Household Food Processing Strategies to Improve Iron and Zinc Bioavailability in Ethiopian Dishes Based on Chickpea (*Cicer arietinum* L.) and Dry Bean (*Phaseolus vulgaris* L.)

A Thesis Submitted to the

College of Graduate and Postdoctoral Studies

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in the

College of Pharmacy and Nutrition,

University of Saskatchewan

Saskatoon, SK

by

Hiwot Abebe Haileslassie

Permission to Use

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professors who supervised my thesis work or, in their absence, by the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other uses of materials in this thesis in whole or part should be addressed to:

Dean of the College of Pharmacy and Nutrition

University of Saskatchewan

E3134 Health Sciences, 104 Clinic Place

Saskatoon, Saskatchewan S7N 2Z4

Canada

OR

Dean College of Graduate and Postdoctoral Studies

University of Saskatchewan

116 Thorvaldson Building, 110 Science Place

Saskatoon, Saskatchewan S7N 5C9

Canada

Abstract

Pulses are major constituents of the human diet. Dry bean and chickpea, commonly grown in Ethiopia, are among the pulses that serve as important sources of energy and nutrients, particularly protein, minerals and folate. However, pulses also contain anti-nutrients which bind minerals, mainly iron and zinc, rendering them less bioavailable or unavailable for absorption. These anti-nutrient contents of pulses are a particular problem in Ethiopia, where the population consumes a plant-based diet and a large percentage of young children and women are affected by micronutrient deficiencies. The nutrient and anti-nutrient contents of raw, cooked, soaked-cooked, germinated-cooked and fermented dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local) varieties grown in Ethiopia were determined with the hypothesis that the iron and zinc bioavailability can be enhanced through processing methods applicable at household scale. In addition, the effect of soaking and germination on cooking time and the acceptability of dishes prepared from dry bean and chickpea were determined. Ferritin formation in the Caco-2 cell intestinal absorption model was used as a proxy for iron bioavailability.

Fermentation of dry bean and chickpea flours significantly reduced the contents of anti-nutrients (phytate, tannin and polyphenols), as well as the phytate:iron molar ratio compared to unfermented samples. For most dry bean and chickpea samples, germination-cooking yielded superior results in terms of reducing cooking time, phytate, tannin, and phytate:iron and phytate:zinc molar ratios compared to cooking and soaking-cooking. Polyphenol contents were lower for soaking-cooking than for germination-cooking. With a few exceptions, the scores for sensory attributes of bean-based and chickpea-based dishes prepared from soaked or germinated

samples were not significantly different than those of dishes prepared from untreated bean and chickpea.

Among the unprocessed dry bean and chickpea varieties, there was significantly higher ferritin formation (better iron bioavailability) in Caco-2 cells exposed to Habru compared to the other samples of dry bean and chickpea varieties used in the study. Overall, soaking (18 h)-cooking resulted in higher ferritin formation for the dry bean samples. On the other hand, soaked (12 h)-cooked and germinated (72 h)-cooked in Habru, soaked (12 and 18 h)-cooked and germinated (72 h)-cooked in Local and germinated (72 h)-cooked in Mastewal chickpea resulted in higher ferritin formation compared to samples cooked without pre-treatment. Fermentation for 72 h was effective in increasing ferritin formation in all dry bean samples, but not in chickpea samples, with the exception of Habru. Although the expected improvements due to the reduced anti-nutrient contents were not confirmed by high ferritin formation in Caco-2 cells or by lowering molar ratios below critical values in all samples, soaking-cooking, germination-cooking and fermentation will still be effective with regards to lowering the total anti-nutrient contents.

Acknowledgments

I would like to express my sincere gratitude and thanks to my supervisors Dr. Carol Henry and Dr. Robert Tyler. Because of their support, guidance and kindness, my journey has been full of learning and growth. I would also like to offer special thanks to my thesis advisory committee members Dr. Jane Alcorn, Dr. Bunyamin Ta'ran and Dr. Derek Peak, whose insightful and constructive comments have enriched my doctoral study and thesis. Special thanks also go to Dr. Susan Whiting, my advisory committee chair, for the guidance and support she provided during my PhD program. I would like to thank my external examiner, Dr. Grace Marquis for the suggestions to improve the thesis.

I appreciate the technical support provided by Barry Goetz, Dr. Gene Arganosa, Mary Bodis, Yongpei Chang and Afework Kebebu Gebrie during laboratory analysis, Caco-2 cell experiments, seed shipment and sensory evaluation. I am grateful for the support provided by Dr. Raymond Glahn from Cornell University, particularly in the Caco-2 cell study.

I also would like to acknowledge both the College of Pharmacy and Nutrition and the College of Graduate and Postdoctoral Studies for providing me with travel awards and all the necessary tools during my studies. I am highly appreciative of the financial support received through the University of Saskatchewan Dean`s scholarship for three years, the Saskatchewan Innovation and Opportunity Scholarship, the Elizabeth Helen McLeod Scholarship, as well as the support for the research from Global Affairs Canada (GAC) and the International Development Research Centre (IDRC) through the Canadian International Food Security Research Fund (CIFSRF). Special thanks also go to Shawna Bieber, the project manager, for the support she provided during my PhD study.

Finally, I would like to thank my family and friends. Zene, your presence in our family is what draws me to academia. I am grateful. Thanks also go to Enda and his family who were always there for me. I also would like to extend my appreciation and thanks to Geni, a very caring individual, and to Kassu, who built the foundation of my educational journey. Thanks also go to my friends (Get, Bez, Bire, Meazi, Sis, Tsi, Gete and Renee) for making Saskatoon feel like home. I would like to acknowledge Grandma, who, despite lacking formal education, always encouraged me to pursue academia. Grandpa was always proud of me, which inspired me to work harder. I also would like to send a big thank you to Asnu for the care and support she provided by taking time away from her home. Thanks also are due to my parents, Abe and Z, who gave me more than they have. I also send a big shout out to my brother, Kidus and, my son, Nana, my constant sources of smiles. Thanks is due to my unborn child, who becomes the motivation to finish the thesis. Last but not least, I cannot express how fortunate and grateful I am for Bini, my husband, who gave up everything to make my dream come true, and for his unreserved love and support.

Dedication

To my beloved hubby, Bini, my son, Nana, and my parents, Z and Abe.

To the two most beautiful and strong women that I know in my life, Tete and Fru.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AEC	Anion-exchange Chromatography
AI	Adequate Intake
ANOVA	Analysis of Variance
BC	Before Christ
CaCo-2	Colon Adenocarcinoma Cell Line
CSA	Central Statistics Authority
EAR	Estimated Average Requirement
EDHS	Ethiopian Demographic and Health Survey
EDTA	Ethylenediamineteraacetic Acid
EPHI	Ethiopian Public Health Institute
FAO	Food and Agriculture Organization
FMOH	Federal Ministry of Health
g	Gram
h	Hour
HC	Hydration Capacity
HCP1	Heme Carrier Protein
HI	Hydration index
HPLC	High Performance Liquid Chromatography
IP	Inositol phosphate
IZiNCG	International Zinc Nutrition Consultative Group
L	Litre

LMIC	Low and Middle Income Countries
MEM	Minimum Essential Medium
min	Minutes
mg	Milligram
mo	Month
MT	Metric Tonnes
MWCO	Molecular Weight Cut-off
NIST	The National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NS	Non-Significant
Phytate:iron	Phytate-to-iron
Phytate:zinc	Phytate-to-zinc
RDA	Recommended Dietary Allowance
SC	Swelling Capacity
SI	Swelling Index
SNNPR	Southern Nations, Nationalities, and Peoples' Region
USDA-ARS	United States Department of Agriculture-Agriculture Research Service
WHO	World Health Organization
w/v	Weight to Volume Ratio
Y	Year

Chapter 1

Introduction

1.1 Background Information and Rationale

Iron and zinc are among the micronutrients with the greatest impacts on health, including physical and mental development (Algarin, Peirano, Garrido, Pizarro, & Lozoff, 2003; Caulfield & Black, 2004). Iron and zinc deficiencies tend to occur together since both are affected by similar inhibitors, which are highly present in plant-based diets. These inhibitors are collectively termed anti-nutrients. Studies have found deficiencies in iron and zinc in many parts of Africa, particularly in countries such as Ethiopia where people consume a diet high in plant-based foods. A study conducted in two agro-ecological zones of rural Ethiopia showed that 32.7% of children aged 6-23 months and 19.1% of lactating mothers were deficient in both nutrients (Roba, O'Connor, Belachew, & O'Brien, 2016). Another study in a rural village in southern Ethiopia reported that 21.3% of pregnant women suffered from deficiencies of both micronutrients (Gebremedhin, Enquselassie, & Umeta, 2011). Studies in Ethiopia also have found evidence of zinc deficiency among women and children. One study reported 72% prevalence of zinc deficiency among pregnant women in their third trimester (Abebe et al., 2008). Another found zinc deficiencies in Ethiopian pre-school children, school-age children and women of reproductive age of 35%, 35.7% and 33.8%, respectively (Ethiopian Public Health Institute, 2016). Studies of iron deficiency in Ethiopia have had varying results, with some indicating that iron deficiency is rare and others indicating its public health significance. Among these studies of iron deficiency, Haidar and Pobocik (2009) reported 18% prevalence of iron deficiency anemia among women of reproductive age. Their study covered a wide geographic area and had a large sample size. In another 2005 cross-

sectional study involving women of 15 to 49 years, Haidar (2010) found that more than half were deficient in iron.

Despite studies with varying results, these mostly high percentages of deficiencies found in women and young children are not a surprise, given that most families in Ethiopia cannot afford to regularly consume foods from animal sources. The causes of nutritional deficiency are multifactorial, with inadequate dietary intakes and infections considered to be immediate causes (UNICEF, 1990). Although inadequate dietary intake could be attributed to a lack of sufficient food, micronutrient deficiency also can occur due to poor bioavailability of nutrients (Thavarajah & Thavarajah, 2012) and not merely to the absence of food. Due to the presence of anti-nutrients, such as phytate, tannin and polyphenols, nutrients such as iron and zinc obtained from plant-based foods are not as bioavailable as those obtained from animal-source foods. This lack of bioavailability is a great concern in most developing countries that largely depend on plant-based staple diets (Hotz & Gibson, 2001). Ethiopia is no exception. Adish et al (1999) reported that, despite adequate dietary iron intake from cereals, 43% of preschool children in northern Ethiopia had a serum ferritin of less than 12 μg per l, indicative of iron deficiency. Adish et al.'s findings were supported by a longitudinal study (Zimmermann, Chaouki, & Hurrell, 2005), which found that children aged 6 to 10 had a higher iron intake than required but absorbed only 2% of the iron consumed. To alleviate nutrient deficiencies through food-based approaches, it is thus important to assess and understand the nutrient composition of available diets and factors that affect their bioavailability (Lönnerdal, 2000).

In Ethiopia, diets mainly comprise cereals, starchy roots and tubers (Food and Agriculture Organization, 2008; Mesfin, Henry, Girma, & Whiting, 2015; Roba et al., 2015). Next to cereal, roots and tubers, pulses play an important role in the diet of Ethiopians, serving as the top source of protein intake. The term pulse refers to dry grain legumes, including bean, pea, lentil and

chickpea, but excluding legumes used for oil extraction (soybean and ground nut) or those consumed green (green pea and green bean) (Asif et al., 2013; Singh & Basu, 2012). Ethiopia is the number one producer of chickpea in Africa (Kassie et al., 2009) and among the top ten producers in the world. Ethiopia is also among the top producers of dry bean in Africa (International Food Policy Research Institute, 2010). Pulses in Ethiopia have a dual role, being important from both an agricultural and a food security perspective. Since the majority of Ethiopians depend on subsistence farming, cultivating pulses has a great impact on improving productivity and income for the population (Rashid, Yirga, Behute, & Lemma, 2010). Pulses are environmentally friendly crops, as they grow even with small amounts of water and improve the fertility of soil through their nitrogen fixing capacity. From a food security perspective, pulses serve as a cheap alternative protein source, while also providing high amounts of micronutrients such as zinc, iron and folate. Studies carried out in Ethiopia that supplemented cereal-based dishes with pulses have demonstrated an improvement in nutritional quality. For example, improved amino acid profiles (Abebe et al., 2006) and increased protein content (Kebebu, Whiting, Dahl, Henry, & Kebede, 2013) have been reported.

Pulses have the capacity to provide micronutrients, but micronutrient bioavailability, particularly iron and zinc bioavailability, tend to be lower in plant-based diets. Studies have shown that calcium concentrations in plant-based diets contributed to poor iron bioavailability. It also was thought that calcium could result in low zinc bioavailability, however recent studies indicated no effect of calcium on zinc absorption (Hunt and Beiseigel, 2009). Food processing strategies, applicable at the household level, have been found to be useful in improving zinc and iron bioavailability (Khalil et al., 2007; Luo, Gu, Han, & Chen, 2009; Nergiz & Gökgöz, 2007; Shimelis & Rakshit, 2007). These strategies include soaking, germination and fermentation. However, there is no universal recommended period of soaking, germination or fermentation to reduce the levels

of anti-nutrients in plant-based diets. Information on the effect of such strategies on dry bean- and chickpea-based Ethiopian dishes is limited. The optimal conditions that result in the highest reduction differ based on the species and the food processing strategy used. There is a need to determine the appropriate strategy before promoting it at the community level. In addition, research linking the impact of household-level food processing strategies with changes in bioavailability of minerals measured using *in vitro* techniques are scarce. Therefore, this study was designed to fill these gaps in research.

1.2 Hypotheses and Objectives

1.2.1 Hypotheses

The study was carried out to test the following hypotheses:

- Flours from fermented dry bean and chickpea are lower in polyphenols, tannin and phytate than are raw dry bean and chickpea flours.
- Cooked dry bean and chickpea prepared from pre-soaked and pre-germinated seed are lower in polyphenols, tannin and phytate than are cooked dry bean and chickpea prepared from untreated seeds.
- The sensory attributes of dishes prepared from pre-soaked and pre-germinated dry bean and chickpea seed are acceptable to consumers and panelists as dishes prepared from untreated seed.
- Cooked dry bean and chickpea prepared from pre-germinated and pre-soaked seed are higher in iron bioavailability than are cooked dry bean and chickpea prepared from untreated seeds.

- Flour from fermented dry bean and chickpea are higher in iron bioavailability than are raw bean and chickpea flours.

1.2.2 Objectives

The general objective of the current study was to improve the bioavailability of iron and zinc in dry bean and chickpea grown in Ethiopia through food processing strategies applicable at the household level, and to evaluate the impact of these strategies on anti-nutrient levels and iron uptake using the Caco-2 cell intestinal absorption model coupled with *in vitro* digestion.

1.2.3 Specific Objectives

Objective 1: To determine the effect of fermentation for 24, 48 and 72 h on nutrient (iron, zinc and calcium) and anti-nutrient (phytate, polyphenols, tannin) levels of dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local);

Objective 2: To determine the effect of soaking for 6, 12 and 18 h combined with cooking on nutrient (iron, zinc and calcium) and anti-nutrient (phytate, polyphenols, tannin) levels of dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local);

Objective 3: To determine the effect of germination for 24, 48 and 72 h combined with cooking on nutrient (iron, zinc and calcium) and anti-nutrient (phytate, polyphenols, tannin) levels of dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local);

Objective 4: To determine the acceptability of dishes prepared from soaked and germinated dry bean and chickpea as compared to dishes prepared from untreated seed;

Objective 5: To analyze iron bioavailability of soaked-cooked, germinated-cooked and fermented dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local) using the Caco-2 cell intestinal absorption model coupled with *in vitro* digestion.

1.3 Significance of the Study

This study will help to determine the effect of soaking, germination and fermentation on the iron and zinc bioavailability of dry bean and chickpea. The results will be instrumental for designing simple interventions applicable at the household level with minimal resources. Not only will the information generated from this study help to identify the best strategy for processing pulses at the household level, it also will help to compare the nutrient composition of samples of local and improved varieties of dry bean and chickpea, knowledge that will be useful in selecting varieties with high levels of nutrients of interest. The sensory evaluation of dishes prepared after applying different food preparation strategies also will be useful in determining the acceptance of the strategies before promoting them at the community level.

1.4 Definition of Terms

Bioavailability: the proportion of a nutrient in a food that could be absorbed and used by the body (Hurrell, 2002).

Chickpea: *Cicer arietinum* L. that include Desi and Kabuli type (Common name: bengal gram and garbanzo).

Dry Bean: *Phaseolus vulgaris* L. *(*Common name: common bean, kidney bean, haricot bean, black bean and white bean).

Improved varieties: varieties that are released from agriculture research centres.

Local varieties: varieties that are commonly known and used by local communities, either purchased from markets or from farmers.

1.5 Organization of the Thesis

The main objective of the study was to improve the bioavailability of iron and zinc in dry bean and chickpea-based Ethiopian dishes through food processing strategies applicable at the household level, and to evaluate the impact of these strategies on anti-nutrient levels and iron uptake using the Caco-2 cell intestinal absorption model coupled with *in vitro* digestion. This thesis is divided into seven chapters. Chapter 1 presents the background and rationale for the study. Chapter 2 presents a broad overview of the literature. Chapter 3 describes a scoping review published in 2016 in the *International Journal of Food Science and Technology* of food processing strategies and their impact on the anti-nutrient content of dry bean and chickpea. The materials and methods used are included as sections of Chapters 4 to 6 of the thesis. Chapter 4 provides insights on the impact of fermentation on the nutrient and anti-nutrient content of dry bean and chickpea grown in Ethiopia. Chapter 5 describes the key findings of a study on the impact of soaking and germination combined with cooking (soaked-cooked and germinated-cooked) on nutrient and anti-nutrient contents, cooking time and the acceptability of cooked dry bean and chickpea. Chapter 6 explores iron bioavailability of soaked-cooked, germinated-cooked and fermented dry bean and chickpea samples measured using the *in vitro* digestion/Caco-2 cell culture intestinal absorption model. The thesis concludes with Chapter 7, the general discussion, which summarizes the key findings and compares them with those in the literature. This section is informed by the literature presented in Chapters 2 and 3. Chapter 7 also outlines the strengths of the study, limitations and areas for future research.

Chapter 2

Literature Review

This chapter presents a general comprehensive review, beginning with an overview of micronutrient malnutrition at the global level followed by a discussion of the Ethiopian situation. The review focuses on iron and zinc, providing details on dietary sources, mechanisms of absorption and consequences of deficiency. Also discussed are anti-nutrients in pulses, with a focus on dry bean and chickpea, their impact on iron and zinc bioavailability, assessment techniques and interventions to help tackle iron and zinc deficiency.

2.1 Micronutrient Deficiency

Micronutrient deficiency, also known as "hidden hunger," is a major public health problem in most developing countries, including Ethiopia (Chakravarty & Sinha, 2002). Micronutrients are required in minute amounts for normal physiological function. However, the vast majority of the world's people do not regularly consume adequate levels of these nutrients, leading to the development of deficiencies. The micronutrients vitamin A, iron, iodine and zinc are of greatest public health concern (World Health Organization, 2007) since deficiencies in these micronutrients have serious health consequences, affecting many individuals across the globe.

2.1.1 Iron and Zinc Deficiency: The Global Situation

More than two billion individuals worldwide suffer from micronutrient deficiency (World Health Organization, 2007). Among the micronutrient deficiencies, iron is the most common nutritional problem reported in both developing and developed countries. Regarding zinc,

approximately 17.3% of the global population is estimated to be at risk of inadequate intake (Wessells & Brown, 2012). Iron and zinc deficiencies contribute to the global burden of disease, resulting in approximately 2.4% and 2.9%, respectively, losses of healthy life years (World Health Organization, 2002). The highest prevalence of zinc deficiency is reported in sub-Saharan Africa, South and Central America and South Asia (Black et al., 2008). Both iron and zinc deficiencies are among the top 10 risk factors for morbidity in developing countries (World Health Organization, 2002). Some segments of the population in these countries are at high risk for these deficiencies, particularly women, the elderly, infants and young children (Hotz & Brown, 2004). The prevalence of iron deficiency anaemia is estimated to be 56% and 41% in pregnant and non-pregnant women, respectively, in developing countries (Denic & Agarwal, 2007). Micronutrient deficiency contributes to about 10% of deaths and Disability Adjusted Healthy Life Years (DALYS) in children under five years of age (Bhutta et al., 2008).

2.1.2 The Ethiopian Situation

Poor nutritional status of women and young children in Ethiopia has been reported for years, with high rates of stunting, underweight, wasting and micronutrient deficiency (vitamin A, iodine, zinc and iron). A national survey carried out in Ethiopia in 2011 indicated that 44%, 29% and 10% of children under five years of age were stunted (height for age <-2SD), underweight (weight for age <-2SD) and wasted (weight for height <-2SD), respectively (Central Statistical Agency [Ethiopia] and ICF International, 2012). The same study found that 27% of women were thin or undernourished (Body Mass Index< 18.5 kg/m^2). Poor diet quality and diversity has been reported in Ethiopia, with the diets of more than half of infants and young children in two rural communities in Ethiopia comprising up to only two food groups (Ersino, Henry, & Zello, 2016), the diets of about 80% of school-aged children comprising less than three food groups (Herrador

et al., 2015) and the diets of 56% of lactating mothers comprising less than 3.4 food groups (Weldehaweria et al., 2016). An Ethiopian national food consumption survey (EPHI, 2013) found that the protein intake of approximately half the children in Ethiopia is less than estimated average requirements. A study carried out in third-trimester pregnant women in the southern part of Ethiopia reported a 72% prevalence of zinc deficiency based on plasma zinc concentration (Abebe et al., 2008). In other study conducted in a similar set up, a lower prevalence (53.0%) was reported, unsurprisingly, as it included pregnant women in all trimesters (Gebremedhin et al., 2011). The same 2013 national food consumption survey mentioned above (EPHI, 2013) found that the zinc and calcium intake of Ethiopian women and children was lower than the recommended amount. The Ethiopian Demographic and Health Survey (EDHS) report also showed that a large percentage of children (38%) under five years are stunted (Central Statistical Agency [Ethiopia] and ICF, 2016b). Height- or length-for-age is considered a functional outcome associated with risk for zinc deficiency in populations (Walker, Young, Black, Fontaine, & Black, 2009). Using WHO criteria, a national stunting rate of ≥ 20% is considered a public health concern and such high rates of stunting also suggest that zinc deficiency is a public health problem, according to International Zinc Nutrition Consultative Group (IZiNCG) (Hotz & Brown, 2004).

Anemia is a low level of circulating hemoglobin, which can be caused by iron deficiency, other micronutrient deficiencies and non-nutritional causes such as infection, blood loss and genetic disorders. Findings from studies on the prevalence of anemia in Ethiopian women and children have been uneven, but, in general, studies have found a higher prevalence of anemia in infants and children under five years. According to government statistical reports, the prevalence of anaemia among Ethiopian children increased from 44% in 2011 to 57% in 2016 (Central Statistical Agency [Ethiopia] and ICF, 2016b). An earlier study (Adish, Esrey, Gyorkos, & Johns, 1999) also showed a high prevalence rate of anemia (42%) among children 6 to 60 months of age.

Prevalence rates of anemia in older children seem to be lower. A national survey of school children in 2008 reported a prevalence of anemia of 9.79% among these children (Hall, Kassa, Demissie, Degefie, & Lee, 2008). Another more recent survey, however, reported much different results, indicating that 34.4% and 25.6% of pre-school and school children, respectively, were anemic (Ethiopian Public Health Institute, 2016).

Findings from studies of anemia prevalence among women of child-bearing age have also varied. The 2016 EDHS reported that 23% of women aged 15-49 years were anaemic (Central Statistical Agency [Ethiopia] and ICF, 2016b), whereas an earlier study conducted in nine administrative regions of Ethiopia reported 30.4% prevalence of anemia in women of reproductive age (Umeta, Haidar, Demissie, Akalu, & Ayana, 2008). Another recent survey found a much lower prevalence of anemia: 17.7% among women of reproductive age (Ethiopian Public Health Institute, 2016). In general, then, studies have consistently found 37 to 57% rates of anemia among children under five years of age, rates of about 10 to 15% among older children and rates of about 18 to 30% among women of reproductive age. According to the World Health Organization (WHO), a population prevalence of anemia above or equal to 40% is considered a major public health problem, whereas a prevalence of 20.0 to 39.9% of anemia is considered a moderate public health problem (World Health Organization, 2001).

These findings, especially for infants and young children, indicate that anemia has public health importance in Ethiopia. Although, according to the World Health Organization (2011), about half of the world's anemia cases are estimated to be due to iron deficiency, in Ethiopia, health care workers and researchers dispute whether iron deficiencies or other conditions are at the root of the condition's prevalence and inconclusive and mixed results have been reported (Umeta et al., 2008). Some of these studies, however, have linked anemia to iron deficiency. It is important to note that when anemia occurs due to iron deficiency, it is the manifestation of severe form of iron deficiency,

which reflects the status of only a few segments of the population affected by iron deficiency, indicating that the majority of the population may present as sub-clinical cases (Umbreit, 2005). In the above-mentioned 1999 study reporting 42% prevalence of anemia among children 6 to 60 months of age, a subsample of 43% of children had serum ferritin levels below 12 µg per litre, indicating that iron deficiency greatly contributed to the manifestation of anemia in these children (Adish et al., 1999). Other studies have also measured serum ferritin levels to specifically identify iron deficiency in children and women. A recent study in the southwestern part of Ethiopia reported 37.5% prevalence of iron deficiency in school children (Desalegn et al., 2014). A 1999 study indicated 18.7% prevalence rate of iron deficiency in pregnant and lactating women (Haidar et al., 1999). In a later study Haider and colleagues (2003) reported a 22.3% prevalence of iron deficiency in lactating mothers in urban slum areas in Addis Ababa (Haidar et al., 2003). A study in a small village in the southern part of Ethiopia reported 13% and 33% prevalence of iron deficiency and depleted iron stores in pregnant women, respectively (Gibson et al., 2008). The evidence from all these studies suggests that iron deficiency is likely one of the causes of anemia in Ethiopia, or at least a contributing factor.

The intake of iron rich foods varies in the Ethiopian population. For example 84% and 6% prevalence of inadequate iron intake was reported in the Somali and Amhara regions, respectively (EPHI, 2013). The EDHS study, which found 36.9% prevalence of anemia in children aged 6-59 months in Southern Nations, Nationalities, and Peoples' Region (SNNPR), also found that children in SNNPR were less likely to eat iron-rich foods; only 6% of children 6-23 months of age consumed iron-rich foods in the 24-hour period preceding the survey (Central Statistical Agency [Ethiopia] and ICF International, 2012). The first national food consumption survey conducted in all nine regions of Ethiopia reported that iron intakes are lower than the recommended amount in children (EPHI, 2013). The same study reported an excessive intake of iron rich foods in adult populations

in Ethiopia. Despite the debate about whether iron is the main cause of anemia in Ethiopia, iron deficiency anemia ranked as the 15th and 16th cause of under-5 mortality rate in 1990 and 2013, respectively (Deribew et al., 2016).

2.2 Zinc

Even though the importance of zinc has long been recognized for plants, animals and microorganisms, the importance of zinc for humans was not understood until the 1960s (Prasad, 2003). The abundant presence of zinc in biological systems and the lack of specific signs and symptoms of its deficiency had misled scientists, who believed that zinc could not be deficient in humans (Prasad, 2012). It was not until 1958 that investigations into zinc deficiency in humans began after a 21-year-old male presented in Iran with growth retardation, dry and rough skin and hypogonadism (Prasad, Halsted, & Nadimi, 1961). The patient's diet was mainly plant-based and an additional 10 patients with a similar dietary history and comparable clinical manifestations were seen in the two years following the initial case. Prasad et al. (1961) indicated that zinc deficiency might be responsible for the growth retardation and hypogonadism of the 11 patients in their study. Other cases with a similar clinical picture and dietary history were examined in Egypt and they responded well to zinc supplementation (Prasad, 2012). This research confirmed that zinc deficiency is possible in humans. Since the discovery of the importance of zinc for humans, extensive studies have been carried out to understand its impact on different health outcomes. In 2002, zinc deficiency was included as a major risk factor for the global burden of disease (Ezzati, Lopez, Rodgers, Vander Hoorn, & Murray, 2002). In Ethiopia, zinc has received similar attention in recent years.

More than 300 enzymes in the human body are zinc-dependent; hence, zinc takes part in and influences a large number of biochemical pathways (Prasad, 2012). Zinc's function can be categorized as catalytic, structural and cellular regulatory (Maret, 2013) and it has a role in protein synthesis, collagen formation and energy metabolism. The zinc content in the human body ranges from 1.5 to 2.5 g (Hotz & Brown, 2004). The major factors in the etiology of zinc deficiency are inadequate dietary intake, an increased physiological requirement and increased loss or impaired use due to disease conditions (Gibson, 2006). Zinc is considered as a type-2 nutrient; deficiencies of type-2 nutrients result in reduction of body growth and urinary excretion, as the body attempts to conserve its stores.

2.2.1 Dietary Sources of Zinc

Zinc is ubiquitously available in different foods, most of which are associated with protein and nucleic acid (Gropper, Smith, & Groff, 2005). Animal-source foods such as organ meats, flesh meats, poultry and seafood are rich sources of zinc. Among plant-based food sources, whole grains, legumes and leafy vegetables are relatively rich sources. The Food and Nutrition Board of the National Research Council of the USA National Academy of Sciences established a recommended dietary allowance (RDA) for zinc for the first time in 1974 (Food and Nutrition Board, 1974). The EAR (Estimated Average Requirement) and RDA for zinc by age group are presented in Table 2-1.

Table 2-1. Dietary reference values for zinc.

Age	EAR*		RDA**	
	Male (mg/day)	Female (mg/day)	Male (mg/day)	Female (mg/day)
0-6 mo			2***	2***
7-12 mo	2.5 mg/day	2.5	3	3
1-3 y	2.5	2.5	3	3
4-8 y	4.0	4.0	5	5
9-13 y	7.0	7.0	8	8
14-18 y	8.5	7.3	11	9
19-50 y	9.4	6.8	11	8
≥ 59 y	9.4	6.8	11	8

Source: Otten, Hellwig, & Meyers (2006)
* EAR=Estimated Average Requirement, ** RDA=Recommended Dietary Allowance ***AI= Adequate intake

2.2.2 Zinc Absorption

Absorption is the process of influx of nutrients into enterocytes at the apical membrane and efflux across the basolateral membrane into the portal circulation (Krebs, 2000). The gastrointestinal system plays a major role in the homeostasis of zinc (Krebs, 2000; Lopez, Leenhardt, Coudray, & Remesy, 2002). After zinc is liberated into ionic form through digestion, it is absorbed through either a saturable carrier-mediated process or a nonsaturable uptake process (diffusion). In addition to being ingested, zinc is released endogenously into the intestinal tract from pancreatic and biliary secretions. Part of the endogenous zinc is either absorbed or excreted (Gropper et al., 2005).

The main mechanism of zinc absorption depends on the concentration in the consumed meal; it is absorbed passively with high dietary intake and actively with low intake (Roohani, Hurrell, Kelishadi, & Schulin, 2013). Zinc transporters, namely ZnT (SLC30) and ZIP (SLC39), are involved in zinc influx and efflux. The latter leads to the influx of extracellular or organellar zinc into the cytoplasm, whereas ZnT does the opposite (Prasad, 2012). Ten ZnTs and 14 ZIPs have been identified to date (Lichten & Cousins, 2009). ZIP4 is localized in the apical membrane

of intestinal cells, stomach and colon and is principally responsible for zinc influx (King, 2010). Its expression increases with low dietary zinc intake. ZnT5, believed to have a bidirectional role, is also localized in the apical membrane of intestine and Caco-2 cells (Cousins, 2010). Researchers have speculated that ZIP11 is expressed in the apical membrane, but this needs further investigation. ZIP14 is found in both the apical and basal membrane: however, its role remains to be determined. Zinc that enters enterocytes can be used by the intestinal cells themselves, stored in the enterocyte, or transported across the basolateral membrane to be used by other body tissues. ZnT2 and ZnTs 4 to 7 are involved in zinc transport into intracellular organelles (Cousins, 2010). ZnT1, localized in the basolateral membrane of the enterocyte, facilitates the movement of zinc across the basolateral membrane of the enterocyte into the portal circulation (McMahon & Cousins, 1998). ZIP5, localized at the basolateral membrane, was degraded with zinc depletion, suggesting that ZIP5 could serve as a monitor for body zinc status, but its role has yet to be studied (Dufner-Beattie, Kuo, Gitschier, & Andrews, 2004).

Zinc that crosses the basolateral membrane is then transported to the liver and released into systematic circulation, which leads to distribution to different tissues. In the blood, zinc is transported mostly by albumin, but 15 to 40% of zinc in the blood is transported by transferrin, α-2 macroglobulin, or immunoglobulin G (Gropper et al., 2005). A low molecular weight protein, termed thionein, binds and stores zinc not immediately required. Thionein, with zinc bound to it, is known as metallothionein (MT). MT serves as a source of zinc when the level of available zinc is low (Maret, 2000).

The absorption of zinc is affected by both dietary and systemic factors. Physiological conditions are among the systemic factors that affect the amount of intestinal zinc absorption. It was previously thought that absorption was linked to zinc status; however, a recent review indicated that absorption is linked more to dietary intake than to status (King, 2010). Zinc is

absorbed more efficiently when dietary intake is low and vice versa. Zinc homoeostasis is maintained largely by changes in absorption and excretion. It has been reported that such an adaption occurs in low-phytate diets but not in high-phytate diets (Hunt et al., 2008).

Among the dietary factors that affect zinc absorption negatively is the phytate, polyphenols and tannin content of meals, with phytate exerting the greatest negative influence (Lopez et al., 2002). The amount and quality of protein and zinc in meals also influences zinc absorption (Lönnerdal, 2000). Even when the dietary zinc supply and absorption is insufficient, the level of zinc is maintained in some parts of the body, such as the skeletal muscles, brain, lungs and heart. However, there is no reserve for zinc that could sustain long-term inadequate intake. If dietary intake and absorption progressively decline to meet the body's requirements for a long period, symptoms of zinc deficiency start to manifest.

2.2.3 Consequences of Zinc Deficiency

Since several metabolic processes in humans are zinc-dependent, many systems are affected by zinc deficiency (Hambidge, 2000). Meta-analysis reviews have demonstrated the role of zinc on growth (Brown, Peerson, Baker, & Hess, 2009; Brown, Peerson, Rivera, & Allen, 2002; Imdad & Bhutta, 2011). Zinc also affects immunity, thereby increasing morbidity in zinc- deficient humans (Fraker, King, Laakko, & Vollmer, 2000).

Manifestations of moderate zinc deficiency include growth retardation, loss of appetite, delayed wound healing, male hypogonadism and skin changes. Some of the clinical manifestations of severe zinc deficiency are alopecia, mental disturbance, recurrent infections and dermatitis (Prasad, 2012). Zinc deficiency also increases the risk of diarrhoeal disease, pneumonia and malaria, due to the significant role of zinc in the immune system (Bhandari, Bahl, Hambidge, & Bhan, 1996; Rink & Gabriel, 2001; Shankar et al., 2000). Zinc deficiency can occur at any age;

however, deficiency during pregnancy can lead to irreversible health consequences on both the mother and the fetus. The child could suffer from cognitive impairment and congenital malformations (Bhatnagar & Taneja, 2001). Among the common consequences for the mother are pregnancy-induced hypertension, miscarriage and prolonged and preterm labour. A recent study conducted in Southern Ethiopia confirmed that zinc deficiency was a major factor influencing cognitive abilities (Stoecker et al., 2009). About 16% of repetitions in primary school are associated with stunting in Ethiopia. In addition, a higher school dropout rate was noted in stunted children (Dione, Omamo, Diop, & Kaloko, 2013).

2.3 Iron

Iron is one of the essential trace elements required for the body's proper function. Iron plays a major role in different metabolic activities and functions as part of important human body structures, including hemoglobin and myoglobin. It is involved in oxygen transport, DNA synthesis and energy generation in the mitochondria (Montalbetti, Simonin, Kovacs, & Hediger, 2013; Pietrangelo, 2002). The human body contains 2 to 5 grams of iron, the majority of which is found in haemoglobin.

2.3.1 Dietary Sources of Iron

Dietary iron is present in two forms: heme and non-heme iron. The only source of heme iron is animal-source foods, whereas non-heme iron can be found in both plant- and animal-source foods. Organ meats, e.g., liver, are rich sources of heme iron from animal-source foods and red meat contains more iron than fish and poultry (Sharp, 2010). Among plant-based foods, bean, nuts

and dark green leafy vegetables are rich sources of non-heme iron. The EAR and RDA for iron are presented in Table 2-2.

Table 2-2. Dietary reference values for iron.

Age	EAR*		RDA**	
	Male (mg/day)	Female (mg/day)	Male (mg/day)	Female (mg/day)
0-6 mo			0.27***	0.27***
7-12 mo	6.9	6.9	11	11
1-3 y	3.0	3.0	7	7
4-8 y	4.1	4.1	10	10
9-13 y	5.9	5.7	8	8
14-18 y	7.7	7.9	11	15
19-50 y	6.0	8.1	8	18
51-70 y	6.0	5.0	8	8
>70 y	6.0	5.0	8	8

Source: Otten, Hellwig, & Meyers (2006)

* EAR=Estimated Average Requirement, ** RDA=Recommended Dietary Allowance ***AI= Adequate intake

2.3.2 Iron Absorption

Although there is no active and regulated excretion of iron, approximately 0.5-2 mg of iron is lost daily (Sharp, 2010). On the other hand, the majority of iron (20-25 mg per day) is recycled through the reticuloendolethelial system (Montalbetti et al., 2013; Srai & Sharp, 2012). However, to maintain iron balance in the body, the lost iron needs to be replaced (Hurrell & Egli, 2010). Iron imbalance occurs when the intake is lower than the requirement for a long period (Tapiero, Gate, & Tew, 2001). The consumption of an adequate amount of bioavailable iron is essential since absorption is one of the key regulatory mechanisms for iron hemostasis. The type and amount of iron, dietary factors, iron stores and the rate of erythropoiesis all determine the amount of iron to be absorbed (Srai & Sharp, 2012). Most of the absorption occurs in the duodenum, the upper part of the intestine. Three steps are involved in the absorption: uptake by intestinal cells across the

apical membrane, intracellular processing and transport to the basolateral membrane and release into the portal circulation.

Heme iron is well absorbed compared to non-heme iron. The absorption of heme iron present in meat is about 20-30%, whereas non-heme iron absorption is only 1-10% (Sharp, 2010). There is no clear understanding of heme iron uptake; however, studies indicate that it is absorbed intact through a pathway independent of non-heme iron absorption via a heme carrier protein (HCP1) (Shayeghi et al., 2005). HCP1 is localized in duodenal enterocytes and its expression has been found to be upregulated by iron deficiency and hypoxia. Once inside the cell, heme iron is liberated from the porphyrin ring by hemeoxygenase and joins the non-heme iron pool (Andrews & Schmidt, 2007). Unlike non-heme iron, its absorption is not affected by dietary factors, with the exception of calcium.

Non-heme iron absorption is primarily affected by inhibitors in food (Sharp, 2010). It exists in two oxidation states in food and the human body: as ferrous (Fe^{+2}) and ferric (Fe^{+3}) ions. Ferric iron is the most common form of iron that enters the intestine (McKie et al., 2001). Before it is absorbed, ferric iron needs to be reduced to a ferrous, more soluble form. This process can be facilitated by dueodenal cytochrome b (Dcytb), a protein with a ferric reductase property, localized in the duodenum (McKie, 2008). Dietary factors such as ascorbic and other organic acids also are believed to enhance iron absorption (Gillooly et al., 1983; Salovaara, Sandberg, & Andlid, 2002; Teucher, Olivares, & Cori, 2004). Ascorbic acid is a more potent enhancer of iron absorption than other organic acids since it has the capacity to keep iron in a more soluble form due to its reducing potential (Teucher et al., 2004). Some ferric iron can also be absorbed as is, but the mechanism is not clearly understood. The reduced form of iron (Fe^{+2}) is transported into the enterocyte via a divalent metal transporter (DMT1), also referred to as a divalent cation transporter (DCT1), SLC11A2, or NRAMP2 (Zimmermann & Hurrell, 2007). The expression of DMT1 is linked to

body iron status and its expression is increased with body iron depletion. Part of the iron that enters the enterocytes can be used by the intestine itself, stored, or transported into the circulatory system to be used by other tissues.

Ferroportin (FPN), also termed iron regulated transporter 1 (IREG1), SLC40A1, or MTP1, is a protein responsible for the efflux of iron from the enterocytes to the circulation (McKie et al., 2000). The expression of FPN is linked to body iron status. In the intestine, its expression is increased with body iron depletion. Before iron is transported into the blood, ferrous iron carried by FPN needs to be converted to the ferric form, which is facilitated by membrane-bound ferrioxidase, hephaestin (HEPH) (Anderson & Vulpe, 2009). Transferrin is a plasma protein that binds two iron atoms (Tapiero et al., 2001) and then carries ferric iron and transports it to different parts of the body for use or storage (Montalbetti et al., 2013). Part of the iron stored in the enterocytes and not transferred across the basolateral membrane is sloughed off after two to three days (Andrews & Schmidt, 2007). Liver-derived hepcidin controls the efflux of iron into the circulation (Srai & Sharp, 2012). It inhibits the efflux of iron into the circulation when the liver iron level is high and vice versa, by binding to ferroportin and degrading it (Andrews & Schmidt, 2007).

About 3-4 mg of transferrin-bound iron circulates in the blood (Srai & Sharp, 2012). Most of the transferrin-bound iron is transported to the liver and reticuloendothelial system (Montalbetti et al., 2013). Cells can take up iron bound by transferrin in different ways, but the best known method is via the transferrin receptor (TfR). The expression of the transferrin receptor is dependent on the cellular iron need (Gropper et al., 2005). The transferrin receptor complex enters the endosomal compartment of the cytoplasm via endocytosis (Tapiero et al., 2001). DMT1 is important for the endosomal release of transferrin-bound iron into the cytoplasm (Srai & Sharp, 2012). Iron taken up by cells but not needed is stored in ferritin (Srai & Sharp, 2012; Tapiero et al.,

2001). Ferritin can store up to 4,500 atoms of iron in its central cavity (Anderson & Vulpe, 2009). When there is more iron than can be stored as ferritin, part of it is degraded to hemosiderin (Koorts & Viljoen, 2007). Major storages sites are the liver, spleen and bone marrow. The reticuloendothelial system contributes mainly to the circulatory iron by recycling iron. When the level of stored iron declines and fails to meet the body's iron requirement, the consequences of iron deficiency start to manifest.

2.3.3 Consequences of Iron Deficiency

Iron is important for proper bodily functions and its deficiency has been associated with adverse health and social consequences. For example, iron deficiency has been associated with low levels of hemoglobin, a compromised immune system, poor brain development (Radlowski & Johnson, 2013) and reduced productivity and work performance (Haas & Brownlie, 2001). Iron deficiency that occurs during pregnancy can affect both the mother and the fetus with adverse long-term health consequences. Among the adverse consequences of iron deficiency in pregnant women are pre-term births, infants with low birth weights and still births (Coad & Conlon, 2011). Although the biological mechanism is not clear, studies have shown an association between iron deficiency anemia and lower cognitive development scores of children, as measured using standard techniques (Grantham-McGregor & Ani, 2001).

2.4 Pulses

Plant foods provide almost 80% of the energy and 65% of the protein supply worldwide (Sathe, 2002). Plant-based foods are relatively cheap compared to animal-source foods and serve as an alternative source of nutrients for low-income groups (Huma, Anjum, Sehar, Khan, &

Hussain, 2008; Kebebu et al., 2013; Tharanathan & Mahadevamma, 2003). In addition to their benefits as food for humans, pulses serve as feed for livestock production. They also contribute to household income as a cash crop and have valuable impacts in sustainable agriculture. Soil nutrient depletion is an issue for many households that depend on subsistence farming (Graham & Vance, 2003), as they cannot afford to purchase fertilizers. However, soil fertility can be improved by intercropping pulses with cereals since pulses have nitrogen fixing capacity. Studies have shown that the rotation of pulses could reduce the use of chemical fertilizers (Kumar & Bourai, 2012). The United Nations has recognized the versatile benefits of pulses and declared 2016 as the International Year of Pulses.

2.4.1 Dry Bean

Dry bean (*Phaseolus vulgaris* L.) is a polymorphic crop with 150-200 species (Sathe, 2002). Beans are consumed in different parts of the world, especially in Africa and Asia (de Almeida Costa, da Silva Queiroz-Monici, Pissini Machado Reis, & de Oliveira, 2006). Different names are used to refer to the dry bean, including French bean, kidney bean, navy bean, pinto bean, field bean, haricot bean and common bean. Ethiopia is the ninth producer of dry bean in the world (Food and Agriculture Organization, 2012). Dry bean is the second most important pulse in Ethiopia, accounting for 17% of the total pulse production (Rashid et al., 2010). Mostly produced by smallholder farmers, dry bean has a significant role in food security and in the export sector (Asfaw & Blair, 2014). Among the regional states in the country, the Oromia (43%), SNNPR (30%) and Amhara (24%) regions produced the highest percentage of dry bean in 2009/10. The majority of the production is used for household consumption, mostly in the rural area (Mulugeta, 2010). Dry beans are mostly consumed by boiling as *nifro*, a local word used to describe boiled grains or grains cooked as stew (Ministry of Agriculture and Rural Development, 2009). Different varieties of dry

bean are being improved and released by agriculture research centres. These varieties are usually adapted for different conditions such as yield, resistance to disease, seed size and drought resistance (Shimelis & Rakshit, 2005a). A recent study indicated that the Hawassa Dume variety of dry bean performed well in drought (Asfaw & Blair, 2014).

2.4.2 Chickpea

Chickpea (*Cicer arietinum* L.) is among the most widely consumed pulses across the world. Chickpea is referred to by different names, including bengal gram and garbanzo. Chickpea is one of the oldest grain legumes and is believed to have originated in an area around Turkey and Syria (Singh, 1997; Tanno & Willcox, 2006). In Ethiopia, the earliest record of chickpea dates back to 1520 BC (Joshi et al., 2001). Two types of chickpea exist: Kabuli and Desi. The Kabuli-type chickpea is larger in size and brighter in color than the Desi-type chickpea.

Chickpeas are cultivated in the semi-arid tropics and in temperate climatic zones, mainly in Asia, north and east Africa, southern Europe, Australia and North and South America (Singh, 1997). With an annual production of 409,733 metric tons, Ethiopia is the fourth largest producer of chickpea in the world, after India, Australia and Turkey (Food and Agriculture Organization, 2012). As the leading producer in Africa, Ethiopia accounts for 46% of chickpea production on the continent (Kassie et al., 2009). Among pulse varieties, chickpea is third in terms of production in Ethiopia, accounting for 16% of total pulse production (Rashid et al., 2010). For the farmers in Ethiopia, chickpea offers a unique opportunity because it can grow in residual moisture following the harvest of a main crop and provide a reasonable yield with minimal input.

2.4.3 Nutritional Importance of Pulses

Pulses are good sources of protein, folate and minerals (Sandberg, 2010), with protein content ranging from 16.89 to 34.7% (Boye, Zare, & Pletch, 2010). Pulses have the added benefit of providing lysine, a limiting amino acid in cereals. In developing countries, pulses greatly contribute to energy intake, second only to cereals (Juliano, 1999; Siddiq & Uebersax, 2012). Their demand is on the increase all over the world due to their high fibre content, low glycemic index and low calorie content (Asif et al., 2013). Pulses also exhibit important characteristics such as water holding, foaming and fat binding, all of which are required for pulse-based food products (Boye et al., 2010). The chemical composition of pulses, including minerals and phytate, varies based on climatic conditions, the nutrient content and pH of soil, location and genotype (Bueckert, Thavarajah, Thavarajah, & Pritchard, 2011; Ray et al., 2014; Reddy, 2002; Ribeiro et al., 2012).

2.4.4 Factors Limiting Pulse Consumption

Pulse consumption has been limited because they are hard to cook and take many hours to prepare (Huma et al., 2008; Wang & Daun, 2005). In Ethiopia, it is common practice to store pulses on farms before they are sold or consumed (Rashid et al., 2010). However, storage conditions are often poor. Long storage times and high temperatures can reduce water absorption capacity and exacerbate the hard-to-cook phenomenon (Laurent, Ousman, Dzudie, Mbofung, & Emmanuel, 2010; Reyes-Moreno, Okamura-Esparza, Armienta-Rodelo, Gomez-Garza, & Milan-Carrillo, 2000).

Pulses are deficient in sulphur-containing amino acids, methionine and cysteine (Asif et al., 2013). They also contain high amounts of anti-nutrients, which hinder their nutritional and health benefits. Anti-nutrients with a significance presence in pulses include proteinase inhibitors, e.g., trypsin inhibitors, oxalate, lectins, raffinose oligosaccharides, saponins, amylase inhibitor,

polyphenols and phytate (de Almeida Costa et al., 2006; El-Adawy, 2002; Sandberg, 2002; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010). Since most of the anti-nutrients affecting protein digestibility are heat sensitive, cooking alone has been found to improve protein digestibility in pulse-based diets (Nergiz & Gökgöz, 2007; Tharanathan & Mahadevamma, 2003). On the other hand, phytate, tannin and polyphenols present in pulses can withstand heat and thus are categorized as heat-stable anti-nutrients. When it comes to mineral bioavailability, these three anti-nutrients are of great concern because they form complexes with minerals, rendering them less available or unavailable for absorption.

2.5 Bioavailability of Nutrients

Bioavailability was first recognized as a result of animal studies that found not all consumed nutrients are used (Hurrell, 2002). The term "bioavailability" has been used extensively in nutrition (e.g., Benito & Miller, 1998; Berhanu, Mesfin, Kebebu, Whiting, & Henry, 2014; Gibson, 2007; Gibson, Perlas, & Hotz, 2006; Hotz & Gibson, 2007; Hurrell, 2002; Hurrell & Egli, 2010; Prakash & Jamuna, 2014; Sandberg, 2002; Sharp, 2005; Tako, Vandenberg, Thavarajah, Thavarajah, & Glahn, 2011; Campen & Glahn, 1999). However, Etcheverry, Grusak and Fleige (2012) argue that bioaccessibility is a better term since bioavailability cannot be measured entirely by *in vitro* methods. On the other hand, these authors note that Caco-2 cell intestinal absorption models, described below in section 2.6.2, can measure bioavailability. There is no universally accepted definition for nutrient bioavailability; it is defined variously by different researchers presented below.

- "The amount of nutrient that is available for absorption in a form that is physiologically useful" (Campen & Glahn, 1999, p. 94)
- "Proportion of the total amount of a mineral element present in a nutrient medium that is potentially absorbable in a metabolically active form"(House, 1999, p. 116)
- "The proportion of dietary nutrient that is absorbed and utilized through normal metabolic pathways" (Hurrell, 2002, p. 5)
- "Proportion of a given nutrient in a given food or diet which the body can actually utilize" (Benito & Miller, 1998, p. 586)
- "Measure of proportion of an ingested nutrient that is utilized for normal body functions"(Fairweather-Tait et al., 2005, p. 158)
- "Fraction of absorbed and utilized micronutrients (utilization includes storage and absorbed micronutrients) (Davidsson & Haskell, 2011, p. S24)

The first two definitions provided above by Campen and Glahn (1999) and House (1999) do not consider the physiological factors that can impact the absorption of minerals, making these definitions closer to that of bioaccessibility. According to Etcheverry et al. (2012), bioaccessibility refers to "the amount of ingested nutrient that is potentially available for absorption," and that this depends "only on digestion and release from the food matrix" (p.1). The remaining bioavailability definitions acknowledge that absorption can be affected by host factors.

As the above definitions suggest, a chain of events occurs following the ingestion of food, including the release of the nutrient from the food matrix, transport into intestinal cells, efflux across the basolateral membrane of intestinal cells, retention or excretion in urine and feces and transport to tissues for utilization and storage. As the net effect of different factors influences the

chain of events, bioavailability is critical for iron and zinc (Davidsson & Haskell, 2011). These factors are not totally related to the food that we consume and thus can be broadly classified as diet- and host-related factors (Hunt, 2010). Host-related or physiological factors include gastric acidity, intestinal secretions, levels of nutrients in tissues and body stores, the body's demand in different physiological states and life stages, such as infancy, pregnancy and lactation and gut microflora (Gibson, 2007). In addition to the importance of bioavailability in the etiology of micronutrient deficiency, poor environmental conditions under which the plants are grown also play a large role in the process. For example, the amount of micronutrients, such as zinc, selenium and iodine in plants, depends on the soil content (Cakmak, 2009).

Although host-related factors can impact nutrient absorption, the proportion of nutrients potentially available for uptake is related mainly to dietary composition (Fairweather-Tait, Phillips, Wortley, Harvey, & Glahn, 2007). Diet-related factors include the physiochemical form of the nutrient, food matrix, presence of enhancers and inhibitors of absorption, food processing and interaction among nutrients and other compounds found in the food (Gibson et al., 2006). Enhancers of iron include ascorbic acid and meat. Since the constituent of meat that enhances iron absorption is unknown, it is referred to as the "meat factor." Before promoting a particular food to tackle malnutrition, bioavailability should be assessed to determine how much of the nutrient is going to be used by the body. The conceptual framework in Figure 2-1 describes the types of research needed in designing a food-based approach as a public health intervention.

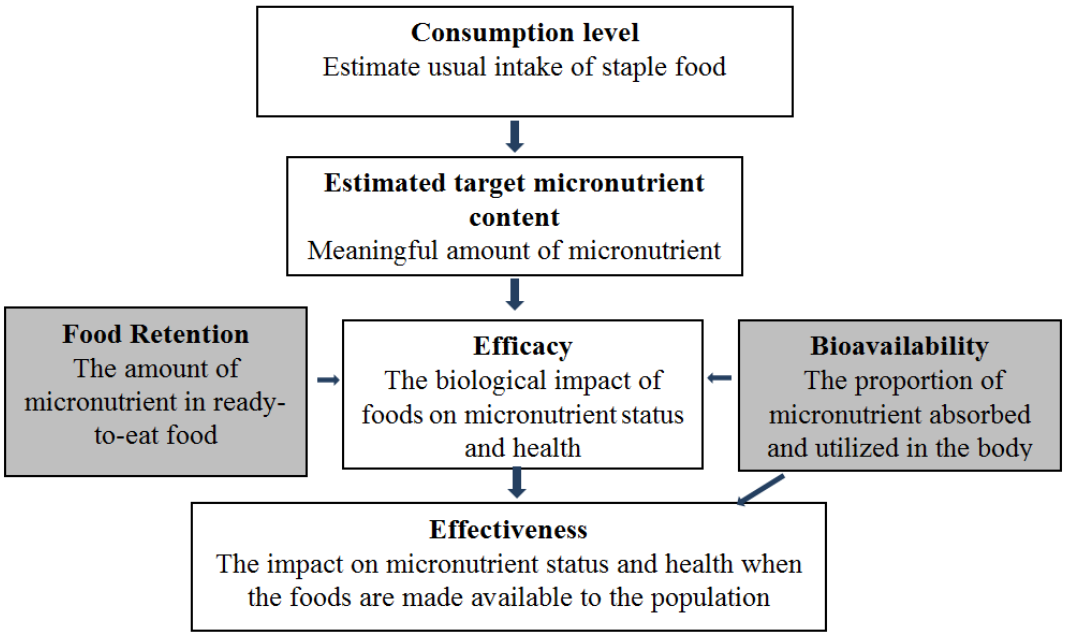

Figure 2-1. Nutrition research required for developing food based interventions.
From harvest to health: Challenges for developing biofortified staple foods and determining their impact on micronutrient status by Hotz & McClafferty, 2007, reprinted with permission (See Appendix 1 for copyright).

2.5.1 Phytate

Phytate (myo-inositol hexakisphosphate, IP-6) was initially recognized in 1855 (Oatway, Vasanthan, & Helm, 2001). The chemical formula is $C_6H_{18}O_{24}P_6$. Myo-inositol phosphate refers to an inositol ring with at least one phosphate group and 'myo' indicates the conformation of the attached hydroxyl group (Bohn, Meyer, & Rasmussen, 2008). Phytate is the salt form, whereas the acid form is termed phytic acid. Calcium or magnesium salt forms are specifically referred to as phytin (Ali, Shuja, Zahoor, & Qadri, 2010).

Phytate, the main storage unit for phosphate, minerals and inositol, is essential for seed germination and plant growth (Raboy, 2003). Phytate starts to develop after flowering and accumulates until the seed reaches maturity (Bohn et al., 2008; Gupta, Gangoliya, & Singh, 2013). Phytate accounts for 60 to 80% of the phosphorus content of cereals, legumes and oil-seeds. Understanding the biosynthesis of phytate is complicated because the location in which it

accumulates differs based on species. However, it is known that the biosynthesis of phytate starts with the conversion of D-glucose-6-P to Ins(3)P1, which is further processed to form different inositol phosphates (Loewus & Murthy, 2000). Phytate is mostly stored in the cotyledon of pulses and in the aleurone layer of cereals, with the exception of maize, where it is stored in the germ (Bohn et al., 2008). Phytate concentrations in plants vary depending on the location, year of cultivation, genotype, wet or dry conditions (rain or drought), temperature and pathogens (Bohn et al., 2008; Bueckert et al., 2011).

The negatively charged phosphate groups (Figure 2-2) in phytate give it the capacity to chelate minerals (calcium, magnesium, zinc, iron), leading to a phytate mineral complex (Bohn et al., 2008; Lopez et al., 2002). The complex formed between phytate and minerals has low solubility in the pH of the intestine (Persson, Turk, Nyman, & Sandberg, 1998). Since the minerals are bound to phytate and no longer in ionic form, carrier proteins located on the intestinal cell membrane cannot bind and transport them (Lopez et al., 2002). To be able to use the mineral bound with the phytate, the complex must be hydrolysed, but this process needs an acidic environment as opposed to the alkaline environment of the upper part of the small intestine. Moreover, the upper part of the intestine has a limited microbial population to contribute to the breakdown of phytate. Consequently, the insoluble complex is excreted via the stool (Lönnerdal, 2000).

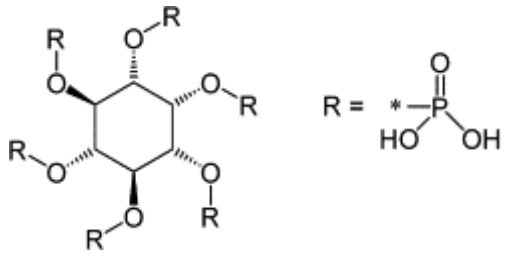

Figure 2-2. Chemical structure of phytate.
Dietary roles of phytate and phytase in human nutrition: A review by Kumar, Sinha, Makkar and Becker (2010), reprinted with permission (See Appendix 1 for copyright).

An earlier study reported that there is no intestinal adaptation associated with a high intake of phytate (Brune, Rossander, & Hallberg, 1989) or mechanism to absorb phytate. In a later study, Hunt et al. (2008) reported that humans could adapt to absorb more zinc in low-phytate low-zinc diet but not in high-phytate diets. However, a recent study carried out on non-heme iron absorption in young women with suboptimal iron store indicated that regular consumption of a high phytate diet may reduce the inhibitory effect of phytate (Armah, Boy, Chen, Candal, & Reddy, 2015). Partial adaptation of iron absorption also was noted in men consuming a low-bioavailability diet, however it is not clear if the adaptation is specific to inhibitors and enhancers of non-heme iron absorption (Hunt & Roughead, 2000). Despite some adaptation in iron absorption, the impact of phytate on mineral bioavailability is dose dependent (Hurrell & Egli, 2010); thus, reduction of phytate in food helps to increase bioavailability. Phytate also has a negative impact on the environment. Phytate excreted by animals during livestock production can result in the accumulation of phosphorus in the soil, which may contaminate surface water (Bohn et al., 2008). On the other hand, a systematic review of 28 studies reported the benefits of phytate in the prevention of cancer (Fox & Eberl, 2002).

Different techniques have been used to extract and quantify phytate from plants, which include anion-exchange chromatography (AEC), High-Performance Liquid Chromatography (HPLC), precipitation and colorimetric procedures. Synchrotron X-ray and Nuclear Magnetic Resonance (NMR) have also been used in some cases (Thavarajah & Thavarajah, 2014). HPLC, AEC and NMR have better accuracy; however, they are expensive and require a trained operator and sophisticated instruments (Gao et al., 2007).

Most analytical methods for measuring phytate are based on phytate's ability to solubilize in an acidic medium and form a complex with iron. To quantify phytate, researchers analyze phosphorus or iron in ferric phytate or use residual iron in the solution following the precipitation

of ferric phytate from a known concentration of ferric salt. The original methods that involved precipitation and titration suffered from the inability to detect the exact endpoint of titration and from lack of specificity, as other chemicals may also precipitate (Young, 1936). Another method that involved the use of ion-exchange resin to avoid interference with inorganic phosphate was reported by Harland & Oberleas (1977). Since this method was time consuming, several attempts were undertaken to further modify it (Fruhbeck, Alonso, Marzo, & Santidrian, 1995; Latta & Eskin, 1980). A simple and low cost modification of the method for measuring phytate was reported by Latta and Eskin (1980). Because phytate cannot absorb ultraviolet-visible light, they identified phytate by a colorimetric method using coloring reagents. Wade's reagent, a combination of ferric chloride and sulfosalicylic acid, was used in their study. Latta and Eskin (1980) reported that since iron has more affinity to phytate than to sulfosalicylic acid, when the reagent is added to a digest containing phytate in an acidic medium, sulfosalicylic acid is replaced by the phytate, which leads to the formation of an insoluble complex. This process was found to reduce the pink colour of the reagent, with the intensity of this reduction depending on the amount of phytate present in the digest. The absorbance of the samples, along with that of standards and a blank measured using a spectrophotometer were then used to develop a standard curve for determining phytate concentration.

The Latta and Eskin (1980) method was later modified by Vaintraub and Lapteva (1988). These modifications included changes in extraction time and centrifugation temperature and the addition of cleaning steps with NaCL (Vaintraub & Lapteva, 1988). Their modified method resulted in the underestimation of phytate (Gao et al., 2007). Further modifications were done on the method by Gao et al. (2007) and compared with AEC, HPLC and NMR. The results revealed good agreement between phytate measurements of 42 soybean samples carried out by the different method. Overall, the modified Wade's reagent method is used most frequently; however, it is not

without limitations. For example, phenolic compounds and organic acids can bind with iron, resulting in overestimation of results (Gao et al., 2007; Thavarajah & Thavarajah, 2014).

Phytase, which is chemically known as myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolases, could help to remove phosphate groups from the inositol ring of phytate and create inorganic orthophosphates and intermediate myo-inositol phosphates (IP-5, IP-4, IP-3, IP-2 and IP-1). To increase iron absorption, phytate (Ip-6) needs to be degraded to inositol phosphates lower than IP-3 (Sandberg et al., 1999). As the number of phosphate groups on myo-inositol decreases, so does the stability and solubility of the myo-inositol phosphate-mineral complex, which improves bioavailability (Greiner & Konietzny, 2006). Phytase can be obtained from four different sources: plants, microbes, small intestinal mucosa and gut microbial flora (Kumar et al., 2010). However, phytase activity in most plants in the dry or dormant stage is negligible. In addition, phytase is not significantly present in the human gut (Iqbal, Lewis, & Cooper, 1994).

2.5.2 Polyphenols

Plant polyphenols are a wide range of secondary metabolites (Robards, 2003) ubiquitously present in varying amounts in plant foods such as cereals, legumes, vegetables, fruits, tea, coffee and wine. Plant phenols include simple phenols, coumarins, flavonoids, phenolic acids, stilbenes and tannins (Naczk & Shahidi, 2004). Tannins are further subdivided into hydrolyzable tannins and condensed tannins (proanthocyanidins). The word tannin is developed from the definition of tanning, which is the process of converting animal hides and skin to leather with the application of plant extracts (Salunkhe & Chavan, 1989). Tannins are water-soluble, high molecular weight phenolic compounds with the ability to form insoluble complexes with certain proteins, making them less available or unavailable for digestion (Champ, 2002; Gilani, Wu Xiao, & Cockell, 2012). They have a large number of hydroxyl groups, which explains their capacity to form complexes

with carbohydrate and protein (Bravo, 1998). Most polyphenols are capable of binding iron and rendering it less bioavailable (Sandberg, 2002) as shown in Figure 2-3. Gilani and colleagues (2012) reported that different types of bean contain large amounts of tannin (up to 72 g/kg). Despite their anti-nutritive impacts, polyphenols are useful for plants as they have a role in defending them against pathogens and pests (War et al, 2012).

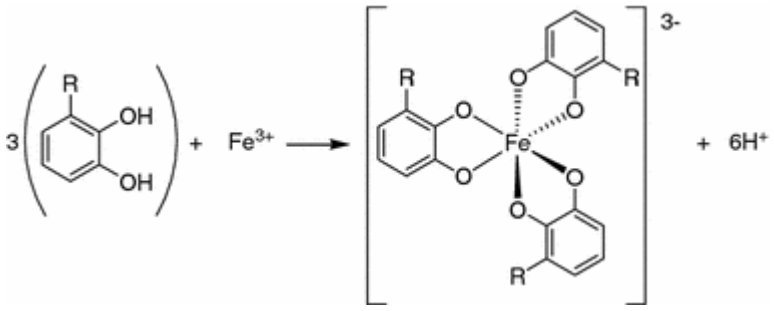

Figure 2-3. Expected iron–polyphenol complexes (Gallols, R=OH; Catechols, R=H).
A review of the antioxidant mechanisms of polyphenol compounds related to iron binding by Perron and Brumaghim (2009), reprinted with permission (See Appendix 1 for copyright)

There is no standardized procedure for the analysis of phenols; some methods detect specific phenolic groups, whereas others detect the total content of phenols (Salunkhe & Chavan, 1989). Different techniques, including gas chromatography, HPLC and spectrophotometry, have been used to quantify phenols (Naczk & Shahidi, 2004). Vanillin-HCl and Folin-Ciocalteu are common methods used to determine the tannin and total polyphenol contents of foods, respectively. They are most commonly extracted in organic solvents such as methanol, ethanol, acetone, or ethyl acetate. The blue colour formed in the Folin-Ciocalteu method is measured spectrophotometrically and is expressed in molar equivalents of the most commonly used standards, gallic acid, or quercetin (Robards, 2003). The Folin-Ciocalteu method is not specific and thus detects total phenols. One of the limitations of this method is the interference of reducing agents (Naczk & Shahidi, 2004; Robards, 2003). The vanillin-HCL method is specific to condensed tannins with catechin most commonly used as a standard. In the presence of mineral acid, vanillin can react with

tannin. The hydroxyl group of tannin is substituted with aldehyde (vanillin), yielding a red color. The hydroxyl group is the electron donor and enhances the substitution reaction (Salunkhe & Chavan, 1989). The method is simple, specific and sensitive (Naczk & Shahidi, 2004).

2.5.3 Calcium

Pulses contain higher amounts of calcium than do cereals, roots and tubers (Frossard, Bucher, Mächler, Mozafar, & Hurrell, 2000). Calcium is not only affected by the anti-nutrients in plants that reduce zinc and iron bioavailability but also contributes to their bioavailability. It was initially thought that calcium has a synergistic effect on zinc bioavailability; in other words, it has the potential to bind with the already formed phytate zinc complex in the presence of high phytate and further reduce zinc bioavailability (Morris & Ellis, 1980; Zemel & Shelef, 1982). For this reason, the molar ratio of phytate*calcium:zinc was proposed to predict zinc bioavailability (Bindra, Gibson, & Thompson, 1986). It was later reported that phytate*calcium:zinc is a better predictor of zinc in similarly processed foods but not when the food processing alters the interaction of zinc with phytate and other food components (Fordyce, Forbes, Robbins, & Erdman, 1987). However, Lönnerdal (2000) reported a contradictory result, finding that adding higher calcium levels to a soy-based infant formula increased zinc absorption. Lönnerdal (2000) also stated that a phytate-to-zinc (phytate:zinc) molar ratio may have better predictive ability than phytate*calcium:zinc in estimating zinc bioavailability from the diet. In a randomized control trial of healthy women who consumed high and low phytate diets, either fortified with moderate or high calcium levels, Hunt and Beiseigel (2009) demonstrated that dietary phytate reduces zinc absorption but calcium has no effect on zinc absorption. The issue of calcium remains complex. Although some studies used the phytate*calcium:zinc molar ratio to predict zinc bioavailability (Frontela, Scarino, Ferruzza, Ros, & Martínez, 2009; Ma et al., 2005; Umeta, West, & Fufa, 2005),

no consensus exists on the critical phytate*calcium:zinc molar ratio, thus, the ratio is not widely used.

Even though the mechanism on how calcium impacts iron bioavailability is not yet fully clear, the effect of calcium on iron bioavailability has been reported. Initially, calcium was thought have an inhibitory effect on iron absorption at the apical membrane of the enterocytes, but later calcium was reported to have an effect at both apical and basolateral membranes. The proposed mechanisms of inhibition include competitive inhibition (Etcheverry, Wallingford, Miller, & Glahn, 2005), a decrease in DMT-1 (Thompson, Sharp, Elliott, & Fairweather-Tait, 2010) and interference with gastrointestinal transit time (Hallberg, Brune, Erlandsson, Sandberg, & Rossander-Hulten, 1991). There are contradictory reports on the effect of calcium on Caco-2 cell ferritin formation, a measure of iron bioavailability. A recent study reported that ferritin formation in Caco-2 cells was significantly decreased due to calcium, which altered the cellular localization of DMT-1 down regulating iron transport into the cell (Thompson et al., 2010). On the other hand, an earlier study observed that the addition of different calcium salts did not affect ferritin levels (Etcheverry et al., 2005). It should be noted that most of the studies showing the effect of calcium on iron bioavailability are single-meal studies, which may have exaggerated its impact (Grinder-Pedersen, Bukhave, Jensen, Højgaard, & Hansen, 2004).

2.6 Techniques to Assess Iron and Zinc Bioavailability

Different methods have been designed to determine the bioavailability of nutrients. These methods include using algorithms and conducting *in vitro* and *in vivo* studies. The latter involves preparing a meal labelled with either a radioisotope or staple isotope nutrient provided to study participants accompanied by follow-up to assess the uptake of the nutrient of interest. Indirect non-

isotopic methods, such as chemical balance, changes in hemoglobin, serum ferritin and zinc, have also been used to assess bioavailability in human studies. *In vivo* studies are expensive and ethical issues are of great concern. Animal models such as rat pups have been used to determine zinc bioavailability because they have lower levels of phytase than adult rats; however, their immature gut limits their full use (Hotz, 2005). To assess iron bioavailability, rat models are not as useful as they are for zinc bioavailability since rodents can synthesise ascorbic acid, unlike humans. *In vitro* methods, including solubility, dialyzability gastrointestinal models and Caco-2 cells, are the most popular methods used to assess iron and zinc bioavailability. Assessing the solubility of iron at a pH simulating gastrointestinal digestion can be unreliable because it does not replicate all the physiological conditions of the intestine and results have not been well correlated with human studies (Pynaert et al., 2006). Dialyzability, a measure of dialyzable mineral, is a better predictor of bioavailability than solubility. However, it has low reproducibility and does not measure uptake (Fairweather-Tait et al., 2005, 2007). Algorithms and Caco-2 cell methods are discussed in the sections below.

2.6.1 Algorithms and Ratios to Estimate Bioavailability

To provide information about bioavailability, researchers have developed different algorithms (Hallberg & Hulthén, 2000; Hunt, 2010) based on the amounts of inhibitors and enhancers and the form and amount of iron and zinc present in food. Bioavailable zinc and iron from the diet can be estimated from the phytate:zinc molar ratio and phytate-to-iron (phytate:iron) molar ratio, respectively. The phytate:zinc molar ratio can be calculated by dividing the molar concentration of phytate (molecular weight, 660) by the molar concentration of zinc (molecular weight, 65.4). The phytate:iron molar ratio can be calculated in the same way, using 55.85 as the atomic weight of iron. To improve bioavailability, the phytate:iron molar ratio needs to be reduced

below 1:1; if possible, a reduction to below 0.4:1 is most desirable (Hurrell, 2004). Pulses, cereals and nuts have the highest phytate:zinc molar ratios, ranging from 22 to 88, whereas other plant foods have phytate:zinc molar ratios in the range of 0 to 42 (IZiNCG, 2004). One study based on modeling of data from stable-isotope studies reported that dietary phytate may not have significant effect in children as opposed to adults; thus, the use of phytate:zinc molar ratio for children might be limited (Miller, Hambidge, & Krebs, 2015). Umeta and colleagues (2005) reported that the phytate:zinc molar ratios of most commonly consumed dishes in Ethiopia were >20 for non-fermented cereal foods, >15 for legumes and >12 for fermented cereals, starchy tubers and roots. Their results show that most foods do not meet daily requirements, as a phytate:zinc molar ratio of >15 is indicative of poor bioavailability of zinc (Hotz & Brown, 2004). The phytate:iron and phytate:zinc molar ratios of selected commonly consumed dishes in Ethiopia are presented in Table 2-3 and Figure 2-4, respectively.

Table 2-3. Phytate:iron molar ratio of commonly consumed foods in Ethiopia.

Type of dishes	Phytate:iron molar ratio
Tef injera, unfermented	1.1
Tef injera, fermented	0.3
Maize bread	3.1
Maize porridge	4.8
Sorghum, boiled	6.4
Maize, boiled	8
Ground chickpea stew	2.5
Split kidney bean stew	4.9
Split lentil stew	3.5
Whole kidney bean stew	10.6
Ground pea stew	2.6

Source of data:Umeta, West, & Fufa (2005)

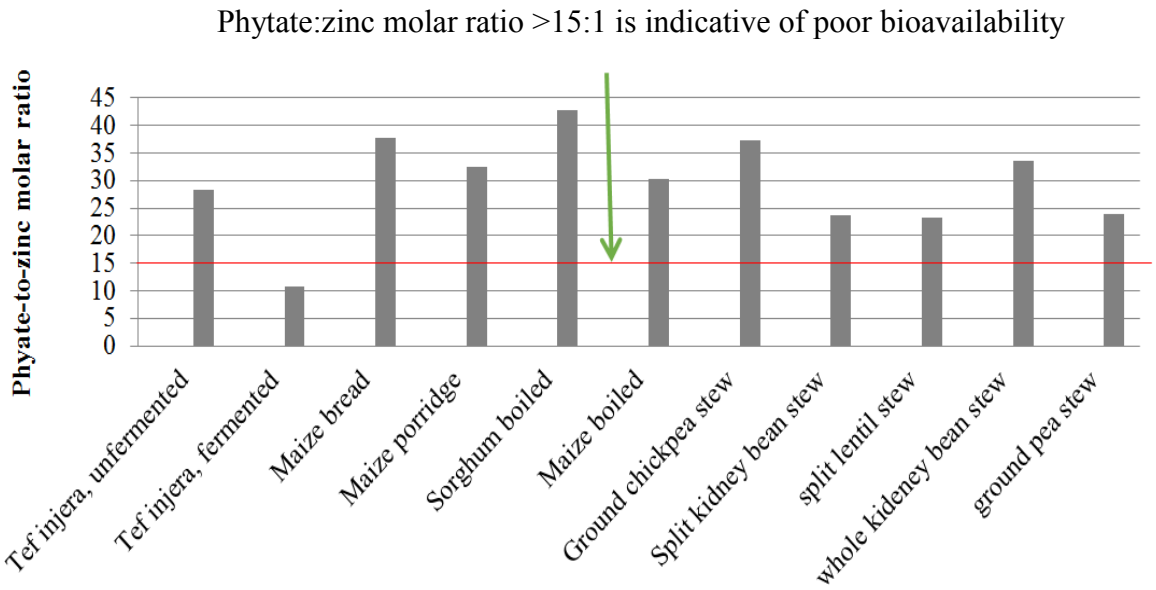

Figure 2-4. Phytate:Zn molar ratio of commonly consumed foods in Ethiopia.
Source of data:Umeta, West, & Fufa (2005)

2.6.2 Caco-2 Cell Intestinal Absorption Model Coupled with *in Vitro* Digestion

Caco-2 cell is the short name for polarized, human colon carcinoma cell lines. Although these cells originate as colon cells, they differentiate into polarized, monolayer cells that exhibit

features of intestinal cells when they are cultured. The Caco-2 cell model is a cost-effective, rapid and useful tool for determining iron bioavailability (Glahn, Wortley, South, & Miller, 2002).

Dietary factors known to affect iron absorption in human studies have shown similar effects on iron uptake in studies that used Caco-2 cells (Follett, Suzuki, & Lönnerdal, 2002; Glahn et al., 2002; Kalgaonkar & Lönnerdal, 2008; Yun, Habicht, Miller, & Glahn, 2004). Yun and colleagues (2004) reported that the absorption ratio increased with an increase in ascorbic acid, whereas it decreased with tannin. The inhibitory effect of phytate on iron absorption also was demonstrated using Caco-2 cells (Glahn et al., 2002). Heme absorption in Caco-2 cells have been found to correlate with findings from human studies (Follett et al., 2002). In addition, the fact that Caco-2 cells originate in humans give this method an advantage over rodent models, since, unlike humans, rodents can produce ascorbic acid (Sharp, 2005). Caco-2 cells also exhibit a similar uptake mechanism as human enterocytes; prior to absorption, iron should be reduced to the ferrous form in both cases (Han, Failla, Hill, Morris, & Smith, 1995).

Iron bioavailability of lentil measured using Caco-2 cells showed similar results in an *in vivo* study (Tako et al., 2011). In this model, the prepared food was subjected to *in vitro* peptic and intestinal digestion and the digest was added to the cells, as shown in Figure 2-5. The amount of ferritin formed in the Caco-2 cells exposed to the food digest was then used as a surrogate for iron bioavailability. The enzymes to be used for digestion need be treated using Chelex 100 and a cell culture medium with ≤ 8 μg iron per litre should be used so that the level of contaminant iron and zinc can be minimized (Glahn, Lee, Yeung, Goldman, & Miller, 1998). Since transepithelial resistance and the efficiency of iron uptake can be influenced by passage numbers, it is advisable to use Caco-2 cells at passage 30-40 and 10-14 days post-confluence as the cells become mature and iron transport molecules reach their maximum level (Fairweather-Tait et al., 2007; Sandberg, 2002). However, the model has limitations in its ability to determine bioavailability; it cannot

determine the effect of an individual's physiological status (Sharp, 2005). It is important to consider that large molecular weight iron complexes like ferritin could be prevented by the dialysis membrane from reaching the cells (Fairweather-Tait et al., 2007). Poor agreement of results in different laboratories has been reported (Sandberg, 2010). To help compare the results of different laboratories, researchers have recommended expressing ferritin in ng/mg cell protein and using ferrous sulphate during the experiment (Fairweather-Tait et al., 2007).

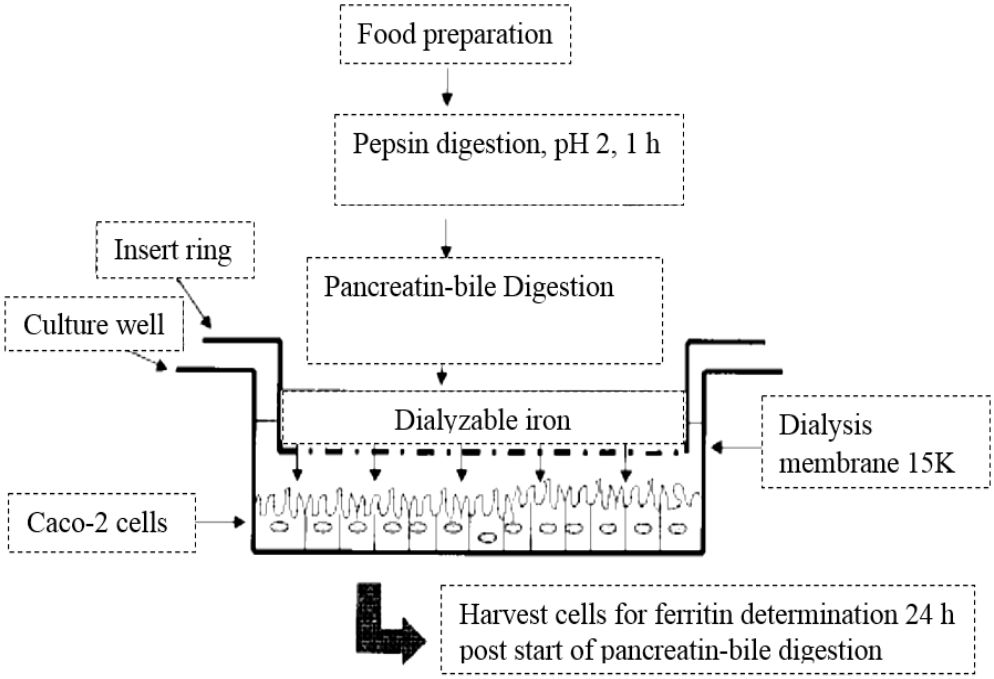

Figure 2-5. Caco-2 cell coupled with *in vitro* digestion to measure iron bioavailability.
Caco-2 Cell Ferritin Formation Predicts Nonradiolabeled Food Iron Availability in an In Vitro Digestion/Caco-2 Cell Culture Model by Glahn et al., 1998, reprinted with permission (See Appendix 1 for copyright).

2.7 Interventions to Alleviate Iron and Zinc Deficiency

Among the recommended strategies to alleviate nutrient deficiencies are supplementation, fortification, biofortification and dietary diversification and modification. As each of these strategies has associated limitations, they are designed to complement each other.

Supplementation, fortification and dietary diversification are considered to be short-term, mid-term and long-term approaches, respectively. Biofortification, a strategy aimed at improving the micronutrient content of staple crops through plant breeding or the use of micronutrient-containing fertilizers, has gained attention in recent years (Bouis, Graham, & Welch, 2000). The next section discusses each of the four strategies.

2.7.1 Supplementation

Supplementation is the provision of pharmaceutically prepared vitamins and minerals in the form of capsules, tablets, or injections administered to specific target groups in a community. It has an advantage in that the supplements are readily available, but cost, accessibility and delivering them to the most vulnerable groups are difficult most of the time. Iron and folic acid supplementation is recommended for all pregnant women as a strategy to prevent low birth weight, iron deficiency and maternal anaemia (World Health Organization, 2012). However, studies have indicated that compliance with iron supplementation is generally low (Habib et al., 2009; Haidar et al., 2003; Haidar & Pobocik, 2009). The 2011 EDHS report showed that only 17% of Ethiopian women took iron supplements during their last pregnancy (Central Statistical Agency [Ethiopia] and ICF International, 2012). A recent study in Ethiopia indicated that only 15% of pregnant women fully complied with iron supplementation orders in the four weeks preceding the survey (Gebremedhin et al., 2011). Although zinc supplementation is recommended for children with diarrhea, only a few children have access to supplementation (Walker, Young, Black, Fontaine, & Black, 2009).

2.7.2 Fortification

According to the United Nations Codex Alimentarius Commission (1987), food fortification refers to "the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups" (p. 2). Unlike supplementation, this method does not add a burden on women or caregivers and clinical trials have indicated its efficacy; however, food fortification may not benefit communities that depend on unprocessed local production. Among the factors that determine the effectiveness of fortification are the presence of inhibitory factors and the selection of the proper fortificant in particular situations. There is an initiative to fortify flour and oil in Ethiopia, although it has not yet been implemented (Federal Ministry of Health, 2011).

2.7.3 Biofortification

Biofortification is the development of plant varieties rich in vitamin and mineral content. Proven agricultural strategies improve the nutrient contents of plants, including fertilizers, plant breeding and genetic engineering (Welch & Graham, 2004). Progress has been made in breeding for better micronutrient levels. For example, iron-biofortified bean and pearl millet, zinc-biofortified rice and wheat, vitamin A-biofortified cassava, maize and orange sweet potato are currently available.

With regards to iron and zinc biofortification, the focus is on high mineral concentration, low anti-nutrient concentration, or a combination of both. A nine-month feeding trial of biofortified rice increased serum ferritin in Philippines (Haas et al., 2005). Another feeding trial conducted over 128 days using iron-biofortified bean improved the iron status of Rwandan women (Haas et al., 2016). However, a similar earlier study reported that the absorption of iron from biofortified

bean varieties, measured using a stable isotope technique, had low bioavailability (Petry et al., 2012). This result was explained by the presence of high amounts of phytate and polyphenols (Petry et al., 2014), which need to be addressed for the biofortification to be successful. Breeding low phytate and low polypehnol plants is suggested as an option to improve iron bioavailability; however, yield and long-term health impacts of low phytate and low polyphenol plants need to be considered. Research is needed on the safety of iron-fortified crops, particularly in countries with high rates of infectious disease such as malaria. According to the literature, the next challenges in biofortification are demonstrating its effectiveness and scaling up (Ruel & Alderman, 2013).

2.7.4 Dietary Diversification

Dietary diversification is considered to be a long-term strategy in which individuals access and utilize different food items to meet their daily nutrient requirements. A diversified diet with enhancers of absorption and rich sources of nutrients like animal source foods is the best strategy to alleviate nutrient deficiencies. Food-based strategies are generally more acceptable and sustainable than supplementation, fortification and biofortification. However, when designing effective food-based approaches to alleviate micronutrient deficiencies, individuals need to understand the nutrient and anti-nutrient contents of food, preferences and beliefs, as well as cope with the resulting workload from paying attention to such details (Gibson, 2011). Not all households have the ability or purchasing power to ensure their members consume diversified diets. As well, for most households, improving bioavailability through food-based and food processing strategies requires behavioural change. Once new dietary patterns are established, however, they are not necessarily difficult to maintain and these patterns can become more sustainable as they are handed down from one generation to the next.

2.7.5 Household-level Food Processing Strategies and Consumer Acceptance

In Ethiopia, pulses are prepared and consumed in various forms. The most common cooking methods for bean and chickpea are boiling and roasting (Berhanu et al., 2014; Kabata et al., 2016). The type of cooking method employed to prepare pulses could change the nutrient composition of the cooked pulse-based food products compared to raw pulses; however, some nutrients could be retained better than others (Pujolà, Farreras, & Casañas, 2007). Among the common household-level food processing strategies employed to improve mineral bioavailability are soaking, malting, fermentation, germination, dehulling and milling. These processing techniques can play a major role in the hydrolysis of phytate, either through activation of phytase, naturally present in plants and microorganisms, through synthesis of phytase, through diffusion of phytate, or a combination of these.

Dehulling and milling has been found to be helpful in reducing phytate in monocotyledon seeds such as cereals in which the phytate is located in the outer layer of the endosperm (aleurone). Although dehulling and milling can help reduce polyphenols in legumes, they cannot help reduce phytate as it is located within the cotyledon. Another disadvantage of milling is that it may remove some amounts of minerals and fibre (Bohn et al., 2008; Gupta et al., 2013). Reducing the amount of phytate has the potential to increase iron absorption 2- to 12-fold depending on the level of reduction (Hurrell, 2003, 2004).

Processing methods such as soaking, germination and fermentation may not result in complete dephosphorylation of phytate (Kumar et al., 2010); however, a large percentage (73% to 90%) of phytate reduction has been reported in germinated chickpea and bean (Khalil et al., 2007; Shimelis & Rakshit, 2007). Additional improvements reported due to soaking, germinating and fermenting pulses include reduced cooking time (Ghavidel & Prakash, 2007; Khalil et al., 2007), increased protein digestibility (Alonso, Aguirre, & Marzo, 2000; Nergiz & Gökgöz, 2007) and the

production of ascorbic acid (Masood, Shah, & Zeb, 2014). Consumers will benefit from improvements obtained by soaking, germinating and fermenting pulses if they are willing to carry out these methods. Thus, it is important to conduct sensory evaluation to determine acceptance and sensory characteristics of the products developed by applying such strategies.

2.7.5.1 Consumer Acceptance

Sensory evaluation is defined as "a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing" (Anonymous, 1975, as cited in Stone & Sidel, 2004, p. 15). Sensory evaluation is an invaluable tool used to understand if a food product will be used by end users. For example, if a local recipe is modified by soaking, germination, or fermentation of the ingredients, sensory evaluation could be carried out to determine if the modified recipe is still acceptable.

Different sensory evaluation techniques can be conducted using humans as a measuring instrument and relying on their sensory perception. These techniques are generally classified into three major groups: discrimination or difference, descriptive and affective (Lawless & Hildegarde, 2010). A discrimination or difference test can be an overall difference test or an attribute difference test. In an overall difference test, panellists are asked to detect if there is any difference between food samples, whereas in an attribute difference test, panellists are asked to measure a difference between food samples by concentrating on a single attribute such as sweetness. A number of discrimination tests exist, including triangle, duo-trio, two-out-of-five, paired comparison and ranking test (Larmond et al, 1999). A descriptive test involves the detection and description of the perceived sensory characteristics of a product. Panellists are requested to evaluate food samples and provide information on the intensity of sensory attributes. The descriptive test is the most

expensive form of sensory evaluation because of the need to train panellists over long periods of time. An affective test is the preferred technique when the objective is to determine how well a product is liked by end users (Meilgaard et al., 2006). Affective tests can be further categorized into preference and acceptance tests. In a preference test, the main objective is to understand which one of the samples is preferred by the target consumer. The preference test is usually presented as a choice question. It could be a paired preference where the respondent is requested to choose one sample over another, or a rank preference where respondents are required to rank the samples in their relative order of preference. The other form of an affective test is an acceptance test, which helps to determine if the product is acceptable to consumers or how well a product is liked.

The findings from affective tests are useful in product development because they have end users choose between different products using different scales. A hedonic scale can be used to determine the degree of acceptability. A 9-point hedonic scale (from 1=dislike extremely, 5=neither like nor dislike, to 9=like extremely) is most common; however, modifications are carried out depending on the context (Berhanu et al., 2014; Kebebu et al., 2013). Tests may be conducted in a central location or in consumers' homes. The former is useful for testing a large number of products and is easier to monitor. Home testing is advantageous for testing products under natural conditions of use, but it is time consuming since it requires repeated testing of the product under daily use conditions, which also increases non-response rates (Meilgaard et al., 2006).

Panels can be grouped into highly trained experts, trained laboratory panels, laboratory acceptance panels and consumer panels (Larmond et al., 1991). Laboratory acceptance panels can be used to screen products to be tasted by consumers and minimize sensory fatigue that may lead to bias. Common attributes of food tested in sensory evaluation are appearance, odour, texture, taste and overall acceptability.

Sensory measurements can be biased by different factors: expectation error, stimulus error, position bias, suggestion effect and identification bias. Expectation error occurs when the panellists have prior information about the test or when individuals with direct involvement in the study are used as panellists. Stimulus error occurs when the food tested differs in size and or has irrelevant characteristics, which may influence the testers. Position bias occurs when panellists assign ratings based on the order in which the products are presented. This problem can be addressed through random presentation of the products and the use of random number tables. Suggestion effect can occur if one panellist influences another. To avoid this problem, researchers should ensure privacy. Identification bias can occur if the panelists can identify the product. This problem can be addressed by coding, such as blinding the product by labelling it with three digit numerical codes.

Sensory studies have been used to determine the effects of food processing on sensory attributes and to understand the acceptance of products. Sensory evaluation of germinated and control chickpea samples measured using a 9-point hedonic scale revealed that sprouting has a significant effect on appearance, with the highest appearance score noted during a short duration (24 h) of germination (Khalil et al., 2007). The same study reported that taste was significantly affected by germination time; however, there was no significant effect on texture and odor. A study carried out to determine the effect of adding germinated pulses to rice pudding, measured by 11 panelists using a 5-point hedonic scale, showed that the addition of germinated pulses either was not significantly different from the control, or, when the proportion of pulses was higher, was not acceptable (Sattar, Ali, & Hasnain, 2017). The acceptability of porridge prepared by adding germinated beans measured using a 5-point hedonic scale by 36 mother-child pairs showed no significant difference in sensory attributes compared to control porridges (Berhanu et al., 2014). Kinfe et al. (2015) reported moderate overall acceptability of boiled and roasted Habru and Local chickpea varieties evaluated by 15 panelists using a 9-point hedonic scale.

Chapter 3

Impact of Household Food Processing Strategies on Anti-nutrient (Phytate, Tannin and Polyphenol) Contents of Dry Bean (*Phaseolus vulgaris* L.) and Chickpea (*Cicer arietinum* L.): A Review

The first chapter presented the rationale for conducting the study and Chapter 2 provided a comprehensive review on the magnitude of the problem, the mechanism of iron and zinc absorption along with factors contributing to absorption and their techniques of measurement, as well as potential interventions. To further explore the impact of the intervention, household food processing strategies, a scoping review was carried out and provided in this chapter. The review focused mainly on determining the effect of household food processing strategies, namely soaking, germination and fermentation, on anti-nutrient contents of dry bean and chickpea.

This review is published on International Journal of Food Science and Technology. In preparation of this chapter, the published manuscript has been reformatted for the thesis and a section on fermentation was added (See Appendix 1 for documentation of permission to use in this thesis).

Haileslassie, H. A., Henry, C. J. and Tyler, R. T. (2016), Impact of household food processing strategies on antinutrient (phytate, tannin and polyphenol) contents of chickpea (*Cicer arietinum* L.) and bean (*Phaseolus vulgaris* L.): a review. Int J Food Sci Technol, 51: 1947–1957. doi:10.1111/ijfs.13166

Contribution statement:

Hiwot Abebe Haileslassie contributed to this manuscript by designing the study, reviewing literature, data acquisition, and manuscript drafting.

Abstract

Pulses, which include dry bean and chickpea, are major constituents of the human diet. They are important sources of energy and nutrients, particularly protein, folate and minerals. However, they also contain anti-nutrients which bind minerals, mainly iron and zinc, rendering them less bioavailable or unavailable for absorption. The levels of these anti-nutrients may be reduced by food processing techniques such as soaking and germination. Researchers have used these techniques in a number of studies; however, there is no consensus regarding the optimum processing conditions for reduction of these anti-nutrients. Thus, this review was conducted to describe the results of studies on soaking and germination of dry bean and chickpea. A systematic search was carried out utilizing Food Science and Technology Abstracts (FSTA) (1969 to present), Web of Science (1899 to present) and Scopus (1823 to present). A total of thirty-eight articles was reviewed. Both soaking and germination resulted in significant but variable degrees of reduction of levels of anti-nutrients in most studies.

3.1 Introduction

Pulses are an essential part of the human diet, among which common bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) are the most commonly consumed (Al-Numair, Ahmed, Al-Assaf, & Alamri, 2009; Alajaji & El-Adawy, 2006; Xu & Chang, 2008). In developing countries, the contribution of pulses to energy intake is second only to that of cereals (Juliano, 1999; Siddiq & Uebersax, 2012). The demand for pulses is on the increase worldwide due to their high fibre content, low glycaemic index and low calorie content (Asif et al., 2013). They also are good sources of protein, folate and minerals (Sandberg, 2002). Pulses contain 17-35% protein (Boye et al., 2010) with the added benefit of providing lysine, a limiting amino acid in cereals. In addition, pulses are relatively inexpensive compared to animal-source foods and serve as an

alternative source of nutrients for low income groups (Huma et al., 2008; Tharanathan & Mahadevamma, 2003).

However, pulses contain significant levels of anti-nutritive factors which hinder their nutritional and health benefits. These anti-nutrients include trypsin and chymotrypsin inhibitors, oxalate, lectin, raffinose family oligosaccharides, saponins, phytate, tannin and polyphenols (de Almeida Costa et al., 2006; El-Adawy, 2002; Sandberg, 2002; Wang et al., 2010). Most of the anti-nutrients that affect protein digestibility are heat sensitive, thus cooking alone improves protein digestibility in pulse-based diets (Nergiz & Gökgöz, 2007; Tharanathan & Mahadevamma, 2003). Phytate, tannin and polyphenols are heat stable anti-nutrients of greatest concern with respect to mineral bioavailability which refers to the proportion of a mineral that is absorbed and utilized through normal metabolic pathways (Hurrell, 2002). The aforementioned anti-nutrients form complexes with minerals, especially with divalent metals such as iron, zinc and calcium, rendering them less bioavailable or unavailable. Of the inositol phosphates (phytate), inositol phosphate 6 (IP6) and inositol phosphate 5 (IP5) exert a higher chelating effect on minerals than do lower inositol phosphates. Petry et al. (2010) reported that both polyphenols and phytate inhibit iron absorption in women. Ironically, studies have reported the importance of phytate in the prevention of cancer (Vucenik & Shamsuddin, 2003, 2006) and the role of polyphenols in the prevention of cardiovascular diseases (Vita, 2005).

Food processing strategies applicable at the household level have been found useful in the reduction of levels of anti-nutrients such as phytate, tannin and polyphenols (Hotz & Gibson, 2007). Soaking and germination of seeds are among the most commonly implemented strategies. These strategies lead to diffusion of the water soluble anti-nutrients and hydrolysis of anti-nutrients due to activation or *de novo* synthesis of enzymes (Hotz and Gibson, 2007). A large body of evidence supports the benefits of these strategies. However, there is no consensus regarding the

duration and optimal conditions required for maximal reduction of anti-nutrients. This review focuses on the impact of soaking and germination on phytate, tannin and polyphenols levels of chickpea and bean.

3.2 Literature Search Methodology

A systematic literature search was conducted utilizing Food Science and Technology Abstracts (FSTA) (1969 to present), Web of Science (1899 to present) and Scopus (1823 to present). Only original articles written in the English language and that reported the effects of either soaking or germination of chickpea or bean on levels of phytate, tannin or polyphenols were considered. The search was carried out separately for bean and chickpea using terminologies presented in Table 3-1. Terms listed within each category such as "seeds", "food process" and "anti-nutrients" were combined with "or", whereas "and" was used to combine terms across categories. Regarding studies on soaking and germination of bean, 336 articles were found in FSTA, 713 in Web of Science and 514 in Scopus. A similar search was carried out for chickpea and a total of 204, 546 and 372 articles were found in FSTA, Web of Science and Scopus, respectively. For studies on bean fermentation, 95, 591, 335 articles were found in FSTA, Web of Science and Scopus, respectively. For studies on chickpea fermentation, 57, 528, 214 articles were found in FSTA, Web of Science and Scopus, respectively. Titles and abstracts were read to determine the articles that would be included in the review. Duplicate articles were deleted. Ultimately a total of thirty-three articles were reviewed. The review is organized by food processing strategy and pulse type. The methodology and materials used in the reviewed articles are described first, followed by a discussion of the impact of the experiments on phytates, tannins and/or polyphenols.

Table 3-1. List of synonymous terms used in literature searches.

Seeds	Food process	Anti-nutrients
Chickpea	Germination/germinate	Anti-nutrient/antinutritional
Cicer arietinum	Sprout	Tannin
Bengal gram	Soak/soaking	Phytate
Garbanzo	Ferment/fermentation	Phytic acid
Legume		Bioavailability
Pulse		Bioaccessibility
Bean/common bean		Polyphenols
Haricot bean/kidney bean		Bioavailability
Phaseolus vulgaris		Bioaccessibility
Legume		
Pulse		

3.3 Soaking of Chickpea

Soaking involves steeping seeds in water for a predetermined period of time. Twelve studies tested the impact of soaking on anti-nutrients in chickpea. The type of water, volume of water used and soaking temperature are summarized in Table 3-2. Researchers used weight to volume (w/v) ratios ranging from 1:3 to 1:10 for soaking. Most of the studies reported using distilled water as the soaking medium (Alajaji & El-Adawy, 2006; El-Adawy, 2002; El Tinay, Mahgoub, Mohamed, & Hamad, 1989; Rehman & Shah, 2005). Other soaking mediums used included tap water (Khandelwal et al., 2010), tap water with EDTA (Máñez, Alegría, Farré, & Frígola, 2002), water purified by reverse osmosis (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002) and soaking medium prepared using 0.01 N NaOH and 0.01 N HCL (Ertas & Turker, 2014), citric acid solution and sodium carbonate solution (Nestares et al., 1999). Egli et al. (2002) carried out the soaking experiments in the dark. The duration of soaking in the reviewed studies ranged from 2 to 24 h.

Table 3-2. Summary of conditions employed for soaking of chickpea.

Source	Soaking medium	Seed to water ratio	Soaking temperature
Bains et al., 2014	-	-	-
Ertas and Turker, 2014	Soaking water prepared using 0.01 N NaOH and 0.01 N HCL	1:5	-
Wang et al., 2010	-	1:4	-
Khandelwal et al., 2010	Tap water	1:5	Room temperature
Alajaji & El-Adawy, 2006	Distilled water	1:10	25^{o}C
Rehman & Shah, 2005	Distilled water	1:5	-
El-Adawy, 2002	Distilled water	1:10	25^{o}C
Egli et al., 2002	Water purified by reverse osmosis	1:5	25^{o}C
Manez et al., 2002	Tap water and water with (EDTA) (250 mg/kg)*	1:3.5	-
Nestares et al., 1999	Distilled water, citric acid solution and sodium carbonate solution*	1:3	Room temperature
El Tinay et al., 1989	Distilled water	1:5	30^{o}C ±2
Khan et al., 1988	Water	-	

-, not reported; *, experiments were carried out separately for each medium

3.3.1 Effect of Soaking with and without Cooking on the Anti-nutrient Contents of Chickpea

All of the studies analysed phytate, whereas only five analysed tannin. Phytate reduction ranged from no significant effect to 59% (Table 3-3). The latter was reported in chickpea that were soaked in water and EDTA for 12 h, blanched and cooked under pressure (Manez et al, 2002). The shortest duration of soaking (2 h) combined with autoclave cooking resulted in a 47% reduction of phytate (Ertas & Turker, 2014). Nestares et al. (1999) carried out soaking for 9 h in water, acid or basic medium followed by boiling. A significant phytate reduction (20%) was found only in the acid-soaked and cooked seeds. Alajaji and El-Adawy (2006) used a 1:10 (w/v) seed to water ratio, whereas Rehman and Shah (2005) used 1:5 (w/v); reductions in phytate were 29% and 35%, respectively. Chickpea samples in the Egli et al. (2002) study were not cooked, which may have contributed to the lower reduction in phytate (14%) compared to other studies that used a similar

seed to water ratio. In addition, they considered only IP-6 and IP-5, whereas other studies used analytical techniques that did not differentiate among inositol phosphates. The smallest reduction (5%) was reported by Bains et al. (2014) in seed soaked for 12 h. Manez et al. (2002) analysed specific inositol phosphates; the reductions were not significant. Wang et al. (2010) found a significant effect of pulse variety on phytate content; however, they did not find a significant effect of cooking soaked chickpea. Reductions in tannins concentration due to soaking were highest in the studies by Alajaji and El-Adawy (2006) and El-Adawy (2002). Khandelwal et al. (2010) reported a 20% reduction in polyphenols as a result of 12 h of soaking.

A variety of heat treatments have been applied after soaking of chickpea, including boiling, pressure cooking and microwave heating. Alajaji and El-Adawy (2006) reported a significant effect of soaking and cooking on phytate and tannin levels. However, there were no significant differences in phytate and tannin levels among different cooking treatments, which included pressure cooking, microwaving and boiling. Ertas and Turker (2014) reported greater phytate reduction in autoclaved chickpea with an increase in soaking time. Rehman and Shah (2005) reported that compared to ordinary boiling, cooking in an autoclave at 121°C or 128°C resulted in greater reductions in phytate and tannin levels. Manez et al. (2002) compared traditional, microwave and pressure cooking. Traditional cooking or boiling did not result in a significant reduction in phytate content. In a study that compared the effect of pressure cooking, soaking and germination, the greatest reduction in polyphenols and tannin content was reported for germination, followed by soaking and pressure cooking (Khandelwal et al., 2010).

Not all of the studies determined the effect of soaking on levels of nutrients. However, Bains et al. (2014) and Wang et al. (2010) reported that zinc and iron were not significantly affected. Alajaji and El-Adawy (2006) and El-Adawy (2002) noted a significant reduction in riboflavin, thiamine, niacin and pyridoxine contents due to soaking.

Table 3-3. Effect of soaking alone or with cooking on the anti-nutrient contents of chickpea.

Source	Type of chickpea	Hours of soaking	Cooking	Phytate reduction (%)	Tannins reduction (%)
Ertas & Turker, 2014	Gokce variety from Turkey	2	Autoclaved (1:2, w/v)	47	-
Rehman and Shah, 2005	CP-98 variety from Pakistan	4	Boiled with water (1:5, w/v)	35	21
		4	Autoclaved at 121°C for 10, 20, 40, 60, and 90 min	31-46	33–40
		4	Autoclaved at 128°C for 20 min	44	37
Ertas and Turker, 2014	Gokce variety from Turkey	8	Autoclaved (1:2, w/v)	50	-
Nestares et al., 1999	Lechosa variety from southern Spain	9	Not cooked	NS	-
		9	Boiled with distilled water (1:6.67, w/v) for 35 min	NS in S and SB 20 in SA	-
Ertas and Turker, 2014	Gokce variety from Turkey	12	Autoclaved (1:2, w/v)	55	-
Alajaji and El-Adawy, 2006	Local variety purchased from local market, Egypt	12	Boiled in tap water (1:10, w/v) on a hot plate for 90 min	29	48
		12	Autoclaved at 121°C in tap water (1:10, w/v) for 35 min	41	50
		12	Cooked in microwave oven with a tap water (1:10, w/v), on high for 15 min	38	48
Bains et al., 2014	PBG 1 variety purchased from Punjab Agricultural University, India	12	Not cooked	5	-
El-Adawy, 2002	Variety purchased from the local market, Shibin El-Kom City	12	Boiled in tap water (1:10, w/v) on a hot plate for 90 min	29	48

Manez et al., 2002	-	12	Blanched at 90°C for 5 min and cooked under pressure at a temperature higher than 121°C for 23 min **	59	-
Khandelwal et al., 2010	Pusa B-72, B-372, B-362, B-1103, and B-391 varieties from New Delhi	12	Not cooked	-	22
El Tinay et al., 1989	NEC 2527, NEC 2695, NEC 1031 B, Baladi, Rabat, 103 1 CP 5-1,8294-BG-203,249 1 N6C, L-550,2486 NEC, and 5250 CL-629 varieties from Shambat Research Station, Sudan.	12	Not cooked	20	-
		12	Boiled for 60 min	30	-
Khan et al., 1988	Brown gram (brown and white varieties)	12	Boiled in water until ready to be eaten	25	-
Khandelwal et al., 2010	Pusa B-72, B-372, B-362, B-1103, and B-391 varieties obtained from the Indian Agricultural Research Institute, New Delhi		Boiled in water (1:5, w/v) at 15 lb psi at 121°C until soft	-	35
Egli et al., 2002	Seeds were purchased from Switzerland and local markets in Ivory Coast.	16	Not cooked	14	-
Manez et al., 2002	-	24	60 min traditional cooking	NS	-
	-	24	Cooked in the microwave oven for 25 min	28	-
Wang et al., 2010	Desi and Kabuli from Manitoba and Saskatchewan, Canada.	24	Cooked in 700 mL distilled water in a 2-L metal beaker placed on a hotplate for its predetermined cooking time	NS	NS

-, Not reported; NS, No significant effect; Soaked in distilled water (S), Soaked in citric acid (SA) and soaked in sodium carbonate solution (SB); ** Soaked in water with EDTA

3.4 Soaking of Bean

The processing conditions for fifteen studies that applied different periods of soaking to bean are presented in Table 3-4. The seed to water ratio used in the experiments ranged from 1:2 to 1:10. Most of the experiments were carried out at room temperature using distilled water as the soaking medium (Chimmad, Naik, & Rao, 2005; El Tinay et al., 1989; Nergiz & Gökgöz, 2007; Piecyk, Wołosiak, Druzynska, & Worobiej, 2012; Rehman & Shah, 2005; Ribeiro et al., 2011; Wang et al., 2010). Other studies used deionized water (Alonso et al., 2000), tap water (Khattab & Arntfield, 2009; Xu & Chang, 2008), plain water and sodium carbonate (Shimelis & Rakshit, 2007) as soaking medium. The duration of soaking ranged from 4 to 48 h.

Table 3-4. Summary of conditions employed for soaking of bean.

Source	Soaking medium	Seed to water ratio	Soaking temperature
Filipiak-Florkiewiz et al., 2012	Water	1:4	Room Temperature
Piecyk et al., 2012	Distilled water	1:3	-
Ribeiro et al., 2011	Sterile distilled water	-	-
Wang et al., 2010	Distilled water	1:4	Room temperature
Khattab & Arntfield, 2009	Tap water	1:5	Room temperature
Yasmin et al., 2008	Distilled water	1:5	Room temperature 25-29°C
Xu & Chang, 2008	Tap water	1:3	Room temperature
Shimelis & Rakshit, 2007	Plain water and sodium carbonate	1:3	-
Nergiz & Gokgoz, 2007	Distilled water	1:5	Room temperature
ElMaki et al., 2007	-	1:5	30°C ±2
Rehman & Shah, 2005	Distilled water	1:5	Room temperature
Chimmad et al., 2005	Distilled water	1:10	-
Alonso et al., 2000	Double deionized water	1:5	-
Greiner and Konietzny, 1998	Water	-	25°C
El Tinay et al., 1989	Distilled water	1:5	30°C±2

-, Not reported

3.4.1 Effect of Soaking with and without Cooking on the Anti-nutrient Contents of Bean

Results for soaking of bean are presented in Table 3-5. Khattab and Arntfield (2009) were able to reduce phytate levels by 58% with 4 h of soaking. In another study, 4 h of soaking resulted in a 24% reduction in phytate (Rehman & Shah, 2005). Shimelis and Rakshit (2007) used a seed to water ratio of 1:3 (w/v), a lower amount of soaking water than reported by other authors that analysed phytate, but reported a 61-65% reduction in phytate in three bean varieties. Yasmin et al. (2008) found no significant effect on phytate levels after 9h of soaking, but 82% and 61% reductions in the levels of tannin and polyphenols, respectively. Greiner and Konietzny (1998) also reported that soaking alone did not affect the level of phytate significantly. However, they did note a significant reduction after cooking. The greatest tannins reduction (99%) was reported by Khattab and Arntfield (2009) after 4 h of soaking. El Maki et al. (2007) found greater reductions in phytate and polyphenols with an increase in soaking time; however, a similar trend was not observed across the reviewed studies.

Soaking, boiling after soaking, autoclaving and microwave cooking resulted in significant reductions in tannin content in bean, with the greatest reduction reported for soaked and boiled bean (Khattab & Arntfield, 2009). All treatments reduced phytate levels significantly; however, there was no significant difference between soaking alone and boiling after soaking. In contrast, El-Tinaya et al. (1989) reported a significant reduction in phytate content with soaking and cooking, but not with soaking alone. Rehman and Shah (2005) reported a significant reduction in both tannin and phytate levels with boiling and autoclaving. On the other hand, a significant reduction in the phytate content of bean was reported with cooking after soaking in citric acid solution, but not with soaking in water and sodium bicarbonate solution alone, or with cooking after soaking in water or sodium bicarbonate solution (Yasmin et al., 2008). Shimelis and Rakshit (2007) compared soaking in water and sodium carbonate solution with boiling and autoclave

cooking of soaked and unsoaked bean. All treatments led to significant reductions in phytate and tannin content compared to raw bean, with the highest reductions observed with cooking after soaking and with autoclaving of unsoaked bean.

3.5 Germination of Chickpea

Germination is carried out by soaking the seed of interest in water, draining the water, and subsequently letting the seed sprout for a desired period of time. Emergence of radicles indicates the completion of germination (Hernandez-Nistal, Martin, Labrador, & Dopico, 2010). A range of techniques have been used for germination, including moist paper, cheese cloth, incubator, petri dish, seed germinator, wet jute bag covered with moist cotton or cloth and wet muslin cloth. The samples of the germinated chickpea in the reviewed studies are not cooked.

3.5.1 Effect of Germination on the Anti-nutrient Contents of Chickpea

Results from twelve studies that examined the effect of germination of chickpea on levels of phytate, tannin and polyphenols are summarized in Table 3-6 and Table 3-7. Among the studies that applied a shorter duration of germination (24-48 h), Agte et al. (1998) reported the highest reduction in phytate (47%) with a 24-h germination period. Conversely, Hemalatha et al. (2007) found no significant reduction in phytate with germination; this may have been due to the lower seed to water ratio (1:2.5, w/v). Among the studies that used a longer duration of germination, the maximum reduction of phytates (73%) was observed in kabuli-type chickpea germinated for 96 h. However, the same treatment resulted in only a 32% reduction of phytate in desi-type chickpea (Khalil et al., 2007). Bains et al. (2014) noted that the reduction in phytate increased with the duration of germination. Egli et al. (2002) reported that phytase activity in bean and chickpea

remained steady during the first 24 h of germination, but increased thereafter. Forty eight hour germination resulted in 93 and 82% reduction in tannin and polyphenols, respectively (Mittal, Nagi, Sharma, & Sharma, 2012).

3.6 Germination of Dry Bean

The conditions used for germination of bean are provided in Table 3-8 and Table 3-9; it ranges from use of moist cotton cloth to incubator and germinators. The samples of the germinated bean in the reviewed studies are not cooked.

3.6.1 Effect of Germination on the Anti-nutrient Contents of Dry Bean

The effects of germination of bean on levels of phytate, tannin and polyphenols are summarized in Table 3-8 and Table 3-9. Among germination experiments that used a 48-h or shorter duration of germination, three varieties of bean germinated for 48h exhibited reduction in phytate of 62-87% and reduction in tannin of 74-80% (Shimelis and Rakshit, 2007). Only three of the studies that carried out short-duration germination experiments analysed polyphenols, and reductions of 27-52% were reported. Phytates and tannins reductions ranged from 30 to 96% and from 19 to 77%, respectively, in bean germinated for more than 48 h (Table 3-9). Alonso et al. (2000) noted that the longer the duration of germination, the greater was the reduction of phytate, tannin and polyphenol levels. The maximum phytates reduction (79-96%) was reported in bean germinated for 96 h (Shimelis and Rakshit, 2007).

Table 3-5. Effect of soaking alone or with cooking on the anti-nutrient contents of bean.

Source	Type of bean	Hours of soaking	Cooking	Phytate reduction (%)	Tannins reduction (%)	Polyphenols reduction (%)
Khattab and Arntfield, 2009	Red kidney bean from Manitoba, Canada	4	Cooked in a boiling water (1:5, w/v)	58		-
			Cooked in microwave with a tap water (1:5, w/v) for 20 min on high	40	49	-
			Autoclaved	63		-
Khattab and Arntfield, 2009	White kidney bean cultivated in Egypt	4	Cooked in a boiling water (1:5, w/v)	58	99	
			Cooked in microwave with a tap water (1:5, w/v) for 20 min on high	54	92	-
			Autoclaved	69	95	
Rehman and Shah, 2005	Red kidney bean (Chkwa199) and white kidney bean (WK-70) from Pakistan	4	Cooked with water (1:5, w/v)	24	24-27	-
Yasmin et al., 2008	Red kidney bean from local market of Peshawar city	9	Not cooked	NS	NS	13
		9	Cooked in tap water (1:3, w/v) on a hot plate	NS	82	61
		9	Citric acid soaking	NS	63	45
		9	Citric acid soaking and cooked in tap water (1:3, w/v) on a hot plate	6	93	67
		9	Sodium bicarbonate soaking alone	NS	68	51
		9	Sodium bicarbonate soaking and cooked in water (1:3, w/v) on a hot plate	3*	90	79
EL Tinaya et al., 1989	White bean (R0/1/25, 9B, 8R, H.R.S.545, and Var Pi) from Sudan	12	Not cooked	NS	-	-
EL Tinaya et al., 1989	White bean (R0/1/25, 9B, 8R, H.R.S.545, and Var Pi) from Sudan	12	Boiled for 90 min	25	-	-

Alonso et al., 2000	Kidney bean (Athropurpurea) from Spain	12	Not cooked	6	24	21
Nergiz and Gokgoz, 2007	Bean (Dermason, Cali and Horoz) purchased from local market	12	Cooked in a low setting in a stove for 40 min	57-58	81- 82	73- 78
Shimelis and Rakshit, 2007	Kidney bean (Awash, Roba and Beshbesh) from Ethiopia	12	Soaking alone	17- 19	23-25	-
		12	Sodium bicarbonate soaking	14- 15	25-27	
		12	Cooked (1:2, w/v) at 97°C for a previously determined time	61-65	58-70	-
		12	Sodium bicarbonate soaking and cooked	61-64	55-68	-
		12	Autoclaved pre-soaked seeds with a temperature of 121°C in plain water (1:3, w/v) for 20 min	62-66	62-75	-
		12	Autoclaved pre-soaked seeds (sodium bicarbonate) with a temperature of 121°C in plain water (1:3 w/v) for 20 min	62-65	62-75	-
Greiner and Konietzny, 1998	Preto, Macacar and Carioca obtained from IPEN (Sao Paulo, Brazil)	15	After soaking, water was heated to boiling and simmered for 2 h to complete cooking	16-24	-	-
			Not cooked	NS	-	-
Xu and Chang, 2008	Black bean (Turtle Eclipse) from Kimberly, Idaho, U.S.A.	16	Boiled by Mattson cooker until tender enough to be penetrated by the rod	-	-	26-77
Piecyk et al., 2012	White bean (Raba and Warta) from the Institute of Plant Breeding and Acclimatisation	16	Raba cooked for 30 min Warta cooked for 40 min	-	-	11 and 54
			Autoclaved	-	-	25 and 18
Khattab and Arntfield, 2009	Red kidney bean purchased from Manitoba, Canada	18	Not cooked	49	59	-
	White kidney bean cultivated in Egypt	20	Not cooked	48	86	-

El Maki et al., 2007	White bean (Serege, Giza and RO21)	24	Cooked in a round-mouthed tall beakers fitted with condensers until they felt soft between fingers	2-9	-	1-16
Wang et al., 2010	Seven bean samples (black turtle, cranberry, dark red kidney, great northern, pinto, small red and white pea bean) from pulse processors in Manitoba and Saskatchewan.	24	Cooked in 700 mL distilled water in a 2-L metal beaker placed on a hotplate for its predetermined cooking time	NS	71- 80	-
Chimmad et al., 2005	Black bean purchased from local market	24	Not cooked	13	16	43
		24	Cooked at 1.05 kg/cm^2 pressure for 20 min (1:2, w/v) along with the cooking broth	22	39	59
El Maki et al., 2007	White bean (Serege, Giza and RO21)	36	Cooked in a round-mouthed tall beakers fitted with condensers until they felt soft between fingers	4-16	-	13-23
		48	Cooked in a round-mouthed tall beakers fitted with condensers until they felt soft between fingers.	3-12	-	6-22

-, Not reported; NS, No significant effect; *, increase

Table 3-6. Effect of short period (≤ 48 h) of germination on the anti-nutrient contents of chickpea.

Source	Type of chickpea	Germination conditions	Hours of germination	Phytate reduction (%)	Tannin reduction (%)	Polyphenols Reduction (%)
Agte et al., 1998	Variety from local market in India	25-30°C	24	47	-	-
		25-30°C	24	52	-	-
Trugo et al., 1999	Variety from local market in Chile	30°C in dark	24	15	-	-
Ghavidel and Prakash, 2007	Variety from local market	Wet muslin cloth	24	21	17	-
		Wet muslin cloth	24	50	43	
Hemalatha et al., 2007	Variety from local market	In incubator at 25°C	24	NS	47	-
Egli et al., 2002	Seeds were purchased from Switzerland and local markets in Ivory Coast	Household germination system in dark at 25°C	24	5*	-	-
Khandelwal et al., 2010	Pusa B-72, B-372, B-362, B-1103 and B-391 varieties from New Delhi	Moist cloth	24	-	43	52
Bains et al., 2014	PBG 1 from India	Seed germinator at 25°C	36	26	-	-
Egli et al., 2002	Seeds were purchased from Switzerland and local markets in Ivory Coast	Household germination system in dark at 25°C	48	2	-	-
Hemalatha et al., 2007	Variety purchased locally	In incubator at 25°C	48	NS	52	-
Mittal et al., 2012	Variety from India	22°C	48	3	93	82
Trugo et al., 1999	Variety from local market in Chile	30°C in dark	48	15	-	-
Bains et al., 2014	PBG 1 variety from India	Seed germinator at 25°C	48	34	-	-

-, Not reported; NS, No significant effect;*, Increase

Table 3-7. Effect of long period (> 48 h) of germination on the anti-nutrient contents of chickpea.

Source	Types of chickpea	Germination conditions	Hours of germination	Phytate reduction (%)	Tannin reduction (%)	Polyphenols reduction (%)
Bains et al., 2014	PBG 1 from India	Seed germinator at 25°C and 70% RH using petri dish	60	46	-	-
Chitra et al., 1996	chickpea from India	Petri dish lined with wet filter paper	72	64	-	-
Egli et al., 2002	Seeds were purchased from Switzerland and local markets in Ivory Coast	Household germination system in dark at 25°C	72	16	-	-
El-Adawy, 2002	Local chickpea from the local market in Shibin El-Kom, Egypt	Thick layers of cotton cloth in dark	72	56	23	-
Khalil et al., 2007	Kabuli (HASSAN-2K) type chickpea variety from Peshawar	Petri dish lined with filter paper in dark	96	73	-	-
	Desi (NIFA-88) type chickpea variety from Peshawar	Petri dish lined with filter paper in dark	96	32	-	-

-, Not reported

Table 3-8. Effect of short period (24-48 h) of germination on the anti-nutrient contents of bean.

Source	Types of bean	Germination conditions	Hours of germination	Phytate reduction (%)	Tannin reduction (%)	Polyphenols reduction (%)
Agte et al., 1998	Black bean from local market in India	25-30°C	24	31	-	-
Agte et al., 1998	Black bean from local market in India	25-30°°C	24	35	-	-
Trugo et al., 1999	Black bean from Brazil	30°C in dark	24	37	-	-
Alonso et al., 2000	Kidney bean (Athropurpurea) from Spain	Petri dish lined with wet filter paper in aired dark incubator	24	9	44	32
Shimelis and Rakshit, 2007	Kidney bean (Awash, Roba and Beshbesh) from Ethiopia	Filter paper lined petri dish covered with aluminium foil in dark at 25°C	24	28-73	32-35	-
Trugo et al., 1999	Black bean from Brazil	30°C in dark	48	11	-	-
Alonso et al., 2000	Kidney bean (Athropurpurea) from Spain	Petri dish lined with wet filter paper in aired dark incubator	48	19	64	41
Shimelis and Rakshit, 2007	Kidney bean (Awash, Roba and Beshbesh) from Ethiopia	Filter paper lined petri dish covered with aluminium foil in dark at 25°C	48	62-87	74-80	-
AL-Numair et al., 2009	White bean (Serge, Giza and Ro21) from Sudan	Moist cotton cloth at 32 ± 3°C	48	23-29	-	27-52

-, Not reported

Table 3-9. Effect of long period (> 48 h) of germination on the anti-nutrient contents of bean.

Source	Types of bean	Germination conditions	Hours of germination	Phytates reduction (%)	Tannins reduction (%)	Polyphenols reduction (%)
Alonso et al., 2000	Kidney bean (Athropurpurea) from Spain	Petri dish lined with wet filter paper in aired dark incubator	72	30	72	53
Shimelis and Rakshit, 2007	Kidney bean (Awash, Roba and Beshbesh) from Ethiopia	Filter paper lined petri dish covered with aluminium foil in dark at 25ºC	72	79-92	75-77	-
Yasmin et al, 2008	Red kidney bean from local market of Peshawar city	Moisture adherent flax cloth in dark at 22ºC	96	43	69	55
Shimelis and Rakshit, 2007	Kidney bean (Awash, Roba and Beshbesh) from Ethiopia	Filter paper lined petri dish covered with aluminium foil in dark at 25ºC	96	79-96	75-76	-
AL-Numair et al., 2009	White bean (Serge, Giza and Ro21 varieties) from Sudan	Moist cotton cloth at 32 ± 3ºC	96	23-36	30-52	-
Sangronis and Machado, 2005	Black and white bean from local market	Perforated aluminium trays un dark at 25 ± 2ºC	120	45	36	-
		Perforated aluminium trays un dark at 25 ± 2ºC	120	53	19	-
AL-Numair et al., 2009	White bean (Serge, Giza and Ro21) from Sudan	Moist cotton cloth at 32 ± 3ºC	144	26-38	36-53	-

, Not reported

3.7 Fermentation of Dry Bean

Solid-state and liquid fermentation using either the naturally present lactic acid bacteria or by inoculating lactobacilli have been reported for dry bean (Curiel et al., 2015; Limón et al., 2015). The seed to water ratio used in the reviewed studies were 1:2 (w/v) for solid-state fermentation and 1:5 (w/v) for liquid fermentation.

3.7.1 Effect of Fermentation on the Anti-nutrient Contents of Bean

Both natural and accelerated fermentation initiated by inoculating a culture resulted in a reduction of phytate and tannin in beans (Shimelis & Rakshit, 2008). Sourdough fermentation resulted in a significant increase in the concentration of total phenols on four bean varieties, however, no significant effect were noted on other four bean varieties used in the same study (Curiel et al., 2015). Phenolic compounds were increased significantly in 48 and 96 h solid-state fermentation, whereas slight decrease or no significant change was observed in liquid fermentation (Limón et al., 2015). Another study reported more phenols in fermented bean than bean flour (Reyes-Bastidas et al., 2010).

3.8 Fermentation of Chickpea

As noted above for bean, chickpea fermentation was carried through natural fermentation or accelerated fermentation, sometimes referred as controlled fermentation, by adding lactic acid bacteria. The seed to water ratio used in chickpea fermentation ranged from 1:2 (w/v) to 1:5 (w/v).

3.8.1 Effect of Fermentation on the Anti-nutrient Contents of Chickpea

Solid-state fermentation of chickpea reduced levels of tannin and phytate (Reyes-Moreno, Cuevas-Rodríguez, Milán-Carrillo, Cárdenas-Valenzuela, & Barrón-Hoyos, 2004). A significant reduction of phytate was reported following a fermentation of 24 h (Chitra, Singh, & Venkateswara Rao, 1996). Sourdough fermentation increased the concentration of total phenols in two chickpea varieties (Curiel et al., 2015).

3.9 Summary

Based on the majority of the articles reviewed, soaking, germination and fermentation resulted in significant but variable degrees of anti-nutrient reduction. It should be noted that even for a particular treatment time and seed type, the level of reduction was variable. The highest phytate reduction for chickpea (73%) was reported for a 96-h germination of kabuli-type chickpea. However, the same process led to only a 32% reduction of phytates in desi-type chickpea. The highest reduction of phytate in bean was reported for a 96-h germination period. Germination of chickpea for 48 h resulted in the highest reduction of tannins, whereas soaking for only 4 h resulted in the highest reduction in bean. One would expect to see a reduction in phytate proportional to the amount of soaking water, but this was not noted. Although a longer soaking period would be expected to be beneficial, this also was not observed in the studies.

It is important to consider the loss of nutrients associated with soaking, germination and fermentation. Not all of the studies reported changes in zinc or iron concentration, which made it difficult to calculate and comment on iron or zinc to phytate molar ratios. However, based on the available data, the effect of soaking and germination on iron and zinc content is mixed. Three studies reported the presence of no significant effect (Trugo et al., 1999; Bains et al., 2014; Wang

et al., 2010), two studies reported an increase (El-Aldawy, 2002, Sangronis and Machado, 2007) and one study reported a decrease (El Maki et al., 2007) in zinc content. Regarding iron content, Trugo et al. (1999) and Wang et al. (2010) reported no significant effect, whereas El-Aldawy (2002) reported an increase, and others noted decrease (El Maki et al., 2007, Bains et al., 2014, Sangronis and Machado, 2005) in iron content. Thus, the comparison was made only on the relative reductions in the levels of anti-nutrients that occurred during processing. Despite the variation in the percentage of reduction, most studies supported the usefulness of soaking, germination and fermentation to improve the nutritional quality of chickpea and bean.

Chapter 4

Impact of Fermentation on Nutrient and Anti-Nutrient Contents of Dry Bean (*Phaseolus vulgaris* L.) and Chickpea (*Cicer arietinum* L.) Grown in Ethiopia

The literature review presented in Chapters 2 and 3 provided evidence for the potential of household food processing strategies to improve the nutritional composition of dry bean and chickpea. Also discussed in these chapters were the reasons for focusing on micronutrient deficiency, particularly of iron and zinc. As noted in Chapter 1, the overall objective of this thesis was to improve the bioavailability of iron and zinc in dry bean and chickpea grown in Ethiopia through food processing strategies applicable on a household scale. To attain this objective, the study was divided into three sub-studies. In the first study, reported in this chapter, dry bean and chickpea were subjected to natural fermentation, and selected nutrients and anti-nutrients were analysed. Different times of fermentation were explored to address the first objective of the thesis: to determine the effect of fermentation at 24, 48 and 72 h on the nutrient (iron, zinc and calcium) and anti-nutrient (phytate, polyphenols and tannin) contents of dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local) varieties.

Abstract

Dry bean and chickpea are the most widely consumed pulses in the world. Fermentation is one of the oldest traditional food processing techniques practised to improve the nutritional profile of grains and pulses. Fermentation for different times (24, 48 and 72 h) was carried out on samples of three dry bean and three chickpea varieties grown in Ethiopia to determine the fermentation

time that would improve their nutritional profiles. The protein contents of the dry bean and chickpea samples ranged from 16.2 g/100 g to 23.5 g/100 g, with Mastewal exhibiting the lowest protein content of all of the samples. There were no significant differences in the mineral contents of most of the dry bean samples. The Local chickpea sample had the highest zinc (4.93 mg/100 g) and calcium (240 mg/100 g) contents. The iron contents of the dry bean samples were significantly higher than those of the chickpea samples. Fermentation of dry bean and chickpea flours reduced their anti-nutrient contents, with significant reductions in phytate, tannin and polyphenol contents compared to unfermented samples. The results revealed that fermentation could be applied to improve the iron bioavailability of dry bean and chickpea flours with minimal loss of mineral content, as determined by the phytate:iron ratio. Conversely, fermentation did not significantly reduce the phytate:zinc molar ratio. The results from this study could be useful in the formulation of novel, fermented, pulse-based food products.

4.1 Introduction

Dry bean and chickpea are the most widely consumed pulses in the world (Saleh & El-Adawy, 2006). A large percentage of individuals in East Africa and Latin America depend on dry bean as their major source of nutrients. Chickpea is an ancient crop consumed mainly in African and Asian countries (Jukanti, Gaur, Gowda, & Chibbar, 2012).

Fermentation is one of the oldest and most commonly practised traditional food processing techniques. Examples of fermented food products used in different parts of the world include *gari* in west Africa, *idli* in southeast Asia, *injera* in Ethiopia and *tempeh* in Indonesia (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; Kostinek et al., 2005). Whole seed or flour can be fermented either naturally or through the addition of a starter culture. Fermentation improves not

only the nutritional profile of pulse-based foods (Granito et al., 2002; Reyes-Moreno et al., 2004) but also the nutritional and sensory characteristics of cereal-based food products to which fermented pulse has been added (Coda, Varis, Verni, Rizzello, & Katina, 2017; Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014). In addition, fermentation can serve as a food preservation mechanism since most pathogens are unable to survive at the low pH created during the process (Gibson, Bailey, Gibbs, & Ferguson, 2010).

Previous studies have shown that the absorption of iron and zinc is significantly lower in plant-based diets high in phytate, tannin and polyphenols (Davidsson et al., 1994; Fredlund, Isaksson, Rossander-Hulthén, Almgren, & Sandberg, 2006; Gillooly et al., 1983; Hart, Tako, Kochian, & Glahn, 2015; Sandberg, 2002; Tako, Beebe, Reed, Hart, & Glahn, 2014). Natural fermentation has been used as a low-cost process applicable on a household scale to reduce anti-nutrient contents and thus improve mineral bioavailability. Umeta et al. (2005) reported the reduction of the phytate:zinc and phytate:iron molar ratios from 28 and 1.1 in unfermented *injera* [flat bread made mostly from *tef*] to 11 and 0.31 in fermented *injera*, respectively. Other studies (Abebe et al., 2007; Granito et al., 2002; Ibrahim et al., 2002) also have demonstrated the benefits of fermentation in improving nutritional composition. The effect of fermentation depends on pH, type and time of fermentation (Granito et al., 2002; Porres, Aranda, López-jurado, & Urbano, 2003). The current study was conducted to determine the effect of fermentation for 24, 48 and 72 h on nutrient (iron, zinc and calcium) and anti-nutrient (phytate, polyphenol and tannin) levels of samples of dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local) varieties grown in Ethiopia.

4.2 Materials and Methods

4.2.1 Samples

Samples of two recently developed dry bean varieties (Hawassa Dume and Nasir) and two recently developed chickpea varieties (Habru and Mastewal) were obtained from Hawassa Agricultural Research Centre, Hawassa, Ethiopia, and Debre Zeit Agricultural Research Centre, Bishoftu, Ethiopia, respectively. Samples of local varieties of dry bean (Red Wolaita) and chickpea (Local) were purchased from a market in Hawassa, Ethiopia, and a farmer in Wolaita, Ethiopia, respectively (Figure 4-1). The seeds were ground in a laboratory mill (UDY cyclone mill, UDY Corporation, Fort Collins, CO, USA, equipped with a 0.5-mm screen). Portions of each sample, both raw and fermented, were analysed for nutrient and anti-nutrient content.

4.2.2 Fermentation

Natural fermentation was carried out according to the method described by Luo et al. (2013). In brief, three 100-gram ground samples of each seed sample were individually mixed with deionized water (ACS Reagent Grade, ASTM Type I, ASTM Type II, VWR, Mississauga, ON, Canada) at a ratio of 1 to 5 (w/v). The mixtures were allowed to ferment for 24, 48 and 72 h at room temperature (25°C) initiated by microorganisms naturally present on the seeds. Glassware treated with nitric acid, rinsed with deionized water and covered with aluminum foil, as shown in Figure 4-1, was used for the fermentation process. Samples were collected at the beginning of fermentation, and thereafter at 24 h intervals for up to 72 h.

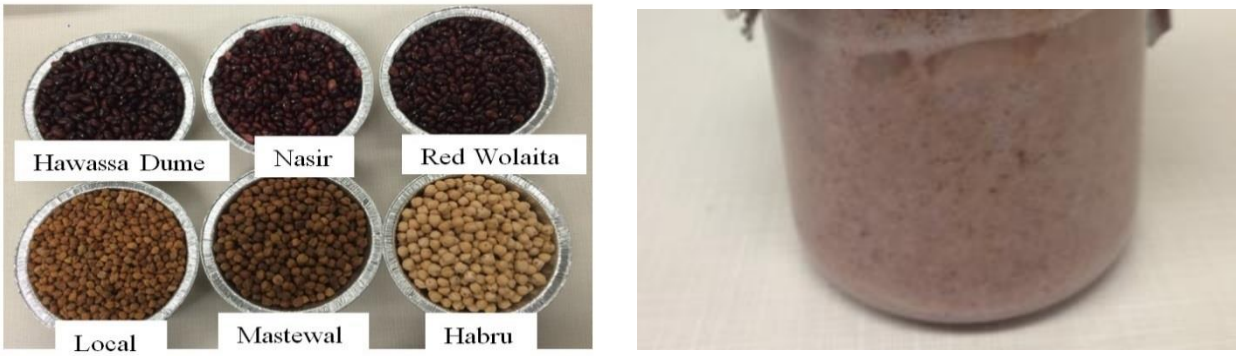

Figure 4-1. A picture showing raw bean and chickpea samples (left) and a fermented sample (right)

4.2.3 Ground Samples

At the end of the fermentation period, the fermented sample was separated from the liquid by sieving and the samples were dried in a convection oven (Sanyo Convection Oven, Model MOV-212F, Sanyo Electric Co. Ltd., Osaka, Japan) at 60°C and ground in a laboratory mill (UDY cyclone mill, UDY Corporation, Fort Collins, CO, USA, equipped with a 0.5-mm screen). The ground samples were stored in air-tight polyethylene bags at 4°C until further analysis. Unfermented flour samples were used as controls.

4.2.4 pH

The pH values of dry bean and chickpea samples were determined with a pH meter (Oakton, Vernon Hills, IL, USA) at 0, 24, 48 and 72 h of fermentation.

4.2.5 Protein analysis

Protein analysis was carried out using AACC International Method 46-30.01 (AACC International, 1999). Briefly, a 250-mg flour sample was weighed and the nitrogen concentration

was determined by the Dumas combustion method with an FP-528 Protein/Nitrogen Analyzer (LECO Corporation, St. Joseph, MI, USA). Protein was calculated by multiplying the nitrogen concentration by a factor of 6.25. Triplicate samples of 200 mg EDTA were used for calibration. To check for accuracy, wheat flour of known protein content was analyzed before the dry bean and chickpea samples were analyzed and after every 20 samples.

4.2.6 Mineral analysis

The mineral (iron, zinc and calcium) contents of unprocessed and fermented samples were determined using a HNO_3-H_2O_2 digestion, as described by Thavarajah and Thavarajah (2012). In brief, 500 mg of flour was placed in 30-mL digestion tubes. Then, 6 mL of concentrated nitric acid (HNO_3) was added to each of the tubes, which were placed in a Vulcan 84 automatic digester (Questron Technology Corporation, Mississauga, ON, Canada). The total digestion procedure required approximately 120 min. After the tubes were heated for 55 min, 3 mL of 30% hydrogen peroxide (H_2O_2) was added, and then another 2 mL of H_2O_2 was added after 5 min. When about 9 min were left, 3 mL of 6M HCl was added to the tubes. At the end of the digestion, the samples were diluted with Millipore water and adjusted to a volume of 25 mL. In each run, 84 tubes were analyzed in the digester, among which blanks and control samples were included. A Flame Atomic Absorption Spectrophotometer (novAA300, Analytik Jena AG, Jena, Germany) was used to determine the minerals (iron, zinc and calcium) in the digest. Lanthanum chloride (1%) was added to food samples to suppress interference from phosphorus in the determination of the calcium. The method used for determining mineral concentrations had been validated previously using NIST standard reference material 1567a (wheat sample). Yellow lentil flour, analyzed after every 10^{th} sample, was used as a laboratory reference material to ensure consistency.

4.2.7 Phytate analysis

Phytate content was analyzed using the Wade reagent method as described by Gao et al. (2007). A ground sample (0.5 g) was mixed with 10 mL of 2.4% HCl in 14-mL Falcon tubes for 16 h using a magnetic stirrer, and then centrifuged at 1000 x *g* at 10°C for 20 min. The crude acid extracts were transferred to 14-mL Falcon tubes and mixed with 1 g NaCl. The mixtures were left to settle at 4°C for 60 min. After an hour, each sample was centrifuged at 1000 x *g* at 10°C for 20 min, and the clear supernatant was collected. One millilitre of the clear supernatant was diluted with 24 mL of deionized water in a 50-mL Falcon tube. Three millilitres of the diluted sample was mixed with 1 mL of Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid). The samples then were mixed using a vortex mixer and centrifuged at 1000 x *g* at 10°C for 10 min. A series of calibration standards containing 0, 1.12, 2.24, 3.36, 5.6 and 11.2 mg per L of sodium phytate was prepared. Samples of normal phytate CDC Redberry lentil and low phytate and normal phytate barley were run along with the samples. The absorbance of samples and standards were read at 500 nm on a UV-Vis spectrophotometer (GENESYS 10S, Thermo Fisher Scientific, Madison, WI, USA).

4.2.8 Polyphenol analysis

Total polyphenols were determined with the Folin-Ciocalteu reagent, according to the method described by Wei et al. (2013). A 2-g ground sample was weighed and transferred to a capped centrifuge tube. Then, 20 mL of methanol:water (50:50, v/v) solution was added. The tube was shaken at room temperature for 1 h and then centrifuged at 1259 x *g* for 15 min. An aliquot (0.2 mL) of the methanol extracts was mixed with 0.8 mL of the Folin-Ciocalteu reagent. After 6

min of incubation, 2 mL of sodium carbonate (10%) was added. The mixture was allowed to stand for 90 min at room temperature. The absorbance of each sample and a blank was measured at 765 nm using a UV-Vis Spectrophotometer (Thermo Fisher Scientific). The total polyphenols content of each extract was calculated using a calibration curve prepared with a gallic acid standard and expressed as mg gallic acid equivalents (GAE) per 100 g.

4.2.9 Tannin analysis

The vanillin-HCl method was used to determine tannin content, as described by Price et al. (1978). One gram of ground sample was measured and mixed with 20 mL of 1% HCL in methanol for 20 min at 30°C in a water-bath. The samples were centrifuged at 490 x *g* for 4 min. One millilitre of supernatant was mixed with 5 mL of freshly prepared vanillin solution (0.5% vanillin + 2% HCL in methanol). Absorbance was read at 500 nm on a UV-Vis Spectrophotometer (Thermo Fisher Scientific). The quantification of tannin was based on a standard curve prepared with 0.05-0.45 mg/mL of catechin, and results were expressed as catechin equivalents per 100 g of sample.

4.2.10 Phytate:zinc and Phytate:iron molar ratios

Phytate:zinc and phytate:iron molar ratios were calculated by dividing the molar concentration of phytate (molecular weight, 660) by the molar concentration of zinc (molecular weight, 65.38) and iron (molecular weight, 55.85), respectively.

4.2.11 Statistical Analysis

SPSS version 20.0 for Windows (IBM Corp. Armonk, NY, USA) was used for data analysis. Results are expressed as mean ± standard deviation. Analysis of variance with Tukey's test at $p < 0.05$ was used to determine means that were statistically different.

4.3 Results

4.3.1 Protein and Mineral Contents of Raw and Fermented Dry Bean and Chickpea

The protein, zinc, iron and calcium contents of the raw dry bean and chickpea samples obtained from Ethiopia are presented in Table 4-1. The protein contents of the dry bean samples ranged from 17.1 g/100 g to 23.5 g/100 g, with Red Wolaita having the lowest protein content of the dry bean samples. The protein contents of the chickpea samples ranged from 16.2 g/100 g to 19.2 g/100 g, with Mastewal exhibiting the lowest protein content of all dry bean and chickpea samples. There was no significant difference in the mineral contents of most dry bean samples. Local chickpea had the highest zinc (4.93 mg/100 g) and calcium (240 mg/100 g) contents of all samples. The iron contents of the dry bean samples were significantly higher than those of the chickpea samples.

The protein, zinc, iron and calcium contents of dry bean and chickpea at different times of fermentation are presented in Table 4-2; statistical comparisons are made between different times of fermentation for the same variety. Compared to the unfermented flour samples, the protein content was significantly lower at: i) 24 h of fermentation for Hawassa Dume; ii) 24 h and 48 h of fermentation for Nasir and Red Wolaita; iii) 24 h and 48 h of fermentation for Habru; iv) all fermentation times for Mastewal samples; and v) 24 h for Local samples.

Table 4-1. Protein, iron, zinc and calcium contents of samples of dry bean and chickpea.

Pulse varieties	Protein (g/100 g)	Zinc (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100 g)
		Bean		
Hawassa Dume	23.5 ± 0.1^{d}	3.78 ± 0.28^{ab}	6.80 ± 0.74^{c}	161.2 ± 12.2^{a}
Nasir	23.5 ± 0.1^{d}	4.22 ± 0.43^{b}	6.68 ± 0.20^{c}	179.3 ± 3.1^{ab}
Red Wolaita	17.1 ± 0.1^{b}	3.52 ± 0.16^{a}	6.78 ± 0.05^{c}	164.4 ± 4.0^{ab}
		Chickpea		
Habru	19.2 ± 0.1^{c}	3.40 ± 0.05^{a}	4.61 ± 0.03^{ab}	126.2 ± 1.9^{a}
Mastewal	16.2 ± 0.2^{a}	3.54 ± 0.19^{a}	4.21 ± 0.03^{a}	154.3 ± 5.1^{b}
Local	19.2 ± 0.1^{c}	4.93 ± 0.03^{c}	5.25 ± 0.11^{b}	239.8 ± 7.3^{c}

Values are mean ± SD (n=3) expressed on a dry weight basis. Means within a column with the same superscript letter are not significantly different ($p > 0.05$).

Zinc contents were significantly lower in fermented dry bean and chickpea samples compared to those of their unfermented counterparts. The iron contents of Hawassa Dume, Nasir and Habru samples were not significantly different from those of the unfermented samples at any of the fermentation times, whereas the iron contents were significantly lower in fermented Red Wolaita, Mastewal and Local samples. The calcium contents of fermented dry bean samples were not significantly different than those of the unfermented flour samples. For chickpea samples, Mastewal and Local samples at 72 h of fermentation had significantly lower calcium contents than did the unfermented samples.

Table 4-2. Changes in protein, iron, zinc and calcium contents of samples of three bean and three chickpea varieties during fermentation.

Food items	Hours of fermentation	Protein (g/100 g)	Zinc (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100 g)
		Bean			
Hawassa Dume	0 h	23.5 ± 0.1^{bc}	3.78 ± 0.28^{c}	6.80 ± 0.74^{ab}	161.2 ± 12.2^{a}
	24 h	20.9 ± 1.3^{a}	2.55 ± 0.18^{a}	7.07 ± 0.10^{ab}	184.7 ± 3.2^{a}
	48 h	21.6 ± 0.1^{ab}	2.53 ± 0.04^{a}	6.49 ± 0.05^{a}	171.6 ± 3.6^{a}
	72 h	23.7 ± 0.4^{c}	2.30 ± 0.05^{a}	7.22 ± 0.15^{b}	168.4 ± 3.7^{a}
Nasir	0 h	23.5 ± 0.1^{b}	4.22 ± 0.43^{c}	6.68 ± 0.20^{a}	179.3 ± 3.1^{a}
	24 h	21.5 ± 0.1^{a}	2.89 ± 0.06^{ab}	6.77 ± 0.34^{a}	172.6 ± 1.8^{a}
	48 h	21.4 ± 0.1^{a}	2.69 ± 0.01^{ab}	6.99 ± 0.06^{a}	176.9 ± 3.3^{a}
	72 h	23.7 ± 0.5^{b}	2.04 ± 0.02^{a}	7.06 ± 0.06^{a}	172.4 ± 3.2^{a}
Red Wolaita	0 h	17.1 ± 0.1^{b}	3.52 ± 0.16^{b}	6.78 ± 0.05^{b}	164.4 ± 4.0^{a}
	24 h	15.6 ± 0.2^{a}	2.24 ± 0.01^{a}	6.24 ± 0.11^{a}	164.7 ± 4.2^{a}
	48 h	15.6 ± 0.4^{a}	2.38 ± 0.14^{a}	6.37 ± 0.10^{a}	169.7 ± 6.5^{a}
	72 h	16.5 ± 0.1^{b}	2.43 ± 0.05^{a}	6.25 ± 0.39^{a}	162.0 ± 1.7^{a}
		Chickpea			
Habru	0 h	19.2 ± 0.1^{b}	3.40 ± 0.05^{d}	4.61 ± 0.03^{a}	126.2 ± 1.87^{a}
	24 h	15.8 ± 0.4^{a}	2.88 ± 0.02^{a}	4.49 ± 0.07^{a}	115.5 ± 2.2^{a}
	48 h	15.6 ± 2.7^{a}	2.63 ± 0.08^{b}	4.68 ± 0.40^{a}	116.7 ± 7.6^{a}
	72 h	17.7 ± 0.1^{ab}	2.16 ± 0.04^{c}	4.63 ± 0.18^{a}	120.2 ± 4.9^{a}
Mastewal	0 h	16.2 ± 0.2^{c}	3.54 ± 0.19^{d}	4.21 ± 0.03^{c}	154.3 ± 5.1^{b}
	24 h	14.1 ± 0.5^{a}	1.40 ± 0.01^{b}	3.78 ± 0.08^{b}	145.6 ± 3.4^{ab}
	48 h	13.9 ± 0.1^{a}	1.64 ± 0.13^{c}	3.48 ± 0.08^{a}	143.1 ± 3.3^{ab}
	72 h	14.9 ± 0.1^{b}	1.15 ± 0.02^{a}	3.64 ± 0.10^{ab}	137.8 ± 10.5^{a}
Local	0 h	19.2 ± 0.1^{b}	4.93 ± 0.03^{b}	5.25 ± 0.11^{c}	239.8 ± 7.3^{b}
	24 h	16.9 ± 0.1^{a}	3.94 ± 0.02^{a}	4.95 ± 0.01^{b}	218.2 ± 2.0^{ab}
	48 h	18.0 ± 0.8^{b}	3.70 ± 0.63^{a}	4.87 ± 0.16^{ab}	221.9 ± 27.0^{ab}
	72 h	18.5 ± 0.1^{b}	3.50 ± 0.04^{a}	4.54 ± 0.27^{a}	198.8 ± 2.8^{a}

Values are mean ± SD (n=3) expressed on a dry weight basis. For each variety, means within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test.

4.3.2 Changes in pH during Fermentation of Dry Bean and Chickpea Samples

The change in pH during the fermentation of samples of three dry bean and three chickpea varieties is shown in Figure 4-2. The pH prior to fermentation ranged from 6.2-6.3 for dry bean samples and from 6.4-6.5 for chickpea samples. The pH dropped significantly during the fermentation process for all dry bean (4.3-4.6) and chickpea samples (4.4-4.7).

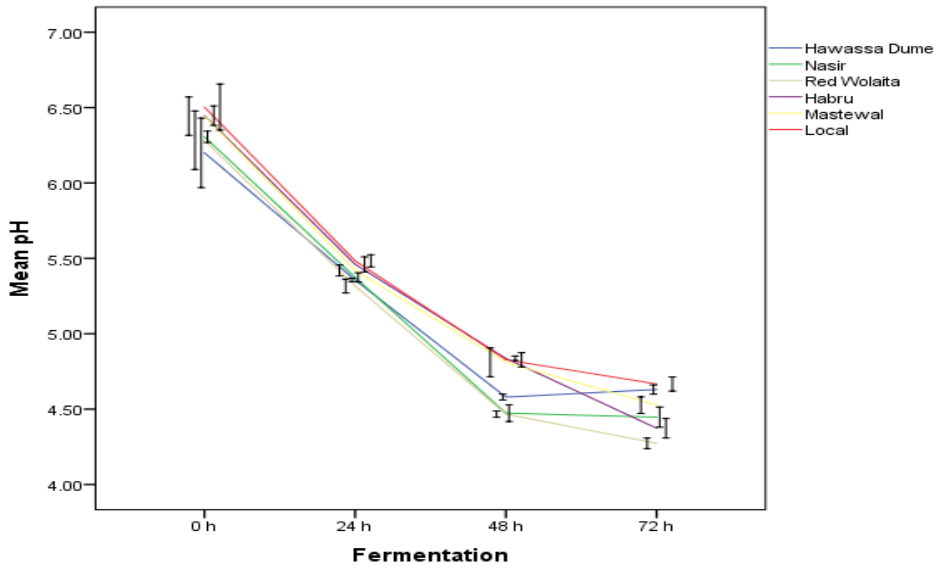

Figure 4-2. Change in pH during fermentation of samples of three dry bean and three chickpea varieties.
Values are means ± SD (n=3).

4.3.3 Changes in Phytate Contents of Bean and Chickpea Samples during Fermentation

The phytate contents of raw and fermented samples of dry bean and chickpea are presented in Figure 4-3. The phytate contents of dry bean and chickpea samples ranged from 689.5-818.5 mg/100 g and 557.3-635.2 mg/100 g, respectively. A significant decrease in phytate content was observed at 24 h of fermentation for all dry bean and chickpea samples. Reductions of phytate during fermentation ranged from 23.5-33.6% at 24 h, 25.4-36.7% at 48 h to 32.2-42.4% at 72 h for chickpea, and 28.3-55.6% at 24 h, 34.6-55.7% at 48 h to 38.9-50.1% at 72 h for dry bean.

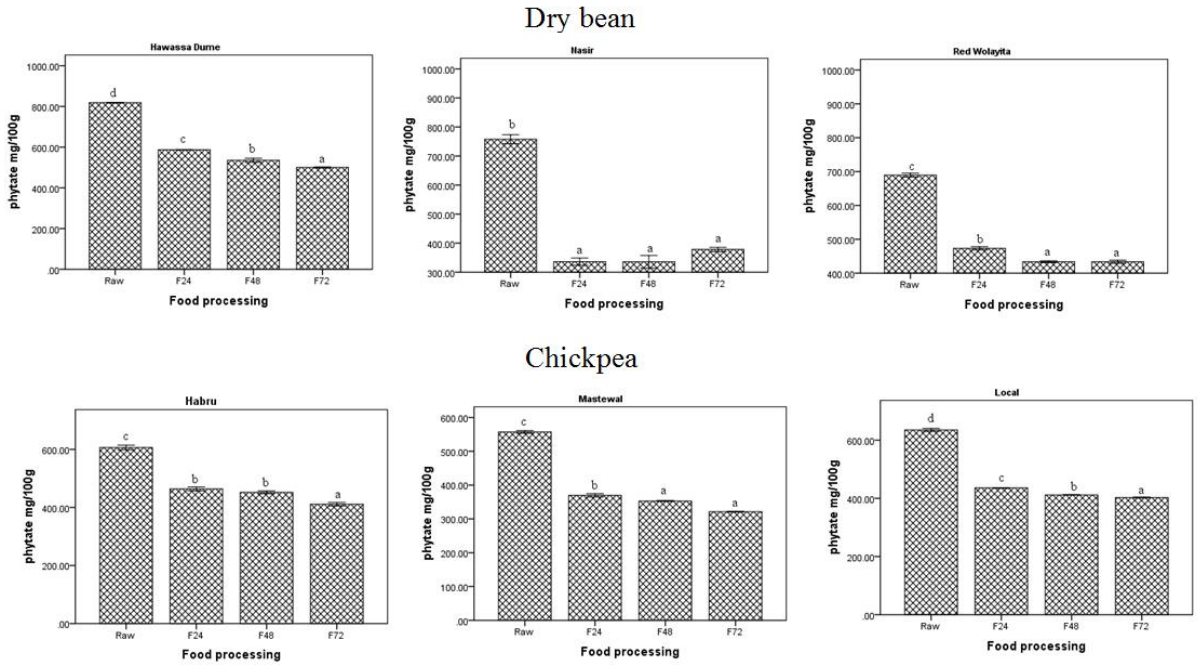

Figure 4-3. Changes in the phytate contents of samples of three dry bean and three chickpea varieties during fermentation.

Values are mean ± SD (n=3) on a dry weight basis. Bars without a common letter (a,b,c,d) are significantly different ($p<0.05$) based on Tukey's multiple comparison test. F24=fermented for 24 h, F48=fermented for 48 h, F72=fermented for 72 h.

4.3.4 Changes in Tannin and Polyphenol Contents and Phytate:zinc and Phytate:iron Molar Ratios of Dry Bean and Chickpea during Fermentation

The tannin and polyphenol contents of raw and fermented dry bean samples are shown in Table 4-3. Unfermented dry bean samples contained tannin and polyphenols ranging in content from 159.3-182.9 mg catechin/100 g and 140.1 to 180.0 mg GAE/100 g, respectively, whereas unfermented chickpea samples contained tannin and polyphenols ranging in content from 10.4-26.1 mg catechin/100 g and 15-27.5 mg GAE/100 g, respectively. The tannin contents decreased significantly with 17-30%, 18-30%, and 20-37% reductions at 24, 48 and 72 h in samples of fermented dry bean, respectively. In samples of chickpea, the tannin contents also decreased significantly with 28-33%, 28-38%, and 30-42% reductions at 24, 48 and 72 h of fermentation,

respectively. Polyphenol reductions also were noted at 24, 48 and 72 h, with 20-28%, 21-29%, and 23-30% reductions in samples of dry bean, and 20-23%, 20-27%, and 20-29% reductions in samples of chickpea. Table 4-3 also presents the phytate:zinc and phytate:iron molar ratios for fermented and non-fermented dry bean and chickpea samples. The phytate:zinc molar ratios were not significantly different in fermented and unfermented Hawassa Dume, Red Wolaita, Mastewal and Local samples, whereas reductions were observed in Nasir fermented for 24 and 48 h and Habru fermented for 24 h. The phytate:iron molar ratios of all fermented dry bean and chickpea samples were significantly lower than values for their unfermented counterparts.

4.4 Discussion

Fermentation is one of the oldest traditional food processing techniques used to reduce nutrient absorption inhibitors and improve the nutritional profile of grains and pulses. It is an inexpensive process that can be carried out with minimal resources. In this study, the effect of natural fermentation on nutrient (protein, zinc, iron and calcium) and anti-nutrient (phytate, tannin and polyphenol) contents of samples of dry bean and chickpea grown in Ethiopia were investigated. The nutrient and anti-nutrient contents of the dry bean and chickpea samples were assessed initially and after 24, 48 and 72 h of fermentation.

The protein contents of unfermented dry bean samples in the current study were comparable to those of eight bean varieties grown in Ethiopia (18-22.1 g/100 g), as reported by Shimelis and Rakshit (2005b). However, the protein contents of the unfermented chickpea samples were lower than those (19.6-20.9 g/100 g) reported by Kinfe et al. (2015).

Table 4-3. Changes in polyphenols, tannin, phytate:zinc and phytate:iron molar ratios of samples of three dry bean and chickpea varieties during fermentation.

Hours of Fermentation	Tannin (mg catechin/100 g)	Polyphenols (mg GAE/100 g)	Phytate:zinc molar ratio	Phytate:iron molar ratio
		Hawassa Dume		
0 h	159.3 ± 0.1^{c}	140.1 ± 0.1^{c}	21.4 ± 1.5^{a}	10.3 ± 1.1^{c}
24 h	124.7 ± 0.0^{b}	108.6 ± 0.4^{b}	23.4 ± 1.5^{a}	7.1 ± 0.1^{b}
48 h	122.5 ± 0.2^{a}	108.4 ± 0.2^{b}	21.1 ± 0.7^{a}	7.0 ± 0.2^{ab}
72 h	122.3 ± 0.2^{a}	105.3 ± 0.4^{a}	21.5 ± 0.8^{a}	5.8 ± 0.1^{a}
		Nasir		
0 h	167.5 ± 0.1^{d}	156.7 ± 0.1^{d}	18.3 ± 1.6^{b}	9.8 ± 0.2^{b}
24 h	138.8 ± 0.7^{c}	125.6 ± 0.3^{c}	11.5 ± 0.8^{a}	4.2 ± 0.5^{a}
48 h	137.3 ± 0.4^{b}	123.9 ± 0.8^{b}	12.4 ± 1.3^{a}	4.1 ± 0.5^{a}
72 h	134.6 ± 0.3^{a}	120.4 ± 0.2^{a}	18.3 ± 0.8^{b}	4.5 ± 0.2^{a}
		Red Wolaita		
0 h	182.9 ± 0.1^{d}	180.0 ± 0.1^{d}	19.3 ± 1.0^{ab}	8.5 ± 0.1^{d}
24 h	128.9 ± 0.2^{c}	130.3 ± 0.2^{c}	20.9 ± 0.5^{b}	6.5 ± 0.1^{c}
48 h	127.8 ± 0.5^{b}	128.2 ± 0.2^{b}	17.8 ± 1.0^{a}	6.1 ± 0.1^{b}
72 h	113.5 ± 0.1^{a}	126.4 ± 0.3^{a}	17.6 ± 0.4^{a}	5.7 ± 0.1^{a}
		Habru		
0 h	10.4 ± 0.3^{c}	15.0 ± 0.1^{c}	17.8 ± 0.5^{bc}	11.3 ± 0.3^{b}
24 h	7.2 ± 0.2^{b}	11.6 ± 0.2^{b}	16.0 ± 0.4^{a}	8.8 ± 0.3^{a}
48 h	6.5 ± 0.3^{ab}	10.9 ± 0.1^{b}	17.0 ± 0.4^{ab}	8.1 ± 1.0^{a}
72 h	6.2 ± 0.2^{a}	10.7 ± 0.6^{ab}	18.7 ± 0.4^{c}	7.6 ± 0.5^{a}
		Mastewal		
0 h	22.8 ± 0.1^{b}	24.1 ± 0.9^{b}	25.7 ± 0.9^{a}	11.3 ± 0.1^{c}
24 h	16.4 ± 0.3^{a}	19.3 ± 0.1^{a}	26.2 ± 0.9^{c}	8.3 ± 0.4^{b}
48 h	16.5 ± 0.4^{a}	19.4 ± 0.1^{a}	20.9 ± 1.7^{b}	8.5 ± 0.1^{b}
72 h	16.0 ± 0.0^{a}	19.4 ± 0.1^{a}	27.8 ± 0.7^{c}	7.4 ± 0.2^{a}
		Local		
0 h	26.1 ± 0.1^{d}	27.5 ± 0.1^{c}	12.8 ± 0.3^{a}	10.3 ± 0.1^{b}
24 h	17.5 ± 0.2^{c}	21.6 ± 0.5^{b}	10.9 ± 0.1^{a}	7.4 ± 0.1^{a}
48 h	16.2 ± 0.1^{b}	20.2 ± 0.1^{a}	10.8 ± 2.0^{a}	7.2 ± 0.3^{a}
72 h	15.1 ± 0.1^{a}	20.4 ± 0.2^{a}	11.4 ± 0.2^{a}	7.7 ± 0.5^{a}

Values are mean ± SD on a dry weight basis. Means for the same variety within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test.

In this study, the protein contents of fermented dry bean and chickpea flours tended to be lower after 24 and 48 h of fermentation compared to those of unfermented samples. Reductions in protein content due to fermentation have been reported by others (Granito et al., 2002; Porres et al., 2003). However, in the current study, fermentation for 72 h did not significantly affect the protein content of most dry bean and chickpea samples compared to values for corresponding flours. The apparent increase in protein content that occurred after 24 and 48 h of fermentation could be attributable to microbial processes, e.g., oxidation or degradation of other compounds such as lipids and fibre (Martin-Cabrejas et al., 2004; Reyes-Moreno et al., 2004; Sparringa & Owens, 1999), leading to a relative increase in the concentration of protein as the length of fermentation increased.

The zinc contents of the unfermented dry bean samples were higher than the values (1.7-2.8 mg/100 g and 1.5-2.8 mg/100 g) reported by Ariza-Nieto et al. (2007) and Shimelis and Rakshit (2005b), respectively. The iron contents of unfermented dry bean samples were in the range (4.8-7.4 mg/ 100 g) reported for eight dry bean varieties from different parts of the world (Ariza-Nieto et al., 2007) and comparable to values (6.1-8.3 mg/100 g) reported by Shimelis and Rakshit (2005) for beans grown in Ethiopia. The calcium contents of unfermented dry bean samples in the current study were within the wide range of values (89-203.8 mg/100 g) reported in the literature (Ariza-Nieto et al., 2007; Shimelis & Rakshit, 2005b). The iron contents of unfermented chickpea samples were within the range (4.04-6.47 mg/100 g) reported by Kinfe et al. (2015). However, the zinc contents of unfermented chickpea samples was higher in the current study, whereas the calcium content of unfermented chickpea samples tended to be lower, than values (147-400 mg/100 g) reported for chickpea grown in Ethiopia (Abebe et al., 2007; Kinfe et al., 2015).

The fermentation of dry bean and chickpea flours affected mineral content to various extents. Decreases in mineral content during fermentation have been reported (Granito et al., 2002).

Unlike other studies that reported no significant change or higher zinc content in fermented samples compared to unfermented counterparts (Granito et al., 2002; Porres et al., 2003), zinc loss was observed in all fermented samples in the current study. Iron loss was less pronounced than zinc loss. Lower calcium levels in a few of the fermented samples also were observed. The fermenting water was discarded after each fermentation time which would have contributed to the reductions in mineral content.

All fermented dry bean and chickpea samples had significantly lower pHs than did the corresponding raw flours. Similar results have been reported in natural and controlled fermentation of dry bean and chickpea (Chandra-Hioe, Wong, & Arcot, 2016; Porres et al., 2003). The lowering of pH has been attributed to the microbial production of organic acids, such as lactic and acetic acid, during fermentation (Hemalatha, Platel, & Srinivasan, 2007).

The phytate contents of dry bean and chickpea samples in the current study were in keeping with previously reported values, which varied over wide ranges, 716-1380 mg/100 g (Abebe et al., 2007; Porres et al., 2003; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010) and 57-1060 mg/100 g (Kinfe et al., 2015; Wang et al., 2010). Under the experimental conditions in this study, fermentation led to significant reductions in the phytate contents of the dry bean and chickpea samples. Even though all of the studies are not directly comparable because of differences in the water to flour ratio, the percentage reductions in this study were in agreement with most previously published results (Chitra et al., 1996; Granito et al., 2002; Porres et al., 2003). Porres et al. (2003) reported a 32.5% phytate reduction in dry bean fermented naturally for 48 h in sterilized distilled water at a concentration of 300 g/L. Granito et al. (2002) found that dry bean flour fermented for 48 h at concentrations ranging from 1:4 to 1:12 (w/v) resulted in a 7-39% reduction in phytate, whereas Chitra et al. (1996) observed that phytate was reduced by 40% in pre-soaked and fermented (24 h) chickpea flour. However, reductions of phytate in this study were lower than

those noted by Shimelis and Rakshit (2008), who reported reductions in phytate content of 18-93% due to fermentation (24-96 h) of dry bean varieties. Studies have attributed the reductions in phytate content during fermentation to the activity of a microbial phytase, an endogenous phytase, or a combination of the two. In line with these observations, Curiel et al. (2015) reported an increase in phytase activity in dry bean and chickpea during fermentation. Phytase can induce hydrolysis of phytate to lower inositol phosphates and enhance micronutrient bioavailability (Hotz & Gibson, 2007). It also has been noted that fermentation creates an acidic environment conducive to phytase activity. In addition, organic acids produced during fermentation have the capacity to create soluble ligands with zinc and iron, protecting them from precipitation (Hotz & Gibson, 2007).

As expected, higher polyphenol and tannin contents were observed in the pigmented dry bean samples than in the chickpea samples. Habru chickpea had the lowest polyphenol and tannin contents, unsurprisingly, since it has light-coloured seed compared to the Mastewal and Local chickpea varieties, which are Desi-type. This observation is consistent with several reports (Wang et al., 2010; Sharma et al., 2013; Kinfe et al., 2015) indicating that polyphenol and tannin contents are higher in Desi chickpea than in Kabuli. In agreement with this explanation, a study has shown that the seed coat (where most polyphenols are located) of Kabuli is thinner than that of Desi, comprising 5% of total seed weight compared to 14% in Desi seeds (Wood et al., 2011). In this study, fermentation caused a significant reduction in polyphenol content. This result is in agreement with polyphenol contents reported for naturally fermented bean (Limón et al., 2015) and lentil (Torino et al., 2013). In contrast to the findings in this study, some studies (Curiel et al., 2015; Reyes-Bastidas et al., 2010) have reported an increase in total polyphenols or some particular polyphenol during fermentation. In one study, sourdough fermentation increased the concentration of polyphenols in four bean and two chickpea varieties, but noted no significant effects for four bean varieties used in the same study (Curiel et al., 2015). Another study reported higher levels of

polyphenols in solid-state fermented bean than in raw bean flour (Reyes-Bastidas et al., 2010), while observing a slight increase or no significant change in liquid-state fermentation (Limón et al. (2015). Gan et al. (2016) noted variable effects (increase, decrease or no effect) of fermentation on the polyphenol contents of different pulses. The same study suggested that this diverse response could be due to differences in the polyphenol composition of the pulses, as well as the microbial species involved in the fermentation process. With respect to the tannin contents of the dry bean and chickpea samples in this study, the fermentation process resulted in reduced tannin levels. Other studies (Curiel et al., 2015; Granito et al., 2002; Limón et al., 2015; Shimelis & Rakshit, 2008) have shown similar reductions.

Although ratios do not consider all of the factors affecting mineral bioavailability, phytate:zinc and phytate:iron molar ratios have been used to indicate the potential availability of nutrients for absorption and utilization from fermented food products (Liang, Han, Nout, & Hamer, 2008; Luo, Gu, Han, & Chen, 2009). All fermented samples in the current study had lower phytate:iron molar ratios than their corresponding unfermented counterparts. The lack of improvement in the phytate:zinc molar ratios of fermented samples, despite the lower level of phytate, could be attributable to the reductions in zinc content observed.

4.5 Conclusion

Broadly, the results indicated that fermentation effectively reduced anti-nutrient contents of dry bean and chickpea samples and that fermentation could improve the iron bioavailability of dry bean and chickpea flours with minimal loss of mineral content, as determined by the phytate:iron molar ratios. Even though the expected improvement due to lower phytate contents was not confirmed by lower phytate:zinc molar ratios, the fermentation process was still effective

in reducing the total anti-nutrient contents. The results from this study could be instrumental in the formulation of novel fermented pulse-based food products.

Chapter 5

Impact of Combined Soaking, Germination and Cooking on Nutrient and Anti-nutrient Content, Cooking Time and Acceptability of Dry Bean (*Phaseolus vulgaris* L.) and Chickpea (*Cicer arietinum* L.) Grown in Ethiopia

The previous chapter provided findings on the effect of fermentation on nutrient and anti-nutrient contents of samples of dry bean and chickpea varieties grown in Ethiopia. The fermentation process employed in the previous chapter utilized flour of dry bean and chickpea, whereas the processes presented in this study were carried out on whole seed. The study presented here describes the impact of other traditional food processing strategies, i.e. soaking, germination, cooking and their combinations, on nutrient and anti-nutrient contents, cooking time and sensory acceptability. These strategies would provide households with additional options for recipes requiring whole seed instead of flour. Chapter 5, presented here as a publishable manuscript, describes the results of specific objectives 2-4. The objectives were to determine the effect of soaking-cooking and germination-cooking on mineral (iron, zinc and calcium) content as well as anti-nutrient (phytate, tannin and polyphenols) content of dry bean and chickpea and the acceptability of the dishes prepared after soaking-cooking and germinating-cooking dry bean and chickpea.

Abstract

Pulses are processed in diverse ways prior to consumption. Soaking and germination are among the most common traditional, household-level, food processing strategies. This study was carried out to determine the effects of soaking, germination, cooking and their combinations on the contents of selected nutrients and anti-nutrients of dry bean and chickpea. In addition, the effects of the pre-treatments on cooking time and the acceptability of dishes prepared from dry bean and chickpea were determined. The nutrient compositions (protein, zinc, iron and calcium) of most soaked-cooked and germinated-cooked dry bean and chickpea samples were not significantly different than those of respective samples cooked without pre-treatment. However, soaking-cooking and germination-cooking significantly lowered the phytate and tannin contents of the dry bean and chickpea samples, with a few exceptions, and overall, polyphenol contents were lower after soaking-cooking than after germination-cooking. Most scores for sensory attributes of bean-based and chickpea-based dishes prepared from soaked or germinated samples were not significantly different than those of dishes prepared from samples that had not been pre-treated. For most dry bean and chickpea samples, longer germination times yielded superior results in terms of cooking time, tannin and phytate:zinc and phytate:iron molar ratio reduction.

5.1 Introduction

Micronutrient deficiency is a major public health problem in most developing countries, as it predisposes the population to a wide range of impacts on health and physical and mental development (Umamaheswari, Bhaskaran, Krishnamurthy, Hemamalini, & Kavita, 2011; Viteri & Gonzalez, 2002). Pulses, including dry bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.), can provide essential micronutrients. They also are useful in enriching cereal-based diets as

they provide lysine, which is deficient in cereals. The production of pulses has been increasing in recent years (Joshi & Rao, 2016). Chickpea and dry bean are among the major pulses produced and consumed in Ethiopia (Shiferaw & Teklewold, 2007).

The anti-nutrients, abdominal discomfort and hard-to-cook phenomenon associated with pulses limit their use (Huma *et al*., 2008; Kabata *et al*., 2016; Wang & Daun, 2005). The longer the cooking time, the higher will be the loss of nutrients and fuel use (Shimelis and Rakshit, 2005b; Nakitto *et al*., 2015). Long cooking times also will add to the already burdened life of women, especially in developing countries, where food preparation is the main responsibility of women. Improved plant varieties have been released from agricultural research centres; however, they are selected mainly on agronomic traits with little focus on their nutritional profile (Ray et al., 2014; Shimelis & Rakshit, 2005a). Reducing cooking time and anti-nutrients through simple, household-level, food processing strategies would increase the role of pulses in tackling micronutrient and protein deficiencies.

Pulses are processed at home in a variety ways. Soaking and germination are among the most common traditional, household-level, food processing strategies. These traditional strategies offer several benefits in addition to a reduction in anti-nutrients (Alajaji & El-Adawy, 2006; Khattab & Arntfield, 2009; Yasmin et al., 2008). For example, germination increased ascorbic acid content (Sangronis and Machado, 2007; Masood et al., 2014) and improved protein digestibility (Ghavidel & Prakash, 2007). It has been demonstrated that these processing strategies also improved the nutritional profile of pulses and reduced cooking time (Carmona-García, Osorio-Díaz, Agama-Acevedo, Tovar, & Bello-Pérez, 2007; Correaa et al., 2010; Ghavidel & Prakash, 2007; Khalil et al., 2007). This study was carried out to determine the effect of soaking, germination, cooking and their combinations on selected nutrient and anti-nutrient contents of dry bean and chickpea, thus identifying processing strategies that result in an improved nutritional

profile. Also, the effects of pre-treatments on cooking time and the acceptability of dishes prepared from soaked and germinated dry bean and chickpea were determined.

5.2 Materials and Methods

All chemicals and reagents were of analytical grade. Deionized water (ACS Reagent Grade, ASTM Type I, ASTM Type II) was obtained from VWR, Mississauga, ON, Canada.

5.2.1 Samples

Samples of three pigmented dry bean (Hawassa Dume, Nasir and Red Wolaita) and three chickpea (Habru, Mastewal and Local) varieties obtained from local markets and agricultural research centres in Ethiopia were thoroughly cleaned and used in the experiment. All of the dry bean samples used in the study were of red bean. Mastewal and Local are Desi-type chickpea, whereas Habru is Kabuli-type. Five-kilogram samples of each dry bean and chickpea variety were cleaned by hand sorting to remove dust, extraneous matter, and broken and immature seeds. The seeds were washed thoroughly three times using deionized water and then air dried before further processing.

5.2.2 Cooking Time

Cooking times of raw and processed seeds were determined using a Mattson cooker (Figure 5-1) according to the method described by Wang & Daun (2005). Twenty-five seeds were positioned in each of the 25 saddles of the rack such that the tip of each plunger rested on top of the seed. The rack was then placed in a 2-L metal beaker containing 1.2 L of boiling water. The time at which a plunger drops and penetrates a seed was registered. Cooking time was calculated

as the time to cook 80% of the seeds. The experiment was carried out in triplicate and the average cooking time for each seed sample was reported.

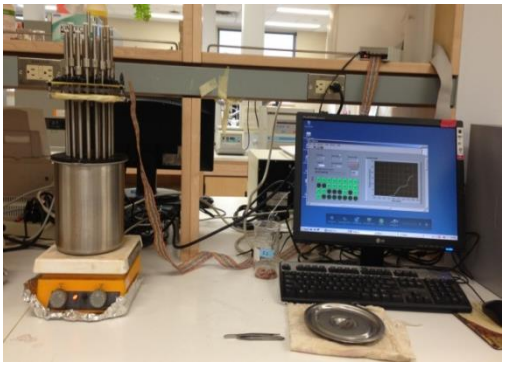

Figure 5-1. A picture of Mattson cooker.

5.2.3 Food Processing Methods

Portions of each seed sample were soaked, germinated and cooked. Processing is described in the following sections.

5.2.3.1 Soaking

Three, 100-gram samples of each seed variety were soaked separately in water (1:3, w/v) for 6, 12 or 18 h at room temperature (25°C). The soak water was decanted at the end of the soaking period.

5.2.3.2 Germination

Three, 100-gram samples of each seed variety were soaked separately in water (1:3, w/v) for 12 h at room temperature (25°C). The soak water was decanted and the seeds were placed in a glass container covered with perforated aluminum foil. The container was positioned upside down to drain any remaining water, then covered with a dark cloth and left to germinate for 24, 48 or 72 h according to the method described by Sangronis & Machado (2007) with slight modifications of

soaking time and the container used for germination. The seeds were sprinkled with water every 12 h until the end of the germination period.

5.2.3.3 Cooking

Three, 100-gram samples of each seed variety, without any pre-treatment, were cooked in water (1:5, w/v); these samples served as controls. The samples were cooked on the stove-top for their pre-determined cooking times. The pre-soaked and pre-germinated seed samples were cooked similarly. The cooked seeds were dried in a convection oven (Sanyo Convection Oven, Model MOV-212F, Sanyo Electric Co. Ltd., Osaka, Japan) at 60°C, milled (UDY cyclone mill, UDY Corporation, Fort Collins, CO, USA, equipped with a 0.5-mm screen), sealed in air-tight polyethylene bags and stored at 4°C. Processing protocols are summarized in Figure 5-2.

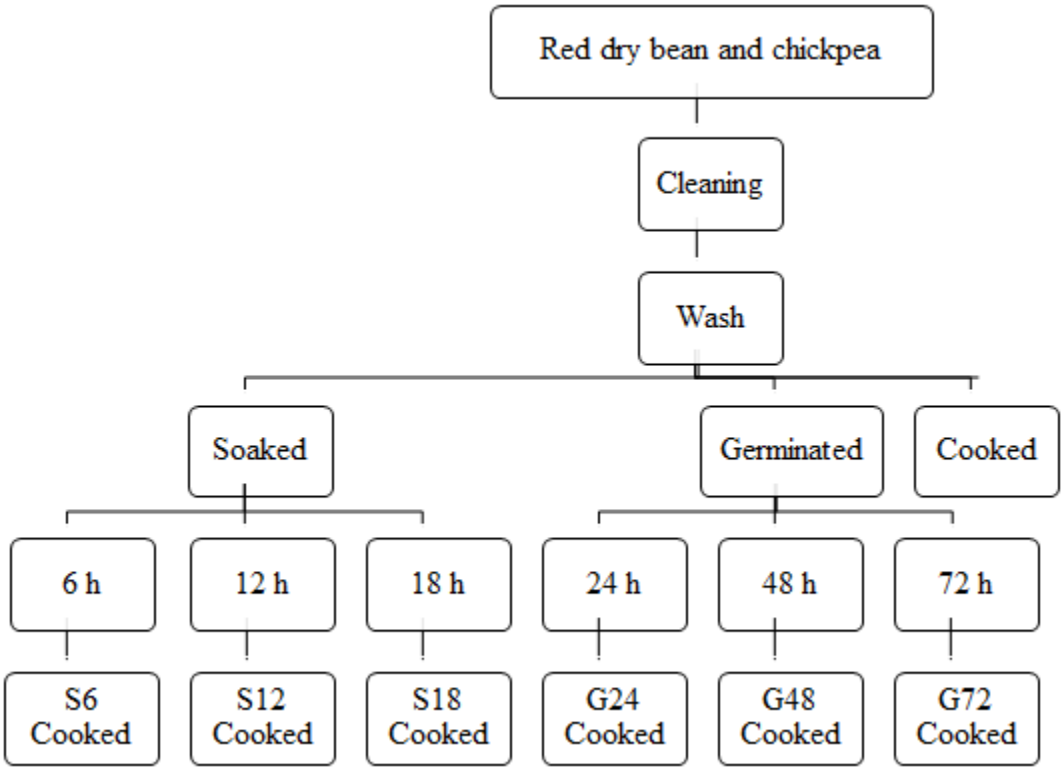

Figure 5-2. Flow chart of household-level food processing strategies employed in this study.

5.2.4 Nutrient and Anti-nutrient Analysis

Total iron, zinc and calcium concentrations were determined using a modified HNO_3-H_2O_2 digestion. The digestion protocol was previously described by Thavarajah & Thavarajah (2012). Approximately 500 mg of flour was digested and Flame Atomic Absorption Spectrophotometry (novAA300, Analytik Jena AG, Jena, Germany) was used for determination of minerals in the digest. Lanthanum chloride (1%) was added to food samples to suppress interference from phosphorus in the determination of calcium. For protein analysis, 250-mg samples were weighed and nitrogen concentration was determined by the Dumas combustion method using a FP-528 Protein/Nitrogen Analyzer (LECO Corporation, St. Joseph, MI, USA) as described in AACC International Method 46-30.01 (AACC International, 1999). Protein was calculated by multiplying the nitrogen concentration by a factor of 6.25. Phytate content was determined using the Wade reagent method as described by Gao et al. (2007).

Total polyphenols were determined with Folin-Ciocalteu reagent according to the method described by Wei et al. (2013). Tannin was analysed using the Vanillin-HCl method as described by Price et al. (1978).

5.2.5 Sensory Evaluation

Dishes were prepared with soaked and germinated seed using modified local recipes (100 g dried bean or chickpea, boiled, mashed, and mixed with salt, onion and sunflower oil). Pre-soaked and germinated bean and chickpea were used for the treatment group, and untreated seed for the controls. Sensory evaluation was carried out according to the method described by Meilgaard et al. (2006). Recruitment was based on willingness to participate, availability for the entire period of evaluation, history of consumption of bean and chickpea, and no related history of allergy. Health conditions such as flu that would affect participants' sensory ability were considered and potential

panelists were disqualified if necessary. Sensory evaluation was carried out at two different levels. The initial evaluation was carried out at Hawassa University. Thirty panelists (*i.e.* students at the School of Nutrition, Food Science and Technology, Hawassa University) divided into two groups tested the food samples; one group tested bean-based dishes, while the other tested chickpea-based dishes. All tests were carried out in triplicate. After recruitment, the panelists were informed about the objectives of the study in detail. They also were instructed on how to complete the questionnaire. A nine-point hedonic scale (from 1=dislike extremely, 5=neither like nor dislike, to 9=like extremely) was used to test appearance, texture, odour, taste and overall acceptability (see Appendix 2).

The second level of sensory evaluation was carried out in dry bean and chickpea growing rural areas, namely the Hawassa Zuriya and Zeway districts. Sixty women with children under five years of age were recruited in each community. Bean-based dishes were tested in Hawassa Zuriya, while chickpea-based dishes were tested in Zeway. A five-point hedonic scale (from "5" = excellent, to "1" = terrible, see Appendix 3), used in similar communities (Berhanu et al., 2014; Kebebu et al., 2013), was selected for this phase since the majority of women in rural Ethiopia have no formal education (Central Statistical Agency [Ethiopia] and ICF, 2016a) This phase of sensory evaluation was interview-administered for the same reason. The participants were informed about the study and the same conditions as described earlier with respect to the initial sensory evaluation, which may affect senses were considered. To avoid sensory fatigue, sub-samples of dry bean- and chickpea-based dishes were selected from soaked and germinated dishes. To ensure the quality of the sensory evaluation, the dishes were coded with three-digit, random numbers and presented in similar utensils. To avoid a carryover effect, water was provided to the participants to rinse their mouths before and between samples.

5.2.6 Ethical Clearance

Ethical approval was obtained from the Behavioural Research Ethics Board, University of Saskatchewan, and the Institutional Review Board of the College of Medicine and Health Sciences, Hawassa University.

5.2.7 Statistical Analysis

Data were analyzed using SPSS version 20.0 for Windows (IBM Corp. Armonk, NY, USA). Results are expressed as the mean ± standard deviation. Analysis of Variance (ANOVA) with Tukey's test at $p<0.05$ was used to determine whether means were statistically different.

5.3 Results

5.3.1 Nutrient Composition of Processed Dry Bean and Chickpea

The protein, zinc, iron and calcium contents of samples of the three dry bean varieties subjected to different processing techniques are presented in Table 5-1; statistical comparisons are made between different processing treatments for the same variety. The protein, zinc, iron and calcium contents of soaked-cooked and germinated-cooked Hawassa Dume samples were not significantly different compared to samples cooked without pre-treatment, with the exception of zinc in germinated (24 and 48 h)-cooked and calcium in germinated (72 h)-cooked samples. The protein, zinc, iron and calcium contents of soaked-cooked and germinated-cooked Nasir samples were not significantly different from that of the sample cooked without pre-treatment, with the exception of protein in germinated (72 h)-cooked, zinc in germinated (48 and 72 h)-cooked and calcium in soaked (18h)-cooked samples. The protein, iron and calcium contents of soaked-cooked and germinated-cooked Red Wolaita samples were not significantly different from that of the

sample cooked without pre-treatment, with the exception of iron in soaked (6 h)-cooked and calcium in soaked (6 and 18 h)-cooked samples. However, the zinc contents of all soaked-cooked and germinated-cooked Red Wolaita samples were significantly lower than in the sample cooked without pre-treatment.

The impact of processing on the protein, zinc, iron and calcium contents of samples of the three chickpea varieties is presented in Table 5-2; statistical comparisons are made between different processing treatments for the same variety. The protein, zinc, iron and calcium contents of soaked-cooked and germinated-cooked Habru samples were not significantly different from that of the sample cooked without pre-treatment, with the exception of protein in germinated (48 h)-cooked, zinc in germinated (24 h)-cooked and calcium in germinated (24 and 48 h)-cooked samples. Protein content was significantly higher in germinated (72 h)-cooked Mastewal and Local samples, but not significantly different among other treatments. The iron contents of soaked-cooked and germinated-cooked Mastewal and Local samples were not significantly different from those of samples cooked without pre-treatment. The zinc and calcium contents of most Mastewal and Local samples [soaked (12 and 18 h)-cooked, germinated (72 h)-cooked] were significantly different from those of samples cooked without treatment.

Table 5-1. Effect of soaking, germination and cooking on protein, zinc, iron and calcium contents of samples of three dry bean varieties.

Treatment	Protein (g/100 g)	Zn (mg/100 g)	Fe (mg/100 g)	Ca (mg/100 g)
		Hawassa Dume		
Cooked	23.9 ± 0.5^{a}	3.75 ± 0.24^{b}	6.45 ± 0.42^{a}	163.5 ± 8.2^{ab}
S6 Cooked	23.6 ± 0.8^{a}	3.33 ± 0.05^{ab}	6.07 ± 0.10^{a}	158.9 ± 3.6^{a}
S12 Cooked	23.9 ± 0.1^{a}	3.27 ± 0.00^{ab}	6.45 ± 0.09^{a}	156.8 ± 6.4^{a}
S18 Cooked	23.3 ± 0.0^{a}	3.46 ± 0.04^{ab}	5.81 ± 0.08^{a}	184.1 ± 7.2^{b}
G24 Cooked	23.5 ± 0.1^{a}	3.18 ± 0.03^{a}	6.06 ± 0.04^{a}	173.4 ± 14.6^{a}
G48 Cooked	22.5 ± 2.5^{a}	3.07 ± 0.02^{a}	6.57 ± 0.80^{a}	164.4 ± 11^{ab}
G72 Cooked	20.8 ± 0.9^{a}	3.41 ± 0.03^{ab}	6.08 ± 0.00^{a}	149.3 ± 5.8^{a}
		Nasir		
Cooked	23.6 ± 0.5^{a}	3.45 ± 0.15^{b}	6.44 ± 0.11^{ab}	166.1 ± 1.4^{ab}
S6 Cooked	23.8 ± 1.1^{ab}	3.36 ± 0.05^{b}	6.31 ± 0.25^{a}	164.5 ± 6.0^{ab}
S12 Cooked	23.9 ± 0.2^{ab}	3.31 ± 0.00^{b}	6.44 ± 0.24^{ab}	173.4 ± 6.6^{b}
S18 Cooked	23.8 ± 0.0^{ab}	3.34 ± 0.05^{b}	5.89 ± 0.21^{a}	205.7 ± 6.5^{c}
G24 Cooked	23.9 ± 0.0^{ab}	3.34 ± 0.06^{b}	6.08 ± 0.01^{a}	157.8 ± 2.6^{a}
G48 Cooked	23.7 ± 0.0^{ab}	3.08 ± 0.02^{a}	5.92 ± 0.08^{a}	176.9 ± 4.0^{b}
G72 Cooked	25.5 ± 0.0^{b}	3.73 ± 0.04^{c}	6.92 ± 0.07^{b}	171.4 ± 4.6^{b}
		Red Wolaita		
Cooked	17.5 ± 1.1^{bc}	3.13 ± 0.01^{c}	6.40 ± 0.02^{b}	156.3 ± 1.1^{a}
S6 Cooked	17.6 ± 0.1^{bc}	2.73 ± 0.05^{ab}	5.62 ± 0.15^{a}	165.0 ± 2.5^{b}
S12 Cooked	17.1 ± 0.0^{ab}	2.73 ± 0.01^{ab}	6.18 ± 0.03^{b}	161.4 ± 2.4^{ab}
S18 Cooked	18.4 ± 0.1^{c}	2.79 ± 0.08^{b}	6.36 ± 0.07^{b}	222.7 ± 6.8^{c}
G24 Cooked	16.9 ± 0.2^{ab}	2.82 ± 0.01^{b}	6.15 ± 0.30^{b}	157.4 ± 0.1^{a}
G48 Cooked	16.7 ± 0.4^{ab}	2.69 ± 0.04^{a}	6.08 ± 0.19^{b}	158.1 ± 0.1^{a}
G72 Cooked	16.4 ± 0.1^{ab}	2.69 ± 0.01^{a}	6.07 ± 0.16^{b}	157.8 ± 0.0^{a}

Values are mean ± SD (n=3) expressed on a dry weight basis. For each variety, means within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S6 Cooked=soaked for 6 hours and cooked, S12 Cooked=soaked for 12 hours and cooked, S18 Cooked=soaked for 18 hours and cooked, G24 Cooked=germinated for 24 hours and cooked, G48 Cooked=germinated for 48 hours and cooked, G72 Cooked=germinated for 72 hours and cooked.

Table 5-2. Effect of soaking, germination and cooking on protein, zinc, iron and calcium contents of samples of three chickpea varieties.

Treatment	Protein (g/100 g)	Zn (mg/100 g)	Fe (mg/100 g)	Ca (mg/100 g)
		Habru		
Cooked	17.6 ± 0.6^{b}	3.10 ± 0.09^{b}	4.00 ± 0.28^{b}	115.5 ± 25.9^{bc}
S6 Cooked	18.9 ± 0.4^{b}	2.59 ± 0.33^{ab}	3.45 ± 0.20^{ab}	114.2 ± 37.1^{bc}
S12 Cooked	17.7 ± 1.6^{b}	3.00 ± 0.45^{ab}	3.90 ± 0.31^{ab}	120.3 ± 13.0^{c}
S18 Cooked	18.7 ± 3.1^{b}	2.80 ± 0.14^{b}	3.98 ± 0.13^{b}	113.5 ± 11.2^{c}
G24 Cooked	16.6 ± 2.9^{ab}	2.15 ± 0.28^{a}	3.77 ± 0.87^{ab}	110.8 ± 22.8^{a}
G48 Cooked	12.4 ± 0.9^{a}	2.55 ± 0.59^{ab}	2.92 ± 0.55^{ab}	80.4 ± 12.5^{a}
G72 Cooked	14.9 ± 3.7^{ab}	2.28 ± 0.55^{ab}	3.41 ± 0.10^{ab}	79.1 ± 2.1^{ab}
		Mastewal		
Cooked	16.2 ± 0.3^{a}	1.83 ± 0.04^{a}	3.75 ± 0.08^{a}	128.2 ± 1.6^{a}
S6 Cooked	15.7 ± 0.3^{a}	1.92 ± 0.10^{a}	3.87 ± 0.10^{a}	134.0 ± 5.3^{ab}
S12 Cooked	15.8 ± 0.0^{a}	1.90 ± 0.01^{a}	4.02 ± 0.02^{a}	141.5 ± 1.4^{bc}
S18 Cooked	15.8 ± 0.1^{a}	2.17 ± 0.05^{b}	3.98 ± 0.02^{a}	146.7 ± 2.7^{d}
G24 Cooked	16.1 ± 0.1^{a}	1.95 ± 0.01^{a}	3.99 ± 0.31^{a}	137.6 ± 0.8^{abc}
G48 Cooked	16.1 ± 0.1^{a}	1.95 ± 0.02^{a}	3.99 ± 0.31^{a}	138.1 ± 0.1^{abc}
G72 Cooked	18.3 ± 0.0^{b}	2.52 ± 0.02^{c}	3.78 ± 0.05^{a}	145.8 ± 5.6^{c}
		Local		
Cooked	20.0 ± 0.1^{b}	4.26 ± 0.06^{a}	5.35 ± 0.07^{ab}	214.2 ± 12.2^{a}
S6 Cooked	20.1 ± 0.3^{b}	4.27 ± 0.01^{a}	5.30 ± 0.15^{ab}	214.2 ± 4.2^{a}
S12 Cooked	20.1 ± 0.1^{b}	4.71 ± 0.01^{c}	5.55 ± 0.10^{bc}	230.3 ± 7.8^{ab}
S18 Cooked	19.5 ± 0.4^{b}	4.84 ± 0.02^{c}	5.47 ± 0.03^{ab}	270.8 ± 0.2^{c}
G24 Cooked	19.4 ± 0.5^{b}	4.42 ± 0.02^{b}	5.23 ± 0.06^{a}	224.3 ± 0.4^{ab}
G48 Cooked	19.4 ± 0.5^{b}	4.41 ± 0.04^{ab}	5.27 ± 0.01^{a}	224.3 ± 0.3^{ab}
G72 Cooked	21.1 ± 0.0^{c}	4.99 ± 0.03^{d}	5.32 ± 0.05^{ab}	244.2 ± 0.7^{bc}

Values are mean ± SD (n=3) expressed on a dry weight basis. For each variety, means within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S6 Cooked=soaked for 6 hours and cooked, S12 Cooked=soaked for 12 hours and cooked, S18 Cooked=soaked for 18 hours and cooked, G24 Cooked=germinated for 24 hours and cooked, G48 Cooked=germinated for 48 hours and cooked, G72 Cooked=germinated for 72 hours and cooked.

5.3.2 Anti-nutrient Content and Phytate:zinc and Phytate:iron Molar Ratios of Processed Dry Bean and Chickpea Samples

Changes in the phytate content of cooked dry bean and chickpea samples due to soaking and germination are illustrated in Figure 5-3. Phytate reduction in dry bean samples was 15-35% in Hawassa Dume, 13-69% in Nasir, and 1-39% in Red Wolaita [except in soaked (6 h)-cooked]. For the chickpea samples, a significant reduction in phytate (6-52%) was noted for all processing

treatments for Habru, with the exception of soaked (6 h)-cooked. Both soaking and germination combined with cooking resulted in significant reductions (12-63%) in phytate in Local chickpea samples. Similarly, phytate was reduced significantly (11-61%) in Mastewal.

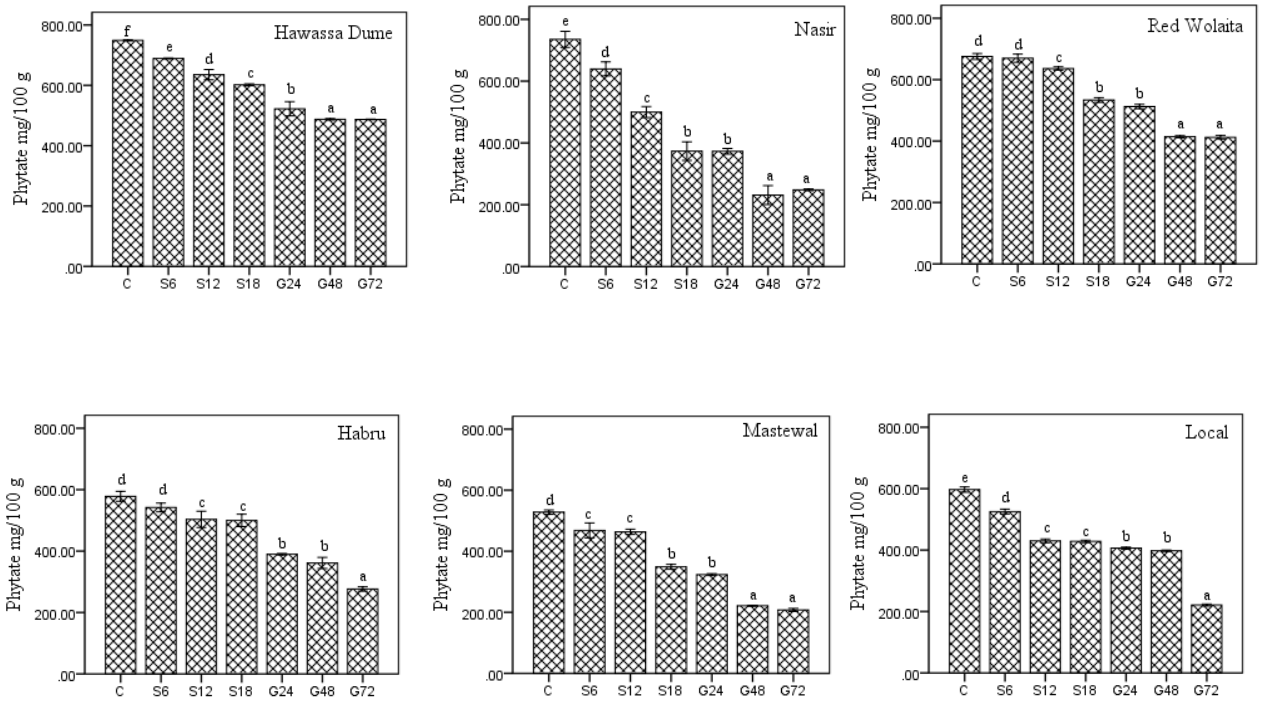

Figure 5-3. Effect of soaking, germination and cooking on phytate contents of samples of three dry bean and three chickpea varieties.

Values are mean ± SD (n=3) expressed on a dry weight basis. Bars without a common letter (a,b,c,d) are significantly different ($p<0.05$) based on Tukey's multiple comparison test. C=control (cooked without pre-treatment), S6=soaked for 6 hours and cooked, S12=soaked for 12 hours and cooked, S18=soaked for 18 hours and cooked, G24=germinated for 24 hours and cooked, G48=germinated for 48 hours and cooked, G72=germinated for 72 hours and cooked.

Changes in the tannin and polyphenol contents of dry bean and chickpea are presented in Table 5-3 and Table 5-4. The tannin content of dry bean samples cooked without pre-treatment ranged from 142.4-146.4 mg catechin/100 g. The tannin content of chickpea samples cooked without pre-treatment ranged from 9.3-21.0 mg catechin/100 g. Soaked-cooked and germinated-cooked dry bean had significantly lower tannin contents compared to samples cooked without pre-

treatment. A similar pattern was observed for soaked-cooked and germinated-cooked chickpea samples, with the exception of the soaked (6 h)-cooked Mastewal sample. The highest tannin reduction was noted in germinated (72 h)-cooked samples for both dry bean and chickpea samples, except for Habru. Polyphenol contents of dry bean and chickpea cooked without pre-treatment ranged from 128.7-165.5 and 13.7-25.3 mg GAE/100 g, respectively. Soaking combined with cooking resulted in significant reductions in the polyphenol content of all dry bean and chickpea samples compared to samples cooked without pre-treatment. Germination combined with cooking resulted in lower polyphenol contents in some dry bean samples (and no significant effect in others) compared to samples cooked without pre-treatment. Germination combined with cooking resulted in significantly lower polyphenol contents in chickpea samples compared to samples cooked without pre-treatment.

The phytate:zinc and phytate:iron molar ratios of processed dry bean and chickpea are presented in Table 5-3 and Table 5-4. In comparison to samples cooked without treatment, the phytate:zinc molar ratio of Hawassa Dume was reduced significantly by germination-cooking, whereas the phytate:iron molar ratio was reduced by soaking (12 and 18 h)-cooking and germination-cooking. For Nasir and Red Wolaita, the lowest phytate:zinc and phytate:iron molar ratios were noted for germination (48 and 72 h)-cooking. The phytate:zinc and phytate:iron molar ratios of soaked-cooked and germinated-cooked chickpea samples were significantly lower than those of the samples cooked without pre-treatment, with the exception of Habru. The lowest phytate:zinc and phytate:iron molar ratios for Mastewal and Local were observed for germination (48 and 72 h)-cooking and germination (72 h)-cooking, respectively.

Table 5-3. Effect of soaking, germination and cooking on tannin and polyphenol contents and phytate:zinc and phytate:iron molar ratios of samples of three dry bean varieties.

Treatment	Tannin (mg catechin/100 g)	Polyphenols (mg GAE/100 g)	Phytate:zinc molar ratio	Phytate:iron molar ratio
		Hawassa Dume		
Cooked	143.2 ± 0.7^{e}	128.7 ± 1.3^{d}	19.8 ± 1.6^{cd}	10.2 ± 0.1^{d}
S6 Cooked	127.3 ± 1.1^{d}	111.9 ± 1.1^{b}	20.5 ± 0.4^{cd}	9.6 ± 0.2^{cd}
S12 Cooked	127.4 ± 0.7^{d}	110.0 ± 0.6^{a}	19.5 ± 0.5^{cd}	8.4 ± 0.1^{bc}
S18 Cooked	127.4 ± 0.7^{d}	112.9 ± 0.7^{c}	17.4 ± 0.3^{abc}	8.8 ± 0.1^{bc}
G24 Cooked	126.6 ± 0.6^{c}	128.3 ± 2.8^{c}	16.3 ± 0.6^{ab}	7.1 ± 0.2^{ab}
G48 Cooked	124.5 ± 1.0^{b}	128.8 ± 0.9^{d}	16.0 ± 1.6^{a}	6.6 ± 0.9^{a}
G72 Cooked	124.2 ± 0.7^{a}	128.7 ± 1.8^{cd}	14.1 ± 0.2^{a}	6.8 ± 0.1^{a}
		Nasir		
Cooked	142.4 ± 0.9^{f}	133.2 ± 0.9^{f}	21.2 ± 0.9^{d}	9.7 ± 0.3^{d}
S6 Cooked	140.7 ± 0.9^{e}	125.4 ± 0.7^{a}	18.9 ± 0.6^{d}	8.7 ± 0.2^{d}
S12 Cooked	139.1 ± 1.4^{d}	126.0 ± 0.9^{c}	14.9 ± 0.7^{c}	6.5 ± 0.6^{c}
S18 Cooked	137.4 ± 0.9^{c}	125.8 ± 0.4^{b}	11.2 ± 0.9^{b}	5.4 ± 0.5^{bc}
G24 Cooked	139.0 ± 0.9^{d}	131.4 ± 0.7^{d}	10.8 ± 0.1^{b}	5.1 ± 0.0^{b}
G48 Cooked	135.7 ± 0.9^{b}	132.8 ± 0.5^{e}	7.4 ± 1.3^{a}	3.3 ± 0.7^{a}
G72 Cooked	134.0 ± 0.6^{a}	133.1 ± 0.5^{f}	6.5 ± 0.1^{a}	3.0 ± 0.0^{a}
		Red Wolaita		
Cooked	146.4 ± 0.6^{f}	165.5 ± 1.0^{g}	21.4 ± 0.4^{c}	8.9 ± 0.1^{c}
S6 Cooked	128.1 ± 0.3^{e}	143.9 ± 0.5^{c}	24.4 ± 0.4^{e}	10.1 ± 0.1^{d}
S12 Cooked	122.6 ± 0.1^{d}	133.3 ± 1.6^{a}	23.1 ± 0.2^{d}	8.7 ± 0.1^{c}
S18 Cooked	122.7 ± 2.2^{d}	135.0 ± 0.1^{b}	18.8 ± 0.8^{b}	7.1 ± 0.1^{b}
G24 Cooked	120.8 ± 0.3^{c}	153.7 ± 1.4^{e}	18.1 ± 0.3^{b}	7.2 ± 0.4^{b}
G48 Cooked	118.9 ± 0.2^{b}	155.0 ± 0.7^{f}	15.1 ± 0.1^{a}	5.9 ± 0.1^{a}
G72 Cooked	113.5 ± 1.1^{a}	153.1 ± 1.4^{d}	15.2 ± 0.2^{a}	5.8 ± 0.1^{a}

Values are mean ± SD (n=3) expressed on a dry weight basis. For each variety, means within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S6 Cooked=soaked for 6 hours and cooked, S12 Cooked=soaked for 12 hours and cooked, S18 Cooked=soaked for 18 hours and cooked, G24 Cooked=germinated for 24 hours and cooked, G48 Cooked=germinated for 48 hours and cooked, G72 Cooked=germinated for 72 hours and cooked.

Table 5-4. Effect of soaking, germination and cooking on tannin and polyphenol contents and phytate:zinc and phytate:iron molar ratios of samples of three chickpea varieties

Treatment	Tannin (mg catechin/100 g)	Polyphenols (mg GAE/100 g)	Phytate:zinc molar ratio	Phytate:iron molar ratio
		Habru		
Cooked	9.3 ± 3.0^{b}	13.7 ± 0.8^{c}	18.5 ± 0.7^{ab}	12.3 ± 0.9^{ab}
S6 Cooked	8.4 ± 4.1^{a}	11.9 ± 1.1^{ab}	20.9 ± 0.6^{b}	13.5 ± 0.7^{b}
S12 Cooked	8.1 ± 2.0^{a}	11.8 ± 0.7^{a}	17.4 ± 2.1^{ab}	11.2 ± 0.3^{ab}
S18 Cooked	8.0 ± 0.6^{a}	12.2 ± 1.3^{b}	15.9 ± 1.4^{ab}	10.6 ± 0.7^{ab}
G24 Cooked	8.0 ± 0.7^{a}	12.0 ± 0.6^{ab}	17.1 ± 1.5^{ab}	9.8 ± 1.7^{ab}
G48 Cooked	7.8 ± 0.6^{a}	12.0 ± 0.7^{b}	13.6 ± 3.8^{a}	11.7 ± 3.1^{ab}
G72 Cooked	7.7 ± 0.9^{a}	12.1 ± 1.4^{b}	14.5 ± 1.1^{a}	8.4 ± 0.4^{a}
		Mastewal		
Cooked	19.2 ± 1.6^{d}	20.5 ± 0.4^{c}	28.7 ± 1.1^{d}	11.8 ± 0.4^{d}
S6 Cooked	19.1 ± 1.0^{cd}	19.3 ± 0.7^{a}	23.8 ± 1.8^{c}	10.1 ± 0.6^{c}
S12 Cooked	19.0 ± 0.4^{c}	19.4 ± 0.9^{a}	24.4 ± 0.1^{c}	9.9 ± 0.3^{c}
S18 Cooked	19.0 ± 0.4^{c}	19.4 ± 0.4^{a}	15.8 ± 0.2^{b}	7.4 ± 0.2^{b}
G24 Cooked	18.7 ± 0.0^{b}	19.8 ± 0.4^{b}	16.4 ± 0.3^{b}	6.9 ± 0.5^{b}
G48 Cooked	18.5 ± 0.6^{b}	19.8 ± 0.9^{b}	11.2 ± 0.2^{a}	4.7 ± 0.4^{a}
G72 Cooked	18.1 ± 0.8^{a}	19.7 ± 0.9^{b}	8.2 ± 0.2^{a}	3.7 ± 0.4^{a}
		Local		
Cooked	21.0 ± 1.7^{f}	25.3 ± 0.43^{e}	13.9 ± 0.3^{d}	9.4 ± 0.1^{d}
S6 Cooked	18.2 ± 0.4^{e}	22.0 ± 0.33^{c}	12.3 ± 0.3^{c}	8.4 ± 0.2^{c}
S12 Cooked	17.5 ± 0.4^{d}	20.6 ± 0.08^{b}	9.0 ± 0.1^{b}	6.5 ± 0.2^{b}
S18 Cooked	16.9 ± 0.5^{b}	20.3 ± 0.13^{b}	8.7 ± 0.1^{b}	6.6 ± 0.0^{b}
G24 Cooked	17.3 ± 1.3^{cd}	22.2 ± 0.58^{d}	9.1 ± 0.1^{b}	6.6 ± 0.2^{b}
G48 Cooked	17.0 ± 1.7^{bc}	22.3 ± 1.41^{d}	8.9 ± 0.1^{b}	6.4 ± 0.0^{b}
G72 Cooked	16.1 ± 0.8^{a}	22.4 ± 0.84^{d}	4.4 ± 0.1^{a}	3.2 ± 0.0^{a}

Values are mean ± SD (n=3) expressed on a dry weight basis. For each variety, means within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S6 Cooked=soaked for 6 hours and cooked, S12 Cooked=soaked for 12 hours and cooked, S18 Cooked=soaked for 18 hours and cooked, G24 Cooked=germinated for 24 hours and cooked, G48 Cooked=germinated for 48 hours and cooked, G72 Cooked=germinated for 72 hours and cooked.

5.3.3 Cooking Time and Sensory Attributes of Cooked Dry Bean and Chickpea

The cooking times of dry bean and chickpea samples are presented in Table 5-5. The cooking time of unhydrated samples of dry bean varieties ranged from 38-107 min. Nasir and Hawassa Dume cooked much more quickly than did Red Wolaita, which took more than twice as long. The cooking times of unhydrated samples of chickpea varieties were significantly different and ranged from 45-106 min; the shortest cooking time was noted for Habru. Red Wolaita and Local had the longest cooking times of all samples.

For both dry bean and chickpea, cooking time was significantly lower for soaked and germinated seed, with the exception of soaking (6 h), compared to seed cooked without pre-treatment. For processed dry bean, cooking times ranged from 24-38 min for Hawassa Dume, 21-36 min for Nasir and 81-99 min for Red Wolaita. Cooking times for processed chickpea ranged from 18-41 min, 28-62 min and 56-103 min for Habru, Mastewal and Local, respectively.

The results of the initial sensory evaluation are presented in Table 5-6 and Table 5-7. The overall acceptability of dishes prepared from dry bean ranged from 5.8-6.7. The sensory attributes of most bean-based dishes were not significantly different from those of dishes prepared without pre-treatment, except for a lower appearance score for germinated (72 h)-cooked Red Wolaita. For chickpea-based dishes, the overall acceptability ranged from 5.6-7.1, *i.e.* from “neither like nor dislike” to “like moderately”. The sensory attributes of chickpea-based dishes were not significantly different from those of dishes prepared without pre-treatment, except for a lower appearance score for soaked (6 h)-cooked Mastewal and a lower taste score for germinated (72 h)-cooked Habru.

Table 5-5. Impact of soaking and germination on cooking time of samples of three red dry bean and three chickpea varieties.

Treatment	Cooking time (min)		
	Bean		
	Hawassa Dume	**Nasir**	**Red Wolaita**
Cooked	42 ± 1^{c}	38 ± 0.1^{c}	107 ± 4^{d}
S6 Cooked	38 ± 1^{c}	36 ± 0.2^{c}	99 ± 2^{cd}
S12 Cooked	31 ± 5^{b}	28 ± 1^{b}	93 ± 11^{bc}
S18 Cooked	30 ± 1^{ab}	26 ± 0.3^{b}	85 ± 1^{bc}
G24 Cooked	26 ± 2^{ab}	21 ± 1^{a}	81 ± 1^{a}
G48 Cooked	24 ± 1^{a}	21 ± 1^{a}	81 ± 1^{a}
G72 Cooked	25 ± 1^{ab}	21 ± 2^{a}	81 ± 1^{a}
	Chickpea		
	Habru	**Mastewal**	**Local**
Cooked	45 ± 3^{b}	63 ± 2^{b}	106 ± 2^{c}
S6 Cooked	41 ± 5^{b}	62 ± 2^{b}	103 ± 2^{c}
S12 Cooked	19 ± 3^{a}	30 ± 4^{a}	65 ± 5^{b}
S18 Cooked	18 ± 2^{a}	30 ± 1^{a}	61 ± 1^{ba}
G24 Cooked	19 ± 2^{a}	28 ± 2^{a}	63 ± 2^{ba}
G48 Cooked	18 ± 2^{a}	28 ± 2^{a}	61 ± 5^{ba}
G72 Cooked	18 ± 2^{a}	28 ± 2^{a}	56 ± 1^{a}

Values are mean ± SD (n=3). For each variety, means within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S6 Cooked=soaked for 6 hours and cooked, S12 Cooked=soaked for 12 hours and cooked, S18 Cooked=soaked for 18 hours and cooked, G24 Cooked=germinated for 24 hours and cooked, G48 Cooked=germinated for 48 hours and cooked, G72 Cooked=germinated for 72 hours and cooked.

Table 5-6. Effect of soaking, germination and cooking on sensory attributes of samples of three dry bean varieties (n=15).

Dry bean	Processing						
	Cooked	**S6 Cooked**	**S12 Cooked**	**S18 Cooked**	**G24 Cooked**	**G48 Cooked**	**G72 Cooked**
			Appearance score				
Hawassa Dume	6.2 ± 2.1^{ab}	5.9 ± 2.6^{ab}	6.4 ± 1.6^{b}	5.7 ± 2.0^{ab}	6.2 ± 2.1^{ab}	4.9 ± 2.2^{ab}	4.8 ± 1.9^{a}
Nasir	5.8 ± 2.1^{a}	5.6 ± 1.9^{a}	6.3 ± 1.3^{a}	5.6 ± 1.5^{a}	6.3 ± 1.3^{a}	6.0 ± 1.9^{a}	6.1 ± 1.8^{a}
Red Wolaita	6.5 ± 2.0^{b}	6.0 ± 1.7^{ab}	5.7 ± 1.8^{ab}	5.8 ± 2.0^{ab}	5.7 ± 1.8^{ab}	5.2 ± 2.2^{a}	5.7 ± 2.0^{ab}
			Texture score				
Hawassa Dume	6.7 ± 1.8^{ab}	7.2 ± 1.8^{b}	5.9 ± 2.0^{a}	5.8 ± 1.5^{a}	6.7 ± 1.8^{ab}	5.9 ± 2.0^{a}	5.7 ± 1.6^{a}
Nasir	6.1 ± 1.9^{a}	6.0 ± 1.9^{a}	6.3 ± 1.6^{a}	6.0 ± 2.0^{a}	6.3 ± 1.6^{a}	6.3 ± 2.0^{a}	6.2 ± 2.0^{a}
Red Wolaita	6.2 ± 1.8^{a}	6.3 ± 1.8^{a}	6.4 ± 2.0^{a}	6.6 ± 2.0^{a}	6.4 ± 2.0^{a}	6.2 ± 1.8^{a}	6.5 ± 1.8^{a}
			Odour score				
Hawassa Dume	6.3 ± 2.0^{a}	6.9 ± 1.6^{a}	6.2 ± 1.8^{a}	6.1 ± 2.0^{a}	6.3 ± 2.0^{a}	6.1 ± 2.1^{a}	5.8 ± 2.4^{a}
Nasir	6.6 ± 2.1^{a}	6.4 ± 2.0^{a}	6.6 ± 1.6^{a}	6.5 ± 1.6^{a}	6.6 ± 1.6^{a}	6.1 ± 1.4^{a}	6.6 ± 1.6^{a}
Red Wolaita	6.3 ± 1.8^{a}	6.0 ± 2.3^{a}	6.0 ± 2.1^{a}	5.8 ± 1.8^{a}	6.0 ± 2.1^{a}	5.7 ± 2.5^{a}	6.4 ± 1.8^{a}
			Taste score				
Hawassa Dume	6.8 ± 2.1^{ab}	7.4 ± 1.9^{b}	6.0 ± 2.3^{a}	5.9 ± 1.8^{a}	6.8 ± 2.1^{ab}	5.9 ± 1.9^{a}	5.7 ± 2.0^{a}
Nasir	5.9 ± 2.0^{a}	6.4 ± 1.8^{a}	6.5 ± 1.5^{a}	6.2 ± 1.6^{a}	6.5 ± 1.5^{a}	6.0 ± 1.8^{a}	6.2 ± 2.0^{a}
Red Wolaita	6.1 ± 1.9^{a}	6.2 ± 1.9^{a}	6.4 ± 2.1^{a}	6.2 ± 2.2^{a}	6.4 ± 2.0^{a}	6.4 ± 2.0^{a}	6.2 ± 2.1^{a}
			Overall acceptability score				
Hawassa Dume	6.6 ± 2.0^{a}	6.7 ± 1.8^{a}	6.6 ± 1.5^{a}	5.9 ± 1.8^{a}	6.6 ± 2.0^{a}	5.9 ± 2.0^{a}	5.8 ± 1.8^{a}
Nasir	6.3 ± 1.8^{a}	6.2 ± 1.6^{a}	6.3 ± 1.4^{a}	6.2 ± 1.8^{a}	6.3 ± 1.4^{a}	6.1 ± 1.8^{a}	6.0 ± 1.8^{a}
Red Wolaita	6.3 ± 1.6^{a}	6.4 ± 2.0^{a}	6.2 ± 1.8^{a}	6.3 ± 1.8^{a}	6.2 ± 1.8^{a}	6.1 ± 2.0^{a}	6.0 ± 1.8^{a}

Values are mean ± SD (n=3). Means within a row with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S6 Cooked=soaked for 6 hours and cooked, S12 Cooked=soaked for 12 hours and cooked, S18 Cooked=soaked for 18 hours and cooked, G24 Cooked=germinated for 24 hours and cooked, G48 Cooked=germinated for 48 hours and cooked, G72 Cooked=germinated for 72 hours and cooked.

Table 5-7. Effect of soaking, germination and cooking on sensory attributes of samples of three chickpea varieties (n=15).

Chickpea	Processing						
	Cooked	**S6 Cooked**	**S12 Cooked**	**S18 Cooked**	**G24 Cooked**	**G48 Cooked**	**G72 Cooked**
				Appearance score			
Habru	6.5 ± 1.4^{ab}	6.4 ± 1.4^{ab}	7.1 ± 1.4^{ab}	7.3 ± 1.53^{b}	7.1 ± 1.5^{b}	6.8 ± 1.8^{ab}	6.2 ± 1.5^{a}
Mastewal	6.2 ± 1.9^{b}	5.1 ± 1.5^{a}	6.5 ± 1.3^{b}	6.2 ± 1.8^{b}	6.1 ± 1.6^{b}	6.1 ± 1.6^{b}	6.3 ± 1.4^{b}
Local	6.3 ± 1.6^{a}	5.9 ± 1.6^{a}	6.7 ± 1.0^{a}	6.6 ± 1.9^{a}	6.5 ± 1.5^{a}	6.0 ± 1.8^{a}	6.2 ± 1.6^{a}
				Texture score			
Habru	6.5 ± 1.5^{ab}	6.4 ± 1.4^{ab}	6.9 ± 1.5^{ab}	7.1 ± 1.5^{b}	7.1 ± 1.3^{b}	6.8 ± 1.3^{ab}	5.9 ± 1.5^{a}
Mastewal	6.2 ± 1.7^{a}	5.5 ± 1.8^{a}	6.4 ± 1.3^{a}	6.3 ± 1.6^{a}	6.4 ± 1.3^{a}	6.2 ± 1.7^{a}	6.0 ± 1.4^{a}
Local	6.4 ± 1.3^{a}	6.5 ± 1.3^{a}	6.1 ± 1.1^{a}	6.3 ± 1.8^{a}	6.4 ± 1.5^{a}	5.8 ± 1.8^{a}	5.8 ± 1.3^{a}
				Odour score			
Habru	6.8 ± 1.6^{ab}	6.4 ± 1.5^{ab}	7.1 ± 1.2^{ab}	7.3 ± 1.6^{b}	7.2 ± 1.2^{b}	6.8 ± 1.6^{ab}	6.0 ± 1.6^{a}
Mastewal	6.3 ± 1.9^{ab}	5.6 ± 1.6^{a}	6.4 ± 1.4^{ab}	6.6 ± 1.4^{b}	6.4 ± 1.4^{ab}	6.5 ± 1.3^{b}	6.6 ± 1.3^{b}
Local	6.4 ± 1.9^{a}	6.5 ± 1.6^{a}	6.8 ± 1.4^{a}	6.9 ± 1.5^{a}	6.8 ± 1.6^{a}	6.3 ± 1.7^{a}	6.6 ± 1.8^{a}
				Taste score			
Habru	6.7 ± 1.6^{b}	6.4 ± 1.6^{ab}	6.2 ± 1.8^{ab}	6.8 ± 1.7^{b}	6.6 ± 1.6^{ab}	6.9 ± 1.6^{b}	5.6 ± 1.8^{a}
Mastewal	6.5 ± 1.7^{a}	5.9 ± 1.5^{a}	6.6 ± 1.3^{a}	6.6 ± 1.5^{a}	6.7 ± 1.4^{a}	6.6 ± 1.3^{a}	6.0 ± 1.7^{a}
Local	6.3 ± 1.4^{ab}	6.5 ± 1.1^{ab}	6.3 ± 1.4^{ab}	6.4 ± 1.5^{ab}	6.5 ± 1.5^{ab}	6.7 ± 1.4^{b}	5.7 ± 1.8^{a}
				Overall acceptability score			
Habru	6.8 ± 1.4^{ab}	6.3 ± 1.4^{ab}	6.9 ± 1.5^{ab}	7.1 ± 1.5^{b}	7.0 ± 1.4^{b}	6.9 ± 1.3^{ab}	6.1 ± 1.5^{a}
Mastewal	6.3 ± 1.6^{ab}	5.6 ± 1.6^{a}	6.6 ± 1.3^{ab}	6.5 ± 1.5^{ab}	6.7 ± 1.1^{b}	6.6 ± 1.3^{b}	6.1 ± 1.6^{ab}
Local	6.3 ± 1.5^{a}	6.5 ± 1.2^{a}	6.1 ± 1.6^{a}	6.6 ± 1.4^{a}	6.8 ± 1.2^{a}	6.5 ± 1.4^{a}	6.0 ± 1.7^{a}

Values are mean ± SD (n=3). Means within a row with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S6 Cooked=soaked for 6 hours and cooked, S12 Cooked=soaked for 12 hours and cooked, S18 Cooked=soaked for 18 hours and cooked, G24 Cooked=germinated for 24 hours and cooked, G48 Cooked=germinated for 48 hours and cooked, G72 Cooked=germinated for 72 hour sand cooked.

Sub-samples of bean-based and chickpea-based dishes were evaluated in a second phase of sensory evaluation. The longest soaked (18 h)-cooked and germinated (72 h)-cooked were selected for the second phase of sensory evaluation, since the results from the initial evaluation indicated that the scores for the sensory attributes of most dishes were comparable. Results for dishes prepared from dry bean that had been soaked (18 h)-cooked or germinated (72 h)-cooked and which were evaluated in a dry bean growing community, Hawassa Zuriya, are presented in Figure 5-4. The bean-based dishes prepared from soaked-cooked or germinated-cooked dry bean received either significantly higher or not significantly different sensory attribute scores compared to controls, except for Nasir germinated (72 h)-cooked. Results for sensory evaluation (carried out in Ziway Zuriya) of dishes prepared from chickpea which had been soaked (18 h)-cooked or germinated (72 h)-cooked are presented in Figure 5-5. Scores for appearance, texture, taste and overall acceptability of dishes prepared from soaked (18 h)-cooked Habru were significantly lower than those of the controls, as were scores for texture, taste and overall acceptability of soaked (18 h)-cooked Local.

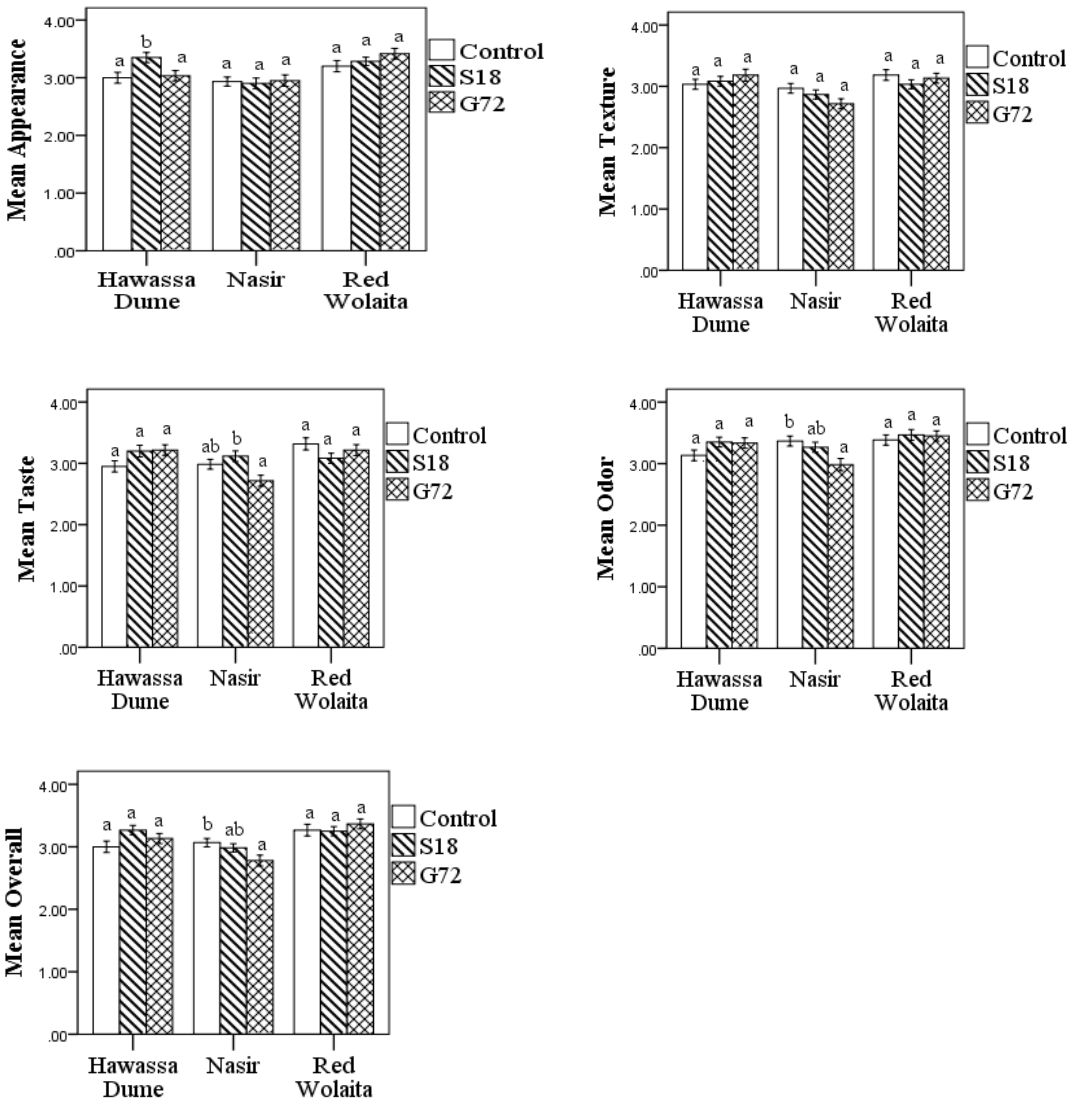

Figure 5-4. Effect of processing on consumer sensory scores of samples of three dry bean-based dishes at Hawassa Zuriya (n=60).

Values are mean ± SD. Bars without a common letter (a,b) are significantly different ($p<0.05$) based on Tukey's multiple comparison test.

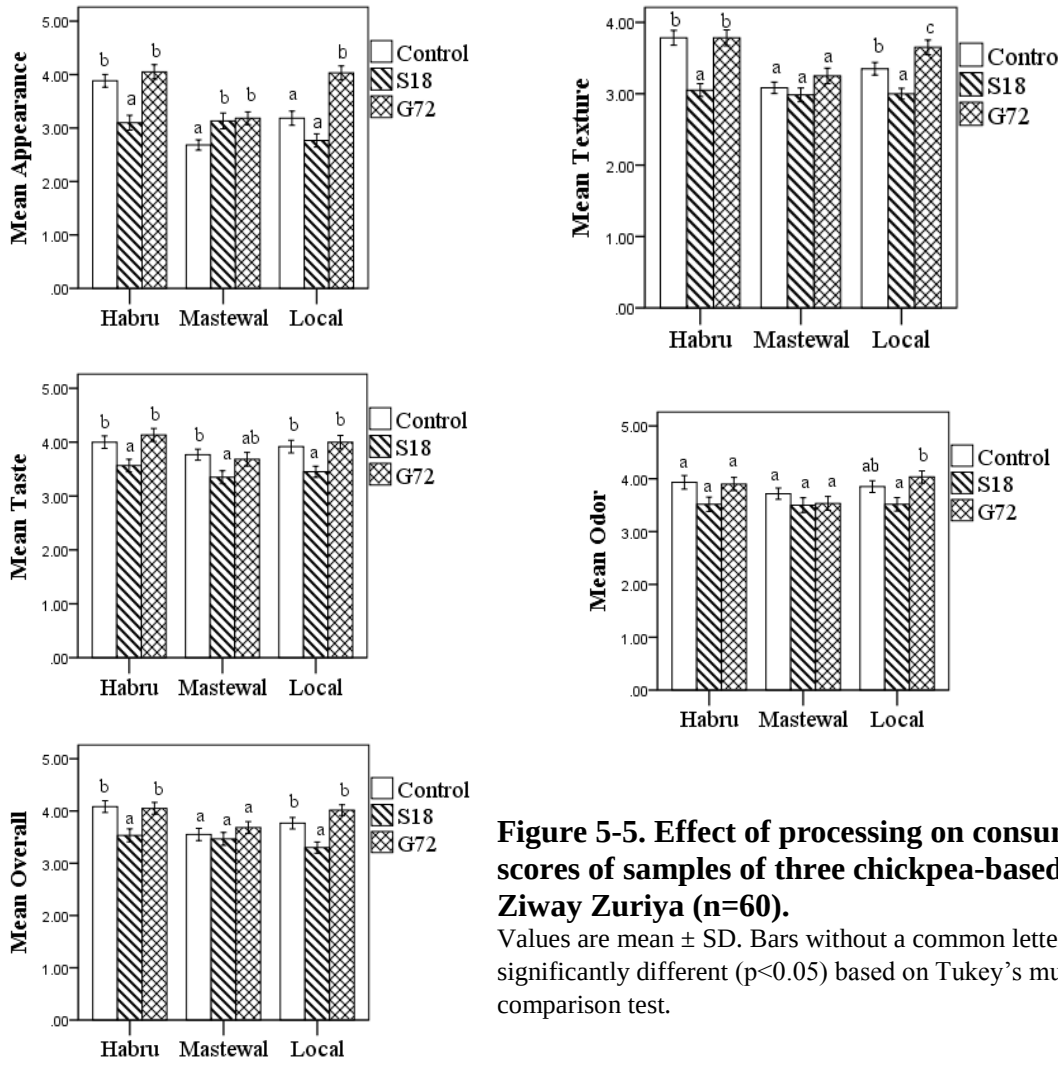

Figure 5-5. Effect of processing on consumer sensory scores of samples of three chickpea-based dishes at Ziway Zuriya (n=60).

Values are mean ± SD. Bars without a common letter (a,b,c) are significantly different ($p<0.05$) based on Tukey's multiple comparison test.

5.4 Discussion

In this study, the effect of soaking, germination, cooking and their combinations on nutrient and anti-nutrient contents, cooking time and acceptability of samples of three dry bean and three chickpea varieties grown in Ethiopia were investigated. As dry bean and chickpea go through different processing and cooking regimes prior to consumption, understanding the changes in nutrient and anti-nutrient composition which occur during common, household-level processing is important for selection of the best processing strategies.

5.4.1 Protein and Mineral Contents of Raw and Fermented Dry Bean and Chickpea

In the current study, an increase in protein content was observed during germination (72 h)-cooking of Local and Mastewal samples. An increase in protein content during germination and cooking has been reported by others (Alonso et al., 2000; El-Adawy, 2002; Khalil et al., 2007; Masood et al., 2014; Wang et al., 2010). The increase in protein content could be due to the utilization of major storage compounds such as carbohydrate, leading to changes in the relative concentrations of other components (Khalil et al., 2007). A longer processing time may lead to a higher loss of dry matter, as evidenced in a recent study which reported a 16.4% increase in protein in chickpea germinated for 171 h (Domínguez-Arispuro et al., 2018). In line with this, the increase in protein in this study was noted particularly in germinated (72 h)-cooked samples, which could be attributed to a higher dry matter loss as compared to the other processing regimes. A similar increase in protein was not observed for dry bean samples, except for Nasir germinated for 72 h. This was not surprising, as changes in protein content have been shown to be species specific (Ahmed, Abdel-Rahim, Abdel-Fatah, Erdmann, & Lippmann, 1995; Alonso et al., 2000; Kuo, Rozan, Lambein, Frias, & Vidal-Valverde, 2004).

Germination, soaking and cooking of pulses have been reported to affect mineral contents. However, the direction of the effect is not consistent in literature reports. Some studies reported that iron content increased during germination and soaking (El-Adawy, 2002). On the contrary, others reported a reduction (ElMaki *et al.*, 2007; Sangronis and Machado, 2007; Bains *et al*., 2014), and other groups have reported a non-significant effect (Berhanu et al., 2014; Trugo et al., 1999; Wang et al., 2010). A similar pattern was observed for zinc content; other studies have reported an increase (El-Adawy, 2002; Sangronis & Machado, 2007), a decrease (ElMaki et al., 2007) or a non-significant effect (Bains et al., 2014; Berhanu et al., 2014; Trugo et al., 1999; Wang et al.,

2010). Part of the explanation for the effect of germination and soaking on the mineral contents of dry bean and chickpea is the leaching into the soaking water that occurs at rates which vary among pulses and minerals (El-Adawy, 2002). The type of water used also was reported to influence mineral content. Differences in the effect on mineral content for the same food processing treatment could be due to differences in the location of minerals in the seed (Raes, Knockaert, Struijs, & Van Camp, 2014), or differences in seed coat thickness, as this would affect water absorption capacity which would in turn affect the leaching of nutrients. For example, a previous study noted that Desi-type chickpea had a 1.2 to 2 times thicker seed coat than Kabuli-type chickpea (Wood, Knights, & Choct, 2011).

5.4.2 Anti-nutrient Content and Phytate:zinc and Phytate:iron Molar Ratios of Processed Dry Bean and Chickpea Samples

Nearly all of the processing regimes employed reduced the levels of phytate significantly. Similar findings have been reported for both dry bean (Khattab & Arntfield, 2009; Nergiz & Gökgöz, 2007; Rehman & Shah, 2005; Shimelis & Rakshit, 2008) and chickpea (Alajaji & El-Adawy, 2006; Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002b; Rehman & Shah, 2005). The reasons stated for the reductions were activation of phytase, an enzyme that degrades phytate, and leaching due to the water soluble nature of phytate (Egli et al., 2002; Khalil et al., 2007). In the current study, the most pronounced phytate reductions were observed in germinated (48 and 72 h)-cooked samples, presumably due to higher phytase activity in germinated (48 and 72 h)-cooked samples than in germinated (24 h)-cooked samples. These findings are consistent with the increase in phytase activity of pulses after 48 h germination reported by Egli *et al.* (2002).

Under the experimental conditions in this study, almost all processing resulted in reductions in tannin levels, with the maximum reduction observed for germination-cooking for both dry bean and chickpea. Reductions in tannin contents of dry bean and chickpea during soaking and germination, with or without cooking, have been reported by others (Alajaji & El-Adawy, 2006; Khandelwal, Udipi, & Ghugre, 2010; Khattab & Arntfield, 2009; Rehman & Shah, 2005).

Most germinated-cooked samples had significantly lower polyphenol contents than did their counterparts which were cooked without pre-treatment, but these reductions were less than for most soaked-cooked samples of the same variety. A study that assessed the effect of processing on individual phenolic compounds in bean, lentil and pea reported a decrease in phenolic compounds during soaking and the appearance of phenolic compounds, which were not detected in raw samples, during germination (López-Amorós, Hernández, & Estrella, 2006). *De novo* synthesis and transformation were reported to cause changes in phenolic contents during germination (Gan et al., 2017). This may explain why a larger reduction of polyphenols was observed in soaked-cooked than in germinated-cooked samples. In addition, the reduction in polyphenol contents during soaking could be attributed to leaching. Another study carried out to assess the impact of germination and cooking on polyphenols in dietary fibre fractions of bean and lentil reported increased polyphenol contents due to germination (Dueñas et al., 2016). Other studies (Domínguez-Arispuro et al., 2018; Mamilla & Mishra, 2017) also have reported increased polyphenol contents upon germination. On the other hand, reductions in polyphenol content due to these processing techniques have been reported (Alonso et al., 2000; Khandelwal et al., 2010; Yasmin et al., 2008). A recent review pointed out that the inconsistency could be partly due to results being expressed on a wet weight vs. a dry weight basis (Gan et al., 2017). However, this may not solely explain the reductions reported by all studies mentioned above, as differences in

the techniques used for germination and quantification of polyphenols may have contributed as well.

Phytate:zinc and phytate:iron molar ratios have been used to predict mineral bioavailability in plant-based diets (Bel-Serrat et al., 2014; Hotz & Gibson, 2007). For example, a study that investigated the effects of soaking and germination for different times (36, 48 and 60 h) reported maximum reductions in the phytate:iron molar ratio from 11.04-6.26 and in the phytate:zinc molar ratio from 31.66-16.84 during germination (Bains et al., 2014). In this study, germination (48 and 72 h)-cooking resulted in lower phytate:zinc and phytate:iron molar ratios than did cooking without pre-treatment for most dry bean and chickpea samples.

5.4.3 Cooking Time and Sensory Attributes of Cooked Dry Bean and Chickpea

Cooking time is one of the important parameters that impact the preferences of consumers. The Kabuli variety, Habru, was superior in terms of having the shortest cooking time and large seed size. Red Wolaita and Local had the longest cooking times, and even though the community has been using these varieties, they probably would not be the preferred choice of consumers if offered alongside newer, faster cooking varieties. In a study carried out in Southern Ethiopia, approximately 80% of consumers indicated that they were influenced by grain size (Tefera, 2013). Similarly, a study carried out to determine consumer preference and its relationship to sensory and physico-chemical properties reported that large-seeded pulses were the most preferred (Mkanda *et al.*, 2007). ElMaki *et al.* (2007) argued that germination will add to women's workloads and change organoleptic properties. However, a reduction in cooking time due to soaking and germination of chickpea and dry bean has been reported (Correaa et al., 2010; Khattak et al., 2007). The results in this study are in agreement, except for 6 h of soaking. In this study, sensory

evaluation revealed that scores for the pre-treated samples remained within the acceptable range of “neither like nor dislike” to “like moderately”, with only a few exceptions. Previous studies have indicated consumer acceptance of germinated pulses used in various recipes (Berhanu et al., 2014; Sattar et al., 2017). On the other hand, Khalil *et al*. (2007) reported a deleterious effect of sprouting on the appearance of cooked seed, where a lower score was given to sprouted (96 h) samples. Such an impact was not apparent in the current study, probably because of the shorter germination time employed.

5.5 Conclusion

Overall, the nutrient composition (protein, zinc, iron and calcium) of most soaked-cooked and germinated-cooked dry bean and chickpea samples was not significantly altered as compared to samples cooked without pre-treatment. Generally, longer germination (48 and 72 h)-cooking for dry bean (Hawassa Dume, Nasir, Red Wolaita) and chickpea (Mastewal) samples and germination (72 h)-cooking for the Local chickpea sample were satisfactory processes in terms of cooking time, tannin, and phytate:zinc and phytate:iron molar ratio reduction. These food processing strategies can be used without a deleterious influence on sensory attributes. The results also reinforced that these strategies could improve cooking time, thus the longest cooking times noted for Red Wolayita dry bean and Local chickpea may not influence consumer preference if cooked after soaking or germination. These observations are relevant to designing food-based strategies for tackling micronutrient deficiencies.

Chapter 6

Iron Bioavailability of Soaked-cooked, Germinated-cooked and Fermented Dry Bean and Chickpea Measured Using the Caco-2 Cell Intestinal Absorption Model

In continuation of the determination of nutrient and anti-nutrient contents of processed dry bean and chickpea as presented in Chapters 4 and 5, *in vitro* digestion coupled with Caco-2 cell culture was used to determine iron bioavailability, and the findings are presented in this chapter. The index used for iron bioavailability was the formation of ferritin in Caco-2 cells following exposure to food digests. The food digests used in this experiment were prepared from samples of dry bean and chickpea varieties by applying the household-level food processing strategies described in Chapters 4 and 5. The purpose of this study was to meet objective 5 of the thesis: to determine the iron bioavailability of soaked-cooked, germinated-cooked and fermented samples of three dry bean and chickpea varieties using the Caco-2 cell intestinal absorption model.

Abstract

Iron deficiency, common in communities consuming diets without much diversity and dependent mainly on plant-source foods, has severe health consequences. Soaking, germination and fermentation have the potential to influence iron bioavailability because they impact absorption inhibitors. The objective of this study was to compare iron bioavailability in soaked-cooked, germinated-cooked and fermented dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Habru, Mastewal and Local) samples using Caco-2 cell culture coupled with *in vitro* digestion. Among the unprocessed dry bean and chickpea samples, there was significantly higher ferritin

formation (better iron bioavailability) in Caco-2 cells exposed to Habru digests compared to digests of the other samples of dry bean and chickpea used in the study. Overall, soaking (18 h)-cooking resulted in higher ferritin formation for the dry bean samples. On the other hand, soaked (12 h)-cooked and germinated (72 h)-cooked for Habru, soaked (12 and 18 h)-cooked and germinated (72 h)-cooked for Local, and germinated (72 h)-cooked for Mastewal chickpea resulted in higher ferritin formation compared to samples cooked without pre-treatment. Fermentation for 72 h was effective in increasing ferritin formation in all dry bean samples but not in chickpea samples, with the exception of Habru. Soaking, germination and fermentation are promising methods for improving iron bioavailability. However, since their effectiveness varies for different types and varieties of pulses, processing strategies must be carefully selected.

6.1 Introduction

Iron deficiency has severe health consequences, including reduced productivity and work performance, poor development, low birth weight, premature birth and increased risk of infections (Coad & Conlon, 2011; Deribew et al., 2016; Grantham-McGregor & Ani, 2001; Haas & Brownlie, 2001). Studies have reported iron deficiency in communities that consume a non-diversified diet dependent mainly on plant-source foods, such as cereal- and pulse-based foods (Desalegn et al., 2014; Zimmermann et al., 2005). Among plant-source foods, pulses are key contributors to alleviating micronutrient deficiency due to their nutritional quality (Costa, Queiroz-Monici, Reis, & Oliveira, 2006; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010). Largely due to their nutrient and economic potential, the production of pulses is increasing worldwide (Chandra-Hioe et al., 2016). Several specific factors can be attributed to this growth: first, the rich nutritional profile of pulses makes them suitable for populations in both developed and developing countries

(Tharanathan & Mahadevamma, 2003); second, pulses are favoured by many agricultural operations and communities because their nitrogen fixing capability helps improve soil fertility and yield; third, pulses have high dietary fibre content and low glycemic index, making them appropriate for overweight and obesity, as well as various metabolic disorders such as diabetes and hyperlipidemia (Ramdath, Renwick, & Duncan, 2016); finally, their high protein and mineral contents make them suitable for tackling undernutrition. However, to improve the impact of pulses, the bioavailability of minerals contained within them needs to be improved. As demonstrated in a longitudinal study in rural Africa, the low bioavailability of iron from a pulse- and cereal-based diet can be a cause of iron deficiency in children (Zimmermann et al., 2005). Studies have shown that phytate, calcium and tannin and other iron-binding polyphenols are major inhibitors of iron absorption (Sandberg, 2010). Other researchers have demonstrated that these iron bioavailability inhibitors can be modified by household-level food preparation techniques such as soaking, germination, fermentation and cooking (Rahman, Sana, Hasan, Huque, & Shaha, 2008; Sangronis & Machado, 2007; Shimelis & Rakshit, 2008; Wang et al., 2010).

In vivo and *in vitro* studies have been used to determine iron bioavailability in pulses and pulse-based food products. *In vivo* studies are expensive, time-consuming and associated with ethical constraints (Scheers, Almgren, Sandberg, & Sofie, 2014). However, *in vitro* methods serve as important tools for screening samples before carrying out human or animal studies. A Caco-2 cell intestinal absorption model coupled with *in vitro* digestion was developed and used successfully to determine iron bioavailability (Glahn, Lee, Yeung, Goldman, & Miller, 1998). Although the Caco-2 cell model can not measure the impact of systemic factors such as human iron status (DellaValle & Glahn, 2014; Sandberg, 2010), the high-throughput, sensitivity and validity of the model have made it useful (Glahn et al., 2017). Compared to human studies, the results obtained on dietary factors that are known to affect iron absorption also showed similar effects on

iron uptake in Caco-2 cells (Follett, Suzuki, & Lönnerdal, 2002; Glahn et al., 2002; Kalgaonkar & Lönnerdal, 2008; Yun, Habicht, Miller, & Glahn, 2004). As described in several reports, the synthesis of ferritin in Caco-2 cells following exposure to food digests expressed as ng ferritin/mg cell protein has been used as an index for iron bioavailability of pulses (Ariza-Nieto et al., 2007; Dellavalle, Vandenberg, & Glahn, 2013; Tako, Blair, & Glahn, 2011; Tako, Vandenberg, et al., 2011).

Even though several studies have assessed the impact of household-level food processing on nutrient and anti-nutrient contents of pulses, studies that have taken an approach comparing the effect of these different processes over a period of time using a Caco-2 cell bioavailability assessment technique are limited. The objective of this study was to determine the iron bioavailability of soaked-cooked, germinated-cooked and fermented dry bean (Red Wolaita, Nasir and Hawassa Dume) and chickpea (Habru, Mastewal and Local) samples using Caco-2 cell culture coupled with *in vitro* digestion.

6.2 Materials and Methods

All chemicals and reagents used were of analytical grade. Deionized water (ACS Reagent Grade, ASTM Type I, ASTM Type II) was obtained from VWR, Mississauga, ON, Canada.

Samples of two dry bean (Hawassa Dume and Nasir) and two chickpea (Habru and Mastewal) varieties were obtained from Hawassa Agricultural Research Centre, Hawassa, Ethiopia, and from Debre Zeit Agricultural Research Centre, Bishoftu, Ethiopia respectively. Samples of local varieties of dry bean (Red Wolaita) and chickpea (Local) were purchased from a farmer in Wolaita, Ethiopia, and from a market in Hawassa, Ethiopia, respectively. The dry bean and chickpea samples were subjected to several types of food processing: soaking-cooking, germination-cooking, and fermentation. In brief, portions of the dry bean and chickpea samples

were soaked for 6, 12, or 18 h in deionized water (1:3, w/v) at room temperature in closed containers. The germinated-cooked samples were prepared by soaking for 12 h, followed by germination for 24, 48 or 72 h. All soaked and germinated samples, as well as unprocessed seed, were cooked by being boiled in water (1:5, w/v) on a stove top. The dry bean and chickpea flour were mixed with deionized water at a solids to water ratio of 1: 5 (w/v) and left to naturally ferment in a flask covered with aluminum foil for 24, 48 or 72 h.

6.2.1 Sample Preparation

The food samples (0.5 g) were weighed in triplicate, and controls were prepared for analysis, along with the food samples. Blank digests were prepared in triplicate without any food added to the tubes to monitor baseline levels of cell ferritin. Additional controls were prepared with $FeCl_3$ and $FeCl_3$/AA (ascorbic acid) separately in triplicate. CDC lentil was used in all experiments as an internal quality control.

6.2.2 Caco-2 Cell Culture

Iron bioavailability was determined by Caco-2 cell culture coupled with *in vitro* digestion, as described by Glahn et al. (1998). The method is described briefly in the sections below. The Caco-2 cell experiment was conducted in the laboratory of Dr. Raymond Glahn, United States Department of Agriculture-Agriculture Research Service (USDA-ARS), Robert W. Holley Center for Agriculture and Health, Ithaca, New York, USA. Caco-2 cells obtained from the American Type Culture Collection (Rockville, MD, USA) at passage 17 were used at passage 34-36 in the current study. Cells were seeded at a density of 50,000 cells/cm^2 in collagen-treated, 6-well plates and grown in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY, USA) with 10% (v/v) fetal calf serum (GIBCO), 25 mmol/L HEPES and 1% antibiotic-antimycotic solution

(GIBCO). The cells were maintained at 37°C in an incubator with a 5% CO_2-95% air atmosphere at 90% constant humidity. The medium was changed every second day. The cells were used for the experiment 13 days post-seeding. A day before the experiment was conducted, the medium was removed from the plates containing cells, and 2 mL of warmed Minimum Essential Medium (MEM, #41500, GIBCO, Grand Island, NY, USA) was added. The plates were then returned to the incubator.

6.2.3 *In vitro* Digestion

Pepsin and pancreatin-bile solutions were freshly prepared on the day of the experiment. Pepsin solutions were prepared by weighing 1 g pepsin (Sigma #P-7000, ≥250 units/mg solid, Saint Louis, MO, USA) in 25 mL of 0.1 M HCl. Chelex-100 (142-2832, Bio-Rad Laboratories, Life Science Group, Hercules, CA, USA) was added to the solution and mixed by shaking for 30 min. The mixture was then poured into a 1.6-cm filtration column (VWR #KT420400-1520, Atlanta, GA, USA) and the filtrate was collected. Pancreatin-bile solution was prepared by weighing 0.35 g pancreatin (Sigma #P-1750, 4 X USP specification) and 2.1 g bile extract (Sigma #B-8631) and mixing in 175 mL of 0.1M $NaHCO_3$. Chelex-100 (Bio-Rad Laboratories, 87.5 g) was poured into the mixture by shaking for 30 min. The slurry was then poured into a filtration column (#KT420400-1530, VWR), and the filtrate was collected.

Immediately after the solutions were prepared, 10 mL of 140 mM NaCL, 5 mM KCL solution was added to each food sample. Pepsin solution (0.5 mL) was added to each of the samples, which were then placed on a rocker in an incubator at 37°C with a 5% CO_2-95% air atmosphere for 1 h. Before the termination of the pepsin digestion, the cells were prepared for the next steps. Medium was removed from the cells, and 1 mL of MEM was added to each well. An insert (Corning Life Sciences, Tewksbury, MA, USA) was then carefully placed into each well, avoiding

bubbles, which created upper and lower chambers. Inserts were prepared in advance by cutting 66 pieces of dialysis membrane (Spectrum Laboratories, #132123, 15,000 MWCO, Rancho Dominguez, CA, USA) of approximately 2 inches in length, and soaking and rinsing in 18.2 MΩ water. The dialysis membrane was then held in place with silicone O-rings (Web Seal, Rochester, NY, USA).

After a 1-h incubation, samples were removed from the incubator and the pH was adjusted to 5.5 -6.0 with 1.0 M $NaHCO_3$. Pancreatin-bile solution (2.5 mL) was added to the samples. The pH was readjusted to pH 6.9-7.0 by dropwise addition of 1.0 M $NaHCO_3$. The volume of the samples was then adjusted to tube weight plus 15 g using a 140 mM NaCl, 5 mM KCL, pH 6.7 solution. One and one-half millilitres of the solution was transferred to the inserts on the plates. The plates were then placed on the rocker in the incubator for 2 h, after which the inserts were carefully removed and 1 mL of MEM was added to each well. The plates were then returned to the incubator.

6.2.4 Harvesting of Caco-2 Cells

After 18 h, the media was aspirated from the cells. The cells were then rinsed twice with 2 mL of rinse solution (130 mM NaCl, 5 mM KCL, 5 mM PIPES, pH 6.7). The solution was aspirated from the cells, and 2 mL of 18.2 MΩ water was added. The plates were sonicated for 15 minutes at 4°C. Finally, the cells were harvested for protein and ferritin assays.

6.2.5 Ferritin and Protein Analysis

The Caco-2 cell ferritin content was measured using an enzyme immunoassay (SPECTRO FERRITIN, RAMCO Laboratories, Stafford, TX, USA) according to the manufacturer's

instructions. Caco-2 cell protein was measured using a Bio-Rad DC protein assay kit (Bio-Rad Laboratories) following the manufacturer's instructions. The index for iron bioavailability was calculated as ng ferritin/mg cell protein.

6.2.6 Statistical Analysis

Data were analyzed using SPSS version 20.0 for Windows. Results are expressed as mean ± standard deviation. Analysis of Variance with Tukey's test was used to determine means that were statistically different. A Pearson correlation analysis was conducted to determine the relationships between iron bioavailability and phytate, polyphenol, tannin, iron and calcium.

6.3 Results

6.3.1 Iron Bioavailability of Samples of Bean and Chickpea

Statistical comparisons of iron bioavailability were made between samples of the raw dry bean and chickpea varieties, between soaked-cooked, germinated-cooked, and cooked without pre-treatment dry bean and chickpea samples, and between fermented and unfermented dry bean and chickpea samples. Iron bioavailability following exposure of Caco-2 cells to raw dry bean and chickpea is presented in Figure 6-1. There was significantly higher ferritin formation in cells exposed to Habru compared to any other sample dry bean or chickpea sample used in the study.

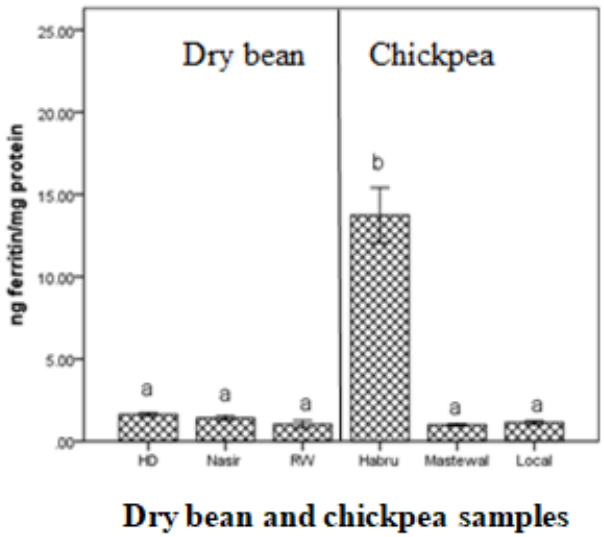

Figure 6-1. Comparison of ferritin formation in Caco-2 cells exposed to raw samples of three dry bean and three chickpea varieties.
Values are mean ± SD (n=3). Bars without a common letter (a, b) are significantly different ($p<0.05$) based on Tukey's multiple comparison test. HD=Hawassa Dume, RW=Red Wolaita.

6.3.2 Effect of Soaking, Germination and Cooking on Iron Bioavailability of Samples of Three Dry Bean and Three Chickpea Varieties as Measured by Caco-2 Cell Ferritin Formation

The effects of household-level food processing on iron bioavailability in samples of cooked, soaked-cooked, and germinated-cooked dry bean are shown in Figure 6-2. As evidenced in the figure, soaked (12 and 18 h)-cooked resulted in significantly higher ferritin formation in Hawassa Dume and Red Wolaita, whereas soaked (18 h)-cooked resulted in significantly higher ferritin formation in Nasir.

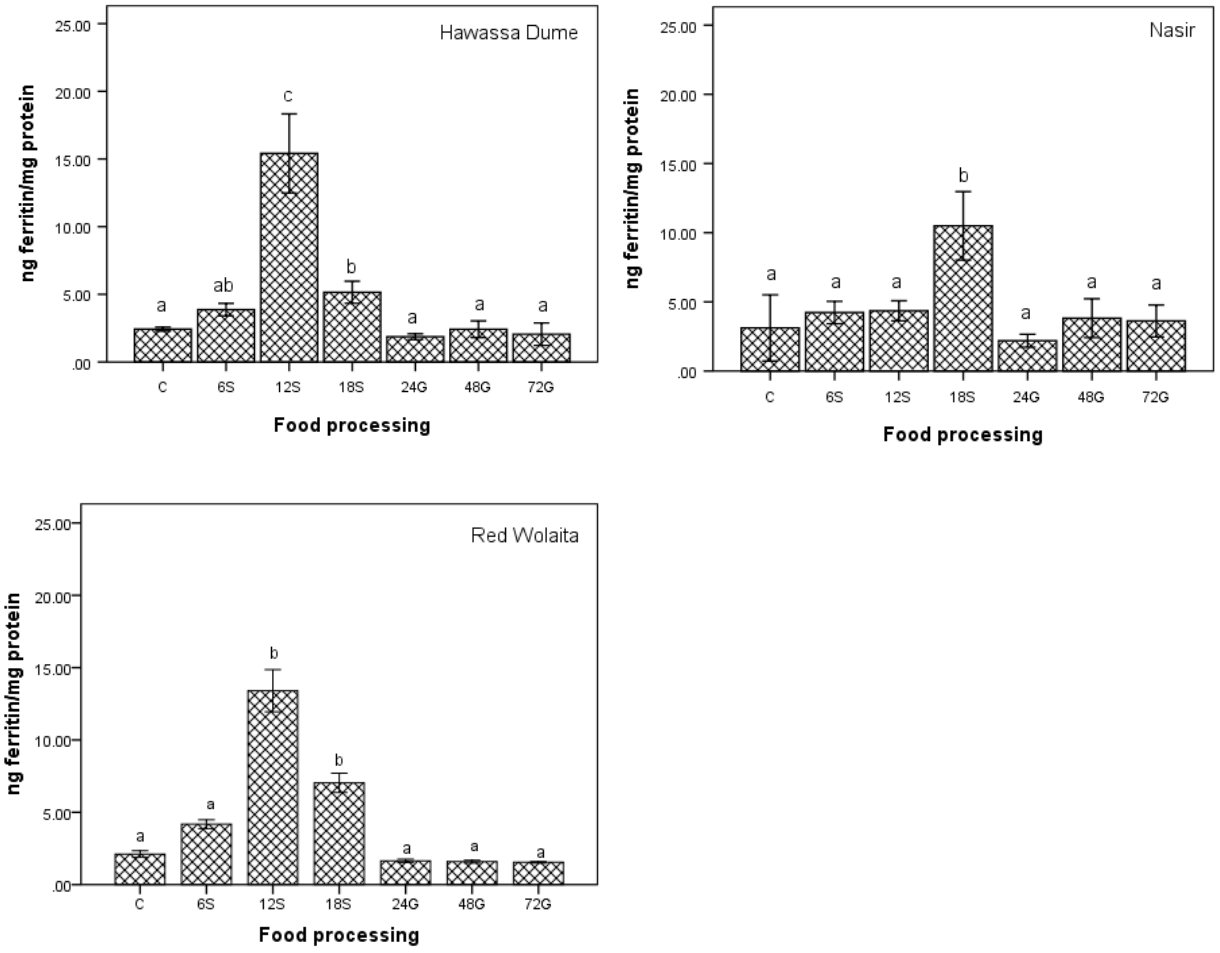

Figure 6-2. Effect of soaking, germination and cooking on iron bioavailability of samples of three bean varieties as measured by Caco-2 cell ferritin formation.
Values are mean ± SD (n=3). Bars without a common letter (a,b,c,d) are significantly different ($p<0.05$) based on Tukey's multiple comparison test. C=cooked without pre-treatment, S6=soaked for 6 h and cooked, S12=soaked for 12 h and cooked, S18=soaked for 18 h and cooked, G24=germinated for 24 h and cooked, G48=germinated for 48 h and cooked, G72=germinated for 72 h and cooked.

The effects of household-level food processing on iron bioavailability in samples of chickpea are shown in Figure 6-3. The highest iron bioavailability was observed in soaked (12 h)-cooked and germinated (72 h)-cooked for Habru, soaked (12 and 18 h)-cooked and germinated (72 h)-cooked for Local, and germinated (72 h)-cooked for Mastewal chickpea samples.

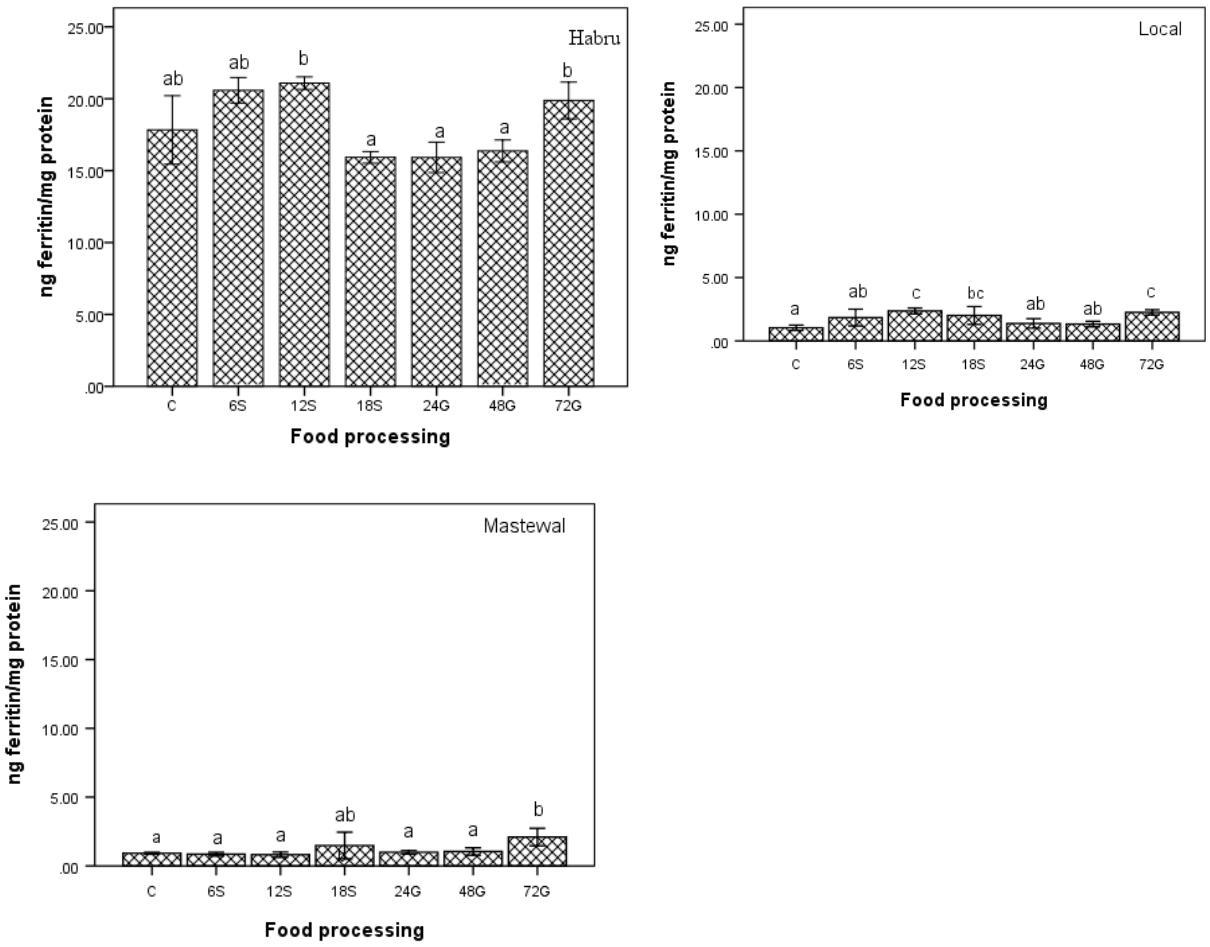

Figure 6-3. Effect of soaking, germination and cooking on iron bioavailability of samples of three chickpea varieties as measured by Caco-2 cell ferritin formation.
Values are mean ± SD (n=3). Bars without a common letter (a,b,c,d) are significantly different ($p<0.05$) based on Tukey's multiple comparison test. C=cooked without pre-treatment, S6=soaked for 6 h and cooked, S12=soaked for 12 h and cooked, S18=soaked for 18 h and cooked, G24=germinated for 24 h and cooked, G48=germinated for 48 h and cooked, G72=germinated for 72 h and cooked.

6.3.3 Effect of Fermentation on Iron Bioavailability of Samples of Three Bean and Three Chickpea Varieties as Measured by Caco-2 Cell Ferritin Formation

The effects of fermentation on iron bioavailability in samples of dry bean are shown in Figure 6-4. Fermentation for 72 h resulted in a significant increase in ferritin formation in all dry

bean samples compared to the unfermented counterparts, whereas 48 h of fermentation also resulted in significantly higher ferritin formation in Hawassa Dume.

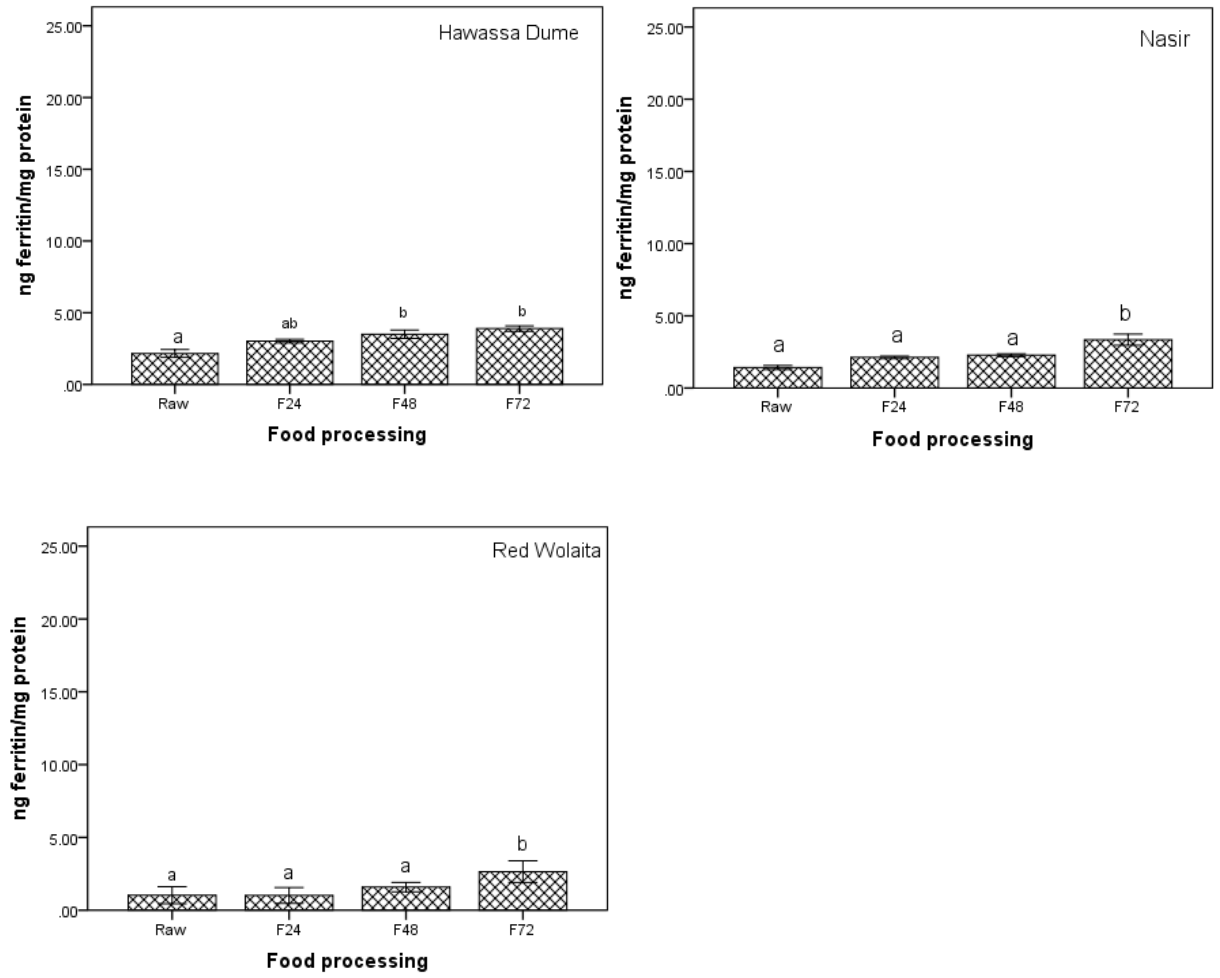

Figure 6-4. Effect of fermentation on iron bioavailability of samples of three bean varieties as measured by Caco-2 cell ferritin formation.
Values are mean ± SD (n=3). Bars without a common letter (a,b,c,d) are significantly different ($p<0.05$) based on Tukey's multiple comparison test. F24=fermented for 24 h, F48=fermented for 48 h, F72=fermented for 72 h.

The effects of fermentation on iron bioavailability in samples of chickpea are shown in Figure 6-5. Fermentation for 72 h resulted in a significant increase in ferritin formation in Habru compared to the unfermented counterparts. However, the different fermentation times applied to samples of Mastewal and Local sample did not affect ferritin formation.

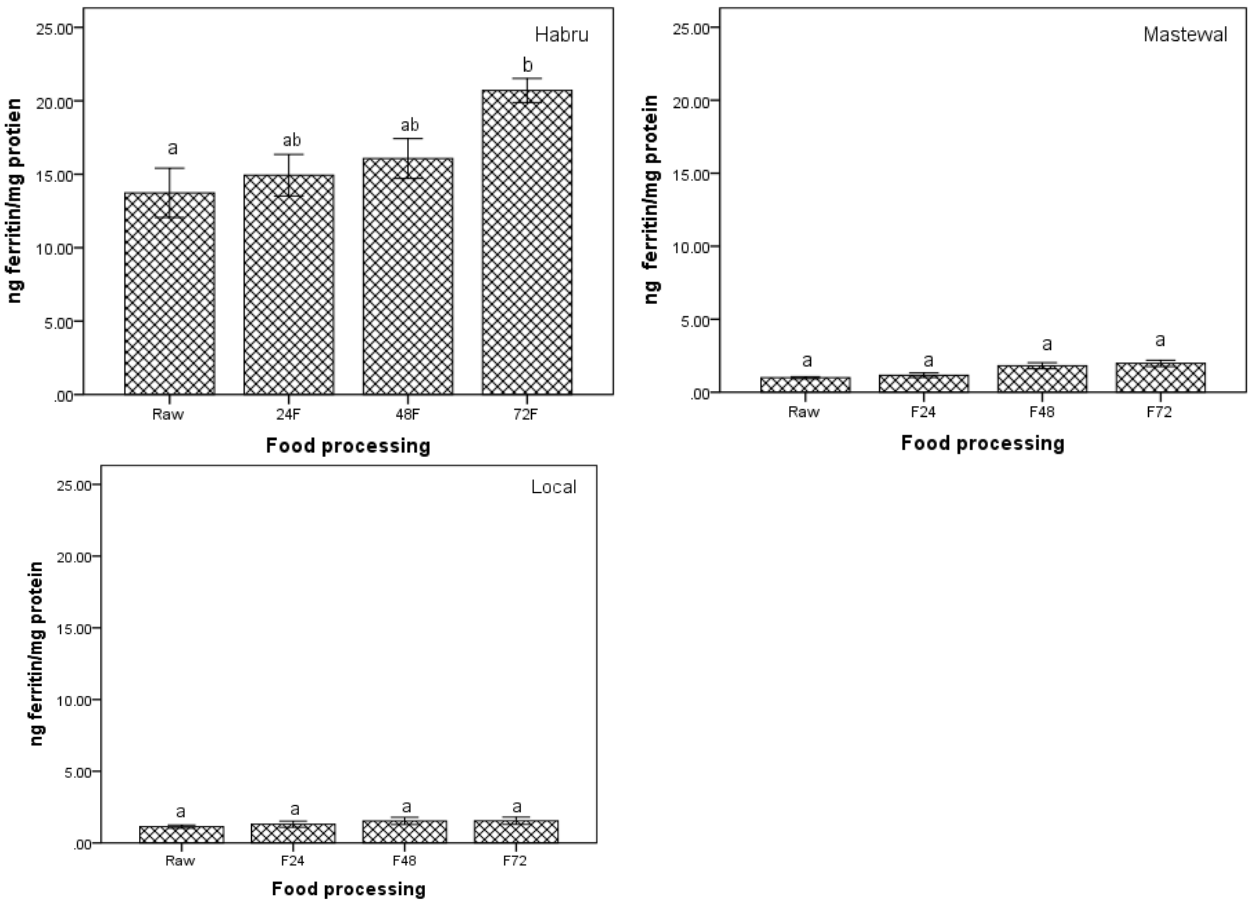

Figure 6-5. Effect of fermentation on iron bioavailability of samples of three chickpea varieties as measured by Caco-2 cell ferritin formation.
Values are mean ± SD (n=3). Bars without a common letter (a,b,c,d) are significantly different ($p<0.05$) based on Tukey's multiple comparison test. F24=fermented for 24 h, F48=fermented for 48 h, F72=fermented for 72 h.

6.3.4 Correlation between Iron Bioavailability Measured by Caco-2 Cell Ferritin Formation versus Selected Nutritional Factors in Processed Three Dry Bean and Three Chickpea Samples

Correlation coefficients for the iron bioavailability as measured by the Caco-2 cell model versus nutrient and anti-nutrient of interest (phytate, polyphenols, tannin, iron and calcium) are presented in Table 6-1. The results indicated significant negative association between iron

bioavailability and polyphenols, iron bioavailability and tannin, and iron bioavailability and calcium.

Table 6-1. Pearson correlation coefficients between iron bioavailability (ng ferritin/mg protein) versus phytate, polyphenols, tannin, iron, and calcium contents of processed three bean and three chickpea samples.

	Iron bioavailability versus				
	Phytate	**Polyphenols**	**Tannin**	**Iron**	**Calcium**
Chickpea samples					
Habru	0.116	-0.524**	-0.497**	0.136	-0.328*
Mastewal	0.115	-0.520**	-0.496**	0.131	-0.328*
Local	0.172	-0.654**	-0.439**	0.132	-0.328*
Dry bean samples					
Hawassa Dume	0.068	-0.444**	-0.282*	0.165	-0.300*
Nasir	0.057	-0.596**	-0.295*	0.135	-0.290*
Red Wolaita	0.090	-0.612**	-0.202*	0.128	-0.291*

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

6.4 Discussion

The dietary iron present in any food consumed has to pass through multiple stages before its utilization or storage in the body; these stages include release from the food matrix, uptake into enterocytes and transepithelial transport into the circulatory system (Sandberg, 2010). Caco-2 cell culture on Transwell inserts coupled with *in vitro* digestion was designed to measure those stages that occur prior to the storage and utilization of iron. The term "iron bioavailability" has been used extensively in the nutrition literature and in the Caco-2 cell model in reference to the stages described earlier (Bangar et al., 2017; Beiseigel et al., 2006; Dellavalle, Vandenberg, & Glahn, 2013; Flores, Dobbs, & Dunn, 2015; Glahn et al., 2017; Tako, Vandenberg, Thavarajah, Thavarajah, & Glahn, 2011; Vaz-Tostes, Verediano, de Mejia, & Costa, 2016). However, there is no consensus on the terminology, and some studies have used the term "iron bioaccessibility". In

this study, the most common terminology, 'iron bioavailability', was used to be consistent with the current literature. Pulses such as dry bean and chickpea are good sources of iron but also contain substantial amounts of iron bioavailability inhibitors such as phytate and polyphenols (Sandberg, 2002). Iron bioavailability inhibitors are known to exert their impact on iron available for uptake into the enterocytes. In this study, it is hypothesised that soaking-cooking, germination-cooking, and fermentation applied to dry bean and chickpea would reduce the levels of these inhibitors and thus impact iron bioavailability measured with the Caco-2 cell culture model.

Among the unprocessed dry bean and chickpea flours, cells exposed to Habru (Kabuli-type chickpea) showed higher ferritin formation, indicating better iron bioavailability than any other dry bean and chickpea flour used in the study. This higher iron bioavailability could be due to Habru's relatively low level of polyphenols. Habru is a Kabuli-type chickpea, which is known to have lower levels of polyphenols than the Desi-type chickpea (Segev et al., 2010) and the pigmented dry bean. Polyphenol contents were 15 mg GAE/100 g in Habru and 24.1- 180 mg GAE/100 g in the other bean and chickpea samples (see Chapter 4). In this study, polyphenol would be expected to impact bioavailability since whole seeds were used. Several authors also have reported the role of polyphenols in inhibiting iron absorption (Dellavalle et al., 2013; Hart, Tako, & Glahn, 2017; Sandberg, 2002; Sharma, Singh, Sharma, Kumar, & Yadav, 2016; Tako et al., 2014; Tako & Glahn, 2010).

No significant difference in ferritin formation was observed among the unprocessed dry bean varieties. All bean varieties were red in colour, and the lack of significant difference in ferritin formation among the dry bean varieties could be due to their relatively similar nutrient and anti-nutrient profiles. Similar results were reported by Ariza-Nieto et al. (2007). They assessed ferritin formation in eight pigmented, whole, cooked bean varieties and found that only one variety had

significantly different ferritin levels than the other four,, and no significant differences were noted among the remaining varieties.

Contrary to what was expected, dry bean samples subjected to soaking (12 and 18 h)-cooking but not germination-cooking caused an increase in ferritin formation. In a review of studies that assessed the impact of soaking and germination on bean and chickpea, in general, germination caused greater reduction in phytate than did soaking (Haileslassie, Henry, & Tyler, 2016). Higher iron bioavailability was thus expected to be found in germinated-cooked than in soaked-cooked samples. A recent study noted that phytate reduction resulted in a decrease in iron uptake by Caco-2 cells and suggested that the phytate reduction could have increased the potential of polyphenols to form complexes with iron (Glahn et al., 2017). This could partly explain the increase in bioavailability in soaked (18 h)-cooked rather than in germinated-cooked dry bean samples. Ariza-Nieto et al. (2007), in their study of eight bean varieties, concluded that polyphenols had higher inhibitory impact on iron bioavailability measured using the Caco-2 cell model than did phytate. The other explanation for the absence of an increase in iron bioavailability in the germinated-cooked beans in the current study could be the production of polyphenols during germination (see Chapter 5). Similar findings were reported by Gan et al. (2017) and López-Amorós et al. (2006). Petry et al. (2015) has reported that a slight increase in polyphenols has the potential to reduce iron bioavailability by 18%. An approximately 4-mg difference between white bean and red bean with similar phytate contents, but significantly lower iron content in the red bean, resulted in a higher ferritin level in Caco-cells exposed to white bean (Tako & Glahn, 2010). Similar results have been noted in germinated rice in which both solubility and iron bioavailability were measured (Wei et al., 2013). Their findings indicated that the increased iron solubility during germination was not adequate to increase iron bioavailability as measured in the Caco-2 cell model.

Soaked (12 h)-cooked and germinated (72 h)-cooked for Habru, soaked (12 and 18 h)-cooked and germinated (72 h)-cooked for Local, and germinated (72 h)-cooked for Mastewal chickpea resulted in higher ferritin formation compared to samples cooked without pre-treatment. Unlike dry bean, germination of chickpea led to higher ferritin formation. This could be related to differences in the polyphenol profiles of dry bean and chickpea and thus different inhibitory potentials. A recent study demonstrated that different polyphenol compounds have promoting and inhibitory potential (Hart et al., 2017). The analysis of the composition of polyphenols, rather than just the total polyphenol content, would be needed to confirm the amount of promoter and inhibitor polyphenols in the seed coats of the dry bean and chickpea samples used in the current study.

In the current study, a longer duration of fermentation (72 h) generally resulted in significant increases in ferritin formation in all dry bean and one chickpea (Habru) sample. This may be due to the combined effect of reduction of anti-nutrients as well as the organic acid production noted during the fermentation process (Chitra et al., 1996; Hemalatha et al., 2007; Porres et al., 2003). The enhancing effect of fermentation may be attributed mostly to acidification from natural lactic acid production rather than to phytate reduction (Proulx & Reddy, 2007). Shorter durations of fermentation were not effective in improving bioavailability, possibly due to higher pH and anti-nutrient content after 24 h and 48 h of fermentation compared to 72 h of fermentation. A lower pH and higher loss of anti-nutrients have been reported as the period of fermentation progresses (Shimelis & Rakshit, 2008). To the best of my knowledge, the effect of natural fermentation of dry bean and chickpea on iron bioavailability has not been extensively studied using the Caco-2 cell model. However, a study has been conducted to determine the effect of fermentation of carrot juice, and an increase was reported in the efficiency of iron uptake in lactic-acid-fermented juice compared to regular juice (Bergqvist, Andlid, & Sandberg, 2006).

The negative association between iron bioavailability and selected nutritional factors (polyphenols, tannin and calcium) in this study was expected due to their inhibitory effect. Inconsistent results have been reported in previous studies. For example, iron bioavailability in rice varieties studied using the Caco-2 cell model showed no correlation with iron and phytate levels (Glahn, Cheng, Welch, & Gregorio, 2002). In contrast, a positive correlation was noted between iron and iron bioavailability in dehulled, lentil-based food products (DellaValle & Glahn, 2014). A previous study of cooked lentil reported that iron content was not correlated with iron bioavailability in whole and dehulled lentil (Dellavalle et al., 2013). In a study of three pea varieties, a significant correlation between iron and iron bioavailability was noted in only one variety, whereas significant correlations between phytate and iron bioavailability and between phytate:iron molar ratio and iron bioavailability were noted in two of the varieties (Bangar et al., 2017). A negative correlation between iron bioavailability and calcium content also was reported by Frontela et al. (2009).

6.5 Conclusion

Habru had the best iron bioavailability of all unprocessed dry bean (Hawassa Dume, Nasir and Red Wolaita) and chickpea (Mastewal and Local) varieties used in the current study. Despite attempts to decrease the level of mineral absorption inhibitors using soaking-cooking, germination-cooking and fermentation, the reduction was not high enough to improve bioavailability at all processing durations, and variable impacts were noted for the different samples of dry bean and chickpea. Soaking (12 and 18 h)-cooking of Hawassa Dume and Red Wolaita and soaking (18 h)-cooking of Nasir, soaking (12 h)-cooking, and germination (72 h)-cooking of Habru, soaking (12 and 18 h)-cooking and germination (72 h)-cooking of Local, and germination (72 h)-cooking of

Mastewal were effective in improving iron bioavailability. Among the different durations of natural fermentation, 72 h was effective in increasing iron bioavailability in all dry bean samples but not chickpea samples, with the exception of Habru. Soaking-cooking, germination-cooking and fermentation are promising food processing techniques for improving iron bioavailability. However, since their effectiveness varies for different types and varieties of pulses, processing strategies must be selected carefully.

Chapter 7

General Discussion

7.1 General Discussion

As discussed in Chapters 1 and 2, despite efforts and programs conducted in different parts of the world, many people remain affected by micronutrient deficiency, predominantly women and children (Coad & Conlon, 2011). The magnitude of the problem is higher in low- and middle-income countries (LMIC) such as Ethiopia (McLean, Cogswell, Egli, Wojdyla, & de Benoist, 2009; Wessells, Singh, & Brown, 2012). In LMIC, the greatest contributors to high rates of hidden hunger or micronutrient deficiency are low dietary diversity, poor bioavailability and high rates of infection. Of particular concern in countries such as Ethiopia, which have low levels of dietary diversity, is the heavy reliance on plant-based diets, such as cereals and pulses with their high levels of anti-nutrients and, hence, poor bioavailability (Zimmermann et al., 2005). Although pulses, particularly dry bean and chickpea, are commonly produced and consumed in Ethiopia (International Food Policy Research Institute, 2010; Kassie et al., 2009), their anti-nutrient qualities have hindered their use as nutritious foods that could counter high levels of iron and zinc deficiency, particularly among young children.

Despite their high levels of anti-nutrients, pulses are nutritious foods known to be rich sources of protein and minerals. Non-governmental and governmental food agencies have long recognized them as low-cost sources of nutrition. Additionally, pulse crops are known to be environmentally sound because of their ability to improve the quality of the soil they are grown in. To recognize the importance of pulses worldwide, particularly in countries with high levels of malnutrition, the 68th United Nations General Assembly declared 2016 to be the International Year

of Pulses. The theme "Pulses: nutritious seeds for a sustainable future" was chosen to raise awareness, highlighting the contribution of pulses in addressing food security and environmental challenges.

Although there are good reasons for the UN and other organizations to be promoting pulses, research is required to enhance their profile and mineral bioavailability. The chemical composition of the raw pulse alone is not a good predictor of the chemical composition of the pulse after processing (Pujolà et al., 2007), and since dry pulses are not usually consumed raw, it is important to determine the effect of food processing techniques used at the household level. According to a review of studies, the mineral bioavailability of pulses can be improved by reducing anti-nutrient contents through food processing strategies such as soaking, germination and fermentation (Haileslassie et al., 2016). However, the percentage reductions were variable in these studies. For example, phytate reduction due to soaking or germination ranged from no significant effect to 96% in dry bean and chickpea.

The current study was designed to explore the role of household-level food processing in improving the bioavailability of iron and zinc in samples of three dry bean and three chickpea varieties grown in Ethiopia. Household-level food processing techniques, which mimicked the conditions commonly practised in Ethiopian households and which required minimal resources, were employed in the study. The water used in the experiments was limited to a minimum amount based on amounts used in previous studies (Piecyk et al., 2012; Shimelis & Rakshit, 2007; Xu & Chang, 2008). In addition, the utensils and procedures used for soaking, germination, fermentation and cooking were selected to reflect those that were or could be employed in resource-limited households in Ethiopia, since the prevalence of nutrition-related problems is high and the consumption of micronutrient-rich foods is low in such households (Central Statistical Agency [Ethiopia] and ICF, 2016b). The results presented in Chapters 4, 5 and 6 will contribute to the

design of food-based interventions by identifying food processing strategies and their impact on nutrient and anti-nutrient contents and mineral bioavailability of dry bean and chickpea grown in Ethiopia.

Chapter 4 explored the impact of fermentation for different times (24, 48 and 72 h) on three dry bean and three chickpea samples. Fermentation is one of the oldest processing techniques practised in Ethiopia. The nutrient contents of the unfermented dry bean and chickpea samples used in the current study were mostly comparable to published data for dry bean and chickpea grown in Ethiopia and elsewhere (Ariza-Nieto et al., 2007; Kinfe et al., 2015; Shimelis & Rakshit, 2005b). As reported in other studies, the current study demonstrated that natural fermentation applied to dry bean and chickpea can result in significant reductions in phytate, tannin and polyphenol contents (Chitra, Singh, & Venkateswara Rao, 1996; Granito et al., 2002; Limón et al., 2015; Porres, Aranda, López-jurado, & Urbano, 2003; Shimelis & Rakshit, 2008; Torino et al., 2013). The reductions were evaluated to determine if they led to improvements in bioavailability using phytate:iron and phytate:zinc molar ratios. The data indicated that in most of the fermented samples, the phytate:iron molar ratio improved, whereas the phytate:zinc molar ratio did not improve significantly. These findings are consistent with another study that reported an improvement in the phytate:iron molar ratio in faba bean (Luo et al., 2009). The same study compared the phytate:iron molar ratio in soaked, germinated and fermented samples and reported the lowest phytate:iron molar ratio in fermented samples. In another study that assessed zinc bioavailability, in contrast to the findings regarding phytate:zinc molar ratio in the current study, the ratio was reduced from 63 to 32 after natural fermentation of rice (Liang et al., 2008).

Chapter 5 presented the results of soaking, germination, cooking and their combinations on the contents of selected nutrients and anti-nutrients in dry bean and chickpea samples. In addition, the effects of these processes on the cooking time and acceptability of dishes prepared from dry

bean and chickpea were determined. Changes in the levels of iron, zinc and calcium also were noted in this study. The scoping literature review (Chapter 3) revealed some inconsistencies in the results reported on the effect of soaking and germination on iron and zinc contents of pulses. These results ranged from no effect, to significantly lower or higher iron and zinc contents after processing (Bains, Uppal, & Kaur, 2014; Berhanu et al., 2014; El-Adawy, 2002; ElMaki et al., 2007; Sangronis & Machado, 2007; Trugo et al., 1999b; Wang et al., 2010). It was found that soaking-cooking and germination-cooking had different efficacies in reducing the contents of phytate, tannin and polyphenols. Similar results have been reported for pulses and grains (Ibrahim, Habiba, Shatta, & Embaby, 2002; Liang et al., 2008; Luo et al., 2013). Germination with cooking of dry bean and chickpea, particularly germination (48 and 72 h)-cooking and germination (72 h)-cooking, were the most effective in reducing phytate and tannin contents, respectively. During germination, a decrease in phytate that progressed with time has been reported (Liang et al., 2008; Shimelis & Rakshit, 2007). Overall, polyphenol contents were lower after soaking-cooking than after germination-cooking.

The current study was intended not only to select household-level food processing strategies to reduce anti-nutrient contents but also to investigate the effect of the processing strategies on sensory characteristics. The results indicated that dry bean- and chickpea-based dishes can be prepared from soaked and germinated seed without significantly altering sensory characteristics (odour, appearance, taste, texture and overall acceptability) compared to dishes prepared without pre-treatment of the dry bean or chickpea. Other studies also have reported that sensory ratings of food products formulated with germinated pulses were not significantly different from those of products prepared with unprocessed pulses (Simons & Hall, 2018).

Previous studies on bioavailability of iron in Ethiopian diets (Abebe et al., 2007; Amare, Mouquet-Rivier, Rochette, Adish, & Haki, 2016; Umeta et al., 2005) have focused on assessment

of the phytate to mineral molar ratio without considering the effect of polyphenols and potential interactions between nutrients and anti-nutrients. However, it is critical to analyze bioavailability using either *in vitro* or *in vivo* studies, since nutrient composition alone does not provide the full picture. As there is no accepted biomarker for zinc in the Caco-2 cell model (Cheng, Tako, Yeung, Welch, & Glahn, 2012; Dias, Costa, Nutti, Tako, & Martino, 2017a), the bioavailability analysis in Chapter 6 focused on iron bioavailability. Thus, to evaluate the impact of the processing techniques on bioavailability, the formation of ferritin in Caco-2 cells, a proxy for iron bioavailability, was assessed. Despite the iron content of the dry bean samples being significantly higher than those of the chickpea samples, the formation of ferritin (indicator of iron bioavailability) was significantly higher in the Habru chickpea sample. Although the highest reductions in phytate and tannin for dry bean were observed after germination-cooking, the Caco-2 cell experiments showed that these reductions do not necessarily improve bioavailability. Baye et al. (2014) also found that reduction of phytate alone was unlikely to improve iron bioavailability, suggesting that interactions with other absorption inhibitors could be responsible. There was a 5-20 mg/100 g increase in polyphenol levels in germinated-cooked and soaked-cooked dry bean samples in this study, making the level of polyphenols in the germinated-cooked samples closer to the values in the cooked samples without treatment (128.7-165.5 mg GAE/100 g in dry bean and 13.7-22.4 mg GAE/100 g in chickpea, Chapter 5). This change may have contributed to the lack of significant difference in iron uptake by Caco-2 cells in germinated bean samples. In agreement with the relationship between lower levels of polyphenols and improved iron bioavailability, Petry et al. (2015) reported that a small increase in polyphenol content has the potential to reduce iron bioavailability.

Generally in the current study, fermentation for 72 h offered the highest degree of anti-nutrient content (phytate, tannin and polyphenol) reduction. The impact of fermentation was further

evaluated using the Caco-2 cell model coupled with *in vitro* digestion (Chapter 6). In agreement with the highest reduction in anti-nutrients noted at 72 h in this study (Chapter 4), the results from the Caco-2 cell model indicated that a longer duration of fermentation, *i.e*., 72 h, improved iron bioavailability of all bean samples and one chickpea sample. The findings also showed that the improvement in iron bioavailability after 72 h of fermentation could have been due to the production of organic acids as evidenced by the change in pH (from 6.2-6.3 to 4.3-4.6 in dry bean and from 6.4-6.5 to 4.4-4.7 in chickpea). Thus, the organic acids may have contributed to the formation of soluble iron (Bergqvist et al., 2006; Salovaara et al., 2002; Teucher et al., 2004).

Overall, the household-level food processing strategies (soaking-cooking, germination-cooking and fermentation) were effective in reducing anti-nutrient contents (phytate, tannin and polyphenols). However, these reductions may not always improve iron bioavailability as measured with Caco-2 cells. The investigation suggested that food processing alone was not effective in improving bioavailability in some samples. However, using household-level processing techniques to decrease anti-nutrient levels remains essential for reducing the total anti-nutrient intake of an individual. Despite the adverse effects of anti-nutrients on mineral bioavailability, studies have reported their health benefits (Vita, 2005; Vucenik & Shamsuddin, 2003, 2006). Since there is no consensus regarding the optimal amount of phytate and polyphenols for consumption, dietary intake recommendations of these substances require consideration of dietary habits and health conditions. It is, therefore, necessary to investigate additional processing methods to fully exploit the nutritional potential of pulses. For example, the household-level food processing strategies can be used in conjunction with other approaches, such as consuming foods rich in vitamin C, to bring about a meaningful change in the nutritional status of consumers. The knowledge generated in this study will contribute to the development of programs devoted to promoting and improving pulse consumption in Ethiopia.

7.2 Strengths and Limitations

7.2.1 Strengths

Unlike in most studies that have focused on a specific duration of food processing (Alajaji & El-Adawy, 2006; Bains, Uppal, & Kaur, 2014; Khandelwal et al., 2010; Rehman & Shah, 2005), a strength of the current study was its use of different durations of food processing techniques. Another strength of the study was the use of the Caco-2 cell model. To my knowledge, this was the first study to use this model to assess the effect of soaking and germination in combination with cooking on the bioavailability of iron in dry bean and chickpea grown in Ethiopia. The focus of most studies has been to assess changes in anti-nutrient and nutrient composition during food processing (López-Amorós et al., 2006; Shimelis & Rakshit, 2008; Wang et al., 2010), but using the Caco-2 cell model to analyze iron bioavailability allowed an indirect assessment of the uptake of iron in this study instead of simply assessing changes in composition. Although understanding the composition and iron bioavailability of processed products is important, the study did more. The composition and iron bioavailability will inform us about the potential of the food product in providing adequate nutrients but not whether it will be accepted by the community or not. Thus, it also determined the sensory acceptability of dry bean and chickpea that had been treated by common food processing techniques used by most Ethiopian households. While soaking-cooking and germination-cooking can be effective in reducing anti-nutrient contents, these processing strategies cannot be applied to all pulse-based food products, e.g. flour made from raw dry bean or chickpea seed and used for baking. This study provided fermentation as an alternative strategy. As Hemalatha et al. (2007) have noted, several studies have focused on the effect of accelerated fermentation, but few investigated the effect of natural fermentation. Thus, this study will make a significant contribution to the literature.

7.2.2 Limitations

The pulses used in this study were not grown in a controlled experiment such that genotype/environmental effects could be studied. The current research determined the total polyphenol contents of dry bean and chickpea. However, it could not distinguish between the different types of individual polyphenol components, with the exception of tannin. The study focused on parameters within the food. However, bioavailability also can be affected by physiological status. The other limitation of this research was the use of an *in vitro* study for iron bioavailability which could not fully simulate the full physiological and metabolic response in the human body (Dias, Costa, Nutti, Tako, & Martino, 2017b). Although Caco-2 cells are similar to human intestinal epithelial cells in morphology and function, they lack some features of human intestinal epithelial cells, such as mucus production (Scheers et al., 2014). Despite this limitation, the results from the Caco-2 cell model were comparable to those of human studies (Follett et al., 2002; Kalgaonkar & Lönnerdal, 2008; Yun et al., 2004).

7.3 Implications for Future Research

The findings of the present study clearly indicate that several factors, such as nutrient content, anti-nutrient content and cooking time, should be considered in the design of food-based strategies to alleviate nutritional deficiencies. It also would be worthwhile to investigate the levels of individual polyphenol components following soaking-cooking, germination-cooking and fermentation, since a recent study revealed that not all types of polyphenols are inhibitors of iron bioavailability (Hart et al., 2017). Thus, identification of specific polyphenol components would help reveal changes in the proportion of enhancers and inhibitors as a result of processing of dry bean and chickpea. Warm water soaking could be considered in future studies as a means to

minimize the duration of soaking. Few studies have assessed the effect of food processing and the associated reductions in anti-nutrient contents at a population level. As a result, future studies could focus on determining the impact of household-level food processing strategies on nutritional biomarkers such as serum ferritin, soluble transferrin receptor and plasma zinc levels in human food trial studies, while considering physiological status. Since single meal studies may exaggerate the effect of anti-nutrients, precautions should be taken when interpreting such data. The mineral bioavailability of food products developed by either soaking, germinating or fermenting pulses, when consumed with other meals, needs to be assessed to understand the interactions between nutrients and anti-nutrients and how these affect bioavailability.

References

AACC International. (1999). Approved Methods of Analysis, 11th Ed. Method 46-30.01. *Crude protein combustion method*. St. Paul, MN, U.S.A. https://doi.org/10.1094/AACCIntMethod-46-30.01

Abebe, Y., Bogale, A., Hambidge, K. M., Stoecker, B. J., Arbide, I., Teshome, A., … Gibson, R. S. (2008). Inadequate intakes of dietary zinc among pregnant women from subsistence households in Sidama, Southern Ethiopia. *Public Health Nutrition*, *11*(4), 379–386. https://doi.org/10.1017/S1368980007000389

Abebe, Y., Bogale, A., Hambidge, K. M., Stoecker, B. J., Bailey, K., & Gibson, R. S. (2007). Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability. *Journal of Food Composition and Analysis*, *20*(3), 161–168. https://doi.org///dx.doi.org/10.1016/j.jfca.2006.09.003

Abebe, Y., Stoecker, B. J., Hinds, M. J., Gates, G. E., Stoecker, B. J., Gates, G. E., & Hinds, M. J. (2006). Nutritive value and sensory acceptability of corn- and kocho-based foods supplemented with legumes for infant feeding in southern Ethiopia. *African Journal of Food Agriculture Nutrition and Development*, *6*(1), 1–19.

Adish, A. A., Esrey, S. A., Gyorkos, T. W., & Johns, T. (1999). Risk factors for iron deficiency anaemia in preschool children in northern Ethiopia. *Public Health Nutrition*, *2*(3), 243–252.

Agte, V., Joshi, S., Khot, S., Paknikar, K., & Chiplonkar, S. (1998). Effect of processing on phytate degradation and mineral solubility in pulses. *Journal of Food Science and Technology*, *35*(4), 330–332.

Ahmed, F. A., Abdel-Rahim, E. A., Abdel-Fatah, O. M., Erdmann, V. A., & Lippmann, C. (1995). The changes of protein patterns during one week of germination of some legume seeds and roots. *Food Chemistry*, *52*(4), 433–437. https://doi.org/10.1016/0308-8146(95)93296-4

Al-Numair, K., Ahmed, S. E. B., Al-Assaf, A., & Alamri, M. S. (2009). Hydrochloric acid extractable minerals and phytate and polyphenols contents of sprouted faba and white bean cultivars. *Food Chemistry*, *113*(4), 997–1002. https://doi.org/http://dx.doi.org/10.1016/j.foodchem.2008.08.051

Alajaji, S. A., & El-Adawy, T. A. (2006). Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*, *19*(8), 806–812. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.jfca.2006.03.015

Alajaji, S. A., & El-Adawy, T. A. (2006). Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*, *19*(8), 806–812. https://doi.org/10.1016/j.jfca.2006.03.015

Algarin, C., Peirano, P., Garrido, M., Pizarro, F., & Lozoff, B. (2003). Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. *Pediatric Research*, *53*(2), 217–223. https://doi.org/10.1203/01.pdr.0000047657.23156.55

Ali, M., Shuja, M. N., Zahoor, M., & Qadri, I. (2010). Phytic acid: How far have we come? *African Journal of Biotechnology*, *9*(11), 1551–1554. https://doi.org/10.5897/AJB10.045

Alonso, R., Aguirre, A., & Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney

beans. *Food Chemistry*, *68*(2), 159–165. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/S0308-8146(99)00169-7

Amare, E., Mouquet-Rivier, C., Rochette, I., Adish, A., & Haki, G. D. (2016). Effect of popping and fermentation on proximate composition, minerals and absorption inhibitors, and mineral bioavailability of Amaranthus caudatus grain cultivated in Ethiopia. *Journal of Food Science and Technology*, *53*(7), 2987–2994. https://doi.org/10.1007/s13197-016-2266-0

Anderson, G. J., & Vulpe, C. D. (2009). Mammalian iron transport. *Cellular and Molecular Life Sciences*, *66*(20), 3241–3261. https://doi.org/10.1007/s00018-009-0051-1

Andrews, N. C., & Schmidt, P. J. (2007). Iron homeostasis. *Annual Review of Physiology*, *69*(1), 69–85. https://doi.org/10.1146/annurev.physiol.69.031905.164337

Ariza-Nieto, M., Blair, M. W., Welch, R. M., & Glahn, R. P. (2007). Screening of iron bioavailability patterns in eight bean (*Phaseolus vulgaris* L.) genotypes using the Caco-2 cell *in vitro* model. *Journal of Agricultural and Food Chemistry*, *55*(19), 7950–7956. https://doi.org/10.1021/jf070023y

Armah, S. M., Boy, E., Chen, D., Candal, P., & Reddy, M. B. (2015). Regular consumption of a high-phytate diet reduces the inhibitory effect of phytate on nonheme-iron absorption in women with suboptimal iron stores. *The Journal of Nutrition*, *145*(8), 1735–9. https://doi.org/10.3945/jn.114.209957

Asfaw, A., & Blair, M. (2014). Quantification of drought tolerance in Ethiopian common bean varieties. *Agricultural Sciences*, *5*, 124–139. https://doi.org/10.4236/as.2014.52016.

Asif, M., Rooney, L. W., Ali, R., & Riaz, M. N. (2013). Application and opportunities of pulses in food system: a review. *Critical Reviews in Food Science and Nutrition*, *53*(11), 1168–

1179. https://doi.org/10.1080/10408398.2011.574804

Bains, K., Uppal, V., & Kaur, H. (2014). Optimization of germination time and heat treatments for enhanced availability of minerals from leguminous sprouts. *Journal of Food Science and Technology*, *51*(5), 1016–1020. https://doi.org/10.1007/s13197-011-0582-y

Bains, K., Uppal, V., & Kaur, H. (2014). Optimization of germination time and heat treatments for enhanced availability of minerals from leguminous sprouts. *Journal of Food Science and Technology*, *51*(5), 1016–1020. https://doi.org/10.1007/s13197-011-0582-y

Bangar, P., Glahn, R. P., Liu, Y., Arganosa, G. C., Whiting, S., & Warkentin, T. D. (2017). Iron bioavailability in field pea seeds: Correlations with iron, phytate, and carotenoids. *Crop Science*, *57*(2), 891–902. https://doi.org/10.2135/cropsci2016.08.0682

Beiseigel, J. M., Hunt, J. R., Glahn, R. P., Welch, R. M., Menkir, A., & Maziya-Dixon, B. B. (2007). Iron bioavailability from maze and beans: a comparison of human and Caco-2 cell measurement. *American Journal of Clinical Nutrition*, *86*, 388–396.

Bel-Serrat, S., Stammers, A.-L., Warthon-Medina, M., Moran, V. H., Iglesia-Altaba, I., Hermoso, M., … Lowe, N. M. (2014). Factors that affect zinc bioavailability and losses in adult and elderly populations. *Nutrition Reviews*, *72*(5), 334–352. https://doi.org/10.1111/nure.12105

Benito, P., & Miller, D. (1998). Iron absorption and bioavailability: An updated review. *Nutrition Research*, *18*(3), 581–603. https://doi.org/http://dx.doi.org/10.1016/S0271-5317(98)00044-X

Bergqvist, S. W., Andlid, T., & Sandberg, A. (2006). Lactic acid fermentation stimulated iron absorption by Caco-2 cells is associated with increased soluble iron content in carrot juice. *The British Journal of Nutrition*, *96*(4), 705–711. https://doi.org/10.1079/BJN20061905

Berhanu, G., Mesfin, A., Kebebu, A., Whiting, S., & Henry, C. (2014). Household food processing methods to enhance iron and zinc bioavailability in formulated haricot bean and maize complementary food. *African Journal of Food Science*, *8*(4), 190–195.

Bhandari, N., Bahl, R., Hambidge, K., & Bhan, M. (1996). Increased diarrhoeal and respiratory morbidity in association with zinc deficiency—a preliminary report. *Acta Paediatrica*, *85*, 148–150. https://doi.org/10.1111/j.1651-2227.1996.tb13981.x

Bhatnagar, S., & Taneja, S. (2001). Zinc and cognitive development. *British Journal of Nutrition*, *85*(S2), S139–S145. https://doi.org/10.1079/BJN2000306

Bhutta, Z. A., Ahmed, T., Black, R. E., Cousens, S., Dewey, K., Giugliani, E., … Shekar, M. (2008). What works? Interventions for maternal and child undernutrition and survival. *The Lancet*, *371*(9610), 417–440. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/S0140-6736(07)61693-6

Bindra, G. S., Gibson, R. S., & Thompson, L. U. (1986). [Phytate][calcium]/[zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. *Nutrition Research*, *6*(5), 475–483. https://doi.org/10.1016/S0271-5317(86)80101-4

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., de Onis, M., Ezzati, M., … Rivera, J. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet*, *371*(9608), 243–260. https://doi.org/http://dx.doi.org/10.1016/S0140-6736(07)61690-0

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., & Webb, C. (2003). Cereal-based fermented foods and beverages. *Food Research International*. https://doi.org/10.1016/S0963-9969(03)00009-7

Bohn, L., Meyer, A. S., & Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University SCIENCE B*, *9*(3), 165–191. https://doi.org/- 10.1631/jzus.B0710640

Bouis, H. E., Graham, R. D., & Welch, R. M. (2000). The Consultative Group on International Agricultural Research (CGIAR) micronutrients project: justification and objectives. *Food and Nutrition Bulletin*, *21*(4), 374–384.

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Molecular, Functional and Processing Characteristics of Whole Pulses and Pulse Fractions and Their Emerging Food and Nutraceutical Applications*, *43*(2), 414–431. https://doi.org/http://dx.doi.org/10.1016/j.foodres.2009.09.003

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, *56*(11), 317–333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x

Brown, K. H., Peerson, J. M., Baker, S. K., & Hess, S. Y. (2009). Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. *Food and Nutrition Bulletin*, *30*(1_suppl1), S12–S40. https://doi.org/10.1177/15648265090301S103

Brown, K. H., Peerson, J. M., Rivera, J., & Allen, L. H. (2002). Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition*, *75*(6), 1062–71.

Brune, M., Rossander, L., & Hallberg, L. (1989). Iron absorption: no intestinal adaptation to a high-phytate diet. *The American Journal of Clinical Nutrition*, *49*(3), 542–545.

Bueckert, R., Thavarajah, D., Thavarajah, P., & Pritchard, J. (2011). Phytic acid and mineral micronutrients in field-grown chickpea (*Cicer arietinum* L.) cultivars from western Canada. *European Food Research and Technology*, *233*(2), 203–212. https://doi.org/10.1007/s00217-011-1495-8

Cakmak, I. (2009). Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. *Journal of Trace Elements in Medicine and Biology*, *23*(4), 281–289. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.jtemb.2009.05.002

Carmona-García, R., Osorio-Díaz, P., Agama-Acevedo, E., Tovar, J., & Bello-Pérez, L. A. (2007). Composition and effect of soaking on starch digestibility of *Phaseolus vulgaris* (L.) cv. 'Mayocoba.' *International Journal of Food Science & Technology*, *42*(3), 296–302. https://doi.org/10.1111/j.1365-2621.2006.01218.x

Caulfield, L. E., & Black, R. E. (2004). Zinc deficiency. *Comparative Quantification of Health Risks: Global and Regional Burden of Diseases Attributable to Selected Major Risks*, 257–279.

Central Statistical Agency [Ethiopia] and ICF. (2016a). *Ethiopia Demographic and Health Survey 2016: Key Indicators Report*. Addis Ababa, Ethiopia, and Rockville, Maryland, USA: CSA and ICF.

Central Statistical Agency [Ethiopia] and ICF. (2016b). *Ethiopia Demographic and Health Survey 2016*. Addis Ababa, Ethiopia, and Rockville, Maryland, USA: Central Statistical Agency and ICF International.

Central Statistical Agency [Ethiopia] and ICF International. (2012). *Ethiopia Demographic and Health Survey 2011*. Addis Ababa, Ethiopia and Calverton, Maryland, USA: Central

Statistical Agency and ICF International.

Chakravarty, I., & Sinha, R. K. (2002). Prevalence of micronutrient deficiency based on results obtained from the national pilot program on control of micronutrient malnutrition. *Nutrition Reviews*, *60*(5 Pt 2), S53–S58. https://doi.org/10.1301/00296640260130740

Champ, M. M.-J. (2002). Non-nutrient bioactive substances of pulses. *British Journal of Nutrition*, *88*(S3), 307–319. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1079/BJN2002721

Chandra-Hioe, M. V., Wong, C. H. M., & Arcot, J. (2016). The Potential use of fermented chickpea and faba bean flour as food ingredients. *Plant Foods for Human Nutrition*, *71*(1), 90–95. https://doi.org/10.1007/s11130-016-0532-y

Cheng, Z., Tako, E., Yeung, A., Welch, R. M., & Glahn, R. P. (2012). Evaluation of metallothionein formation as a proxy for zinc absorption in an *in vitro* digestion/Caco-2 cell culture model. *Food & Function*, *3*(7), 732. https://doi.org/10.1039/c2fo10232c

Chimmad, B. V, Naik, R. K., & Rao, M. (2005). Nutritional quality of black bean (Phaseolus) seeds. *Journal of Food Science and Technology*, *42*(1), 53–55.

Chitra, U., Singh, U., & Venkateswara Rao, P. (1996). Phytic acid, in vitro protein digestibility, dietary fiber, and minerals of pulses as influenced by processing methods. *Plant Foods for Human Nutrition*, *49*(4), 307–316.

Coad, J., & Conlon, C. (2011). Iron deficiency in women: assessment, causes and consequences. *Current Opinion in Clinical Nutrition & Metabolic Care*, *14*(6), 625–634. https://doi.org/10.1097/MCO.0b013e32834be6fd

Coda, R., Varis, J., Verni, M., Rizzello, C. G., & Katina, K. (2017). Improvement of the protein quality of wheat bread through faba bean sourdough addition. *LWT - Food Science and Technology*, *82*, 296–302. https://doi.org/10.1016/J.LWT.2017.04.062

Codex Alimentarius Commisision. (1987). *General principles for the addition of essential nutrients to foods CAC/GL 09-1987 (amended 1989, 1991)*. Rome: Joint FAO/ WHO Food Standards, Codex Alimentarius Commision Programme,.

Correaa, M. M., de Carvalhoa, L. M. J., Nuttib, M. R., Luiz, J., de Carvalhob, V., Netob, A. R. H., & Ribeiroa, E. M. G. (2010). Water absorption, hard shell and cooking time of common beans (Phaseolus vulgaris L.). *African Journal of Food Science and Technology*, *1*(1), 13–20.

Cousins, R. J. (2010). Gastrointestinal factors influencing zinc absorption and homeostasis. *International Journal for Vitamin and Nutrition Research.*, *80*(0), 243–248. https://doi.org/10.1024/0300-9831/a000030

Curiel, J. A., Coda, R., Centomani, I., Summo, C., Gobbetti, M., & Rizzello, C. G. (2015). Exploitation of the nutritional and functional characteristics of traditional Italian legumes: The potential of sourdough fermentation. *International Journal of Food Microbiology*, *196*, 51–61. https://doi.org/10.1016/j.ijfoodmicro.2014.11.032

Davidsson, L., Galan, P., Kastenmayer, P., Cherouvrier, F., Juillerat, M. A., Hercberg, S., & Hurrell, R. F. (1994). Iron Bioavailability Studied in Infants: The Influence of Phytic Acid and Ascorbic Acid in Infant Formulas Based on Soy Isolate. *Pediatric Research*, *36*(6), 816–822. https://doi.org/10.1203/00006450-199412000-00024

Davidsson, L., & Haskell, M. (2011). Bioavailability of micronutrients: Stable isotope techniques

to develop effective food-based strategies to combat micronutrient deficiencies. *Food & Nutrition Bulletin*, *32*(1), 24S–30S.

de Almeida Costa, G. E., da Silva Queiroz-Monici, K., Pissini Machado Reis, S. M., & de Oliveira, A. C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry*, *94*(3), 327–330. https://doi.org/10.1016/J.FOODCHEM.2004.11.020

DellaValle, D. M., & Glahn, R. P. (2014). Differences in relative iron bioavailability in traditional Bangladeshi meal plans. *Food and Nutrition Bulletin*, *35*(4), 431–439. https://doi.org/10.1177/156482651403500405

Dellavalle, D. M., Vandenberg, A., & Glahn, R. P. (2013). Seed coat removal improves iron bioavailability in cooked lentils: Studies using an in vitro digestion/caco-2 cell culture model. *Journal of Agricultural and Food Chemistry*, *61*(34), 8084–8089. https://doi.org/10.1021/jf4022916

Denic, S., & Agarwal, M. M. (2007). Nutritional iron deficiency: an evolutionary perspective. *Nutrition*, *23*(7), 603–614.

Deribew, A., Tessema, G. A., Deribe, K., Melaku, Y. A., Lakew, Y., Amare, A. T., … Kassebaum, N. (2016). Trends, causes, and risk factors of mortality among children under 5 in Ethiopia, 1990--2013: findings from the Global Burden of Disease Study 2013. *Population Health Metrics*, *14*(1), 42. https://doi.org/10.1186/s12963-016-0112-2

Desalegn, A., Mossie, A., & Gedefaw, L. (2014). Nutritional iron deficiency anemia: magnitude and its predictors among school age children, southwest Ethiopia: a community based cross-sectional study. *PloS One*, *9*(12), e114059. https://doi.org/10.1371/journal.pone.0114059

Dias, D. M., Costa, N. M. B., Nutti, M. R., Tako, E., & Martino, H. S. D. (2017a). Advantages and limitations of *in vitro* and *in vivo* methods of iron and zinc bioavailability evaluation in the assessment of biofortification program effectiveness. *Critical Reviews in Food Science and Nutrition*, 1–11. https://doi.org/10.1080/10408398.2017.1306484

Dias, D. M., Costa, N. M. B., Nutti, M. R., Tako, E., & Martino, H. S. D. (2017b). Advantages and limitations of *in vitro* and *in vivo* methods of iron and zinc bioavailability evaluation in the assessment of biofortification program effectiveness. *Critical Reviews in Food Science and Nutrition*, 1–11. https://doi.org/10.1080/10408398.2017.1306484

Dione, J., Omamo, S. W., Diop, A., & Kaloko, M. S. (2013). *The cost of hunger in Ethiopia. Implications for the growth and transformation of Ethiopia. The social and economic impact of child undernutrition in Ethiopia: Summary report*. *African Union Commission, World Food Programme, United Nations Economic Commission for Africa* (Vol. 1). Addis Ababa.

Domínguez-Arispuro, D. M., Cuevas-Rodríguez, E. O., Milán-Carrillo, J., León-López, L., Gutiérrez-Dorado, R., & Reyes-Moreno, C. (2018). Optimal germination condition impacts on the antioxidant activity and phenolic acids profile in pigmented desi chickpea (*Cicer arietinum* L.) seeds. *Journal of Food Science and Technology*, *55*(2), 638–647. https://doi.org/10.1007/s13197-017-2973-1

Dueñas, M., Sarmento, T., Aguilera, Y., Benitez, V., Mollá, E., Esteban, R. M., & Martín-Cabrejas, M. A. (2016). Impact of cooking and germination on phenolic composition and dietary fibre fractions in dark beans (*Phaseolus vulgaris* L.) and lentils (*Lens culinaris* L.). *LWT - Food Science and Technology*, *66*, 72–78. https://doi.org/10.1016/J.LWT.2015.10.025

Dufner-Beattie, J., Kuo, Y. M., Gitschier, J., & Andrews, G. K. (2004). The adaptive response to dietary zinc in mice involves the differential cellular localization and zinc regulation of the zinc transporters ZIP4 and ZIP5. *Journal of Biological Chemistry*, *279*(47), 49082–49090. https://doi.org/10.1074/jbc.M409962200

Egli, I., Davidsson, L., Juillerat, M. A., Barclay, D., & Hurrell, R. F. (2002). The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *Journal of Food Science*, *67*(9), 3484–3488. https://doi.org/10.1111/j.1365-2621.2002.tb09609.x

El-Adawy, T. (2002). Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, *57*(1), 83–97. https://doi.org/10.1023/A:1013189620528

El Tinay, A. H., Mahgoub, S. O., Mohamed, B. E., & Hamad, M. A. (1989). Proximate composition and mineral and phytate contents of legumes grown in Sudan. *Journal of Food Composition and Analysis*, *2*(1), 69–78. https://doi.org/10.1016/0889-1575(89)90065-3

ElMaki, H. B., AbdelRahaman, S. M., Idris, W. H., Hassan, A. B., Babiker, E. E., & El Tinay, A. H. (2007). Content of antinutritional factors and HCl-extractability of minerals from white bean (*Phaseolus vulgaris*) cultivars: Influence of soaking and/or cooking. *Food Chemistry*, *100*(1), 362–368. https://doi.org/10.1016/j.foodchem.2005.09.060

EPHI. (2013). *Ethiopia National Food Consumption Survey*. Addis Ababa.

Ersino, G., Henry, C. J., & Zello, G. A. (2016). Suboptimal feeding practices and high levels of undernutrition among infants and young children in the rural communities of Halaba and Zeway, Ethiopia. *Food and Nutrition Bulletin*, *37*(3), 409–424.

https://doi.org/10.1177/0379572116658371

Ertas, N., & Turker, S. (2014). Bulgur processes increase nutrition value: possible role in in-vitro protein digestability, phytic acid, trypsin inhibitor activity and mineral bioavailability. *Journal of Food Science and Technology-Mysore*, *51*(7), 1401–1405. https://doi.org/10.1007/s13197-012-0638-7

Etcheverry, P., Grusak, M. A., & Fleige, L. E. (2012). Application of *in vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Frontiers in Physiology*, *3*, 1–22. https://doi.org/10.3389/fphys.2012.00317

Etcheverry, P., Wallingford, J. C., Miller, D. D., & Glahn, R. P. (2005). The Effect of calcium salts, ascorbic acid and peptic pH on calcium, zinc and iron bioavailabilities from fortified human milk using an in vitro digestion/Caco-2 cell Model. *International Journal for Vitamin and Nutrition Research*, *75*(3), 171–178. https://doi.org/10.1024/0300-9831.75.3.171

Ethiopian Public Health Institute. (2016). *Ethiopian national micro nutrient survey report*. Addis Ababa.

Ezzati, M. M., Lopez, A. D. A. D., Rodgers, A. A., Vander Hoorn, S. S., & Murray, C. J. L. C. J. (2002). Selected major risk factors and global and regional burden of disease. *Lancet*, *360*(9343), 1347–1360.

Fairweather-Tait, S., Lynch, S., Hotz, C., Hurrell, R., Abrahamse, L., Beebe, S., … Trinidad, P. T. (2005). The usefulness of in vitro models to predict the bioavailability of iron and zinc: a consensus statement from the HarvestPlus expert consultation. *International Journal for*

Vitamin and Nutrition Research, *75*(6), 371–374.

Fairweather-Tait, S., Phillips, I., Wortley, G., Harvey, L., & Glahn, R. (2007). The use of solubility, dialyzability, and Caco-2 cell methods to predict iron bioavailability. *International Journal for Vitamin and Nutrition Research*, *77*(3), 158–165.

Federal Ministry of Health. (2011). Assessment of Feasibility and Potential Benefits of Food Fortification in Ethiopia. Retrieved June 10, 2014, from http://www.fao.org/fsnforum/sites/default/files/discussions/contributions/Food_Fortification_Report_1.pdf

Flores, S. R. L., Dobbs, J., & Dunn, M. A. (2015). Mineral nutrient content and iron bioavailability in common and Hawaiian seaweeds assessed by an *in vitro* digestion/Caco-2 cell model. *Journal of Food Composition and Analysis*, *43*, 185–193. https://doi.org/10.1016/J.JFCA.2015.06.008

Follett, J. R., Suzuki, Y. A., & Lönnerdal, B. (2002). High specific activity heme-Fe and its application for studying heme-Fe metabolism in Caco-2 cell monolayers. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *283*(5), G1125–G1131. https://doi.org/10.1152/ajpgi.00443.2001

Food and Agriculture Organization. (2008). *Ethiopia nutrition country profile*. *Nutrition*.

Food and Agriculture Organization. (2012). FAOSTAT. Retrieved from http://faostat.fao.org/site/339/default.aspx

Food and Nutrition Board. (1974). Recommended dietary allowance. In *Trace Elements:Zinc* (8th ed., pp. 99–101). Washington, DC: National Research Council, National Academy of Sciences.

Fordyce, E. J., Forbes, R. M., Robbins, K. R., & Erdman, J. W. (1987). Phytate X calcium zinc molar ratios - Are they predictive of zinc bioavailability. *Journal of Food Science*, *52*(2), 440–444.

Fox, C. H., & Eberl, M. (2002). Phytic acid (IP6), novel broad spectrum anti-neoplastic agent: a systematic review. *Complementary Therapies in Medicine*, *10*(4), 229–234. https://doi.org/http://dx.doi.org/10.1016/S0965-2299(02)00092-4

Fraker, P. J., King, L. E., Laakko, T., & Vollmer, T. L. (2000). The dynamic link between the integrity of the immune system and zinc status. *The Journal of Nutrition*, *130*(5), 1399S–1406S.

Fredlund, K., Isaksson, M., Rossander-Hulthén, L., Almgren, A., & Sandberg, A. S. (2006). Absorption of zinc and retention of calcium: Dose-dependent inhibition by phytate. *Journal of Trace Elements in Medicine and Biology*. https://doi.org/10.1016/j.jtemb.2006.01.003

Frontela, C., Scarino, M.-L., Ferruzza, S., Ros, G., & Martínez, C. (2009). Effect of dephytinization on bioavailability of iron, calcium and zinc from infant cereals assessed in the Caco-2 cell model. *World Journal of Gastroenterology*, *15*(16), 1977–84. https://doi.org/10.3748/WJG.15.1977

Frossard, E., Bucher, M., Mächler, F., Mozafar, A., & Hurrell, R. (2000, May 15). Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture*. John Wiley & Sons, Ltd. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<861::AID-JSFA601>3.0.CO;2-P

Fruhbeck, G., Alonso, R., Marzo, F., & Santidrian, S. (1995). A Modified Method for the Indirect Quantitative Analysis of Phytate in Foodstuffs. *Analytical Biochemistry*, *225*(2), 206–212.

https://doi.org/10.1006/abio.1995.1145

Gan, R. Y., Lui, W. Y., Wu, K., Chan, C. L., Dai, S. H., Sui, Z. Q., & Corke, H. (2017). Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. *Trends in Food Science & Technology*, *59*, 1–14. https://doi.org/10.1016/J.TIFS.2016.11.010

Gan, R. Y., Shah, N. P., Wang, M. F., Lui, W. Y., & Corke, H. (2016). Fermentation alters antioxidant capacity and polyphenol distribution in selected edible legumes. *International Journal of Food Science & Technology*, *51*(4), 875–884. https://doi.org/10.1111/ijfs.13062

Gao, Y., Shang, C., Maroof, M. A. S., Biyashev, R. M., Grabau, E. A., Kwanyuen, P., … Buss, G. R. (2007). A modified colorimetric method for phytic acid analysis in soybean. *Crop Science*, *47*(5), 1797–1803.

Gebremedhin, S., Enquselassie, F., & Umeta, M. (2011). Prevalence of prenatal zinc deficiency and its association with socio-demographic, dietary and health care related factors in Rural Sidama, Southern Ethiopia: A cross-sectional study. *BMC Public Health*, *11*(1), 898.

Ghavidel, R. A., & Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *LWT - Food Science and Technology*, *40*(7), 1292–1299. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.lwt.2006.08.002

Gibson, R. S. (2006). Zinc: the missing link in combating micronutrient malnutrition in developing countries. *Proceedings of the Nutrition Society*, *65*(01), 51–60.

Gibson, R. S. (2007). The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates. *Food And Nutrition Bulletin*, *28*(1), S77–

S100.

Gibson, R. S. (2011). Strategies for preventing multi-micronutrient deficiencies: A review of experiences with food-based approaches in developing countries, in combating micronutrient deficiencies. In F. and CABI (Ed.), *Food-based Approaches* (pp. 7–27). B. Thompson, Amoroso, L.,.

Gibson, R. S., Abebe, Y., Stabler, S., Allen, R. H., Westcott, J. E., Stoecker, B. J., … Hambidge, K. M. (2008). Zinc, gravida, infection, and iron, but not vitamin B-12 or folate status, predict hemoglobin during pregnancy in Southern Ethiopia. *The Journal of Nutrition*, *138*(3), 581–586.

Gibson, R. S., Bailey, K. B., Gibbs, M., & Ferguson, E. L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin*. https://doi.org/doi:10.1007/s00394-006-0637-4 Suppl. S

Gibson, R. S., Perlas, L., & Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *The Proceedings of the Nutrition Society*, *65*(2), 160–168. https://doi.org/http://dx.doi.org/10.1079/PNS2006489

Gilani, G. S., Wu Xiao, C., & Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition*, *108*(Supplement S2), S315–S332. https://doi.org/10.1017/S0007114512002371

Gillooly, M., Bothwell, T. H., Torrance, J. D., MacPhail, A. P., Derman, D. P., Bezwoda, W. R., … Mayet, F. (1983). The effects of organic acids, phytates and polyphenols on the

absorption of iron from vegetables. *British Journal of Nutrition*, *49*(03), 331. https://doi.org/10.1079/BJN19830042

Glahn, R. P., Cheng, Z., Welch, R. M., & Gregorio, G. B. (2002). Comparison of iron bioavailability from 15 rice genotypes: Studies using an *in vitro* digestion/Caco-2 cell culture model. *Journal of Agricultural and Food Chemistry*, *50*(12), 3586–3591. https://doi.org/10.1021/jf0116496

Glahn, R. P., Lee, O. A., Yeung, A., Goldman, M. I., & Miller, D. D. (1998). Caco-2 Cell ferritin formation predicts nonradiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. *The Journal of Nutrition*, *128*(9), 1555–1561.

Glahn, R. P., Wortley, G. M., South, P. K., & Miller, D. D. (2002). Inhibition of iron uptake by phytic Acid, tannic acid, and ZnCl2: Studies using an *in vitro* digestion/Caco-2 cell model. *Journal of Agricultural and Food Chemistry*, *50*(2), 390–395. https://doi.org/10.1021/jf011046u

Glahn, R., Tako, E., Hart, J., Haas, J., Lung'aho, M., & Beebe, S. (2017). Iron bioavailability studies of the first generation of iron-biofortified beans released in Rwanda. *Nutrients*, *9*(7). https://doi.org/10.3390/nu9070787

Graham, P. H., & Vance, C. P. (2003). Legumes: Importance and constraints to greater use. *Plant Physiology*, *131*(3), 872–877. https://doi.org/10.1104/pp.017004

Granito, M., Frias, J., Doblado, R., Guerra, M., Champ, M., & Vidal-Valverde, C. (2002). Nutritional improvement of beans (*Phaseolus vulgaris*) by natural fermentation. *European Food Research and Technology*, *214*(3), 226–231. https://doi.org/10.1007/s00217-001-0450-5

Grantham-McGregor, S., & Ani, C. (2001). A review of studies on the effect of iron deficiency on cognitive development in children. *The Journal of Nutrition*, *131*(2), 649S–668S.

Greiner, R., & Konietzny, U. (2006). Phytase for food application. *Food Technology and Biotechnology*, *44*(2), 123–140.

Grinder-Pedersen, L., Bukhave, K., Jensen, M., Højgaard, L., & Hansen, M. (2004). Calcium from milk or calcium-fortified foods does not inhibit nonheme-iron absorption from a whole diet consumed over a 4-d period. *The American Journal of Clinical Nutrition*, *80*(2), 404–409. https://doi.org/10.1093/ajcn/80.2.404

Gropper, S., Smith, J., & Groff, J. (2005). *Advanced nutrition and human metabolism* (Fourth). Canada: Thomson Wadsworth.

Gupta, R., Gangoliya, S., & Singh, N. (2013). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, *52*(2), 676–684. https://doi.org/10.1007/s13197-013-0978-y

Haas, J. D., Beard, J. L., Murray-Kolb, L. E., del Mundo, A. M., Felix, A., & Gregorio, G. B. (2005). Iron-biofortified rice improves the iron stores of nonanemic Filipino women. *The Journal of Nutrition*, *135*(12), 2823–2830.

Haas, J. D., & Brownlie, T. (2001). Iron deficiency and reduced work capacity: A critical review of the research to determine a causal relationship. *The Journal of Nutrition*, *131*(2), 676S–690S.

Haas, J. D., Luna, S. V., Lung'aho, M. G., Wenger, M. J., Murray-Kolb, L. E., Beebe, S., … Egli, I. M. (2016). Consuming iron biofortified beans increases iron status in Rwandan women after 128 days in a randomized controlled feeding trial. *Journal of Nutrition*, *146*(8), 1586–

1592. https://doi.org/10.3945/jn.115.224741

Habib, F., Habib, Z. A., Alenazy, M., & Nooh, R. (2009). Compliance to iron supplementation during pregnancy. *Journal of Obstetrics and Gynaecology*, *29*(6), 487–492. https://doi.org/10.1080/01443610902984961

Haidar, J. A. (2010). Prevalence of anaemia, deficiencies of iron and folic acid and their determinants in Ethiopian women. *Journal of Health, Population, and Nutrition*, *28*(4), 359–368. https://doi.org/10.3329/jhpn.v28i4.6042

Haidar, J. A., Omwega, A. M., Muroki, N. M., & Ayana, G. (2003). Daily versus weekly iron supplementation and prevention of iron deficiency anaemia in lactating women. *East African Medical Journal*, *80*(1), 11–16.

Haidar, J. A., & Pobocik, R. S. (2009). Iron deficiency anemia is not a rare problem among women of reproductive ages in Ethiopia: a community based cross sectional study. *BMC Blood Disorders*, *9*, 7–14. https://doi.org/10.1186/1471-2326-9-7

Haidar, J., Nekatibeb, H., & Urga, K. (1999). Iron deficiency anemia in pregnant and lactating mothers in rural Ethiopia. *East African Medical Journal*, *76*(11), 618–622.

Haileslassie, H. A., Henry, C. J., & Tyler, R. T. (2016). Impact of household food processing strategies on antinutrient (phytate, tannin and polyphenol) contents of chickpeas (*Cicer arietinum* L.) and beans (*Phaseolus vulgaris* L.): a review. *International Journal of Food Science & Technology*, *51*(9), 1947–1957. https://doi.org/10.1111/ijfs.13166

Hall, A., Kassa, T., Demissie, T., Degefie, T., & Lee, S. (2008). National survey of the health and nutrition of schoolchildren in Ethiopia. *Tropical Medicine & International Health*, *13*(12), 1518–1526. https://doi.org/10.1111/j.1365-3156.2008.02168.x

Hallberg, L., Brune, M., Erlandsson, M., Sandberg, A. S., & Rossander-Hulten, L. (1991). Calcium: Effect of different amounts of nonheme- and heme-iron absorption in humans. *American Journal of Clinical Nutrition*, *53*(1), 112–119. https://doi.org/10.1016/S0022-3182(12)81252-0

Hallberg, L., & Hulthén, L. (2000). Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *The American Journal of Clinical Nutrition*, *71*(5), 1147–1160. https://doi.org/10.1093/ajcn/71.5.1147

Hambidge, M. (2000). Zinc and health: Current status and future directions. *Journal of Nutrition*, *130*, 1344–1349.

Han, O., Failla, M. L., Hill, A. D., Morris, E. R., & Smith, J. C. (1995). Reduction of Fe(III) is required for uptake of nonheme iron by Caco-2 cells. *The Journal of Nutrition*, *125*(5), 1291–1299.

Harland, B. F., & Oberleas, D. (1977). A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. *Cereal Chemistry*, *54*(4), 827–832.

Hart, J. J., Tako, E., & Glahn, R. P. (2017). Characterization of polyphenol effects on inhibition and promotion of iron uptake by Caco-2 cells. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/acs.jafc.6b05755

Hart, J. J., Tako, E., Kochian, L. V., & Glahn, R. P. (2015). Identification of black bean (*Phaseolus vulgaris* L.) polyphenols that inhibit and promote iron uptake by Caco-2 cells. *Journal of Agricultural and Food Chemistry*, *63*(25), 5950–5956. https://doi.org/10.1021/acs.jafc.5b00531

Hemalatha, S., Platel, K., & Srinivasan, K. (2007). Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains. *European Journal of Clinical Nutrition*, *61*(3), 342–348. https://doi.org/10.1038/sj.ejcn.1602524

Hernandez-Nistal, J., Martin, I., Labrador, E., & Dopico, B. (2010). The immunolocation of XTH1 in embryonic axes during chickpea germination and seedling growth confirms its function in cell elongation and vascular differentiation. *Journal of Experimental Botany*, *61*(15), 4231–4238. https://doi.org/10.1093/jxb/erq223

Herrador, Z., Perez-Formigo, J., Sordo, L., Gadisa, E., Moreno, J., Benito, A., … Custodio, E. (2015). Low dietary diversity and intake of animal source foods among school aged children in Libo Kemkem and Fogera districts, Ethiopia. *PLoS ONE*, *10*(7), e0133435. https://doi.org/10.1371/journal.pone.0133435

Hotz, C. (2005). Evidence for the usefulness of in vitro dialyzability, Caco-2 cell models, animal models, and algorithms to predict zinc bioavailability in humans. *International Journal for Vitamin and Nutrition Research*, *75*(6), 423–435. https://doi.org/10.1024/0300-9831.75.6.423

Hotz, C., & Brown, K. H. (2004). International zinc nutrition consultative group (IZiNCG) technical document: Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin*, *25*, 94–203.

Hotz, C., & Gibson, R. S. (2001). Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white Maize (*Zea mays*). *Journal of Agricultural and Food Chemistry*, *49*(2), 692–698. https://doi.org/10.1021/jf000462w

Hotz, C., & Gibson, R. S. (2007). Traditional food-processing and preparation practices to

enhance the bioavailability of micronutrients in plant-based diets. *The Journal of Nutrition*, *137*(4), 1097–1100.

Hotz, C., & McClafferty, B. (2007). From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food and Nutrition Bulletin*, *28*(2), S271–S279.

House, W. A. (1999). Trace element bioavailability as exemplified by iron and zinc. *Field Crops Research*, *60*(1), 115–141. https://doi.org/http://dx.doi.org/10.1016/S0378-4290(98)00136-1

Huma, N., Anjum, M., Sehar, S., Khan, M. I., & Hussain, S. (2008). Effect of soaking and cooking on nutritional quality and safety of legumes. *Nutrition & Food Science*, *38*(6), 570–577. https://doi.org/10.1108/00346650810920187

Hunt, J. R. (2010). Algorithms for iron and zinc bioavailability: are they accurate? *International Journal for Vitamin and Nutrition Research*, *80*(45), 257–262. https://doi.org/10.1024/0300-9831/a000032

Hunt, J. R., & Beiseigel, J. M. (2009). Dietary calcium does not exacerbate phytate inhibition of zinc absorption by women from conventional diets. *The American Journal of Clinical Nutrition*, *89*(3), 839–843. https://doi.org/10.3945/ajcn.2008.27175

Hunt, J. R., Beiseigel, J. M., & Johnson, L. K. (2008). Adaptation in human zinc absorption as influenced by dietary zinc and bioavailability. *The American Journal of Clinical Nutrition*, *87*(5), 1336–1345. https://doi.org/10.1093/ajcn/87.5.1336

Hunt, J. R., & Roughead, Z. K. (2000). Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. *American Journal of Clinical Nutrition*. https://doi.org/10.1093/ajcn/71.1.94

Hurrell, R. F. (2002). Bioavailability - a time for reflection. *International Journal of Vitamin and Nutrition Research*, *72*(1), 5–6.

Hurrell, R. F. (2003). Influence of vegetable protein sources on trace element and mineral bioavailability. *The Journal of Nutrition*, *133*(9), 2973S–2977S.

Hurrell, R. F. (2004). Phytic acid degradation as a means of improving iron absorption. *International Journal of Vitamin and Nutrition Research*, *74*(6), 445–452.

Hurrell, R. F., & Egli, I. (2010). Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition*, *91*(5), 1461S–1467S. https://doi.org/10.3945/ajcn.2010.28674F

Ibrahim, S. S., Habiba, R. A., Shatta, A. A., & Embaby, H. E. (2002). Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. *Food/Nahrung*, *46*(2), 92–95.

Imdad, A., & Bhutta, Z. A. (2011). Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries: a meta-analysis of studies for input to the lives saved tool. *BMC Public Health*, *11 Suppl 3*(Suppl 3), S22. https://doi.org/10.1186/1471-2458-11-S3-S22

International Food Policy Research Institute. (2010). Pulses value chain potential in Ethiopia: Constraints and opportunities for enhancing exports. Retrieved from http://www.ata.gov.et/wp-content/uploads/Ethiopia-Pulses-Value-Chain-Diagnostic-July-2010.pdf

Iqbal, T. H., Lewis, K. O., & Cooper, B. T. (1994). Phytase activity in the human and rat small intestine. *Gut*, *35*(9), 1233–1236. https://doi.org/10.1136/gut.35.9.1233

Joshi, P. K., & Rao, P. P. (2016). *Global and regional pulse economies: Current trends and outlook* (No. 1544). Washington, D.C.

Joshi, P. K., Rao, P. P., Gowda, C. L. L., Jones, R. B., Silim, S. N., Saxena, K. B., & Kumar, J. (2001). *The world chickpea and pigeonpea economies: Facts, trends, and outlook*. Andhra Pradesh, India.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. *British Journal of Nutrition*, *108*, S11–S26.

Juliano, B. O. (1999). Comparative nutritive value of various staple foods. *Food Reviews International*, *15*(4), 399–434. https://doi.org/10.1080/87559129909541197

Kabata, A., Henry, C., Moges, D., Kebebu, A., Whiting, S., Regassa, N., & Tyler, R. (2016). Determinants and constraints of pulse production and consumption among farming households of Ethiopia. *Journal of Food Research*, *6*(1), 41. https://doi.org/10.5539/jfr.v6n1p41

Kalgaonkar, S., & Lönnerdal, B. (2008). Effects of dietary factors on iron uptake from ferritin by Caco-2 cells. *The Journal of Nutritional Biochemistry*, *19*(1), 33–39. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.jnutbio.2007.02.001

Kassie, M., Shiferaw, B., Asfaw, S., Abate, T., Muricho, G., Ferede, S., … Assefa, K. (2009). Current situation and future outlook of the chickpea sub-sector in Ethiopia. Retrieved from http://www.icrisat.org/TropicalLegumesII/pdfs/Current_Situation.pdf

Kebebu, A., Whiting, S. J., Dahl, W. J., Henry, C. J., & Kebede, A. (2013). Formulation of a complementary food fortified with broad beans (*Vicia Faba*) in Southern Ethiopia. *African*

Journal of Food, Agriculture, Nutrition & Development, *13*(3), 7789–7803.

Khalil, A. W., Zeb, A., Mahmood, F., Tariq, S., Khattak, A. B., & Shah, H. (2007). Comparison of sprout quality characteristics of desi and kabuli type chickpea cultivars (*Cicer arietinum* L.). *LWT - Food Science and Technology*, *40*(6), 937–945. https://doi.org/10.1016/j.lwt.2006.05.009

Khandelwal, S., Udipi, S. A., & Ghugre, P. (2010). Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. *Food Research International*, *43*(2), 526–530. https://doi.org/10.1016/j.foodres.2009.09.036

Khattab, R. Y., & Arntfield, S. D. (2009). Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. *LWT - Food Science and Technology*, *42*(6), 1113–1118. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.lwt.2009.02.004

Khattak, A. B., Zeb, A., Khan, M., Bibi, N., Ihsanullah, & Khattak, M. S. (2007). Influence of germination techniques on sprout yield, biosynthesis of ascorbic acid and cooking ability, in chickpea (Cicer arietinum L.). *Food Chemistry*, *103*(1), 115–120. https://doi.org/10.1016/j.foodchem.2006.08.003

Kinfe, E., Singh, P., & Fekadu, T. (2015). Physicochemical and functional characteristics of desi and kabuli chickpea (Cicer arietinum L.) cultivars grown in Bodity, Ethiopia and sensory evaluation of boiled and roasted products prepared using chickpea varieties. *International Journal of Current Research in Biosciences and Plant Biology*, *2*(4), 21–29.

King, J. C. (2010). Does zinc absorption reflect zinc status? *International Journal for Vitamin and Nutrition Research*, *80*(4–5), 300–306.

Koorts, A. M., & Viljoen, M. (2007). Ferritin and ferritin isoforms I: Structure–function

relationships, synthesis, degradation and secretion. *Archives of Physiology and Biochemistry*, *113*(1), 30–54. https://doi.org/10.1080/13813450701318583

Kostinek, M., Specht, I., Edward, V. A., Schillinger, U., Hertel, C., Holzapfel, W. H., & Franz, C. M. A. P. (2005). Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. *Systematic and Applied Microbiology*, *28*(6), 527–540. https://doi.org/10.1016/j.syapm.2005.03.001

Krebs, N. F. (2000). Overview of zinc absorption and excretion in the human gastrointestinal tract. *The Journal of Nutrition*, *130*(5), 1374S–1377S.

Kumar, S., & Bourai, V. A. (2012). Economic analysis of pulses production their benefits and constraints (A case study of sample villages of Assan valley of Uttarakhand, India). *IOSR Journal of Humanities and Social Science*, *1*(4), 41–53.

Kumar, V., Sinha, A. K., Makkar, H. P. S., & Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, *120*(4), 945–959. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.foodchem.2009.11.052

Kuo, Y.-H., Rozan, P., Lambein, F., Frias, J., & Vidal-Valverde, C. (2004). Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. *Food Chemistry*, *86*(4), 537–545. https://doi.org/10.1016/j.foodchem.2003.09.042

Latta, M., & Eskin, M. (1980). A simple and rapid colorimetric method for phytate determination. *Journal of Agricultural and Food Chemistry*, *28*(6), 1313–1315. https://doi.org/10.1021/jf60232a049

Laurent, B., Ousman, B., Dzudie, T., Mbofung, M. F. C., & Emmanuel, T. (2010). Digital camera images processing of hard-to-cook beans. *Journal of Engineering and Technology Research*, *2*(9), 177–188.

Lawless, H. T., & Hildegarde, H. (2010). *Sensory evaluation of food; principles and practices* (2nd ed). New York: Springer. https://doi.org/10.1007/978-1-4419-6488-5

Liang, J., Han, B.-Z., Nout, M. J. R., & Hamer, R. J. (2008). Effects of soaking, germination and fermentation on phytic acid, total and in vitro soluble zinc in brown rice. *Food Chemistry*, *110*(4), 821–828. https://doi.org/10.1016/J.FOODCHEM.2008.02.064

Lichten, L. A., & Cousins, R. J. (2009). Mammalian zinc transporters: Nutritional and physiologic regulation. *Annual Review of Nutrition*, *29*(1), 153–176. https://doi.org/10.1146/annurev-nutr-033009-083312

Limón, R. I., Peñas, E., Torino, M. I., Martínez-Villaluenga, C., Dueñas, M., & Frias, J. (2015). Fermentation enhances the content of bioactive compounds in kidney bean extracts. *Food Chemistry*, *172*, 343–352. https://doi.org/10.1016/j.foodchem.2014.09.084

Loewus, F. A., & Murthy, P. P. (2000). myo-Inositol metabolism in plants. *Plant Science*, *150*(1), 1–19.

Lönnerdal, B. (2000). Dietary factors influencing zinc absorption. *The Journal of Nutrition*, *130*(5), 1378S–1383S.

López-Amorós, M. L., Hernández, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, *19*(4), 277–283. https://doi.org/10.1016/J.JFCA.2004.06.012

Lopez, H. W., Leenhardt, F., Coudray, C., & Remesy, C. (2002). Minerals and phytic acid interactions: is it a real problem for human nutrition? *International Journal of Food Science & Technology*, *37*(7), 727–739. https://doi.org/10.1046/j.1365-2621.2002.00618.x

Luo, Y., Gu, Z., Han, Y., & Chen, Z. (2009). The impact of processing on phytic acid, in vitro soluble iron and Phy/Fe molar ratio of faba bean (Vicia faba L.). *Journal of the Science of Food and Agriculture*, *89*(5), 861–866. https://doi.org/10.1002/jsfa.3525

Luo, Y., Xie, W., Jin, X., Zhang, B., Wang, Q., & He, Y. (2013). The impact of processing on phytic acid, in vitro soluble zinc and Phy/Zn molar ratio of faba bean (Vicia faba L.). *International Food Research Journal*, *20*(3), 1285–1291.

Ma, G., Jin, Y., Piao, J., Kok, F., Guusje, B., & Jacobsen, E. (2005). Phytate, calcium, iron, and zinc contents and their molar ratios in foods commonly consumed in China. *Journal of Agricultural and Food Chemistry*, *53*(26), 10285–10290. https://doi.org/10.1021/jf052051r

Mamilla, R. K., & Mishra, V. K. (2017). Effect of germination on antioxidant and ACE inhibitory activities of legumes. *LWT - Food Science and Technology*, *75*, 51–58. https://doi.org/10.1016/J.LWT.2016.08.036

Máñez, G., Alegría, A., Farré, R., & Frígola, A. (2002). Effect of traditional, microwave and industrial cooking on inositol phosphate content in beans, chickpeas and lentils. *International Journal of Food Sciences and Nutrition*, *53*(6), 503–508. https://doi.org/10.1080/09637480220164343

Maret, W. (2000). The function of zinc metallothionein: A link between cellular zinc and redox state. *The Journal of Nutrition*, *130*(5), 1455S–1458S.

Maret, W. (2013). Zinc biochemistry: From a single zinc enzyme to a key element of life.

Advances in Nutrition, *4*(1), 82–91. https://doi.org/10.3945/an.112.003038

Martin-Cabrejas, M. A., Sanfiz, B., Vidal, A., Molla, E., Esteban, R., & Lopez-Andreu, F. J. (2004). Effect of fermentation and autoclaving on dietary fiber fractions and anti nutritional factors of beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, *52*(2), 261–266. https://doi.org/10.1021/jf034980t

Masood, T., Shah, H. U., & Zeb, A. (2014). Effect of sprouting time on proximate composition and ascorbic acid level of mung bean (*Vigna Radiate* L.) and chickpea (*Cicer Arietinum* L.) Seeds. *Journal of Animal & Plant Sciences*, *24*(3), 850–859.

McKie, A. T. (2008). The role of Dcytb in iron metabolism: an update. *Biochemical Society Transactions*, *36*(Pt 6), 1239–1241. https://doi.org/10.1042/BST0361239

McKie, A. T., Barrow, D., Latunde-Dada, G. O., Rolfs, A., Sager, G., Mudaly, E., … Simpson, R. J. (2001). An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* , *291*(5509), 1755–9. https://doi.org/10.1126/science.1057206

McKie, A. T., Marciani, P., Rolfs, A., Brennan, K., Wehr, K., Barrow, D., … Simpson, R. J. (2000). A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Molecular Cell*, *5*(2), 299–309. https://doi.org/10.1016/S1097-2765(00)80425-6

McLean, E., Cogswell, M., Egli, I., Wojdyla, D., & de Benoist, B. (2009). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutrition*. https://doi.org/10.1017/S1368980008002401

McMahon, R. J., & Cousins, R. J. (1998). Regulation of the zinc transporter ZnT-1 by dietary zinc. *Proceedings of the National Academy of Sciences*, *95*, 4841–4846.

https://doi.org/10.1073/pnas.95.9.4841

Meilgaard, M. C., Carr, B. T., & Civille, G. V. (2006). Sensory evaluation techniques. (4th Edition). Boca Raton: CRC Press.

Mesfin, A., Henry, C., Girma, M., & Whiting, S. J. (2015). Use of pulse crops in complementary feeding of 6-23-month-old infants and young children in Taba Kebele, Damot Gale District, Southern Ethiopia. *Journal of Public Health in Africa*, *6*(1), 357. https://doi.org/10.4081/jphia.2015.357

Miller, L. V, Hambidge, K. M., & Krebs, N. F. (2015). Zinc absorption is not related to dietary phytate intake in infants and young children based on modeling combined data from multiple studies. *The Journal of Nutrition*, *145*(8), 1763–9. https://doi.org/10.3945/jn.115.213074

Ministry of Agriculture and Rural Development. (2009). *Crop variety register*. Addis Ababa.

Mittal, R., Nagi, H., Sharma, P., & Sharma, S. (2012). Effect of processing on chemical composition and antinutritional factors in chickpea flour. *Journal of Food Science and Engineering*, *2*(3), 180–186. https://doi.org/10.17265/2159-5828/2012.03.008

Mkanda, A. V, Minnaar, A., & de Kock, H. L. (2007). Relating consumer preferences to sensory and physicochemical properties of dry beans (*Phaseolus vulgaris*). *Journal of the Science of Food and Agriculture*, *87*(15), 2868–2879. https://doi.org/10.1002/jsfa.3046

Montalbetti, N., Simonin, A., Kovacs, G., & Hediger, M. A. (2013). Mammalian iron transporters: Families SLC11 and SLC40. *Molecular Aspects of Medicine*, *34*(2–3), 270–287. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.mam.2013.01.002

Morris, E. R., & Ellis, R. (1980). Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. *The Journal of Nutrition*, *110*(5), 1037–1045. https://doi.org/10.1093/jn/110.5.1037

Mulugeta, F. (2010). Profile of haricot bean production, supply, demand and marketing issues in Ethiopia.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Food Science*, *1054*(1–2), 95–111. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.chroma.2004.08.059

Nakitto, A. M., Muyonga, J. H., & Nakimbugwe, D. (2015). Effects of combined traditional processing methods on the nutritional quality of beans. *Food Science & Nutrition*, *3*(3), 233–241. https://doi.org/10.1002/fsn3.209

Nergiz, C., & Gökgöz, E. (2007). Effects of traditional cooking methods on some antinutrients and in vitro protein digestibility of dry bean varieties (*Phaseolus vulgaris* L.) grown in Turkey. *International Journal of Food Science & Technology*, *42*(7), 868–873. https://doi.org/10.1111/j.1365-2621.2006.01297.

Oatway, L., Vasanthan, T., & Helm, J. H. (2001). Phytic acid. *Food Reviews International*, *17*(4), 419–431. https://doi.org/10.1081/FRI-100108531

Otten, J. J., Hellwig, J. P., & Meyers, L. D. (2006). *Dietary reference intakes: The essential guide to nutrient requirements.* Washington, DC: National Academies Press.

Perron, N. R., & Brumaghim, J. L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochemistry and Biophysics*, *53*(2), 75–100. https://doi.org/10.1007/s12013-009-9043-x

Persson, H., Turk, M., Nyman, M., & Sandberg, A.-S. (1998). Binding of cu2+, zn2+, and cd2+ to inositol tri-, tetra-, penta-, and hexaphosphates. *Journal of Agricultural and Food Chemistry*, *46*(8), 3194–3200. https://doi.org/10.1021/jf971055w

Petry, N., Boy, E., Wirth, J., & Hurrell, R. (2015). Review: The potential of the common bean (*Phaseolus vulgaris*) as a vehicle for iron biofortification. *Nutrients*, *7*(2), 1144–1173. https://doi.org/10.3390/nu7021144

Petry, N., Egli, I., Gahutu, J. B., Tugirimana, P. L., Boy, E., & Hurrell, R. (2012). Stable iron isotope studies in Rwandese women indicate that the common bean has limited potential as a vehicle for iron biofortification. *Journal of Nutrition.*, *142*(3), 492–497.

Petry, N., Egli, I., Gahutu, J. B., Tugirimana, P. L., Boy, E., & Hurrell, R. (2014). Phytic acid concentration influences iron bioavailability from biofortified beans in Rwandese women with low iron status. *The Journal of Nutrition*, *144*(11), 1681–1687. https://doi.org/10.3945/jn.114.192989

Petry, N., Egli, I., Zeder, C., Walczyk, T., & Hurrell, R. (2010). Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. *The Journal of Nutrition*, *140*(11), 1977–82. https://doi.org/10.3945/jn.110.125369

Piecyk, M., Wołosiak, R., Druzynska, B., & Worobiej, E. (2012). Chemical composition and starch digestibility in flours from Polish processed legume seeds. *Food Chemistry*, *135*(3), 1057–1064. https://doi.org/10.1016/j.foodchem.2012.05.051

Pietrangelo, A. (2002). Physiology of iron transport and the hemochromatosis gene. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *282*(3), G403–G414. https://doi.org/10.1152/ajpgi.00404.2001

Porres, J. M., Aranda, P., López-jurado, M., & Urbano, G. (2003). Effect of natural and controlled fermentation on chemical composition and nutrient dialyzability from beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, *51*(17), 5144–5149. https://doi.org/10.1021/jf0300083

Prakash, R. A. G., & Jamuna. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *LWT - Food Science and Technology*, *40*(7), 1292–1299.

Prasad, A. S. (2003). Zinc deficiency has been known for 40 years but ignored by global health organizations. *BMJ*, *326*(7386), 409–410. https://doi.org/10.1136/bmj.326.7386.409

Prasad, A. S. (2012). Discovery of human zinc deficiency: 50 years later. *Journal of Trace Elements in Medicine and Biology*, *26*(2–3), 66–69. https://doi.org/http://dx.doi.org/10.1016/j.jtemb.2012.04.004

Prasad, A. S., Halsted, J. A., & Nadimi, M. (1961). Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *The American Journal of Medicine*, *31*(4), 532–546. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/0002-9343(61)90137-1

Price, M. L., Scoyoc, S. V, & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, (5), 1214–1218. https://doi.org/10.1021/jf60219a031

Pujolà, M., Farreras, A., & Casañas, F. (2007). Protein and starch content of raw, soaked and cooked beans (Phaseolus vulgaris L.). *Food Chemistry*, *102*(4), 1034–1041. https://doi.org/10.1016/J.FOODCHEM.2006.06.039

Pynaert, I., Armah, C., Fairweather-Tait, S., Kolsteren, P., van Camp, J., & Henauw, S. De. (2006). Iron solubility compared with in vitro digestion–Caco-2 cell culture method for the assessment of iron bioavailability in a processed and unprocessed complementary food for Tanzanian infants (6–12 months). *British Journal of Nutrition*, *95*(04), 721. https://doi.org/10.1079/BJN20051722

Raboy, V. (2003). myo-Inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry*, *64*(6), 1033–1043. https://doi.org/http://dx.doi.org/10.1016/S0031-9422(03)00446-1

Radlowski, E. C., & Johnson, R. W. (2013). Perinatal iron deficiency and neurocognitive development. *Frontiers in Human Neuroscience*, *7*, 585. https://doi.org/10.3389/fnhum.2013.00585

Raes, K., Knockaert, D., Struijs, K., & Van Camp, J. (2014). Role of processing on bioaccessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. *Trends in Food Science and Technology*. https://doi.org/10.1016/j.tifs.2014.02.002

Rahman, M. S., Sana, N. K., Hasan, M. M., Huque, M. E., & Shaha, R. K. (2008). Enzyme activities and degradation of nutrients in chickpea (*Cicer arietinum* L.) seeds during germination. *Journal of Bio-Science* , *16*, 29–34.

Ramdath, D., Renwick, S., & Duncan, A. M. (2016). The Role of Pulses in the Dietary Management of Diabetes. *Canadian Journal of Diabetes*, *40*(4), 355–363. https://doi.org/10.1016/j.jcjd.2016.05.015

Rashid, S., Yirga, C., Behute, B., & Lemma, S. (2010). *Pulses Value Chain in Ethiopia: Constraints and opportunities for enhancing exports* (Globalization and Markets program).

Ifpri. Washington, D.C.

Ray, H., Bett, K., Tar'an, B., Vandenberg, A., Thavarajah, D., & Warkentin, T. (2014). Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. *Crop Science*, *54*(4), 1698–1708. https://doi.org/10.2135/cropsci2013.08.0568

Reddy, N. R. (2002). Occurrence, distribution, content and dietary intake of phytate. In N. R. Reddy & S. K. Sathe (Eds.), *Food phytates* (pp. 30–32). Boca Raton, FL: CRC Press.

Rehman, Z., & Shah, W. H. (2005). Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chemistry*, *91*(2), 327–331. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.foodchem.2004.06.019

Reyes-Bastidas, M., Reyes-Fernández, E. Z., López-Cervantes, J., Milán-Carrillo, J., Loarca-Piña, G. F., & Reyes-Moreno, C. (2010). Physicochemical, nutritional and antioxidant properties of tempeh flour from common bean (Phaseolus vulgaris L.). *Food Science and Technology International*, *16*(5), 427–434. https://doi.org/10.1177/1082013210367559

Reyes-Moreno, C., Cuevas-Rodríguez, E., Milán-Carrillo, J., Cárdenas-Valenzuela, O., & Barrón-Hoyos, J. (2004). Solid state fermentation process for producing chickpea (Cicer arietinum L) tempeh flour: Physicochemical and nutritional characteristics of the product. *Journal of the Science of Food and Agriculture*, *84*(3), 271–278. https://doi.org/10.1002/jsfa.1637

Reyes-Moreno, C., Okamura-Esparza, J., Armienta-Rodelo, E., Gomez-Garza, R. M., & Milan-Carrillo, J. (2000). Hard-to-cook phenomenon in chickpeas (Cicer arietinum L): Effect of accelerated storage on quality. *Plant Foods for Human Nutrition*, *55*(3), 229–241.

https://doi.org/10.1023/A:1008106229189

Ribeiro, E. D. S., Centeno, D. D. C., Figueiredo-Ribeiro, R. D. C., Fernandes, K. V. S., Xavier-Filho, J., & Oliveira, A. E. A. (2011). Free cyclitol, soluble carbohydrate and protein contents in *Vigna unguiculata* and *Phaseolus vulgaris* bean sprouts. *Journal of Agricultural and Food Chemistry*, *59*(8), 4273–4278. https://doi.org/10.1021/jf104979m

Ribeiro, N. D., Maziero, S. M., Prigol, M., Nogueira, C. W., Rosa, D. P., & Possobom, M. T. D. F. (2012). Mineral concentrations in the embryo and seed coat of common bean cultivars. *Journal of Food Composition and Analysis*, *26*(1), 89–95. https://doi.org///dx.doi.org/10.1016/j.jfca.2012.03.003

Rink, L., & Gabriel, P. (2001). Extracellular and immunological actions of zinc. *Biometals*, *14*(3–4), 367–383. https://doi.org/10.1023/A:1012986225203

Rizzello, C. G., Calasso, M., Campanella, D., De Angelis, M., & Gobbetti, M. (2014). Use of sourdough fermentation and mixture of wheat, chickpea, lentil and bean flours for enhancing the nutritional, texture and sensory characteristics of white bread. *International Journal of Food Microbiology*, *180*, 78–87. https://doi.org/10.1016/j.ijfoodmicro.2014.04.005

Roba, A. C., Gabriel-Micheal, K., Zello, G. A., Jaffe, J., Whiting, S. J., & Henry, C. J. (2015). A low pulse food intake may contribute to the poor nutritional status and low dietary intakes of adolescent girls in rural southern Ethiopia. *Ecology of Food and Nutrition*, *54*(3), 240–254. https://doi.org/10.1080/03670244.2014.974593

Roba, K. T., O'Connor, T. P., Belachew, T., & O'Brien, N. M. (2016). Concurrent iron and zinc deficiencies in lactating mothers and their children 6–23 months of age in two agro-ecological zones of rural Ethiopia. *European Journal of Nutrition*, *57*(2), 654–667.

https://doi.org/10.1007/s00394-016-1351-5

Robards, K. (2003). Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *A Century of Chromatography 1903-2003*, *1000*(1–2), 657–691. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/S0021-9673(03)00058-X

Roohani, N., Hurrell, R. F., Kelishadi, R., & Schulin, R. (2013). Zinc and its importance for human health: An integrative review. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences*, *18*(2), 144–157.

Ruel, M. T., & Alderman, H. (2013). Nutrition-sensitive interventions and programmes: How can they help to accelerate progress in improving maternal and child nutrition? *The Lancet*, *382*(9891), 536–551. https://doi.org/http://dx.doi.org/10.1016/S0140-6736(13)60843-0

Salovaara, S., Sandberg, A.-S., & Andlid, T. (2002). Organic Acids Influence Iron Uptake in the Human Epithelial Cell Line Caco-2. *Journal of Agricultural and Food Chemistry*, *50*(21), 6233–6238. https://doi.org/10.1021/jf0203040

Salunkhe, D. K., & Chavan, J. K. (1989). *Dietary tannins: Consequences and remedies*. Boca Raton, FL: CRC Press.

Sandberg, A. S. (2002). Bioavailability of minerals in legumes. *The British Journal of Nutrition*, *88*(S3), S281-5. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1079/BJN/2002718

Sandberg, A. S. (2010). The use of Caco-2 cells to estimate Fe absorption in humans: A critical appraisal. *International Journal for Vitamin and Nutrition Research*, *80*(4–5), 307–313. https://doi.org/10.1024/0300-9831/a000038

Sandberg, A. S., Brune, M., Carlsson, N. G., Hallberg, L., Skoglund, E., & Rossander-Hulthén,

L. (1999). Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *The American Journal of Clinical Nutrition*, *70*(2), 240–246.

Sangronis, E., & Machado, C. J. (2007). Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. *LWT - Food Science and Technology*, *40*(1), 116–120. https://doi.org/10.1016/j.lwt.2005.08.003

Sathe, S. K. (2002). Dry bean protein functionality. *Critical Reviews in Biotechnology*, *22*(2), 175–223. https://doi.org/10.1080/07388550290789487

Sattar, D., Ali, T. M., & Hasnain, A. (2017). Effect of nongerminated and germinated legumes on antioxidant, functional, and sensory characteristics of rice puddings. *Cereal Chemistry Journal*, *94*(3), 417–423. https://doi.org/10.1094/CCHEM-04-16-0103-R

Scheers, N. M., Almgren, A. B., Sandberg, A., & Sofie. (2014). Proposing a Caco-2/HepG2 cell model for *in vitro* iron absorption studies. *The Journal of Nutritional Biochemistry*, *25*(7), 710–715. https://doi.org/10.1016/J.JNUTBIO.2014.02.013

Segev, A., Badani, H., Kapulnik, Y., Shomer, I., Oren-Shamir, M., & Galili, S. (2010). Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). *Journal of Food Science*, *75*(2), S115–S119. https://doi.org/10.1111/j.1750-3841.2009.01477.x

Shankar, A. H., Genton, B., Baisor, M., Paino, J., Tamja, S., Adiguma, T., … Alpers, M. P. (2000). The influence of zinc supplementation on morbidity due to Plasmodium falciparum: A randomized trial in preschool children in Papua New Guinea. *The American Journal of Tropical Medicine and Hygiene*, *62*(6), 663–669.

Sharma, S., Singh, A., Sharma, U., Kumar, R., & Yadav, N. (2016). Effect of thermal processing

on anti nutritional factors and *in vitro* bioavailability of minerals in desi and kabuli cultivars of chick pea grown in North India. https://doi.org/10.18805/LR-3708

Sharp, P. (2005). Methods and options for estimating iron and zinc bioavailability using Caco-2 cell models: Benefits and limitations. *International Journal for Vitamin and Nutrition Research*, *75*(6), 413–421.

Sharp, P. A. (2010). Intestinal iron absorption: Regulation by dietary & systemic factors. *International Journal for Vitamin and Nutrition Research*, *80*(45), 231–242. https://doi.org/10.1024/0300-9831/a000029

Shayeghi, M., Latunde-Dada, G., Oakhill, J. S., Laftah, A. H., Takeuchi, K., Halliday, N., … McKie, A. T. (2005). Identification of an intestinal heme transporter. *Cell*, *122*(5), 789–801. https://doi.org/http://dx.doi.org/10.1016/j.cell.2005.06.025

Shiferaw, B., & Teklewold, H. (2007). *Structure and functioning of chickpea markets in Ethiopia: Evidence based on analyses of value chains linking smallholders and markets* (Improving Productivity and Market Success (IPMS) of Ethiopian Farmers Project Working Paper No. 6). Nairobi, Kenya.

Shimelis, E. A., & Rakshit, S. K. (2005a). Antinutritional factors and *in vitro* protein digestibility of improved haricot bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *International Journal of Food Sciences & Nutrition*, *56*(6), 377–387. https://doi.org/10.1080/09637480500512930

Shimelis, E. A., & Rakshit, S. K. (2005b). Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *LWT - Food Science and Technology*, *38*(4), 331–338. https://doi.org/10.1016/j.lwt.2004.07.002

Shimelis, E. A., & Rakshit, S. K. (2007). Effect of processing on antinutrients and *in vitro* protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chemistry*, *103*(1), 161–172. https://doi.org/10.1016/j.foodchem.2006.08.005

Shimelis, E. A., & Rakshit, S. K. (2008). Influence of natural and controlled fermentations on α -galactosides, antinutrients and protein digestibility of beans (*Phaseolus vulgaris* L.). *International Journal of Food Science & Technology*, *43*(4), 658–665. https://doi.org/10.1111/j.1365-2621.2006.01506.x

Siddiq, M., & Uebersax, M. A. (2012). Dry beans and pulses production and consumption-An overview. In M. Siddiq & M. A. Uebersax (Eds.), *Dry beans and pulses production, processing and nutrition* (pp. 1–22). Ames, IA: Wiley Blackwell. https://doi.org/10.1002/9781118448298.ch1

Simons, C. W., & Hall, C. (2018). Consumer acceptability of gluten-free cookies containing raw cooked and germinated pinto bean flours. *Food Science & Nutrition*, *6*(1), 77–84. https://doi.org/10.1002/fsn3.531

Singh, J., & Basu, P. S. (2012). Non-nutritive bioactive compounds in pulses and their Impact on human health:An overview. *Food and Nutrition Sciences*, *3*(12), 1664–1672. https://doi.org/10.4236/fns.2012.312218

Singh, K. B. (1997). Chickpea (*Cicer arietinum* L.). *Field Crops Research*, *53*(1–3), 161–170. https://doi.org/http://dx.doi.org/10.1016/S0378-4290(97)00029-4

Sparringa, R. A., & Owens, J. D. (1999). Protein utilization during soybean tempe fermentation. *Journal of Agricultural and Food Chemistry*, *47*(10), 4375–4378. https://doi.org/10.1021/jf981279u

Srai, S. K., & Sharp, P. (2012). Proteins of iron homeostasis. In G. J. Anderson & G. D. McLaren (Eds.), *Iron Physiology and Pathophysiology in Humans* (pp. 3–26). New York: Springer Science and Business Media. https://doi.org/10.1007/978-1-60327-485-2

Stoecker, B. J., Abebe, Y., Hubbs-Tait, L., Kennedy, T. S., Gibson, R. S., Arbide, I., … Hambidge, K. M. (2009). Zinc status and cognitive function of pregnant women in Southern Ethiopia. *European Journal of Clinical Nutrition*, *63*(7), 916–918. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1038/ejcn.2008.77

Stone, H., & Sidel, J. L. (2004). Introduction to sensory evaluation. In H. Stone, R. N. Bleibaum, & H. A. Thoma (Eds.), *Sensory Evaluation Practices* (4th ed., pp. 1–19). San Diego, CA: Academic Press. https://doi.org/http://dx.doi.org/10.1016/B978-012672690-9/50005-6

Tako, E., Beebe, S. E., Reed, S., Hart, J. J., & Glahn, R. P. (2014). Polyphenolic compounds appear to limit the nutritional benefit of biofortified higher iron black bean (*Phaseolus vulgaris* L.). *Nutrition Journal*, *13*(1), 28. https://doi.org/10.1186/1475-2891-13-28

Tako, E., Blair, M. W., & Glahn, R. P. (2011). Biofortified red mottled beans (*Phaseolus vulgaris* L.) in a maize and bean diet provide more bioavailable iron than standard red mottled beans: Studies in poultry (Gallus gallus) and an in vitro digestion/Caco-2 model. *Nutrition Journal*, *10*(1), 113. https://doi.org/10.1186/1475-2891-10-113

Tako, E., & Glahn, R. P. (2010). White beans provide more bioavailable iron than red beans: Studies in poultry (*Gallus gallus*) and an *in vitro* digestion/Caco-2 model. *International Journal for Vitamin and Nutrition Research*, *80*(6), 416–429. https://doi.org/10.1024/0300-9831/a000028

Tako, E., Vandenberg, A., Thavarajah, D., Thavarajah, P., & Glahn, R. P. (2011). Iron

bioavailability in lentil based diets: Studies in poultry and *in vitro* digestion/Caco-2 model. *The FASEB Journal*, *25*(1), 607.8.

Tanno, K., & Willcox, G. (2006). The origins of cultivation of *Cicer arietinum* L. and *Vicia faba* L.: Early finds from Tell el-Kerkh, north-west Syria, late 10th millennium b.p. *Vegetation History and Archaeobotany*, *15*(3), 197–204. https://doi.org/10.1007/s00334-005-0027-5

Tapiero, H., Gate, L., & Tew, K. D. (2001). Iron: deficiencies and requirements. *Biomedicine & Pharmacotherapy*, *55*(6), 324–332. https://doi.org/http://dx.doi.org/10.1016/S0753-3322(01)00067-1

Tefera, T. (2013). Market and consumer studies of pulse crops in Southern Ethiopia. Retrieved July 27, 2017, from https://idl-bnc-idrc.dspacedirect.org/bitstream/handle/10625/51358/IDL-51358.pdf?sequence=1

Teucher, B., Olivares, M., & Cori, H. (2004). Enhancers of iron absorption: ascorbic acid and other organic acids. *International Journal for Vitamin and Nutrition Research*, *74*(6), 403–419.

Tharanathan, R. N., & Mahadevamma, S. (2003). Grain legumes—a boon to human nutrition. *Trends in Food Science & Technology*, *14*(12), 507–518. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.tifs.2003.07.002

Thavarajah, D., & Thavarajah, P. (2012). Evaluation of chickpea (*Cicer arietinum* L.) micronutrient composition: Biofortification opportunities to combat global micronutrient malnutrition. *Food Research International*, *49*(1), 99–104. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.foodres.2012.08.007

Thavarajah, P., & Thavarajah, D. (2014). Inaccuracies in phytic acid measurement: Implications

for mineral biofortification and bioavailability. *American Journal of Plant Sciences*, *5*(1), 29–34. https://doi.org/10.4236/ajps.2014.51005.

Thompson, B. A. V, Sharp, P. A., Elliott, R., & Fairweather-Tait, S. J. (2010). Inhibitory effect of calcium on non-heme iron absorption may be related to translocation of DMT-1 at the apical membrane of enterocytes. *Journal of Agricultural and Food Chemistry*, *58*, 8414–8417. https://doi.org/10.1021/jf101388z

Torino, M. I., Limón, R. I., Martínez-Villaluenga, C., Mäkinen, S., Pihlanto, A., Vidal-Valverde, C., & Frias, J. (2013). Antioxidant and antihypertensive properties of liquid and solid state fermented lentils. *Food Chemistry*, *136*(2), 1030–1037. https://doi.org/10.1016/J.FOODCHEM.2012.09.015

Trugo, L. ., Muzquiz, M., Pedrosa, M. ., Ayet, G., Burbano, C., Cuadrado, C., & Cavieres, E. (1999a). Influence of malting on selected components of soya bean, black bean, chickpea and barley. *Food Chemistry*, *65*(1), 85–90. https://doi.org/10.1016/S0308-8146(98)00207-6

Trugo, L. C., Muzquiz, M., Pedrosa, M. M., Ayet, G., Burbano, C., Cuadrado, C., & Cavieres, E. (1999b). Influence of malting on selected components of soya bean, black bean, chickpea and barley. *Food Chemistry*, *65*(1), 85–90. https://doi.org/10.1016/S0308-8146(98)00207-6

Umamaheswari, K., Bhaskaran, M., Krishnamurthy, G., Hemamalini, & Kavita, V. (2011). Effect of iron and zinc deficiency on short term memory in children. *Indian Pediatrics*, *48*(4), 289–293. https://doi.org/10.1007/s13312-011-0060-7

Umbreit, J. (2005). Iron deficiency: A concise review. *American Journal of Hematology*, *78*(3), 225–231. https://doi.org/10.1002/ajh.20249

Umeta, M., Haidar, J., Demissie, T., Akalu, G., & Ayana, G. (2008). Iron deficiency anaemia

among women of reproductive age in nine administrative regions of Ethiopia. *The Ethiopian Journal of Health Development*, *22*(3), 152–158.

Umeta, M., West, C. E., & Fufa, H. (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *Journal of Food Composition and Analysis*, *18*(8), 803–817. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/j.jfca.2004.09.008

UNICEF. (1990). *A strategy for improved nutrition of children and women in developing countries*. New York, USA: UNICEF.

Vaintraub, I. A., & Lapteva, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Analytical Biochemistry*, *175*(1), 227–230. https://doi.org/http://dx.doi.org.cyber.usask.ca/10.1016/0003-2697(88)90382-X

Van Campen, D. R., & Glahn, R. P. (1999). Micronutrient bioavailability techniques: Accuracy, problems and limitations. *Field Crops Research*, *60*(1), 93–113. https://doi.org/http://dx.doi.org/10.1016/S0378-4290(98)00135-X

Vaz-Tostes, M. das G., Verediano, T. A., de Mejia, E. G., & Brunoro Costa, N. M. (2016). Evaluation of iron and zinc bioavailability of beans targeted for biofortification using *in vitro* and *in vivo* models and their effect on the nutritional status of preschool children. *Journal of the Science of Food and Agriculture*, *96*(4), 1326–1332. https://doi.org/10.1002/jsfa.7226

Vita, J. A. (2005). Polyphenols and cardiovascular disease: effects on endothelial and platelet function. *The American Journal of Clinical Nutrition*, *81*(1 Suppl), 292S–297S.

Viteri, F. E., & Gonzalez, H. (2002). Adverse outcomes of poor micronutrient status in childhood

and adolescence. *Nutrition Reviews*, *60*(5), S77–S83. https://doi.org/10.1301/00296640260130795

Vucenik, I., & Shamsuddin, A. M. (2003). Cancer inhibition by inositol hexaphosphate (IP6) and inositol: From laboratory to clinic. *The Journal of Nutrition*, *133*(11), 3778S–3784S.

Vucenik, I., & Shamsuddin, A. M. (2006). Protection against cancer by dietary IP6 and inositol. *Nutrition and Cancer*, *55*(2), 109–125. https://doi.org/10.1207/s15327914nc5502_1

Walker, C. L. F., Young, M. W., Black, R. E., Fontaine, O., & Black, R. E. (2009). Zinc and low osmolarity oral rehydration salts for diarrhoea: a renewed call to action. *Bulletin of the World Health Organization*, *87*(10), 780–786.

Wang, N., & Daun, J. K. (2005). Determination of cooking times of pulses using an automated Mattson cooker apparatus. *Journal of the Science of Food and Agriculture*, *85*(10), 1631–1635. https://doi.org/10.1002/jsfa.2134

Wang, N., Hatcher, D. W. W., Tyler, R. T. T., Toews, R., & Gawalko, E. J. J. (2010). Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Research International*, *43*(2), 589–594. https://doi.org/10.1016/j.foodres.2009.07.012

Wei, Y., Shohag, M. J. I., Ying, F., Yang, X., Wu, C., & Wang, Y. (2013). Effect of ferrous sulfate fortification in germinated brown rice on seed iron concentration and bioavailability. *Food Chemistry*, *138*(2–3), 1952–1958. https://doi.org/10.1016/j.foodchem.2012.09.134

Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, *55*(396), 353–364. https://doi.org/10.1093/jxb/erh064

Weldehaweria, N. B., Misgina, K. H., Weldu, M. G., Gebregiorgis, Y. S., Gebrezgi, B. H., Zewdie, S. W., … Alemu, W. (2016). Dietary diversity and related factors among lactating women visiting public health facilities in Aksum town, Tigray, Northern Ethiopia. *BMC Nutrition, 2*(1), 38. https://doi.org/10.1186/s40795-016-0077-3

Wessells, K. R., & Brown, K. H. (2012). Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PloS One, 7*(11), e50568. https://doi.org/. doi:10.1371/journal.pone.0050568

Wessells, K. R., Singh, G. M., & Brown, K. H. (2012). Estimating the global prevalence of inadequate zinc intake from national food balance sheets: Effects of methodological assumptions. *PLoS ONE, 7*(11), e50565. https://doi.org/10.1371/journal.pone.0050565

Wood, J. A., Knights, E. J., & Choct, M. (2011). Morphology of chickpea seeds (*Cicer arietinum* L.): Comparison of desi and kabuli types. *International Journal of Plant Sciences, 172*(5), 632–643. https://doi.org/10.1086/659456

World Health Organization. (2001). Iron deficiency anaemia assessment, prevention, and control: A guide for programme managers. Retrieved January 20, 2013, from http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf

World Health Organization. (2002). *Reducing risks, promoting healthy life.* The World Health Organization Report 2002.

World Health Organization. (2007). Preventing and controlling micronutrient deficiencies in populations affected by an emergency. Geneva, Switzerland: Joint statement by the World Health Organization, The World Food Program, and the United Nations Children's Fund.

World Health Organization. (2012). Guideline: Daily iron and folic acid supplementation in

pregnant women. Retrieved from http://apps.who.int/iris/bitstream/10665/77770/1/9789241501996_eng.pdf

Xu, B. J., & Chang, S. K. C. (2008). Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. *Journal of Food Science*, *73*(2), H19–H27. https://doi.org/10.1111/j.1750-3841.2007.00625.x

Yasmin, A., Zeb, A., Khalil, A., Paracha, G.-D., & Khattak, A. (2008). Effect of processing on anti-nutritional factors of red kidney bean (Phaseolus vulgaris) grains. *Food and Bioprocess Technology*, *1*(4), 415–419. https://doi.org/10.1007/s11947-008-0125-3

Young, L. (1936). The determination of phytic acid. *Biochemical Journal*, *30*(2), 252–257.

Yun, S., Habicht, J. P., Ierre, Miller, D. D., & Glahn, R. P. (2004). An *in vitro* digestion/Caco-2 cell culture system accurately predicts the effects of ascorbic acid and polyphenolic compounds on iron bioavailability in humans. *The Journal of Nutrition*, *134*(10), 2717–2721.

Zemel, M. B., & Shelef, L. A. (1982). Phytic acid hydrolysis and soluble zinc and iron in whole wheat bread as affected by calcium containing additives. *Journal of Food Science*, *47*(2), 535–537. https://doi.org/10.1111/j.1365-2621.1982.tb10118.x

Zimmermann, M. B., Chaouki, N., & Hurrell, R. F. (2005). Iron deficiency due to consumption of a habitual diet low in bioavailable iron: A longitudinal cohort study in Moroccan children. *American Journal of Clinical Nutrition*, *81*(1), 115–121. https://doi.org/10.1093/ajcn/81.1.115

Zimmermann, M. B., & Hurrell, R. F. (2007). Nutritional iron deficiency. *The Lancet*, *370*(9586), 511–520. https://doi.org/https://doi.org/10.1016/S0140-6736(07)61235-5

Appendix A. Copyright Permissions

Dear Hiwot,

Thank you for your request. You can consider this email as permission to use the material as detailed below in your upcoming thesis. Please note that this permission does not cover any 3rd party material that may be found within the work. We do ask that you properly credit the original source, Food & Nutrition Bulletin. Please contact us for any further usage of the material.

Best regards,

Michelle Binur

Rights Coordinator

SAGE Publications, Inc.

2455 Teller Road

Thousand Oaks, CA 91320

USA

www.sagepub.com

Los Angeles | London | New Delhi

Singapore | Washington DC

Dear sir/madam,

I would like to include a figure from Hotz, C., & McClafferty, B. (2007). From harvest to health: Challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food & Nutrition Bulletin*, *28*(Supplement 2), 271S-279S (FIG. 1. Summary of nutrition research and challenges for developing and evaluating biofortified foods as a viable public health intervention) to my thesis, thus I am requesting you to send me information on how I could get permission. Thanks in advance.

Regards,

Hiwot

ELSEVIER LICENSE
TERMS AND CONDITIONS

Jan 29, 2019

This Agreement between Hiwot Haileslassie ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	4518381311607
License date	Jan 29, 2019
Licensed Content Publisher	Elsevier
Licensed Content Publication	Food Chemistry
Licensed Content Title	Dietary roles of phytate and phytase in human nutrition: A review
Licensed Content Author	Vikas Kumar,Amit K. Sinha,Harinder P.S. Makkar,Klaus Becker
Licensed Content Date	Jun 15, 2010
Licensed Content Volume	120
Licensed Content Issue	4
Licensed Content Pages	15
Start Page	945
End Page	959
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Original figure numbers	Figure 1
Title of your thesis/dissertation	Household Food Processing Strategies to Improve Iron and Zinc Bioavailability in Ethiopian Dishes Based on Chickpea (Cicer arietinum L.) and Bean (Phaseolus vulgaris L.)
Expected completion date	Aug 2019
Estimated size (number of pages)	200
Requestor Location	Hiwot Haileslassie 115 bottomley Ave South Saskatoon, SK S7n 1k5 Canada Attn: Hiwot Haileslassie
Publisher Tax ID	GB 494 6272 12

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

This Agreement between Hiwot Hailelassie ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Sprir Copyright Clearance Center.

License Number	4517851232049
License date	Jan 28, 2019
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Cell Biochemistry and Biophysics
Licensed Content Title	A Review of the Antioxidant Mechanisms of Polyphenol Compounds Related to Iron Binding
Licensed Content Author	Nathan R. Perron, Julia L. Brumaghim
Licensed Content Date	Jan 1, 2009
Licensed Content Volume	53
Licensed Content Issue	2
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Will you be translating?	no
Circulation/distribution	>50,000
Author of this Springer Nature content	no
Title	Household Food Processing Strategies to Improve Iron and Zinc Bioavailability in Ethiopian Dishes Based on Chickpea (Cicer arietinum L.) and Bean (Phaseo
Institution name	n/a
Expected presentation date	Aug 2019
Portions	Fig. 5 Expected octahedral coordination geometry of general iron–polyphenol complexes. Gallols, R=OH; catechols, R=H. Coordination requires deprotonation of th
Requestor Location	Hiwot Hailelassie 115 bottomley Ave South Saskatoon, SK S7n 1k5 Canada Attn: Hiwot Hailelassie
Billing Type	Invoice
Billing Address	Hiwot Hailelassie 115 bottomley Ave South Saskatoon, SK S7n 1k5 Canada Attn: Hiwot Hailelassie
Total	0.00 CAD
Terms and Conditions	

Springer Nature Terms and Conditions for RightsLink Permissions

Springer Nature Customer Service Centre GmbH (the Licensor) hereby grants you a non-exclusive, world-wide licence to reproduce the material and for the purpose and specified in the attached copy of your order form, and for no other use, subject to the conditions below:

1. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of this material. However, you should ensure that the material you are requesting is o Licensor and does not carry the copyright of another entity (as credited in the published version).

   If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek perm source to reuse the material.

2. Where **print only** permission has been granted for a fee, separate permission must be obtained for any additional electronic re-use.

3. Permission granted **free of charge** for material in print is also usually granted for any electronic version of that work, provided that the material is incidental to your work as a electronic version is essentially equivalent to, or substitutes for, the print version.

4. A licence for 'post on a website' is valid for 12 months from the licence date. This licence does not cover use of full text articles on websites.

5. Where '**reuse in a dissertation/thesis**' has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published versi copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

6. Permission granted for books and journals is granted for the lifetime of the first edition and does not apply to second and subsequent editions (except where the first edition per free of charge or for signatories to the STM Permissions Guidelines http://www.stm-assoc.org/copyright-legal-affairs/permissions/permissions-guidelines/), and does not apply f languages unless additional translation rights have been granted separately in the licence.

7. Rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

8. The Licensor's permission must be acknowledged next to the licensed material in print. In electronic form, this acknowledgement must be visible at the same time as the figures or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

AMERICAN SOCIETY FOR NUTRITION LICENSE TERMS AND CONDITIONS

Jun 22, 2015

This is a License Agreement between Hiwot Haileslassie ("You") and American Society for Nutrition ("American Society for Nutrition") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by American Society for Nutrition, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3654360478812
License date	Jun 22, 2015
Volume number	128
Issue number	9
Licensed content publisher	American Society for Nutrition
Licensed content publication	The Journal of Nutrition
Licensed content title	Caco-2 Cell Ferritin Formation Predicts Nonradiolabeled Food Iron Availability in an In Vitro Digestion/Caco-2 Cell Culture Model
Licensed content author	Raymond P. Glahn, Olivia A. Lee, Andrew Yeung, Matthew I. Goldman, Dennis D. Miller
Licensed content date	Sep 1, 1998
Type of Use	Thesis/Dissertation
Requestor type	Student
Portion	Figures/table/illustration
Number of Figures/table/illustration	1
List of figures/table/ illustration	Fig. 1. Diagram of in vitro digestion/Caco-2 cell culture model.
Order reference number	None
Title of your dissertation / thesis	Household Food Processing Strategies to Improve Iron and Zinc Bioavailability in Ethiopian Dishes Based on Chickpea (Cicer arietinum L.) and Bean (Phaseolus vulgaris L.)
Expected completion date	Jun 2016
Estimated size(pages)	200
Total	0.00 USD
Terms and Conditions	

Terms and Conditions for Rightslink Licenses

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Jun 18, 2018

This Agreement between Hiwot Haileslassie ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4372081381373
License date	Jun 18, 2018
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	International Journal of Food Science & Technology
Licensed Content Title	Impact of household food processing strategies on antinutrient (phytate, tannin and polyphenol) contents of chickpeas (Cicer arietinum L.) and beans (Phaseolus vulgaris L.): a review
Licensed Content Author	Hiwot A. Haileslassie, Carol J. Henry, Robert T. Tyler
Licensed Content Date	Jul 13, 2016
Licensed Content Volume	51
Licensed Content Issue	9
Licensed Content Pages	11
Type of Use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	Household Food Processing Strategies to Improve Iron and Zinc Bioavailability in Ethiopian Dishes Based on Chickpea (Cicer arietinum L.) and Bean (Phaseolus vulgaris L.)
Expected completion date	Aug 2018
Expected size (number of pages)	200
Requestor Location	Hiwot Haileslassie 115 bottomley Ave South Saskatoon, SK S7N 1k5 Canada Attn: Hiwot Haileslassie
Publisher Tax ID	EU826007151
Total	**0.00 CAD**
Terms and Conditions	

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.
- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.
- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.
- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto
- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.
- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

v1.10 Last updated September 2015

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Appendix B. Sensory Evaluation Form for Panellists

Code of Participant _____Age_____ Sex_____

You will be provided with food samples and we request you to evaluate the samples for appearance, texture, taste, odor and overall acceptability. Please give your responses based on a 9 point scale by writing the appropriate scale number on the table below

9=like extremely	8=like very much	7=like moderately	6=like slightly	5=neither like nor dislike	4=dislike slightly	3=dislike moderately	2=dislike very much	1=dislike extremely

	Code _____	Code _____	Code _____	Code _____	Code _____	Code _____	Code _____
Odour							
Appearance							
Texture							
Taste							
Overall acceptability							

Comment:

Appendix C. Sensory Evaluation Form for Community Consumers

Code of Participant _____Age_____ Sex _____Date _____

You will be provided with food samples and we request you to evaluate the samples for appearance, texture, taste, odor and overall acceptability. Please give your responses based on a 5 point scale (from "5" – excellent, to "1" - terrible)

Sample code _____

	5=excellent	4=very good	3=good	2= poor	1= terrible
Odour					
Appearance					
Texture					
Taste					
Overall acceptability					

Comment:

……

……

……

……

Appendix D

Table D.1. Nutrient Contents of Samples of Chickpea Grown in Saskatchewan

Pulse Varieties	Protein (g/100 g)	Zinc (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100 g)
Habru	19.9 ± 0.0	3.42 ± 0.00	6.45 ± 0.08	129.4 ± 4.3
Mastewal	22.6 ± 0.1	3.29 ± 0.01	6.47 ± 0.07	140.3 ± 0.5
Local	21.9 ± 0.2	3.06 ± 0.02	5.71 ± 0.13	217.8 ± 11.8
CDC-C	20.3 ± 0.1	3.37 ± 0.23	4.29 ± 0.15	103.4 ± 11.1
CDC-F	22.6 ± 0.1	2.67 ± 0.01	4.46 ± 0.05	103.7 ± 0.52

Values are mean ± SD on a dry weight basis. CDC-C=CDC Cory, CDC-F= CDC Frontier

Appendix E

Table E.1. Inositol Phosphate Contents of Hawassa Dume

Food process	IP3 (mg/100 g)	Reduction* (%)	IP4 (mg/100 g)	Reduction* (%)	IP5 (mg/100 g)	Reduction* (%)	IP6 (mg/100 g)	Reduction* (%)
Raw	0.86 ± 0.77		8.42 ± 2.33^{a}		12.00 ± 4.07^{a}		34.46 ± 9.17^{a}	
Cooked	0.58 ± 0.51		5.39 ± 2.67^{ab}		4.31 ± 2.13^{b}		20.75 ± 6.5^{b}	
S12 cooked	0.53 ± 0.69	9	2.23 ± 1.91^{b}	59	2.06 ± 1.01^{bc}	52	15.01 ± 2.49^{bc}	28
S24 cooked	0.54 ± 0.59	7	2.12 ± 1.28^{b}	61	1.38 ± 0.83^{bc}	68	9.88 ± 1.82^{c}	52
G24 cooked	0.33 ± 0.17	43	2.10 ± 1.33^{b}	61	0.93 ± 0.66^{c}	78	7.20 ± 9.74^{c}	65
G48 cooked	0.22 ± 0.16	62	1.43 ± 1.24^{b}	73	0.69 ± 0.30^{c}	84	6.81 ± 0.96^{c}	67
G72 cooked	0.40 ± 0.35	31	1.93 ± 1.53^{b}	64	0.60 ± 0.36^{c}	86	9.32 ± 1.46^{c}	55

Values are mean ± SD (n=2) on a dry weight basis. Means within a column with the same superscript letter are not significantly different ($p>0.05$) based on Tukey's multiple comparison test. S12=soaked for 12 hours and cooked, S24=soaked for 24 hours and cooked, G24=germinated for 24 hours and cooked, G48=germinated for 48 hours and cooked, G72=germinated for 72 hours and cooked, *percentage reduction calculated in comparison to samples cooked without treatment