and lack of standardized plate size which might have altered estimated weights of foods (Small *et al.*, 2009).

In the study conducted by Faggiano *et al.*, (1992) actual weights of foods consumed during a meal were compared to the participant's next-day memory recall of developed food photographs. Participants underestimated or overestimated portion sizes by more than 20%. The flat slope syndrome was also observed where volunteers underestimated large portions and overestimated smaller portion sizes.

The study conducted by Foster *et al.*, (2006) assessed the accuracy with which children were able to estimate food portion sizes by evaluating the importance of age appropriate

food photographs in portion size estimation. Three original separate datasets were compared and analysed; data on the accuracy of portion size estimates by children using adult photographs, by adults using food photographs, and by children using age-appropriate photographs. The participants included 210 children (4 - 11 years old) and 135 adult participants (18 - 90 years old). Results indicated that children were considerably more accurate in their estimates of portion sizes using age-appropriate food photographs than when they used food photographs designed for adults. For the age-appropriate photographs, they underestimated by an average of 1% as compared to 45% when adult-appropriate photographs were used.

Turconi *et al.*, (2005) on the other hand compared a colour photographic food atlas as a tool for measuring actual portion size eaten with weighed foods, concluded that weights of portion sizes chosen from a set of photographs were significantly associated to weights of eaten portions but were independent of age, gender and BMI.

In the study by Nelson, *et al.*, (1996) portion sizes served to participants were weighed and waste after consumption also weighed. A choice of 4 - 6 foods from three main meals was allowed. Participants were shown eight photographs of varying portions of each food chosen, five minutes after eating and asked to indicate on a visual analogue scale the portion size consumed in relation to the photographs. A large variation between estimation of portion sizes from photographs was observed. Overestimation was identified particularly in small portion sizes whilst large portion sizes were underestimated. Overestimation was also associated with older participants more than with the younger participants.

Robson & Livingstone (2000) used single colour food photographs where the food was pictured in bowls, on plates, and glasses on top of a wooden table. Although some large food quantification errors occurred, single portion size food photographs were effective when used to estimate nutrient intakes at group level (Robson & Livingstone, 2000). Williamson *et al.* (2003) compared direct visual estimation and digital photography methods. They reported comparatively favourable results regarding food intake; plate waste and portion size estimation of selected food; although both methods showed slight underestimation and overestimation when compared to the weighed food method.

The study conducted by Turconi *et al.*, (2005) compared a colour photographic food atlas as a tool for measuring actual portion size eaten with weighed foods. The colour photographic food atlas which consisted of 434 beverages and foods typical of the Italian diet was developed by taking pictures of pre-weighed cooked foods. Trained investigators pre-

weighed all foods eaten at lunch and dinner at the time of serving. Participants were asked within 5 - 10 minutes after the meal to quantify food eaten in relation to one of three photographs of small, medium or large or as close to photographs as possible on a virtual scale. The researchers concluded that weights of portion sizes chosen from the set of photographs were significantly associated to weights of eaten portions.

2.6.4 Factors Affecting Portion Size Photographs

The study conducted by Turconi *et al.*, (2005) concluded that weights of portion sizes chosen from the set of photographs were significantly associated to weights of eaten portions but were independent of age, gender and BMI. According to Burger *et al.*, (2007) reporting from their study that males' choice of portion sizes of certain foods were significantly larger while other researchers reported no effect of subject characteristics on portion size (Byrd- Bredbenner & Schwartz, 2004; Diliberti *et al.*, 2004; Kral *et al.*, 2004; Rolls, *et al.*, 2002; Nelson and Haraldsdóttir, 1998b). In the study by Nelson *et al.*, (1996) the researchers found errors in portion size conceptualization when food photographs were used, therefore concluded that BMI, age, and gender and portion size were all likely important confounders when nutrient intake or food estimation is investigated using food photographs.

- **Age of participants**

Young children and people older than 65 years may over-estimate portion sizes (Nelson *et al.*, 1994; Robinson *et al.*, 1994, Young & Nestle, 1995, Nelson *et al.*, 1998). In a study by Foster *et al.*, (2006), it was found that children in primary school estimated portion sizes more accurately from age-appropriate food photographs than from photographs designed for adults.

- **Gender of participants**

Studies conducted by Robinson *et al.*, 1997 & Venter *et al.*, 2000 have found that females report more accurately than males, while others reported no differences between genders. Males tend to under-estimate portion sizes (Nelson *et al.*, 1994, Venter *et al.*, 2000).

- **Body mass index of participants**

Participants with a body mass index (BMI) > 30 kg/m² tend to under-estimate portion sizes, possibly because they consume larger portions than those with normal BMIs (Nelson *et al.*, 1994, Nelson & Haraldsdóttir, 1998a).

2.8 SUMMARY

The first two years of a child's life are important as optimal nutrition during this period reduces the risk of chronic disease, lowers mortality and morbidity, and encourages better development overall (WHO, 2014b). Optimal nutrition during infancy and childhood is critical for optimal child health, growth and development, and inappropriate infant feeding practices contribute to under-nutrition related conditions such as chronic (stunting) and acute (wasting) under-nutrition (WHO, 2014a).

Exclusive breastfeeding is regarded as the single most effective preventive intervention to avert at least 13- 15% of deaths of children below 5 years of age in developing countries, and is cost effective (WHO, 2003; UNICEF, 2007). The introduction of semisolid complementary food is recommended from the age of six months, while adjusting it gradually into a variety of solid foods until the infant is able to eat the same type of foods as their family members at the age of 12 months. Maize meal is the staple food in the diets of many African populations. Dowswell *et al.*, (1996) stated that maize meal forms the highest percentage of energy in 22 countries in the world, and 16 of these countries are in Africa. A small percentage of the maize crop is eaten as fresh maize, which is roasted on fire or the whole maize crop is boiled as a snack. Maize kernels are stripped from the cob, dried, and then sold or stored for further processing (Nuss & Tanumihardjo, 2011).

Maize is often given as complimentary food and it is often given before the weaning age which is six months (WHO, 1999). This is especially the case in subsistence households such as those in the EC. Maize-based complementary foods often contain considerable levels of fumonisins (FB) (Shephard *et al.*, 1996). FBs have relatively high prevalence in home grown maize in tropical and subtropical countries (Miller, 1995). Aflatoxin (AF) and fumonisin B (FB) is associated with infant and young child (IYC) growth, stunting and under-nutrition especially weight-for-age, height-for-age and weight-for-height z-scores (Gong *et al.*, 2002; Kimanya *et al.*, 2010).

According to Gibson (2005) there are two categories of dietary intake assessment methods; quantitative and qualitative methods. Quantitative methods consist of dietary recalls and food records, and measures the amount of individual foods consumed in a day. Qualitative methods on the other hand consists of patterns of food used during a longer period of time and looks at the frequency and time to assess habitual food intake of specific food items (Gibson, 2005). As stated by Venter *et al.*, (2000) perception is the participants' ability to relate the amount of food which is present at dishing up to the amount depicted in a

photograph. This is one of three courses of action which occurs when photographs are used as PSMA (Nelson, *et al.*, 1994).

According to Nelson and Haraldsdottir, 1998a a photographic food atlas is a portion size estimation aid, defined as “a set of photograph series, usually bound together in a single volume”. The photographic food atlas may contain portion sizes ranging between three to eight different portion sizes of food (Turconi *et al.*, 2005; Food Safety Bulletin, 1997). In the study conducted by Faggiano *et al.*, (1992) actual weights of foods consumed during a meal were compared to the participant’s next-day memory recall of developed food photographs depicting a series of Italian dishes. The researchers found that volunteers underestimated or overestimated portion sizes by more than 20%. The flat slope syndrome was also observed where volunteers underestimated large portions and overestimated smaller portion sizes. The use of food photographs as PSMA to estimate portion sizes of actual foods by participants (perception) was assessed in this study. These portion size photographs should be validated as it provides information on how the participants perceive the portion sizes depicted on the photographs (Lucas *et al.*, 1995). When validating such photographs, actual food should be prepared in exactly the same way as those depicted on the photographs (Lucas *et al.*, 1995).

CHAPTER 3 METHODS

3.1 INTRODUCTION TO THE METHODS

The aim of this chapter is to present the study methods in terms of the development and validation of the age appropriate portion size food photographs. An adult cultural specific QFFQ was previously developed for this area (Lombard *et al.*, 2013). Mothers/caregivers (N = 100) of IYC completed the QFFQ to identify food items and dishes represented on the cultural specific adult QFFQ that is commonly used as food for IYCF (unpublished data). The questionnaire was designed with a mixed methods approach and includes all maize based dishes relevant to infant and young child feeding consumed in the EC. Firstly the questionnaire determines the amount of cooked maize consumed during a month (the food photograph series is used to determine portion sizes). This is then converted to the amount of raw maize (from the recipes used during the development of the food photograph series) consumed during the month. The amount of raw maize consumed during a month is divided by 28 to determine the average raw maize consumed per day. To accurately determine IYC maize consumption, a valid age appropriate food photograph series depicting IYC portions is required.

3.2 STUDY PROCEDURE

The study was conducted in 2 phases as presented in Figure 3.1:

Phase 1: The development of a food photograph series (FPS) was conducted in two separate steps:

Step 1: Identification of maize based food items and dishes used as weaning and complimentary food as well as age appropriate portion sizes to be depicted in the photographs for 6-24 months;

Step 2: The development of the photographs.

Phase 2: The validation of the food photograph series was conducted in two separate steps:

Step 1: Identification of the most appropriate portion sizes for each food item and dish represented in the FPS.

Step 2: Actual dishing up from food items and pre-prepared dishes by caregivers / mothers.

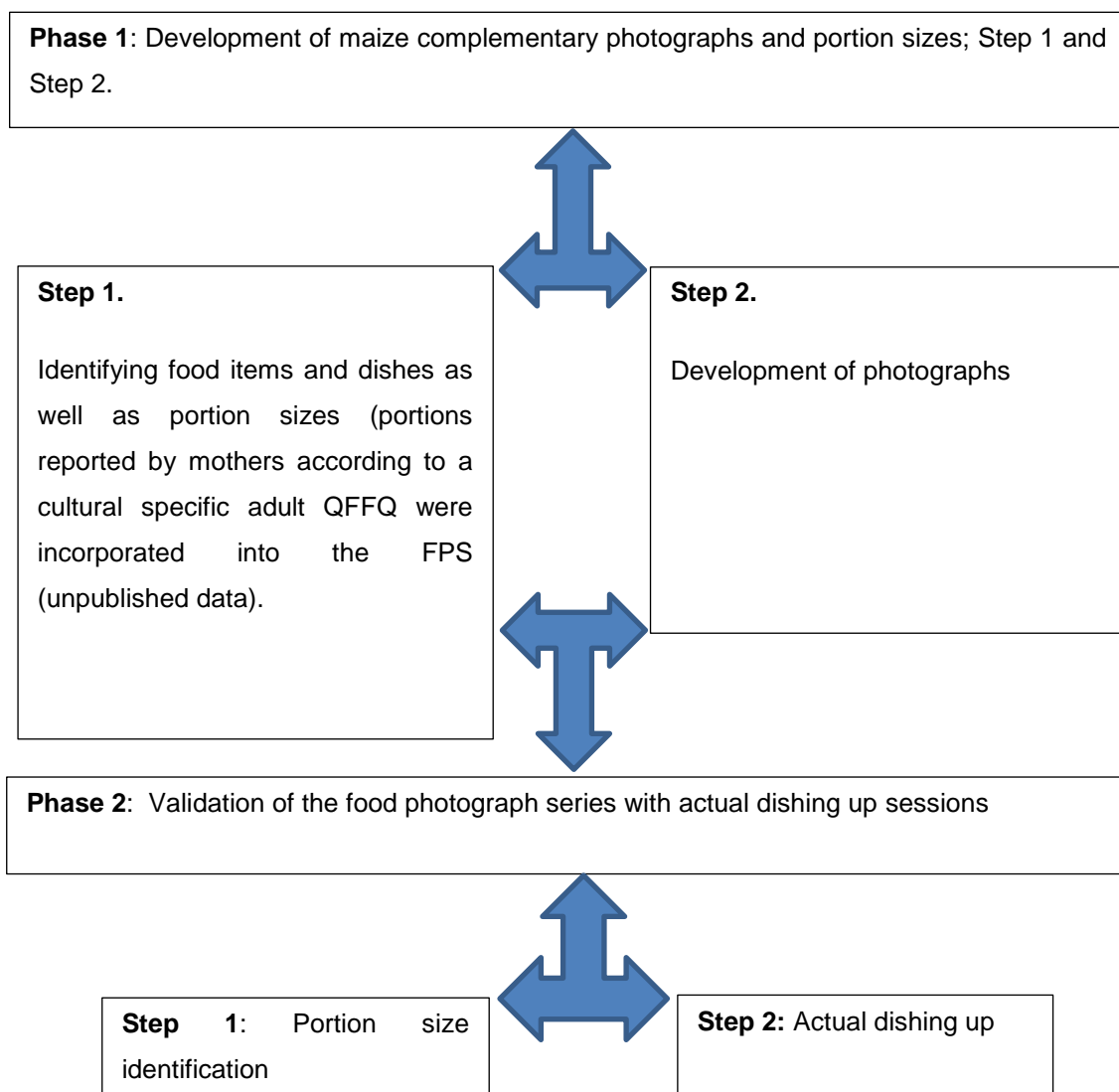


Figure 3.1. Study procedures followed during the development and validation of age appropriate food photograph series.

3.2.1 PHASE 1: DEVELOPMENT OF PHOTOGRAPHS

Phase 1 was completed in two different steps as stipulated in Figure 3.1.

Step 1: Identification of food items and portion sizes to be depicted in the FPS

An adult cultural specific QFFQ was previously developed for this area (Lombard *et al.*, 2013). Mothers / caregivers (N = 100) of IYC completed the QFFQ to identify food items and dishes represented on the cultural specific adult QFFQ that is commonly used as food for

IYCF (unpublished data). This unpublished data was used to identify food items and dishes to be represented on the age appropriate food photograph series. Items not identified as IYC food or dishes were removed from the original adult QFFQ. Only food items and dishes consumed by 80% or more of the infants and young children (0 - 24 months) were included in the FPS.

To accommodate the consumption of different portion sizes during IYCF, the FPS consisted of photos representing portions ranging from teaspoons, tablespoons to large serving ladles. Portion sizes were determined on portions reported from the unpublished QFFQs completed among mothers / caregivers (see above). It was presumed that the portions sizes reported in the cultural specific adult QFFQ were reliable and valid. Ideally the mother must indicate the dishing up method she uses (which spoon) and then report on the number of spoon portion sizes dished up (for instance, three table spoons).

Step 2: Development of food photograph series

A female born and raised in a rural area in the EC prepared the traditional, cultural specific food items and dishes identified during Step 1. Raw ingredients of recipes were weighed (to determine the amount of raw maize used for the relevant portion of cooked maize) and step-by-step preparation and cooking methods were recorded. The following dishes were prepared: soft porridge, crumbly *pap*, stiff *pap*, maize meal and *imifino*, maize meal and pumpkin, maize meal and spinach, maize meal and beans, samp, beans and soup, mealie rice and *imifino*, mealie rice and spinach and mealie rice and pumpkin (Table 4.2).

The ratios depicted in the photographs were obtained from Lombard *et al.*, 2013. Four different ratios were determined for each maize meal combination dish.

The average angle of viewing when a person is seated at a table, (42° above the horizon) was used for all photographs (Nelson *et al.* 1994). Each IYC food item identified in Step 1 was accompanied by a teaspoon, tablespoon and large serving ladle food photograph series.

Dish preparations and the photo shoot took place at the North-West University by an experienced food photographer.

The following factors were taken into consideration while developing the photographs: size of photograph, background of photograph, colour of plate and the type of plate.

3.2.2 PHASE 2: VALIDATION OF THE FOOD PHOTOGRAPH SERIES WITH ACTUAL DISHING UP SESSIONS

Phase 2 was completed in two different steps as stipulated in Figure 3.1.

Participants

The validation part of the study was conducted in two deep rural areas in the Amathole district municipality consisting of 10 different villages of the EC. The study population included isiXhosa speaking mothers and caregivers aged 18 years and older with infants and young children age 6 - 24 months. The study population excluded mothers/caregivers aged 18 years and older not taking care of infant and young children age 6 - 24 months and women or caregivers who do not know how to prepare complementary traditional isiXhosa food.

Due to the widely spread geographical area and the lack of infrastructure such as roads and telephones, the sampling was based on a voluntary, snowball sample.

On the first day of the research project, the relevant traditional leaders including the local chief and headmen, as well as the traditional healers were consulted and the study was explained to them with the aim to obtain goodwill permission to conduct the research. Upon their consent, messengers (mostly young boys) were used to send messages to all households in the relevant area about a community meeting. At the community meeting, which was the following day of the project, the project was explained to the residents (male and female) in the participants' mother tongue (isiXhosa). This task was given to a specially trained, experienced field worker who has conducted similar research projects in the area (with the PROMEC Unit of the MRC as well as with University Stellenbosch) and who is known to the residents and the local traditional leaders.

Once everybody was clear (especially the senior males in the villages as they belong to the tribal council) about the aims and objectives of the study, as well as the inclusion and exclusion criteria and study procedure, a local area (usually the school, shop or outside the clinic) was identified where willing volunteers fitting the inclusion criteria convened the following day. This was the first data collection day. Volunteers (mothers and caregivers) were asked for individual written informed consent and data collection commenced.

The following inclusion criterion was used for the validation process:

- Mothers / caregivers aged 18 years and older taking care of infants and young children (6 - 24 months);
- Mothers / caregivers aged 18 years and older that know how to prepare isiXhosa traditional complementary food;
- Mothers or caregivers aged 18 years and older residing in the preselected research areas.

The following exclusion criterion was used:

- Mothers / caregivers aged 18 years and older not taking care of children 6 - 24 months;
- Women or caregivers who do not know how to prepare complementary traditional isiXhosa feeds.

Step 1: Portion size identification

A sample size of 100 participants was recommended for a validation study (Cade *et al.*, 2002). During the validation process, portion sizes accompanying each food item in the newly developed IYC QFFQ were compared to actual dished up portions of maize dishes.

The mother/caregiver were first shown the FPS. She was asked to identify the portion size most frequently given to the infant/child. This portion size was recorded by the fieldworker (Addendum 3).

Step 2: Actual dishing up from pre-prepared dishes

Volunteers were asked to prepare dishes corresponding to the FPS used at Step 1. The research team provided the ingredients required. Mothers and caregivers of IYC in the area were then asked to actually dish up the normal portion size they would give to the infant or young child in their household.

For the three days, each day two different stations were set up at one of the volunteers' houses. At the first station, participants would sign the consent form, and then fill in the socio-economic questionnaire and lastly would be shown FPS of the different maize dishes. Participants had an opportunity to identify portion size that would usually be consumed by the infants and children. Participants would inform the fieldworker if food was dished with a teaspoon or tablespoon or ladle and the number of the serving spoons given. The

fieldworker would record the portion sizes identified for each dish. Seven dishes were validated: soft porridge, maize meal & *imifino*, maize meal & spinach, maize meal and pumpkin, stiff *pap*, *samp* & beans and crumbly *pap* (Addendum 3).

At the second station, participants were asked to dish up the amount of food usually consumed by the infant or child. If a participant did not give a food item for a specific reason or the dish was not yet age appropriate the participant did not dish up the dish. The interviewer first weighed the empty plate using a digital, precision balance scale (glass electronic kitchen scale (Home elegance) max 5 kg / 1g) Figure 3.2). The mother/caregiver dished up the usual portion size of individual food items consumed by the infant or young child. The empty plate's weight was then subtracted to obtain the actual portion size consumed (Addendum 4).



Figure 3.2. Precision balance scale used to weigh the empty plates and the actual portion sizes consumed.

3.3 QUESTIONNAIRE

A socio-demographic questionnaire was adapted from various sources to measure specific information. This questionnaire elicits information including age, gender, language, and location, sources of domestic water, type of sanitation, crowding (number of household members per sleeping room), occupation and education (Addendum 5).

The questionnaire was completed by trained, fieldworkers and research assistants.

3.4 STATISTICAL ANALYSES

Data of the portion size photographs identified by each mother / caregiver (Step 1) and the actual portion size dished up by the mother/caregiver for each individual dish were captured on an Excel spreadsheet and compared to determine the differences in reported weight and actual weight.

According to Cade *et al.*, (2002) various statistical approaches can be used to assess the validation of the dietary intake of participants. The statistical methods provide validity of the various dietary assessment methods. Each test provide information on a different facet of validity and thus all statistical tests needs to be conducted to provide a complete overview of the validity of the newly developed FPS. Table 3.1 provides a summary of the different statistical tests used in this study as well as the interpretation criteria.

Table 3.1. Summary of identified statistical tests and interpretation criteria for validation of dietary intake assessment methods

Statistical test	Validity	Interpretation Criteria		
		Good	Acceptable	Poor
Paired <i>t</i> -test / Wilcoxon signed rank test (Robinson <i>et al.</i> , 1997, Gibson, 2005)	Agreement at group level	P > 0.05		P ≤ 0.05
Correlation coefficients (Masson <i>et al.</i> , 2003, Gibson, 2005, Easton & McColl, 2010)	Strength and direction of association at individual level	≥ 0.50	0.20 - 0.49	< 0.20
Percent difference (Robinson <i>et al.</i> , 1997, Venter <i>et al.</i> , 2000, Gibson, 2005)	Agreement at group level (size and direction of error)	0.0 - 10.0%	11 – 20%	> 20%
Bland-Altman analyses (Bland & Altman, 1986, Cade <i>et al.</i> , 2002, Bakker <i>et al.</i> , 2003, Lazarte <i>et al.</i> , 2012)				
Correlation between mean and mean difference	Association between size and error	P > 0.05		P ≤ 0.05
% within LOA	Bias and range of agreement	> 95% of N		< 95% of N
Width of LOA	Strength of agreement	< 1 x teaspoon	Between 1 x teaspoon and 1 x table spoon	> 1 x table spoon

LOA = Level of agreement

3.4.1 PAIRED T-TEST / WILCOXON SIGNED RANK TEST

Robinson *et al.*, 1997 stated that the paired *t*-test or Wilcoxon signed rank test indicates agreement between two measures at group level. The size and direction of error at group level, as assessed between the dishing up portion size and the photograph portion size. P-values larger than 0.05 are considered acceptable, P values between 0.05 and 0.001 are considered poor and P values less than 0.001 are considered exceptionally poor (Gibson, 2005) (Table 3.1).

3.4.2 Correlation coefficients

Correlation coefficients measure the strength and direction of the association between the two different measurements at individual level (Gibson, 2005). The strength and direction at individual level of ≥ 0.50 is interpreted as good, 0.20 - 0.49 is acceptable and < 0.20 is poor (Masson *et al.*, 2003) (Table 3.1). According to Easton & McColl, 2010 coefficients of zero reflect no linear relationship between the two measurements, and coefficients with a value 1 reflect positive correlation and -1 value reflect negative correlation (Table 3.1).

3.4.3 Percentage difference

The size and direction of error at group level, assessed between the dishing up portion size and the photograph portion size is the percentage difference (Gibson, 2005). Robinson *et al.*, (1997) stated that to obtain the mean percentage difference the following calculation needs to be performed: dishing up portion size subtracted from photograph portion size, divided by dishing up portion size and multiplied by 100 for each participant. According to Venter *et al.* (2000), the mean percentage difference is considered accurate if it is smaller than 10%, acceptable if between 11% and 20% and poor if larger than 20% (Table 3.1).

3.4.4 Bland-Altman analyses

The Bland-Altman correlations are used to show the differences between the portion sizes identified with the photographs and the actual dished up portion (Lazarte *et al.*, 2012). Bland-Altman analyses are also used to determine the level of agreement (LOA) between two measures and the presence and the extent of bias (Cade *et al.*, 2002). The y-axis of the Bland-Altman plots indicates the mean difference between the photograph portion size and the dished up portion size – while the x-axis indicates the mean of the two portion sizes

[(photograph portion size + dished up portion size / 2)] (Bland & Altman, 1986, Gibson, 2005). Satisfactory Bland-Altman analyses should include (Bland & Altman, 1986, Bakker *et al.*, 2003) mean differences close to zero, data points close to the mean difference, narrow LOA and no bias, as stipulated in Table 3.1.

3.5 ETHICAL APPROVAL

Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the North-West University at the Potchefstroom Campus (NWU-00089-15-S1) (Addendum 1). Goodwill permission was further obtained from the relevant chiefs, headmen and traditional leaders from each village before the onset of the study. Each participant received a detailed, easy-to-understand consent form which was in isiXhosa (participants' first language), provided they could read. The field worker explained the details of the study to the participants who could not read but still give the all participants a consent form (Addendum 2).

CHAPTER 4 RESULTS

4.1 Demographic Information of Participants

All participants were born in the EC Province and isiXhosa speaking. A total of 48 mothers / caregivers participated in the study. Due to logistical constraints a larger sample size was not possible. The average age of the mothers / caregivers was 48 years (SD 13.36) as shown in Table 4.1., and that of their infants was 18 months (SD 4.40) as shown in Table 4.2.

Table 4.1. Mothers / Caregivers age range

Age Range	n	%
15 – 30	15	31
31– 50	24	50
> 51	9	19

Table 4.2. Infants and Children age range

Age Range in months	n	%
6 – 12	21	44
13– 24	27	56

The majority of households used communal taps (N = 20, 41%) with another 19% (N = 9) using river or dam water. All participants had an outside pit toilet at their homes. The majority of the households (N = 24, 50%) used a combination of electricity or paraffin or wood to cook. Only 17% (N = 8) of the households used electricity as the only source of energy for cooking as shown in Table 4.3. All households received financial support from the Department of Social Development, either in the form of child support, old age pension or

social relief. The total income per household ranged from R 500 – R 3 000, depending on the number of beneficiaries.

Table 4.3. Socio-demographic description of participants

Socio-demographic characteristic	n	%
Monthly income		
R 500 – R1 000 (63 - 126 USD)	15	31
> R 1000 (126 USD)	33	69
Number of people contributing to the household		
1 Wage earner	3	6
2 Grants	48	100
Water source		
River water	9	19
Communal tap	20	42
Water tank	19	39
Fuel for cooking		
Wood, Paraffin and Electricity	24	50
Electricity	8	17
Electricity & wood	6	13

Figure 4.1 illustrates the percentage distribution of the different education levels of the participants. The majority of participants (N = 36, 75%) had a secondary school (grades 8 - 12) qualification, with N = 8, (16%) having a primary school qualification and N = 6 (12.5%) having no formal education. Figure 4.2 further illustrates the percentage distribution of employment. The majority of the participants (N = 45, 94%) were unemployed.

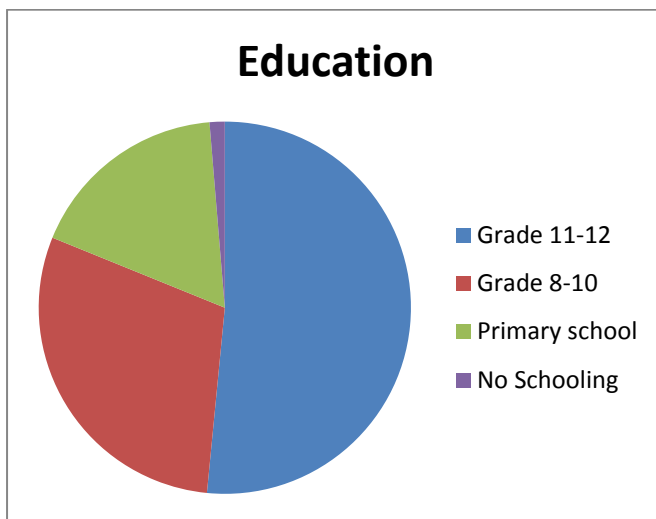


Figure 4.1. Education levels of participants

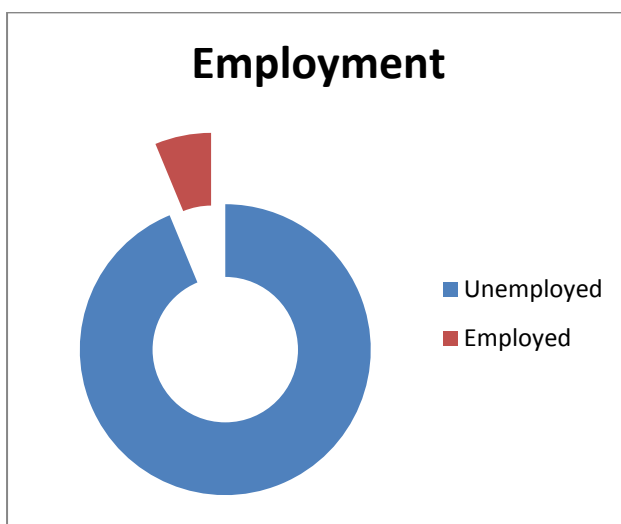


Figure 4.2. Employment distributions of participants

4.2 PHASE 1: DEVELOPMENT OF INFANT FOOD PORTION SIZE PHOTOGRAPHS

The FPS was developed with 2 steps, i) identification of maize based food items and dishes used as weaning and complimentary food as well as relevant age appropriate portion sizes to be depicted on the photographs, and ii) the development of the FPS.

Step 1: Identification of maize dishes and their portion sizes to be depicted in the FPS

Only maize based food items and dishes were included in the age appropriate FPS. Results from the QFFQ conducted amongst 100 mothers / caregivers (2013) indicated that nine food maize dishes were given to IYC including: soft porridge, crumbly *pap*, stiff *pap*, maize meal and *imifino*, maize meal and pumpkin, maize meal and spinach, maize meal and beans, samp, beans and soup, mealie rice and *imifino*, mealie rice and spinach and mealie rice and pumpkin as shown in Table 4.4.

The ratios recipes depicted in the photographs were obtained from Lombard *et al.*, (2013). Four different ratios were determined for each maize meal combination dish. The following standard ratios of maize to vegetables in nine combined dishes were identified as shown in Table 4.5.

Table 4.4. Summary of the different dishes and their different portion sizes

Dish	Portion Size		
	1 Teaspoon (g)	1 Tablespoon (g)	1 Dish up ladle (g)
Soft porridge	20	45	160
Maize meal and <i>imifino</i>	25	50	180
Maize meal and spinach	25	65	215
Maize meal and pumpkin	25	50	200
Stiff pap	25	50	220
Maize meal and dried beans	30	60	210
Samp	30	65	210
Samp and beans	25	55	220
Soup	25	55	185
Crumbly pap	25	60	240
Mealie rice and spinach	25	50	180
Mealie rice and pumpkin	20	40	175
Mealie rice and <i>imifino</i>	25	50	210

Imifino = is a spinach-like vegetable that grows wild in the EC

Table 4.5 Maize meal Ratio Dishes

Dish	Ratio 1	Ingredients	Cooked weight	Ratio 2	Ingredients	Cooked weight	Ratio 3	Ingredients	Cooked Weight	Ratio 4	Ingredients	Cooked weight
Maize meal and <i>imifino</i>	1:2:2	103,7 g Maize meal 207,4 g Cabbage 207,4 g Spinach 33,8 g Onion 732,0 ml Water	945 g	1:1:1	39,95 g Maize meal 39.95 g Cabbage 39,95 g Spinach 10,5 g Onion 227,5 ml Water	375 g	1:5:5	12,2 g Maize meal 60,05 g Cabbage 60,05 g Spinach 6,75 g Onion 140,8 ml Water	335 g	2:1:1	54,3 g Maize meal 27,0 g Cabbage 27,0 g Spinach 7,35 g Onion 149,5 ml Water	380 g
Maize meal and spinach	1:2	72,3 g Maize meal 144,3 g Spinach 552,1 ml Water 10,3 g Onion	630 g	1:1	35,5 g Maize meal 35,5 g Spinach 135,15 ml Water 5,4g Onion	267 g	2:1	44,8 g Maize meal 22,4 g Spinach 98,3 ml Water 3,15 g Onion	227 g	1:5	13,7 g Maize meal 68,65 g Spinach 174,45 ml Water 0,6 g Onion	182 g
Maize meal and pumpkin	1:2	132,2 g Maize meal 264,9 g Pumpkin 578,6 ml Water	804g	1:3	18,1 g Maize meal 80,15 g Pumpkin 234,4 ml Water	305 g	3:1	71,7 g Maize meal 24,15 g Pumpkin 181,7 ml Water	402 g	2:1	69,8 g Maize meal 34,2 g Pumpkin 181.9 ml Water	385 g
Maize meal and dried beans	1:2	114,7 g Maize meal 229,3 g Beans 875,3 ml Water	765 g	2:1	83,3 g Maize meal 41,95 g Beans 270,2 ml Water	382 g	1:3	25,55 g Maize meal 76,35 g Beans 300,7 ml Water	342 g	3:1	93,65 g Maize meal 31,2 g Beans 227,05 ml Water	383 g

Samp and beans	1:2	101,6 g Samp 203,1g Beans 1587,0 ml Water 3,6 g Beef stock 45,3 ml Oil	996 g	2:1	56,1 g Samp 28,05 g Beans 445,10 ml Water 0,9 g Beef stock 11,15 ml Oil	498 g	3:1	63,55 g Samp 21,2 g Bean 415,45 ml Water 0,85 g Beef stock 10,4 ml Oil	398 g	5:1	76,4 g Samp 15,05 g Beans 538,2 ml Water 1,1 g Beef stock 13,45 ml Oil	598 g
Soup	1:2	79,4 g Kernels 158,8 g Beans 992,5 ml Water 2,3 g Beef stock 14,0 ml Oil	894 g	1:1	42,9 g Kernels 42,9 g Beans 311,25 ml Water 0,7 g Beef stock 4,15 ml Oil	347 g	2:1	64,0 g Kernels 42,0 g Beans 271,4 ml Water 0,6 g Beef stock 3,6 ml Oil	307 g	1:3	21 g Kernels 63.2 g Beans 231,0 ml Water 0,35 g Beef stock 2,2 ml Oil	447 g
Mealie rice and spinach	1:2	39,9 g Mealie rice 79,8 g Spinach 451,6 ml Water	894 g	1:2	29,4 g Mealie rice 88,2 g Spinach 210,0 ml Water	447 g	3:1	72,0 g Mealie rice 24,0 g Spinach 191,0 ml Water	387 g	2:1	180,0 g Mealie rice 90,0 g Spinach 370,2 ml Water	487 g
Mealie rice and pumpkin	1:2	137,9 g Mealie rice 275,9 g Pumpkin 757,0 ml Water	844 g	3:1	85.2 g Mealie rice 28.4 g Pumpkin 93,1 ml Water	322 g	1:3	28,8 g Mealie rice 86,0 g Pumpkin 206,0 ml Water	282 g	2:1	59,7 g Mealie rice 30,0 g Pumpkin 141 ml Water	422 g
Mealie rice and imifino	1:2:2	99,0 g Mealie rice 198,7 g	894 g	1:3:3	25,9 g Mealie rice 77,6 g	384 g	3:1:1	78,35 g Mealie rice 26,1 g	344 g	2:1:1	100,5 g Mealie rice 50,25 g	447 g

Cabbage
198,7 g
Spinach
958,8 ml
Water

Cabbage
77,6 g
Spinach
217,35 ml
Water

Cabbage
26,1 g
Spinach
126,7 ml
Water

Cabbage
50,25 g
Spinach
144,35 ml
Water

Step 2: Development of food photograph series

To accommodate the consumption of different portion sizes during IYCF, the FPS consisted of photos representing portions ranging from teaspoons, tablespoons and large serving ladles. Portion sizes were determined on portions reported from the unpublished QFFQs completed among mothers / caregivers (2013). Mothers thus indicate the dishing up method she use (type of spoon) and then report on the number of spoon portion sizes dished up (for instance three table spoons).

A female born and raised in a rural area in the EC prepared the traditional, cultural specific food items and dishes identified during Step 1. Raw ingredients of recipes were weighed (to determine the amount of raw maize used for the amount of cooked maize) and step-by-step preparation and cooking methods recorded in Table 4.6.

Table 4.6. Maize meal dishes

Dish	Ingredients	Cooked weight
Soft Porridge	86 g Maize meal 663,2 ml Water	630 g
Stiff Pap	213 g Maize meal 543, 9 ml Water	640 g
Crumbly Pap	337 g Maize meal 353,2 ml Water	590 g
Samp	192 g Samp 64 g Potatoes 13 g Onion 29 ml Oil 2 g Beef stock	622 g

The average angle of viewing when a person is seated at a table, (42° above the horizon) was used for all photographs (Nelson *et al.* 1994). Each food item chosen from the 100 QFFQ represented on the newly developed infant and young child QFFQ were accompanied by a teaspoon, tablespoon and large serving ladle food photograph series.

The final FPS consisted of 13 food items / dishes and 3 portions per dish (Table 4.6 and Figure 4.3). There are also 40 ratio photos for 10 food items / dishes (Table 4.5 and Figure 4.4).



Soft porridge

Soft porridge

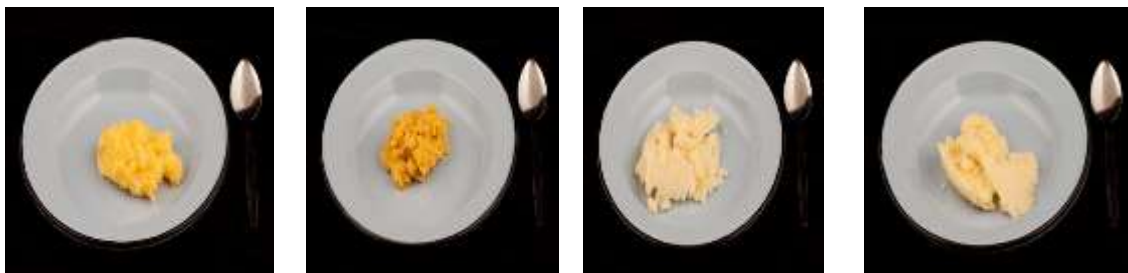
Soft porridge

Teaspoon

Tablespoon

Ladle

Figure 4.3 Portion size photographs for soft porridge



Maize meal and
Pumpkin

Maize Meal and
Pumpkin

Maize meal and
Pumpkin

Maize meal and
Pumpkin

Ratio 1:2

Ratio 1:3

Ratio 3:1

Ratio 2:1

Figure 4.4 Ratio photographs for maize meal and pumpkin

The following factors were considered during the development of portion size photographs:

- **Size of photograph**

According to Nelson *et al.*, (1994) the appearance of the photograph size does not influence accuracy, although the minimum acceptable size is 75 x 100 mm. Actual size of photographs was 42 x 30 cm to provide life-size images of the dishes as per Lombard *et al.*, (2013) recommendation.

- **Background of the photograph**

The background of the photograph seems to have an effect according to Nelson *et al.*, (1994) as he stated that quality black-and-white photographs could provide the same results as colour photographs; just because colour photographs tend to hold the attention of people longer as the photographs are attractive. A dark background was used during the colour photo shoot.

- **Colour of the plate**

According to Lombard *et al.*, (2013) the colour of the plate influences the photograph outcomes: there is little contrast between the white maize dishes and the white plate while the yellow or brown plate and green plate influenced the colour of dishes containing pumpkin and spinach respectively. The light blue enamel plates were used during the colour photo shoot.

- **Type of plate used**

According to Lombard *et al.*, (2014) plates are better to use than bowls especially when determining the depth of the portion size. Plates were used during the colour photo shoot instead of bowls.

4.3 PHASE 2: VALIDATION OF THE FOOD PHOTOGRAPH SERIES WITH ACTUAL DISHING UP SESSIONS

Validation of the FPS was determined by comparing relevant photograph portion size with actual dished up portion size from food items and pre-prepared dishes by caregivers / mothers. Distribution of data was tested with the Shapiro-Wilk's test and found normal. Thus all statistical tests were conducted on parametric data.

Table 4.7 provides a summary of the means, standard deviations, medians, inter-quartile ranges, differences and percentage differences between the identified photograph portion sizes and dished up portion sizes. The highest mean for the photograph portions was for crumbly *pap* (338.5 g) and the lowest for stiff *pap* (235.7 g) (Table 4.7). The dished up portions ranged from 259.0 g (maize meal and spinach) to 152.1 g for crumbly *pap*. Mean differences between the identified photograph portion and the dished up portion ranged between 186.4 g (crumbly *pap*) and 4.9 g (maize meal and pumpkin).

The paired t-test was conducted to determine agreement at group level (Robinson *et al.*, 1997). Results for the paired t-test showed a significant difference ($p < 0.05$) between the photograph portion sizes and the dished up portion sizes for soft porridge and crumbly *pap* (Table 4.7). There were no significant differences between the photograph portion sizes and dished up portion sizes for any of the other dishes.

The percentage difference was used to determine agreement at group level. It is an indication of the size and direction of the error present between the two methods (dishing up portions and the photograph portions) (Gibson, 2005). Percentage differences were highest for crumbly *pap* (14.9 g) indicating that the photographs overestimate the portion size for crumbly *pap*. Photographs underestimated the actual portion size for all other dishes since the percentage differences were all below zero (soft porridge, maize meal and *imifino*, maize meal and spinach, maize meal and pumpkin, stiff *pap* and samp and beans). These percentage differences ranged between -11.0 g (maize meal and pumpkin) and 39.3 g (maize and *imifino*). Percentage differences were considered acceptable (10 - 20% difference) for soft porridge, maize meal and pumpkin, and crumbly *pap*. Percentage differences for maize meal and *imifino*, maize meal and spinach, stiff *pap* and samp and beans were not acceptable ($> 20\%$ differences).

The Pearson correlation coefficients measure the strength and direction of the association between the two different measurements at individual level (Gibson, 2005). Pearson correlation coefficients between the photograph portion sizes and dished up portion sizes were poor (< 0.20) for soft porridge (0.14), maize meal and *imifino* (-0.03), maize meal and spinach (0.14), maize meal and pumpkin (0.10) and crumbly *pap* (0.16). The correlation was acceptable (> 0.20) for only stiff *pap* and samp and beans (0.29) (Table 4.8).

The Bland-Altman analyses were done according to three statistical tests (percentage agreement, Pearson correlation (between the mean and the mean difference between the two portion sizes) and the width of LOA in relation to portion size). Agreement in terms of the number of participants falling between the LOA was acceptable for all dishes ($> 95\%$) as shown in Table 4.9. The width of the LOA was compared against the different portion sizes to determine the clinical relevance of the difference. The width of the LOAs compared to the portion sizes was poor for all dishes as it was larger than the largest portion size (serving spoon). Large bias are present for most of the dishes, including soft porridge (0.20), maize meal and *imifino* (-0.49), maize meal and spinach (0.37), stiff *pap* (0.35), samp and beans

(0.59) and crumbly *pap* (0.64). The only dish where bias was not present was maize meal and pumpkin (-0.10).

The summary of the statistical results (Table 4.10) illustrate that the validity of the food photographs in portion size estimation was overall poor except for the stiff *pap* which was acceptable.

All five statistical tests point to poor validity of the photographs in portion size estimation for the following dishes soft porridge, maize meal and *imifino*, maize meal and spinach, maize meal and pumpkin, *samp* and beans and *crumbly pap* meanwhile the stiff *pap* validity test was acceptable.

Table 4.7. Means, standard deviations, medians, inter-quartile ranges, differences and percentage differences between the photograph weights and dished up weights (N = 48)

Dish	Photograph weights		Dished up weights		Difference (g)			Percentage difference (%)		
	Mean (SD)	Median (IQ Range)	Mean (SD)	Median (IQ Range)	Mean (SD)	Median (IQ Range)	CI	Mean (SD)	Median (IQ Range)	CI
Soft porridge	315.2 (158.6)	320.00 (710.0)	251.4 (129.9)	249.0 (490.0)	63.8* (189.6)	23.0 (395.0)	12.9 – 542.5	-11.5 (49.6)	-18.2 (84.0.0)	-114.7 – 26.4
Maize meal and <i>imifino</i>	237.7 (166.2)	180.0 (670.0)	204.2 (97.5)	208.0 (326.0)	33.6 (195.5)	-27.5 (54.0)	-164.2 – 430.5	-39.3 (33.9)	-48.5 (148.0)	-85.2 – -17.1
Maize meal and spinach	270.3 (149.3)	215.0 (595.0)	259.0 (101.9)	265.0 (335.0)	11.3 (168.9)	-30.5 (89.0)	-159.0 – 309	-26.8 (35.0)	-37.2 (148.0)	-83.2 – 3
Maize meal and pumpkin	252.0 (112.3)	200.0 (300.0)	236.4 (104.2)	235.5 (352.0)	5.9 (163.2)	-1.5 (261.0)	-39.7 – 278.7	-11.0 (56.1)	-30.5 (159.0)	-74.9 – 86.9
Stiff <i>pap</i>	234.7 (156.3)	220.0 (635.0)	221.7 (109.2)	221.0 (476.0)	13.0 (167.0)	-36.0 (231.0)	-32.6 – 292.9	-21.3 (49.9)	-34.9 (115.0)	-68.2 – 66.0
<i>Samp</i> and beans	335.0 (263.1)	220.0 (825.0)	207.4 (136.5)	136.0 (435.0)	127.6 (260.1)	20.0 (546.0)	-167.0 – 519.4	-21.9 (54.9)	-43.6 (127.0)	-95.1 – 46
Crumbly <i>pap</i>	338.5 (199.3)	240.0 (840.0)	152.1 (94.6)	109.0 (307.0)	186.4* (206.8)	156.0 (465.0)	103.8 – 698.2	14.9 (112.3)	-2.8 (180.0)	-125.8 – 85.

Pap and *imifino* = stiff maize meal porridge and spinach,

Soft porridge = thin maize meal porridge,

Stiff *pap* = stiff maize meal porridge,

Samp and beans = dried kernels and sugar beans,

Crumbly *pap* = dry maize meal porridge

*Wilcoxon signed rank t-test significant at $p < 0.05$, **Wilcoxon signed rank t-test significant at $p < 0.01$, *** Wilcoxon signed rank t-test significant at $p < 0.001$

Difference (g) = (Photograph portion – Dishing up portion), Percentage difference (%) = [(Photograph portion – Dishing up portion) / Dishing up portion X 100]

SD = standard deviation, IQ range = Inter quartile range, CI = Confidence interval

Table 4.8 Pearson is correlation coefficients between photograph weights and dished up weights

Dish	Sample Size	Pearson Correlation
	n	R
Soft porridge	41	0.14
Maize meal and <i>imifino</i>	22	-0.03
Maize meal and spinach	26	0.13
Maize meal and pumpkin	25	0.08
<i>Stiff pap</i>	35	0.24
<i>Samp</i> and beans	11	0.28
<i>Crumbly pap</i>	33	0.15

Table 4.9. Bland-Altman analyses for the photograph weights and dished up weights

Dish	Sample Size	Percentage agreement ‡	Limits of agreement		Pearson Correlation	Limit Of Agreement vs portion size #
			Lower limit	Upper limit		
Soft porridge	48	96.5	-315.4	443.0	0.20	> 1 x serving spoon portion
Maize meal and <i>imifino</i>	48	97.3	-357.4	424.6	-0.49	> 1 x serving spoon portion
Maize meal and spinach	48	95.0	-326.7	347.3	0.37	> 1 x serving spoon portion
Maize meal and pumpkin	48	95.3	-320.0	331.9	-0.10	> 1 x serving spoon portion
<i>Stiff pap</i>	48	96.0	-321.0	347.0	0.35	> 1 x serving spoon portion
<i>Samp</i> and beans	48	96.8	-393.6	647.8	0.59	> 1 x serving spoon portion
<i>Crumbly pap</i>	48	95.3	-225.6	598.4	0.64	> 1 x serving spoon portion

Upper limit of agreement = Mean difference + 2 x SD of the difference

Lower limit of agreement = Mean difference – 2 x SD of the difference

Percentage agreement = Number of participants within LOA

r_{BA} = Correlation between mean and difference

LOA is considered clinical acceptable if it is smaller than mean difference ± 1 X small portion

‡ Percentage of data points between the limits of agreement

Table 4.10. Summary of statistical results for the validation of the portion size photographs

Dish	Agreement	Agreement	Strength of association	Percentage agreement	Presence of bias	Limits of Agreement	Final validity*
	Wilcoxon signed rank test	Percentage difference	Spearman correlations	Bland-Altman	Bland-Altman	Bland-Altman (Width of LOA)	> 3 acceptable validation results
Level of validation	Group	Group	Individual	Individual	Individual	Individual	
Soft porridge	Poor	Acceptable	Poor	Good	Poor	Wide LOA	Poor
Maize meal and <i>imifino</i>	Good	Poor	Poor	Good	Poor	Wide LOA	Poor
Maize meal and spinach	Good	Poor	Poor	Good	Poor	Wide LOA	Poor
Maize meal and pumpkin	Good	Acceptable	Poor	Good	Acceptable	Wide LOA	Acceptable
Stiff <i>pap</i>	Good	Poor	Acceptable	Good	Poor	Wide	Acceptable
<i>Samp</i> and beans	Good	Poor	Acceptable	Good	Poor	Wide LOA	Acceptable
<i>Crumbly pap</i>	Poor	Acceptable	Poor	Good	Poor	Wide LOA	Poor

Wilcoxon signed rank test agreement at group level $p < 0.05$ = Good, $p > 0.05$ Poor

Percentage difference: $< 10\%$ = Good, $11 - 20\%$ = Acceptable, $> 20\%$ = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good = > 0.50 , Acceptable $0.21 - 0.50$ and Poor < 0.20

Agreement Bland-Altman: < 1 x small portion = Narrow LOA, ≈ 1 x small portion = Acceptable, > 1 small portion = Wide LOA

Final validity* = Four or more of statistical methods indicate agreement

CHAPTER 5 DISCUSSION

The aim of the study was to develop and validate a portion size FPS to determine maize intake of infants and young children in deep rural areas in the EC. Child development and growth in sub-Saharan African countries such as South Africa are severely affected by chronic and acute malnutrition, especially in rural areas. Deep rural area such as EC are characterised by a high prevalence of poverty and underdevelopment. Subsistence farming is a major source of food and maize consumption is part of an ethnic tradition and culturally distinct dietary pattern (Lombard *et al.*, 2013, Lombard *et al.*, 2014). It has furthermore been well documented that the home grown maize in these rural areas are extremely high in mycotoxins (Burger *et al.*, 2010). Mycotoxins are low-molecular-weight metabolites that are produced by fungi (Miller, 1995) that grow on the maize.

An adult cultural specific QFFQ was previously developed for this area (Lombard *et al.*, 2013). Mothers / caregivers (N = 100) of IYC completed the QFFQ to identify food items and dishes represented on the cultural specific adult QFFQ that is commonly used as food for IYCF (unpublished data). This unpublished data was used to identify food items and dishes to be represented on the age appropriate food photographs series. Items not identified as IYC food or dishes were removed from the original adult QFFQ. Only food items and dishes consumed by 80% or more of the children (0 - 24 months) were included in the FPS. Nelson *et al.*, 1996 stated that measurement error in dietary assessment usually occurs because participants are unable to describe portion sizes accurately. For this reason household measurements (spoons, tablespoons and ladles) that mothers and caregivers mentioned in the adult cultural specific QFFQ were used. As stated by Posner *et al.*, (1992) a two-dimensional model such as photographs and food models have been shown to increase accuracy during portion size estimation in semi- or illiterate populations. A female born and raised in a rural area in the EC prepared the traditional, cultural specific food items and dishes. The average angle of viewing when a person is seated at a table, (420 above the horizon) was used for all photographs (Nelson *et al.* 1994). Each IYC food item identified was photographed, accompanied by a teaspoon, tablespoon and large serving ladle. The final FPS consisted of 13 food items / dishes and 3 portions per dish. There are also 40 ratio photos for 10 food items / dishes.

Lombard *et al.* (2013) stated that the colour of the plate, type of plate, background of the photograph and scale influence the validity of the portion size. Meanwhile Nelson *et al.* (1994)

mentioned that colour; order of presentation and the size of the photographs have an impact on the validation and increase accuracy. On the other hand Lucas *et al.* (1995) mentioned that the type of food items, the containers and preparation methods influence the validation of the complementary food portion sizes.

Agreement at group level was good for all dishes except soft porridge and crumbly *pap* when the t-test was conducted. This indicated that there were no significant differences between the photograph portion sizes and the actual dished up portion size. Agreement for soft porridge and crumbly *pap* was poor but these are both amorphous foods and thus more difficult to identify (Lucas *et al.*, 1995). However, agreement at group level in terms of percentage difference was acceptable for soft porridge, maize meal and pumpkin and crumbly *pap* and not for the other dishes.

The negative percentage difference means the photograph underestimated the actual dished up weight. Venter *et al.*, (2000) found percentage difference of *samp* and beans ranging from -15.6% to 42%, while the percentage difference for soft porridge ranged between 14.2% and 23% and that of stiff *pap* was between -11.4 and 0%.

Poor Pearson correlation coefficients for soft porridge, maize meal and *imifino*, maize meal and spinach, maize meal and pumpkin and crumbly *pap* indicated poor strength and association at individual level. Only two dishes (stiff *pap* and *samp* and beans) had acceptable Pearson correlation coefficients

The width of the LOA were compared against the different portion sizes to determine the clinical relevance of the differences compared to the portion sizes was poor for all dishes. The over estimation of the portion sizes means there was proportional bias. In the study conducted by Venter, *et al.*, (2000) the researchers found errors in estimation for soft porridge, *samp* & beans and stiff *pap* even though the study had adult participants. The agreement at individual level for Bland-Altman analyses indicated good agreement. This was however mostly based on the very wide LOA which is a reflection of the SD. This was the case for all the dishes.

When summarizing the different aspects of validity (agreement, strength of association and bias) results indicate that overall validity is poor for soft porridge, maize meal and *imifino*, maize meal and spinach and crumbly *pap*. Over all validity was acceptable for maize meal and pumpkin, stiff *pap* and *samp* and beans.

Lucas *et al.*, (1995) concluded that participants had difficulties correctly estimating portion sizes when the actual food differed in shape, thickness, number of portion sizes, and distribution on a plate from that in the food photographs. That was true in this study especially since most of the dishes included in the study was amorphous. The error in some of the dishes may due to the fact amorphous foods have different shapes and can also change shapes. The clear signs of portion size estimation such as depth and spread, by which participants could relate actual foods to that in food photograph are absent in amorphous foods (Venter, *et al.*, 2000). Amorphous foods in this study which could easily change shape and depth are stiff *pap*, soft porridge, *samp* and beans, maize meal and *imifino*, maize meal and spinach, maize meal and pumpkin. These foods occur either as grains or powder. The ability of participant's to correctly estimate amorphous foods differ in different studies. In the study by Nelson, *et al.*, (1996) the higher estimation for cornflakes was compared to mashed potatoes, spaghetti and chips.

Other factors could also have influenced portion size estimation results found in this study. Some participants in this study use cups or saucers to dish up and not serving ladles. Even though an attempt was made to match the serving utensils used in the photographs with that used in participants' homes as far as possible it was not always possible due to the wide variety of serving utensils used (Lombard *et al.*, 2013). Furthermore, some participants just decant the soft porridge into the plate and do not measure portion size at all. Current state of satiety and the high levels of poverty could also have influenced the overestimation found in this study. The extremely high rates of food insecurity and poverty in this deep rural community could possibly be because some participants thought they could take the dished up food home. Overestimation on the other hand was not consistent with any specific portion sizes. Some studies observed a flat slope syndrome where participants overestimate smaller portions and underestimate larger portions (Huybregts *et al.*, 2007, Venter *et al.*, 2000, Robinson *et al.*, 1997, Nelson *et al.*, 1994 and Faggiano *et al.*, 1992). In the contrary in our study the participants did not underestimate the large portions or overestimate the smaller portions.

CHAPTER 6 CONCLUSION

Based on the results it can be concluded that the newly developed FPS is valid for groups for soft porridge and crumbly *pap*. Results for the maize meal & *imifino*, maize meal and spinach, maize meal & pumpkin, stiff *pap* and *samp* and beans should be interpreted with caution as group validity for these dishes are questionable. The FPS is further valid for individual use for stiff *pap* and *samp* & beans while results for crumbly *pap*, soft porridge, maize meal and *imifino*, maize meal and spinach and maize meal and pumpkin should be interpreted with caution as the individual validity for these were questionable.

6.1 STRENGTH OF THE STUDY

- The study was part of the longitudinal, cohort study called Philasana, which was advantageous as there were mothers / caregivers who were already part of the study.
- Dishes and portion sizes were based on information obtained from this specific population and are thus considered very relevant.
- A wide variety of statistical tests were conducted to determine validity and thus provided valuable information on the size and direction of error and bias present.

6.2 LIMITATIONS OF THE STUDY

- Due to the widely spread geographical area and the lack of infrastructure the sampling was based on a voluntary, snowball sample which might result in possible selection bias and prevented the use of random sampling.
- The small sample size had a large impact on the results. Although all efforts have been made to increase the sample size, including more participants was not possible due to logistical reasons such as time and funding.

CHAPTER 7 REFERENCES

- Agostoni, C., Decsi, T., Fewtrell, M., Goulet, O., Kolacek, S., Koletzko, B., Fleischer Michaelsen, K., Moreno, L., Puntis, J., Rigo, J., Shamir, R., Szajewska, H., Turck D. & van Goudoever, J. 2008. Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, 46:99–110.
- Alemayehu, A.A., Abebe, Y. & Gibson, R.S. 2011. A 24-h recall does not provide a valid estimate of absolute nutrient intakes for rural women in southern Ethiopia. *Nutrition*, 27(9):919–924.
- Allen, L., de Benoist, B., Dary, O. & Hurrell, R. 2006. *Guidelines on food fortification with micronutrients*. <http://www.who.int/nutrition/publications/micronutrients/9241594012/en/>
Date of access: 10 Oct. 2015.
- Altman, D.G. & Bland, J.M. 1994. Quartiles, quintiles, centiles, and other quantiles. *British Medical Journal*, 309:996.
- Bakker, I., Twisk, J.W., Van Mechelen, W., Mensink, G.B. & Kemper, H.C. 2003. Computerization of a dietary history interview in a running cohort, evaluation within the Amsterdam Growth and Health Longitudinal Study. *European Journal of Clinical Nutrition*, 57:394-404.
- Bembridge, T.J. 1987. Some aspects of household diet and family income problems in Transkei. *South African Medical Journal*, 72:425-428.
- Bennett, J.W. & Klich, M. 2003. Mycotoxins. *Clinical Microbiology Review*, 16:497.
- Beyers, M.J.C., Hammer, M.L. & Groenewald, G. 1979. Foods commonly used by the Xhosa-speaking people of Transkei and Ciskei: weights for various household measures. *Journal of Dietetics and Home Economics*, 7:96–100.
- Black, A.E., Goldberg, G.R., Jebb, S.A., Livingstone, M.B.E., Cole, T.J. & Prentice, A.M. 1991. Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. *European Journal of Clinical Nutrition*, 45:583-599.

- Black, R.E., Allen, L.H., Bhutta, Z.A., Caulfield, L.E., de Onis, M., Ezzati, M., Mathers, C. & Rivera, J. for the Maternal and Child Undernutrition Study Group. 2008. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*, 371:243–260.
- Bland, J.M. & Altman, D.G. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1:307-311.
- Bolger, M., Coker, R.D., DiNovi, M., Gaylor, D., Gelderblom, W., Olsen, M., Paster, N., Riley, R.T., Shephard, G. & Speijers, G.J.A. 2001. Fumonisin. In: Safety evaluation of certain mycotoxins in food. WHO Food Additives Series No. 47, FAO Food and Nutrition Paper No. 74, Prepared for the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Geneva: WHO: 103-279.
- Borrelli, R., Cole, T.J., Biase, G.D. & Contaldo, F. 1989. Some statistical considerations on dietary assessment methods. *European Journal of Clinical Nutrition*, 43:453-463.
- Brown, K.H. & Lutter, K.L. 2000. Potential role of processed complementary foods in the improvement of early childhood nutrition in Latin America. *Food and Nutrition Bulletin*, 21(1):5-11.
- Burger, K., Kern, M. & Coleman, K. 2007. Characteristics of self-selected portion size in young adults. *Journal of the American Dietetic Association*, 107(4): 611-618.
- Burger, H.M., Lombard, M.J., Shephard, G.S., Reeder, J.R., van der Westhuizen, L. & Gelderblom, W.C.A. 2010. Dietary fumonisin exposure in a rural population of South Africa. *Food and Chemical Toxicology*, 8:2103–2108.
- Byrd-Bredbenner, C. & Schwartz, J. 2004. The effect of practical portion size measurement aids on the accuracy of portion size estimates made by young adults. *Journal of Human Nutrition and Dietetics*, 17:351-357.
- Cade, J., Thompson, R., Burley, V. & Warm, D. 2002. Development, validation and utilisation of food frequency questionnaires – a review. *Public Health Nutrition*, 5(4):567-587.
- Carithers, T.C., Talegawkar, S.A., Rowser, M.L., Henry, O.R., Dubbert, P.M., Dogle, M.L., Taylor, H.A. & Tucker, K.L. 2009. Validity and calibration of food frequency

- questionnaires used with African-American adults in the Jackson Heart Study. *Journal of the American Dietetic Association*, 109:1184-1193.
- Chambers, E., Godwin, S.L. & Vecchio, F.A. 2000. Cognitive strategies for reporting portion sizes using dietary recall procedures. *Journal of American Dietetic Association*, 100(8):891–897.
- Davidsson, L. 1996. Iron bioavailability from weaning foods: the effect of phytic acid. In: *Micronutrient Interactions. Impact on Child Health and Nutrition*. Washington, DC: *International Life Sciences Institute*, 21–27.
- Dewey, K.G., & Brown, K.H. 2003. Update on technical issues concerning complementary feeding of young children in developing countries and implication for intervention programs. *Food and Nutrition Bulletin*, 24(1):5-28.
- Diliberti, N., Bordi, P., Conklin, M., Roe, L. & Rolls, B. 2004. Increased portion size leads to increased energy intake in a restaurant meal. *Obesity Research*, 12(3):562-568.
- DOH (Department of Health). 2011a. The *Tshwane declaration of support for breastfeeding in South Africa*. <http://www.gov.za/world-breastfeeding-week-and-tshwane-declaration-support-breastfeeding-south-africa-world>. Date of access: 30 Sep. 2015.
- DOH (Department of Health). 2011b. *National Nutrition Week 2011: Feeding Smart from the Start*. <http://www.nutritionweek.co.za/index1.html>. Date of access: 30 Sep. 2015.
- DOH (Department of Health). 2013. *Roadmap for Nutrition in South Africa 2013-2017*. <http://www.health-e.org.za/2015/06/04/strategy-roadmap-for-nutrition-south-africa-2013-2017/>. Date of access: 30 Sep. 2015.
- Dowswell, C. R., Paliwal, R.L. & Cantrell, R.P. 1996. *Maize in the third world*. Boulder (CO): Westview Press.
- De Waal, A. & Whiteside A. 2003. New variant famine: AIDS and food crisis in southern Africa. *Lancet*, 362(9391):1234-1237.
- De Souza, C. 2001. Education, in: Kane-Berman, J., Henderson, J., De Souza, C., Wilson, F., Schlemmer, L., Laurence, P., Schönteich, M., Dimant, T. & Corrigan, T. (eds.). *South*

Africa survey, 2001/2002. Johannesburg: South African Institute of Race Relations. 239-283.

Easton, V.J. & McColl, J.H. *Statistics glossary*.

http://www.stats.gla.ac.uk/steps/glossary/paired_data.html. Date of access: 10 Oct. 2015.

EC (European Commission). 2000. *Opinion of the Scientific Committee on Food on Fusarium Toxins, Part 3: Fumonisin B1(FB1)*. Health and Consumer Protection Director-General, Directorate C –Scientific Opinions, SCF/CS/CNTM/MYC/24 Rev 4 Final. Brussels: European Commission.

Elwood, P.C. & Bird, G.A. 1983. A photographic method of diet evaluation. *Human Nutrition*, 37(A):474-477.

Engelmann, M.D., Sandström, B. & Michaelsen, K.F. 1998. Meat intake and iron status in late infancy: an intervention study. *Journal of Pediatric Gastroenterology and Nutrition*, 26:26-33.

Faber, M. 2004. Complementary foods consumed by 6–12 month old rural infants in South Africa are inadequate in micronutrients. *Public Health Nutrition*, 8:373–381.

Faber, M. & Benade, A.J.S. 2007. Breastfeeding, complementary feeding and nutritional status of 6 – 12-month-old infants in rural Kwa–Zulu Natal. *South African Journal of Clinical Nutrition*, 20(1):16-24.

Faber, M. 2010. Nutrition in vulnerable communities in economically marginalized societies. *Livestock Science*, 130(1):110-114.

Faggiano, F., Vineis, P., Cravanzola, V., Pisani, P., Xompero, G., Riboli, E. & Kaaks, R. 1992. Validation of a method for the estimation of portion size. *Epidemiology*, 3(4):379-382.

FAO (Food and Agriculture Organization). 2004. *Worldwide regulations for mycotoxins in food and feed in 2003*. Food and Nutrition Paper No. 81.

<http://www.fao.org/docrep/007/y5499e/y5499e00.htm>. Date of access: 25 Sep. 2015.

- Fewtrell, M.S., Morgan, J.B., Duggan, C., Gunnlaugsson, G., Hibberd, P.L., Lucas, A. & Kleinman, R.E. 2007. Optimal duration of exclusive breastfeeding: What is the evidence to support current recommendations? *American Journal of Clinical Nutrition*, 85:635-638.
- Fowles, E.R., Sterling, B.S. & Walker, L.O. 2007. Measuring dietary intake in nursing research. *The Canadian Journal of Nursing Research*, 39:146-165.
- Flegal, K.M. 1999. Evaluating epidemiologic evidence of the effects of food and nutrient exposures. *The American Journal of Clinical Nutrition*, 69:S1339-S1344.
- Flood, V.M., Smith, W.T., Webb, K.L. & Mitchell, P. 2004. Issues in assessing the validity of nutrient data obtained from a food-frequency questionnaire: folate and vitamin B12 examples. *Public Health Nutrition*, 7(6):751-756.
- Foster, E., Matthews, J.N.S., Nelson, M., Harris, J.M., Mathers, J.C. & Adamson, A.J. 2006. Accuracy of estimates of food portion size using food photographs – the importance of using age appropriate tools. *Public Health Nutrition*, 9(4):509-514.
- FSA (Food Standards Agency): Food Safety Information Bulletin 88. 1997. *Food portion sizes*. United Kingdom: MAFF, Department of Health and Scottish Exchange. <http://archive.food.gov.uk/maff/archive/food/bulletin/1997/no88/atlas.htm>. Date of access: 30 Sep. 2015.
- Gibson, R.S. 2005. *Principles of nutritional assessment*. 2nd Ed. Oxford: Oxford University Press.
- Gibson, R.S. & Ferguson, E.L. 1996. Food processing methods for improving the zinc content and bioavailability of home-based and commercially available complementary foods. In: micronutrient interactions. *Impact on child health and nutrition*. Washington, DC: United State Agency for International Development (USAID), 50–57.
- Gibson, R.S. & Ferguson, E. 2008. An interactive 24-h recall for assessing the adequacy of iron and zinc intakes in developing countries. Washington: HarvestPlus Technical Monograph Series 8.

- Gibson, R. & Huddle, J. 1998. Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria. *American Journal of Clinical Nutrition*, 67(4):702–709.
- Glinsmann, W.H., Bartholmey, S.J. & Coletta, F. 1996. Dietary guidelines for infants: a timely reminder. *Nutrition Reviews*, 54:50–57.
- Godwin, S.L., Chambers, E. & Cleveland, L. 2004. Accuracy of reporting dietary intake using various portion-size aids in-person and via telephone. *Journal of the American Dietetic Association*, 104:585-594.
- Gong, Y.Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J. & Wild, C.P. 2002. Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *British Medical Journal*, 325:20–21.
- Gong, Y.Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J., & Wild, C.P. 2003. Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. *International Journal of Epidemiology*, 32:556–560.
- Goyarts, T., Danicke, S., Brussow, K.P., Valenta, H., Ueberschar & K.H., Tiemann, U. 2007. On the transfer of the *Fusarium* toxins deoxynivalenol and zearalenone from sows to their fetuses during days 35–70 of gestation. *Toxicology Letters*, 171:38–49.
- Hess, M.A. 1997. *Portion photos of popular foods*. Chicago: American Dietetic Association.
- Higgins, J.A., LaSalle, A.L., Zhaoxing, P., Kasten, M.Y., Bing, K.N., Ridzon, S.E. & Witten, T.L. (2009). Validation of photographic food records in children: are pictures really worth a thousand words? *European Journal of Clinical Nutrition*, 63(8):1025-1033.
- Hepworth, S.J., Hepworth, L.J., Hardie, L.K., Fraser, V.J., Burley, R.S., Mijal, C.P., Wild, R., Azad, P.A., Mckinney, P.A. & Turner, P.C. 2012. Deoxynivalenol exposure assessment in a cohort of pregnant women from Bradford, United Kingdom. *Food Additives Contaminants*, 29:269-276.
- Horton, R. 2005. Newborn Survival: putting children at the centre. *Lancet Neonatal Survival Series*. <http://www.thelancet.com/series/neonatal-survival>. Date of access: 01 Oct. 2015.

- Howat, P.M., Mohan, R., Champagne, C., Monlezun, C., Wozniak, P. & Bray, G.A. 1994. Validity and reliability of reported dietary intake data. *Journal of American Dietetic Association*, 94(2):169-173.
- HSRC (Human Sciences Research Council). 2004. Food Security in South Africa: Key Policy Issues for the Medium Term. *Integrated Rural and Regional Development, Southern African Regional Poverty Network*. Pretoria. **Error! Hyperlink reference not valid.** Date of access: 30 Sep. 2015
- IARC (International Agency for Research on Cancer). 2002. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. International Agency for Research on Cancer, Lyon, 301–366.
- Kimanya, M.E., De Meulenaer, D., Baert, K., Tiisekwa, B., Van Camp, J., Samapundo, S., Lachat, C. & Kolsteren, P. 2010. Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. *Molecular Nutrition and Food Research*, 54:1659-1667.
- Kimani-Murage, E.W., Kahn, K., Pettifor, J.M., Tollman, S.M., Dunger, D.B., Gómez-Olivé, X.F. & Norris, S.A. 2010. The prevalence of stunting, overweight and obesity, and metabolic disease risk in rural South African children. *BioMed Central Public Health*, 10:158.
- Kohlmeier, L. & Bellach, B. 1995. Exposure assessment error and its handling in nutritional epidemiology. *Annual Review of Public Health*, 16(1):43-59.
- Kral, T.V.E., Roe, L.S. & Rolls, B.J. 2004. Combined effects of energy density and portion size on energy intake in women. *American Journal of Clinical Nutrition*, 79(6):962-968.
- Kramer, M.S. & Kakuma, R. 2002. Optimal duration of exclusive breastfeeding. *Cochrane Database of Systematic Reviews, Issue 1. Art.No.CD003517*.
DOI:10.1002/14651858.CD003517.
- Krebs, N.F., Westcott, J.E., Butler, N., Robinson, C., Bell, M. & Hambidge, K.M. 2006. Meat as a first complementary food for breastfed infants: feasibility and impact on zinc intake and status. *Journal of Paediatric and Gastroenterology Nutrition*, 42(2):207-214.

- Kruger, H.S., Swart, R., Labadarios, D., Dannhauser, A. & Nel J.H. 2007. Anthropometric status. In: Labadarios D (ed)(2007) The National Food Consumption Survey – Fortification Baseline (NFCS-FB): The knowledge, attitude, behaviour and procurement regarding fortified foods, a measure of hunger and the anthropometric and selected micronutrient status of children aged 1 – 9 years and women of child bearing age: South Africa, 2005. Pretoria: Directorate: Nutrition, Department of Health.
- Labadarios, D., Van Middelkoop, A., Coutsooudis, A., Eggers, R., Hussey, G., Ijsselmuiden, C. & Kotze J.P. 1996. South African Vitamin A Consultative Group (SAVACG). Anthropometric, vitamin A, iron and immunization coverage status in children aged 6 - 71 months in South Africa, 1994. *South African Medical Journal*, 86:354-357.
- Labadarios, D., Steyn, N., Maunder, E., MacIntyre U., Swart, R., Gericke G., Huskisson J., Dannhauser A., Voster, H.H. & Nesamvuni A.E. 1999. The National Food Consumption Survey (NFCS): Children aged 1- 9 years, South Africa.
<http://www.sahealthinfo.org/nutrition/foodconsumption.html>. Date of access: 01 Oct. 2015.
- Labadarios, D., Steyn, N., Maunder, E., Macintyre, U., Swart R., Gericke, G., Huskisson, J., Dannhauser, A., Vorster, H.H. & Nesamvuni, E. 2000. The National Food Consumption Survey (NFCS): Children Aged 1-9 Years, South Africa.
<http://www.sun.ac.za/nutrition/nfcs.html>. Date of access: 01 Oct. 2015.
- Lazarte, C.E., Encinas, M,E., Alegre, C. & Granfeldt, Y. 2012. Validation of digital photographs, as a tool in 24-h recall, for the improvement of dietary assessment among rural populations in developing countries. *Nutrition Journal*, 11:61.
- Lombard, M., Steyn, N., Burger, H., Charlton, K. & Senekal, M. 2013. A food photograph series for identifying portion sizes of culturally specific dishes in rural areas with high incidence of oesophageal cancer. *Nutrients*, 5:3118-3130.
- Lombard, M., Steyn, N., Burger, H., Charlton, K. & Gelderblom, W. 2014. A proposed method to determine fumonisin exposure from maize consumption in a rural South African population using a culturally appropriate FFQ. *Public Health Nutrition*, 17(1):131-138.

- Lucas, F., Niravong, M., Villemint, S., Kaaks, R. & Clavel-Chapelon, F. 1995. Estimation of food portion size using photographs: validity, strengths, weaknesses and recommendations. *Journal of Human Nutrition and Dietetics*, 8:65-74.
- Lucas B. 1999. *Normal nutrition from infancy through adolescence*. In: *Handbook of Pediatric Nutrition*, 2nd Ed. Gaithersburg: Aspen Publishers Incorporated.
- MacIntyre, U.E., Venter, C.S. & Vorster, H.H. 2001. A culture-sensitive quantitative food frequency questionnaire used in an African population: 1. Development and reproducibility. *Public Health Nutrition*, 4(1):53-62.
- Mamiro, P.S., Kolsteren, P., Roberfroid, D., Tatala, S., Opsomer, A.S. & Van Camp, J.H. 2005. Feeding practices and factors contributing to wasting, stunting, and iron-deficiency anaemia among 3–23 month old children in Kilosa district, rural Tanzania. *Journal of Health, Population and Nutrition*, 23:222–223.
- Marasas, W.F.O., Jaskiewicz, K., Venter, F.S. & van Schalkwyk, D.J. 1988. *Fusarium moniliforme* contamination of maize in oesophageal cancer areas in Transkei. *South African Medical Journal*, 74:110–114.
- Marjan, Z.M. 1995. Evaluation of food photographs for 24-hour recall method. *Malaysian Journal of Nutrition*, 1(2):95–104.
- Masson, L.F., McNeil, G., Tomany, J.O., Simpson, J.A., Peace, H.S., Wei, L., Grubb, D.A. & Bolton-Smith, C. 2003. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. *Public Health Nutrition*, 6(3):313-321.
- Miller, J.D. 1995. Fungi and mycotoxins in grain – implications for stored-product research. *Journal of Stored Products Research*, 31:1–16.
- Muehlhoff, E., Dirorimwe, C., Huang, S., Kheang, K.M. & Ry, L. K. 2011. *Complementary feeding for children aged 6-23 months. A recipe book for mothers and caregivers*. FAO/European Union food facility project.
- Munkvold, G.P. 2003. Cultural and genetic approaches to managing mycotoxins in maize. *Annual Review Phytopathology*, 41:99–116.

- Nelson, M., Atkinson, M. & Darbyshire, S. 1994. Food photography I: the perception of food portion size from photographs. *British Journal of Nutrition*, 72:649-663.
- Nelson, M., Atkinson, M. & Darbyshire, S. 1996. Food photography II: use of food photographs for estimating portion size and the nutrient content of meals. *British Journal of Nutrition*, 76:31-49.
- Nelson, M. & Haraldsdóttir, J. 1998a. Food photographs: practical guidelines I. Design and analysis of studies to validate portion size estimates. *Public Health Nutrition*, 1(4):219-230.
- Nelson, M. & Haraldsdóttir, J. 1998b. Food photographs: practical guidelines II. Development and use of photographic atlases for assessing food portion size. *Public Health Nutrition*, 1(4):231-237.
- Nuss, E.T. & Tanumihardjo, S.A. 2011. Quality protein maize for Africa: closing the protein inadequacy gap in vulnerable populations. *Advances in Nutrition*, 2:217–224.
- Okoth, S.H. & Ohingo, M. 2004. Dietary aflatoxin exposure and impaired growth in young children from Kisumu District, Kenya: Cross sectional study. *African Journal of Health Sciences*, 11:43–54.
- Ovaskainen, M.L., Paturi, M., Reinivuo, H., Hannila, M.L., Sinkko, H., Lehtisalo, J., Polari Pynnonen, O. & Mannisto, S. 2008. Accuracy in the estimation of food servings against the portions of food photographs. *European Journal of Clinical Nutrition*, 62(5):674-681.
- Poslusna, K., Ruprich, J., de Vries, J.H.M., Jakubikova, M. & van't Veer P. 2009. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. *British Journal of Nutrition*, 101:S73–S85.
- Posner, B.M., Smigelski, C., Duggal, A., Morgan, J., Cobb, J. & Cupples, L.A. 1992. Validation of two dimensional models for estimation of portion size in nutrition research. *Journal of the American Dietetic Association*, 92(6):738-740.
- Rheeder, J.P., Shephard, G. S., Vismar, H.F. & Gelderblom, W.C.A. 2009. Guidelines on mycotoxin control in South African foodstuffs: From the application of the Hazard Analysis

and Critical Control Point (HACCP) system to new national mycotoxin regulations.
PROMEC Unit, Medical Research Council: Cape Town

Robinson, F., Morrirtz, W., McGuinness, P. & Hackett, A. 1997. A study of the use of a photographic food atlas to estimate served and self-served portion sizes. *Journal of Human Nutrition and Dietetics*, 10(2):117– 124.

Robson, P.J. & Livingstone, M.B.E. 2000. An evaluation of food photographs as a tool for quantifying food and nutrient intakes. *Public Health Nutrition*, 3(2):183-192.

Rolls, B.J., Morris, E.L. & Roe, L.S. 2002. Portion size of food affects energy intake in normal weight and overweight men and women. *American Journal of Clinical Nutrition*, 76(6):1207-1213.

Rose, E.F. 1972. Some observations on the diet and farming practices of the people of the Transkei. *South African Medical Journal*, 46:1353-1358.

Schwartz, J. & Byrd-Bredbenner, C. 2006. Portion Distortion: Typical portion sizes selected by young adults. *Journal of the American Dietetic Association*, 106:1412-1418.

Seligson, F.H. 2003. Serving size standards: can they be harmonized? *Nutrition Today*, 38:247-253.

Shephard, G. S., Thiel, P. G., Stockenstrom, S., Sydenham, E. W. 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. *Journal AOAC International*, 79, 671–687

Shephard, G.S., Marasas, W.F.O., Burger, H-M., Somdyala, N.I.M., Rheeder, J.P., Van Der Westhuizen, L., Gatyeni, P. & Van Schalkwyk, D.J. 2007. Exposure assessment for fumonisins in the former Transkei region of South Africa. *Food Additives and Contaminants*, 24(6):621-629.

Shephard, G.S. 2008. The impact of mycotoxins on human health in developing countries. *Food Additives and Contaminants*, 25:146-151.

Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosan, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N.P., Dwane, N., Makoae,

- M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M.G., Jacobs, L., Faber, M. & SANHANES-1 Team. 2012. *South African National Health and Nutrition Examination Survey (SANHANES-1)*. Cape Town: HSRC Press.
- Small, L., Sidora-Arcoleo, K., Vaughan, L., Creed-Capsel, J., Chung, K. & Stevens, C. 2009. Validity and reliability of photographic diet diaries for assessing dietary intake among young. *Infant, Child, & Adolescent Nutrition*, 1(1):27-36.
- Smith, L.E., Stoltzfus, R.J. & Prendergast, A. 2012. Food chain mycotoxin exposure, gut health and impaired growth: a conceptual framework. *Advances in Nutrition*, 3:526–531.
- Steyn, N.P., Senekal, M., Norris, S.A., Whati, L., MacKeown, J.M. & Nel, J.H. 2006. How well do adolescents determine portion sizes of foods and beverages? *Asia Pacific Journal of Clinical Nutrition*, 15(1):35-42.
- Steyn, N.P., Nel, J.H., Parker, W-A., Ayah, R. & Mbithe, D. 2011. *Dietary, social, and environmental determinants of obesity in Kenyan women*. *Scandinavian Journal of Public Health*, 39(1):88–97.
- Subar, A.F., Ziegler, R.G., Thompson, F.E., Johnson, C.C., Weissfeld, J.L., Reding, D., Kavounis, K.H. & Hayes, R.B. 2001. Is shorter always better? Relative importance of questionnaire length and cognitive ease on response rates and data quality for two dietary questionnaires. *American Journal of Epidemiology*, 153(4):404-409.
- Turconi, M., Guarcello, M., Berzolari, F.G., Carolei, A., Bazzano, R. & Roggi, C. 2005. An evaluation of a color food photography atlas as a tool for quantifying food portion size in epidemiological dietary surveys. *European Journal of Clinical Nutrition*, 59(8):923-931.
- Turner, P.C., Collinson, A.C., Cheung, Y.B., Gong, Y., Hall, A.J., Prentice, A.M. & Wild, C.P. 2007. Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology*, 36:1119–1125.
- UNICEF (United Nations Children’s Fund). 2009. *Tracking progress on child and maternal nutrition. A survival and development priority*. New York. United Nations Children’s Fund.

- Van der Merwe, J., Kluyts, M., Bowley, N. & Marais D. 2007. Optimizing the introduction of complementary foods in the infant's diet: a unique challenge in developing countries. *Maternal and Child Nutrition*, 3:259-270.
- Venter, C.S., MacIntyre, U.E. & Vorster, H.H. 2000. The development and testing of a food portion photograph book for use in an African population. *Journal of Human Nutrition and Dietetics*, 13:205-218.
- Victora, C.G., Adai, L., Fall, C., Hallal, P.C., Martorell, R., Richter, L. & Sachdev, H.S., for the Maternal and Child Undernutrition Study Group. 2008. Maternal and child undernutrition: consequences for adult health and human capital. *Lancet*, 371: 340–357.
- Vorster, H.H., Badham, J.B. & Venter, C.S. 2013. An introduction to the revised food-based dietary guidelines for South Africa. *South African Journal of Clinical Nutrition*, 26(3):S1-S164.
- Wild, C.P. 2007. Aflatoxin exposure in developing countries: the critical interface of agriculture and health. *Food and Nutrition Bulletin*, 28:S372-S380.
- Willett, W.C. 1998. *Nutritional epidemiology*. 2nd Ed. New York: Oxford University Press.
- Williamson, D.A., Allen, H.R., Martin, P., Gerald, B. & Hunt, A. 2003. Comparison of digital photography to weighed and visual estimation of portion sizes. *Journal of American Dietetic Association*, 103(9):1139-1145.
- Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. & Aggarwal, D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition*, 80:1106–1122.
- Williamson, D.A., Allen, H.R., Martin, P., Gerald, B. & Hunt, A. 2003. Comparison of digital photography to weighed and visual estimation of portion sizes. *Journal of American Dietetic Association*, 103(9):1139-1145.
- WHO (World Health Organization). 1999. *Complementary Feeding of Young Children in Africa and the Middle East*. World Health Organisation.
<https://extranet.who.int/iris/restricted/handle/10665/66187>. Date of access: 01 Oct. 2015.

- WHO (World Health Organization). 2003. *Global Strategy for infant and Young child feeding*. World Health Organisation.
<http://www.who.int/nutrition/publications/infantfeeding/9241562218/en/>. Date of access: 01 Oct. 2015.
- WHO (World Health Organization). 2004. *Global strategy on diet, physical activity and health*. World Health Organisation. <http://www.int/dietphysicalactivity/strategy/eb11344/en/>.
Date of access: 01 Oct. 2015.
- WHO (World Health Organization). 2005. *Guiding principles for feeding non-breastfed children 6 – 24 months of age*. World Health Organisation.
http://www.who.int/maternal_child_adolescent/documents/9241593431/en/. Date of access: 01 Oct. 2015.
- WHO (World Health Organization). 2007a. *Planning guide for national implementation of the global strategy for infant and young child feeding*. World Health Organisation.
http://www.who.int/maternal_child_adolescent/documents/9789241595193/en/. Date of access: 01 Oct. 2015.
- WHO (World Health Organization). 2007b. *Acceptable, Feasible, Affordable, Sustainable and Safe Principles. Integrated Management of Childhood Illness. Complementary Course on HIV/AIDS; Module 3; Counselling the HIV Positive Mother*. World Health Organisation.
<http://motherchildnutrition.org/info/afass-principles.html>. Date of access: 25 Sep. 2015
- WHO (World Health Organization). 2009. *Infant and young child feeding: Model Chapter for textbooks for medical students and allied health professionals*. World Health Organisation.
<http://www.who.int/nutrition/publications/infantfeeding/9789241597494/en/>. Date of access: 01 Oct. 2015.
- WHO (World Health Organization). 2012a. *10 benefits to breastfeeding*.
<http://www.who.int/features/factfiles/breastfeeding/facts/en/index.html>. Date of access: 01 Oct. 2015.
- WHO (World Health Organization). 2012b. *United Nations International Children Fund & World Health Organization welcomes South Africa's efforts to protect and support breastfeeding*.
http://www.unicef.org/southafrica/media_10469.html. Date of access: 01 Oct. 2015.

- WHO (World Health Organization). 2014a. Children: *reducing mortality*.
<http://www.who.int/mediacentre/factsheets/fs178/en/>. Date of access: 25 Sep. 2015.
- WHO (World Health Organization). 2014b. Infant *and young child feeding*.
<http://www.who.int/mediacentre/factsheets/fs342/en/>. Date of access: 25 Sep. 2015.
- World Bank. 2006. Repositioning Nutrition as Central to Development – A Strategy for Large Scale Action.
<http://web.worldbank.org/WBSITE/EXTERNAL/TOPIC/EXHEALTHNUTRITIONANDPOPULATION/EXNUTRITION/0,,contentMDK:20613959~menuPK:282591~pagePK:210058~piPK:210062~thesitePK:282575,00.html>. Date of Access: 25 Sep. 2015.
- Wrieden, W., Peace, H., Armstrong, J. & Barton, K. 2003. A short review of dietary assessment methods used in National and Scottish research studies. Briefing paper prepared for: Working group on monitoring Scottish dietary targets workshop.
<http://www.food.gov.uk/multimedia/pdfs/scotdietassessmethods.pdf>. Date of access: 30 Sep. 2015.
- Young, L.R. & Nestle, M. 1995. Portion Sizes in Dietary Assessment: Issues and Policy Implications. *Nutrition Reviews*, 53(6):149–158.
- Young, L.R. & Nestle, M. 2002. The Contribution of Expanding Portion Sizes to the US obesity epidemic. *American Journal of Public Health*, 92(2):246-249.

CHAPTER 8 ANNEXURES

ADDENDUM 1: ETHICAL APPROVAL



Private Bag X6001,

Potchefstroom

South Africa

2520

Tel: 018 299-1111/2222

Web: <http://www.nwu.ac.za>

Ethics Office

Tel: 018-299 2092

Fax: 018-299 2088

Email: Minrie.Greeff@nwu.ac.za

Dr M Lombard

Nutrition

Dear Dr Lombard

HREC APPROVAL OF YOUR APPLICATION

Ethics number: NWU-00089-15-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

9 September 2015

Project title: Development and validation of portion size food photographs to determine maize intake of infants and young children in deep rural areas in the Eastern Cape Province of South Africa

Project leader/supervisor: Dr M Lombard

Student: A Rasekhala

Application type: Full Single

Risk level descriptor: Minimal

You are kindly informed that at the meeting held on 14/05/2015 of the HREC, Faculty of Health Sciences, the aforementioned was approved.

The period of approval for this project is from 09/09/2015 to 31/12/2015.

After ethical review:

Translation of the informed consent document to the language's applicable to the study participants should be submitted to the HREC (if applicable).

The HREC requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the protocol or other associated documentation must be submitted to the HREC prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form.

A progress report should be submitted within one year of approval of this study and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC must be notified if the study is temporarily suspended 2

or terminated. The progress report template is obtainable from Carolien van Zyl at Carolien.VanZyl@nwu.ac.za.. Annually a number of projects may be randomly selected for an external audit.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC complies with the South African National Health Act 61 (2003), the regulations on Research with Human Participants of 2014 of the Department of Health and Principles, the Declaration of Helsinki, 2013, the Belmont Report and the Ethics in Health Research: Principles, Structures and Processes (SANS document).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Ethics Office at Carolien.VanZy@nwu.ac.za or 018 299 2089.

Yours sincerely

Prof Minrie Greeff HREC Chairperson

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2015 Applications\Applications 04 - 14 May 2015\NWU-00089-15-S1 (T Lombard-A Rasekhala)\NWU-00089-15-S1 (M Lombard-A Rasekhala) - AL\NWU-00089-15-S1 (M Lombard-A Rasekhala) - AL.docm 9 September 2015

File reference: 9.1.5.3

ADDENDUM 2: CONSENT FORM



HREC Stamp

PARTICIPANT INFORMATION LEAFLET AND WRITTEN CONSENT FORM: (INCUKHACHA ZOMTHABATHI NGXELO NOVUMELWANO OLUBHALIWEYO)

Oomama okanye abanakekeli babantwana abangaphezulu kweminyaka elishumi elinesibhozo

UBUME BEPROJECT YOPHANDO:

Philasana Project: Unxibelelwano phakathi kwe mycotoxin exposure kunye nentsana nabantwana abakhula kwingxondorha zamaphandle ephondo lempumakoloni, eMzantsi Afrika (Pilot study).

UBUME BEZEMFUNDO/UPHANDO:

Uphuhliso kunye nevalidation yesiqingatha kunye nobungakanani bokutya, imifanekiso yokubona ubungakanani bombona wentsana kunye nabantwana abangaphantsi kwenyanga ukuya kwinyanga ezisgamashumi amabini anesine abahlala kwingxondorha zamaphandle ephondo leMpuma Koloni kuMzantsi Afrika.

Development and validation of portion size food photographs to determine maize intake of infants and young children (aged 0 - 24 months) living in deep rural areas in the Eastern Cape Province of South Africa.

REFERENCE NUMBERS: NWU-00207-14-S1

Reference number of sub study: NWU-00089-15-S1

PRINCIPAL INVESTIGATOR:

Dr Martani Lombard

ADDRESS:

Room 149, G16

North-West University

Faculty of Health Sciences

Private Bag X6001

Potchefstroom

2522

CONTACT NUMBER:

018 299-2085

Good day / Molweni

Singabaphandi abasuka kwiDyunivesithi yomntla ntshona yaye sijonga ukondliwa komama kunye nabantwana okusempilweni.

Uyamenywa ukuba uthathe inxaxheba koluphando luquka inxalenye yaPhilasana project. Uyacelwa ukuba uthathe ixesha lokufunda lencukacha ezakunika ubume baleproject. Uyacelwa kanjalo ukuba ubuze umphand ngemibuzo enxulumene nayo nayiphi na into ngaleproject ongayqondiyo. Kubalulekile kakhulu ukuba woneliseke kwaye ucacelwe ukuba oluphando lungantoni na, kwaye ungathatha nxaxheba ni na ngoluphando. Ukuthatha inxaxheba koluphando kuxhomekeke kuwe kwaye uvumelekile ukuba ulandule kwaye ayizonamiphumela mbi into yokulandula kwakho, ukwavumelekile ukurhoxa nokuba ubusovumile.

Oluphando lapasiswe liqumrhu lophando lwezempilo nokuziphatha kwicandelo lezempilo nezenzululwazi kwiDyunivesity yomntla Ntshona (NWU-00207-14-S1) Potchefstroom Campus kwaye luzakuqhutywa phantsi kwemiqathango nemimiselo (yeInternational Declaration of Kelsinki and the ethical guideline) luzwelonke kuphando lwezempilo nokuziphatha. Kungayimfuneko yequmrhu lophando namalungu ekomiti okanye ababandakanyekayo namagunya okuba hahlole incukacha zophando.

Lungantoni oluphando?

Uphando lwePhilisana luzakwenziwa kwilali ezingqonge iMazeppa Bay kunye neQolora by Sea. Kufuneka kuboniwe ukuba ungakanani umlinganiselo owutyisa usana lwakho nokuba lukhula ngaphantsi kokuba kulindelekile na. Abantu abazakwenza oluphando bazakube beqeqeshiwe licandelo lezempilo nophando kulondliwo kunye nezempilo. Omama kunye nabantwana abakwinani elikhulu namashumi amabini bazakubayinxalenye yoluphando.

Injongo zophando:

- Kufunwa ukufunyaniswa ukuba imifanekiso yokutya esetyenziswayo ekuboneni umlinganiselo nomthamo wokutya okutyiwa ngumntwana wakho ungaba ungakanani na kwaye ungolungileyo na.

Kutheni wena nomntwana wakho nimenyiwe ekuthatheni inxaxheba?

Wena kunye nomntwan nimenyelwa ukuthatha inxaxheba ukuba kuba unomntwana ongaphantsi kwenyanga ukuya kwiminyaka emibini kwaye kufuneka kubonakale ukuba umntana umondla kangakanani na kwaye nihlala eMazeppa Bay okanye Qolora by Sea.

Ubandakanyeka ngezizizathu silandelayo :

Ukwiminyaka elishumi elinesibhozo ukuya kwemashumi mane anesihlanu.

Uyinxalenye yophando:

Kuba ungaphantsi kweminyaka elishumi elinesibhozo okanye ungaphezulu kweminyaka emashumi mane aneshlanu okanye undwendwele ingingqi leyo yaye umntwana uzelwe phambi kwexesha.

Zintoni ezilindelekileyo kuwe?

- *Kulindeleke ukuba wenze umboniso wobungakananibokutya okinka umntwana wakho. Okulandelayo, uzakucelwa ukuba ukhethe umfanekiso ozakungqamelana nomlinganiselo owubeka esityeni.*

Ingabe wena nomntwana wakho nizakuzuza ekuthatheni inxaxheba koluphando?

Akuzokuzuza ngokuqondene noluphando kodwa uzakwenza ukuba umsebenzi wabaphandi ubelula kwaye bazi banzi ngezempilo kunbanye abantwana ekuhlaleni.

Oluphando lubalulekile kuba lunika ulwazi olubanzi malunga nokuba kutheni abantwana bengakhuli ngokufanelekileyo. Kuzakuncedakala nabanye abantwana ekuhlaleni kuba abongukazi kunye nabazali

bazakube bexhotyiswe ngoluphando. Ukuba abantwana bakhula kakuhle ayanqaba amathuba okuba bagule kwaye bayakwenza kakuhle esikolweni.

Ingaba bukhona ubungozi ekuthatheni inxaxheba kuluphando?

Ngokokubona kwabaphandi mancinci amathuba obungozi koluphando ukuba uthatha inxaxheba, kuzakudingeka ukuba uthathe uhambo ukuya koluphando yaye kuzakuthatha inxalenye yexesha lakho, kungashushu ngethuba ulindile, ungakhululeki ukuba akukho zitulo zaneleyozokuba uhlale

Kuzakwenzeka ntoni ukuba ungakhululeki ekuthatheni inxaxheba koluphando?

Siyaqonda ukuba xa ungakhululekanga ekusiboneni ukuba ungakanani umthamo wokutya owunika umntwana, asinokwaziukuvumela abanye abantu ukuba babekulo ngingqi., xa usibonisa ukuba ungakanani na xa umlinganiselo ownika umntana wakho.

Eminyeimibuzo kwiphepha Imibuzo ingabangela ukuba ungaphatheki kakuhle, kodwa ukuba akukwazi ukuyiphendula eminye imibuzo khululeka uxelele umntu lo ukubuzayo ukuba akukwazi kuyiphendulakulungile ukenjenjalo.

Ukuba ubona kuyimfuneko ukuba uqhubekeke nengxoxo emveni kotyelelo kuzakubakho ixesha lokuba uthethe nomongikazi okanye umphandi okulo oluphando. .

Ngubani ozakujongana nencukhacha?

Akekho umntu ozakuyazi incazelo osinike yona, Wonke umntu oyinxalenye nomphandikuphela.Kuzakubhalwainombolo leyo hayi igama lakho.

Xa kuxelelwaabanye abantu ngemiphumelaokanye iziphumo zophando bazakuxelelwa ngequmrhu hayi ngeziphumo zakho. Ngumphandi kuphela ozakubanlwazi ngencukacha osinie yona yaye onke amaphepha azabaselugcinweni olukhuselekileyo luka Gqirha Lombard. Naxa kufakwa incukacha kwi computer kuzakufakwa inombolo eyimfihlo ukuze ibengumphathi yedwa onolwaz.

Abachasi bophando (Urhulumente Womzantsi Afrika) kunye nabanye abantu kwisigqeba banganemvume yokujonga amaphepha ukuqinisekisa ukuba abaphandi bawagcina kakuhle. Zonke incukacha ziyakugcinwa iminyaka esixhenxe ukuze asetyenziswe kuphando olunxulumene nokukhula kwabantwana kunye nempilo yabo.

Uzakuhlululwa ekuthatheni inxaxheba?

Akukho ntlawulo uzakuyizuza ekuthabatheni inxaxheba kodwa uzakufumana inxaso kutya ngenjongo yokubulelangexesha lakhoemva kotyelelo rhoqo.

Akuzubakho zindleko xa ungathathanga nxaxheba.

Ingaba ikhona enye into ekufuneka uyenzileokanye uyazile?

Ungaqhakamelishana kunye no Grirha Lombard kwezi nombolo (018 299 2085) ukuba unemibuzo okanye ufumana ingxaki.

Usenako ukuqhakamelishananesisigqeba sophando kwezempilo ngokutsalela umnxeba u Mrs Caroline Van Zylkule nombolo (081 299 2094); Caroline.vanzyl@nwu.ac.za ukuba unezinto nezikhalazo ezingakhange ziaciswe ngumphandi.

Uzakufumana ikopi yezincukacha ukeze ubenazo..

Uzokwazi njani ngemiphumela?

uGqirha Lombard uzakwabelana nawe ngeziphumo rhoqo emva kotyelelo ngalunye..

Declaration by participant / Ufungo lomthabathi nxaxheba

Ngokutyikitya ngezantsi, Indiyavuma ukuba mna nomntwana wam sithatha inxaxheba kuphando lwe PhiliSana project:uphuhliso nobukhobemifanekiso wobungakanani bokutya ekuboneni ubungakanani bombona wentsana kunye nabantwana abangaphantsi kwenyanga ukuya kwiminyaka emibini abahlala kumaphande ephondo Lempuma Koloniyo Mzantsi Africa.

Ndiyavuma ukuthi:

Ndiyifundile yonke incukacha ekuleform yaye ibhalwe ngolwimi endilwaziyo nendiliqondayo

Ndibenalo ixesha lokubuza imibuzo kumntu ocelaimvume yokuba ndithathe inxaxheba kwakunyenakumphandi yaye yonke imibuzo endinayo iphendulekile..

Ndiyayazi ukuba ngothatha inxaxheba kwisifundo sophando kukuzithandela yaye akukho mntu undinyanzelisileyo..

Ndisengakwazi ukuyeka phakathi nangaliphi na ixeshandifuna yaye akukho mntu uzakundibeka ityala.

Umphandi angandikhulula ndisishiye isifundo sophando phambi kokuba siphela xa ebona kuyimfuneko ukuba andiqhubi kakuhle nje ngokuba bekuvunyelwene.

Ndiyavuma / okanye andivumi ukuba umphandi athathe imifanekiso ye project yophando ayisebenzise ngegama lam nelomntana wam ekuboniseni abaphandi ukuba wenze njani kwi project.

_____ (signature)

Signed at (place/indawo) on (date/Umhla) 20.....

Signature of participant (umthathi nxaxheba) Signature of witness (ingqina).....

Declaration by person obtaining consent

I (igama) declare that:

Ndiyicacisilengokufaneleyo incazelo ekuleformku

Ndiye ndamququzelela ukuba abuse imibuzo ndathathaixesha lokuba ndiyiphendule yonke

Ndanelisekiile kuba naye waneslisekilezimekozophando

Aniyisebenzisanga / ndiyisebenzisile itoliki

Signed at (place / indawo) on (date umhla) 20....

Signature of person obtaining consent / (Siyono Lomntu oqhuba Uphando)

Signature of witness / (Usayion lweIngqina)

Declaration by researcher (Ufungo lomphandi)

I (name/igama) declare that (ndafunga ukuthi):

Ndiyicacisile ngokufanelekileyo incazelo ekule form ku

Ndiye ndamquzelela ukuba abuze imibuzo ndathatha nexesha lokuba ndiyiphendule yonke imibuzo.

Ndanelisekile kuba naye wanelisekile zimeko zophando Ndiyisebenzisileitoliki.

Signed at (place/Indawo) On (date/ umhla) 20....

Signature of researcher / (Usayion Lomphandi)

Signature of witness /(Usayino lwengqina)



HREC Stamp

PARTICIPANT INFORMATION LEAFLET AND WRITTEN CONSENT FORM:

Mothers/ Caregivers older than 18 years

TITLE OF THE RESEARCH PROJECT:

The PhilaSana Project: The relationship between mycotoxin exposure and infant and young child growth amongst infants from deep rural areas of the Eastern Cape Province, South Africa – a Pilot Study

TITLE OF SUB STUDY:

Development and validation of portion size food photographs to determine maize intake of infants and young children in deep rural areas in the Eastern Cape Province of South Africa.

REFERENCE NUMBERS: NWU-00207-14-S1

Reference number of sub study: NWU-00089-15-S1

PRINCIPAL INVESTIGATOR:

Dr Martani Lombard

ADDRESS:

Room 149, G16

North-West University

Faculty of Health Sciences

Private Bag X6001

Potchefstroom

2522

CONTACT NUMBER:

018 299-2085

Good day

We are researchers from the North-West University and we look at the health and nutrition of mothers and their babies.

You are being invited to take part in a research study that forms part of the PhilaSana project. Please take some time to read the information in this form, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are happy, that you clearly understand what this research is about, and how you can be part of it. In addition, taking part in the study is completely your choice and you are free to say no. If you say no, this will not affect you negatively in any way. You are also free to stop being part of the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00207-14-S1) (Potchefstroom Campus) and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

The PhilaSana study will be done in villages around Mazeppa Bay and Qolora by Sea. We want to see if there is how much your baby eats and if your baby grows less than he/she should. The people who will do the study will be experienced health researchers trained in nutrition and health. 120 mothers and their babies will be part of the study.

The purpose of this part of the study is to:

- *Find out if the food photographs we use to see how much your baby eats is correct.*

Why have you and your baby been invited to participate?

You and your baby have been invited to participate because you have a child in this age (0-2 years) and we want you to show us how much you give the baby to eat. Also because you and your baby live in either Mazeppa Bay or Qolora by Sea.

You have also been included for the following reasons:

Are in the right age group for the study (18 – 45 years).

You will not be part of the study if:

You are younger than 18 years old or older than 45 years; only visiting the area; your baby is born too early.

What will your responsibilities be?

- ***You will be expected to come and show us how much food you usually give the child. After this, we will ask you to pick a photo that looks almost the same as the amount you have put in the plate.***

Will you and your baby benefit from taking part in this research?

You will not benefit directly from being part of the study but you will help us to do our work right and then we will know more about the health of other babies and children in the area.

This study is important because it will give us more information about why babies are not growing, as they should. This will help all the other babies in your area since we will teach the nurses and mothers in the area about this. If the babies are growing better, they will not get sick so often and they will be doing better at school.

Are there risks involved in your taking part in this research?

As far as we can see, there is very little risk if you take part in this part of the study. You might have to walk to us, this might take some of your time, and it might be hot while you wait your turn and then you may be uncomfortable if there is not a chair available.

What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

You might have to walk to us, this might take some of your time, and it might be hot while you wait your turn and then you may be uncomfortable if there is not a chair available. We understand you may feel uncomfortable to show us how much you food and what food you are giving to the child, so we will not allow any other people to be in the area when you show us the how much and what you are feeding you child.

Some of the questions on the questionnaires may also cause some discomfort, but if you do not want to answer any questions, feel free to say so to the interviewer and that would be fine.

Should you have the need for further discussions after each visit an opportunity will be arranged for you to speak with the nurse on the study or with the researchers.

Who will look at the information?

Nobody will know what information you give us. Everybody that is part of the study will have a special number that only you and the researchers will know. We will always write down the number and not your name.

When we tell other people about what we found in the study we will always tell them about the group and we will never tell them about only your results. Only the researchers will be able to see the information you give us and the papers will be locked in the office of Dr Lombard. When we put the information on the computer we will also put a password on so that only the researchers can get to the information.

Sometimes the sponsors of the study (the South African Government) and the people from the ethics committee might have a look at the papers to make sure that the researchers are keeping them safe.

Data will be stored for 7 years and will be used for research related to the growth of babies and their health.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study but we will give you a small food parcel to say thank you for your time after every visit.

There will thus be no costs involved for you, if you do take part.

Is there anything else that you should know or do?

You can contact Dr Lombard at 018 299-2085 if you have any further queries or encounter any problems.

You can also contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2094; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.

You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

Dr Lombard will share the findings of the research with you after every visit as well as at the end of the visit.

Declaration by participant

By signing below, I agree for myself and my baby to take part in a small study that is part of the the PhilaSana Project: Development and validation of portion size food photographs to determine MAIZE INTAKE of infants and young children (aged 0 - 24 months) living in deep rural areas in the Eastern Cape Province of South Africa.

I declare that:

I have read this information and consent form and it is written in a language with which I understand.

I have had a chance to ask questions to both the person asking permission, as well as the researcher and all my questions have been answered.

I understand that taking part in this study is voluntary and I have not been forced to take part.

I may choose to leave the study at any time and will not be judged in any way.

I may be asked to leave the study before it has finished, if the researcher feels it is the best for me, or if I do not follow the study plan, as agreed to.

I agree / not agree that the researcher may take photos of the research project and that they will use it (without my/my baby's name) to show other researchers what they have done in the project.

_____ ***(signature)***

Signed at (place) on (date) 20....

Signature of participant Signature of witness

Declaration by person obtaining consent

I (name) declare that:

I explained the information in this document to

I encouraged her to ask questions and took adequate time to answer them.

I am satisfied that she adequately understands all aspects of the research, as discussed above

I did/did not use an interpreter.

Signed at (place) on (date) 20....

Signature of person obtaining consent

Signature of witness

Declaration by researcher

I (name) declare that:

I explained the information in this document to

I encouraged her to ask questions and took adequate time to answer them.

I am satisfied that she adequately understands all aspects of the research, as discussed above

I did use an interpreter.

Signed at (place) On (date) 20....

Signature of researcher

Signature of witness

ADDENDUM 3: INFANT DATA COLLECTION SHEET



Development and validation of portion size food photographs to determine maize intake of young children in deep rural areas in the Eastern Cape Province:

Participant Number:

Area:

Age of Child:

Date:

Dish	1 TSP	1 TBS	1 DS
Soft Porridge			
Maize meal and <i>imifino</i>			
Maize Meal and spinach			
Maize Meal and pumpkin			
Stiff <i>pap</i>			
Maize meal and Dried Beans			
<i>Samp</i>			

Samp and Beans			
Soup			
Crumbly pap			
Mealie rice and Spinach			
Mealie rice and pumpkin			
Mealie rice and imifino			

ADDENDUM 4: DISHING UP DATA COLLECTION SHEET



Development and validation of portion size food photographs to determine maize intake of young children in deep rural areas in the Eastern Cape Province:

Participant Number:

Area:

Age of Child:

Date:

Dish	Plate weight	Dished up portion	Portion size
Soft Porridge			
Maize meal and <i>imifino</i>			
Maize Meal and spinach			
Maize Meal and pumpkin			
Stiff <i>pap</i>			
Maize meal and Dried Beans			
<i>Samp</i>			
<i>Samp</i> and Beans			

Soup			
Crumbly <i>pap</i>			
Mealie rice and spinach			
Mealie rice and pumpkin			
Mealie rice and imifino			

ADDENDUM 5: SOCIO-DEMOGRAPHIC QUESTIONNAIRE

Socio-demographic questionnaire

Study No.

Date of interview

Interviewer

1. Who is head of the household	My	Father	Mother	Husband	Grandma	Grandpa	Aunt	Uncle	Brother	Sister	Friend	Self	Other
2. How would you describe yourself in terms of population group (Population group as perceived by the woman herself)									African	Colored	Indian	White	Other (Specify)
3. What is your first language?					Afrikaans	English		Xhosa		Zulu		Other	
4. What is your marital status?	Unmarried	Married	Divorced	Separated	Widowed	Living together	Traditional marriage		Other: Specify				
5. What is your highest formal education level? (Circle one number only)	None				Primary School		Std 6-8 Grade 8-10		Std 9-10 Grade 11-12		Tertiary education		
6. What is your employment status? (Circle one number only)	Un-employed			Home-maker by choice		Self-employed		Wage-earner		Self-employed professional		Other (Specify)	
7. Who decides on what types of food are bought for this household?	My	Father	Mother	Husband	Grandma	Grandpa	Aunt	Uncle	Brother	Sister	Friend	Self	Other
8. Who decides how much money is spent on food for this household?	My	Father	Mother	Husband	Grandma	Grandpa	Aunt	Uncle	Brother	Sister	Friend	Self	Other
9. Who is mainly responsible to buy food for the household?	My	Father	Mother	Husband	Grandma	Grandpa	Aunt	Uncle	Brother	Sister	Friend	Self	Other

10. Who is mainly responsible for food preparation in the house? My	Father	Mother	Husband	Grandma	Grandpa	Aunt	Uncle	Brother	Sister	Friend	Self	Other		
11. Who is mainly responsible for feeding / serving the children? My	Father	Mother	Husband	Grandma	Grandpa	Aunt	Uncle	Brother	Sister	Friend	Self	Other		
Household data														
12. How many people sleep in this house for at least 4 nights per week for most of the year?														
13. How many rooms does this house have? (excluding bathroom, toilet and kitchen if separate)														
14. What is the number of people per living / sleeping room (Tick one)					0-2 persons		3-4 Persons		More than 4 persons					
15. Where do you get drinking water most of the time (Circle one number)		Own tap		Communal tap		River / dam		Borehole / well		Other (Specify)				
16. What type of toilet does this household have? (Circle as many numbers as necessary)		Flush		Pit / VP		Bucket / pot		None		Other (Specify)				
17. What fuel is used for cooking most of the time? (Circle as many numbers as necessary)		Electric		Gas		Paraffin		Wood		Coal		Other (Specify)		
18. Does this home have a working: Refrigerator / Freezer						Fridge		Freezer		Both		None		
19. Stove (oven & hob)				Yes		No		If yes, circle all relevant options Gas Coal Electricity						
20. Primus or Paraffin stove										Yes		No		
21. Microwave										Yes		No		
22. Hot Plate										Yes		No		
23. Radio / television						Radio		TV		Both		None		
24. Telephone						Land line		Cell		Both		None		
25. Do members of this household receive any grants? (You may circle more than one number)				None		Child support		Social relief		Disability		Old age pension		Other (Specify)
26. How many people contribute to the total income (money) in this household?					1 person		2 persons		3-4 persons		5-6 persons		More than 6	

(Circle one number only)							
27. What is the total household income per month (Circle one number only)	None	R1-R500	R501-R1000	R1001-R3000	R3001-R5000	Over R5000	Don't know

ADDENDUM 6: STUDY REGISTER



Development and validation of portion size food photographs to determine maize intake of young children in deep rural areas in the Eastern Cape Province:

Participant Number:	Date:	Name of Participant:	Address of Participant:	Age of Child:
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