EFFECT OF COOKING AND CONCENTRATION OF INHIBITORS ON IRON AND ZINC CONTENT OF BIO-FORTIFIED BEANS

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN HUMAN NUTRITION OF SOKOINE UNIVERSITY OF AGRICULTURE MOROGORO, TANZANIA

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ABSTRACT

Bio-fortified foods usually have high nutrient contents and have potential to improve nutritional status of vulnerable groups; however, increased levels of nutrients do not necessarily translate to enhanced bioavailability due to presence of dietary anti-nutritional factors. The study aimed to determine the effect of cooking and dietary anti-nutritional factors on the content of zinc and iron of bio-fortified beans as compared to non-bio-fortified varieties. Three bio-fortified bean varieties (Selian 13, 14 and 15) and a non-bio-fortified variety (JESCA) which was a benchmark were analyzed by atomic absorption spectrophotometry for the content of iron, zinc and dietary anti-nutritional factors before and after cooking. Determination of anti-nutritional factors (phytic acid) was through phytate precipitation, polyphenols by Follin-Ciocalteu reagent spectrophotometer method and for tannins by the Follin-Dennis reagent titration and spectrophotometric method. Results showed that bio-fortified beans contained a higher concentration of both iron and zinc. Iron and zinc contents are expressed in mg/100g. JESCA contained 6.95 of Fe and 2.37 of Zn; Selian 13 had 15.21 of Fe and 4.07 of Zn; Fe and Zn content of Selian 14 was 10.23 and 5.2, respectively and Selian 15 contained 17.02 of Fe and 4.92 of Zn. There was a significant increase ($p<0.05$) in iron concentration in the bio-fortified bean varieties Selian 13 and 15 and of zinc concentration in the bean variety Selian 14. Cooking of beans at 100 ºC, in distilled water for 35-50 minutes reduced dietary anti-nutritional factors in beans by 64.9 – 97.9%. Bio-fortified beans had higher iron and zinc content and exhibited higher nutrient retention upon cooking. Beans can serve as a vehicle for bio-fortification of iron and zinc, and thus serve as an intervention for reducing micronutrient deficiencies. This study which only highlighted processes and findings before intake has shown that bio-fortification has the potential to enhance bioavailability but this is not guaranteed

without taking into account other factors, therefore recommending further studies in animals and humans. The use of bio-fortified beans as a source of iron should be accompanied with information on handling, storage and preparation.

DECLARATION

I, Maria Sylvester Machilu, declare to the Senate of Sokoine University of Agriculture, that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution for a degree award.

________________________________ __________________

Maria Sylvester Machilu Date

(MSc. Candidate)

The above declaration is confirmed:

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Prof. KINABO, J. Date

(Supervisor)

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Dr. MBWANA, H. Date

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DEDICATION

I would love to dedicate this work to my family, my parents Mr. Sylvester Machilu and Mrs. Zipora Machilu; and to my brothers Michael, McAdams and Mathew Machilu for believing in me, supporting me and their selfless giving to make my education journey a possibility.

I would like to dedicate this to work to my family, friends and society as I hope it brings good to the society, inspires and sheds light to more young individuals to further their studies for their good and the good of society as a whole.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
BNFB	Building Nutritious Food Basket
CIAT	International Centre for Tropical Agriculture
DRI	Daily Recommended Intake
DRI	Dietary Reference Intakes
FAO	Food and Agriculture Organization
Fe	Iron
G	Gram
GNR	Global Nutrition Report
Kg	Kilogram
Mg	Milligram
NaOH	Sodium hydroxide
NH_4OH	Ammonium hydroxide
nm	Nanometer
PA	Phytic acid
RDA	Recommended Dietary Allowance
SD	Standard deviation
SITAN	Situational Analysis Report for Bio-Fortification and Bio-Fortified Crops in Tanzania
SPSS	Statistical Product and Service Solutions
SUA	Sokoine University of Agriculture
TARI	Tanzania Agricultural Research Centre
TBS	Tanzania Bureau of Standards
TDHS-MIS	Tanzania Demographic Health Survey – Malaria Indicator Survey
WHO	World Health Organization
Zn	Zinc
%	Percentage

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Bio-fortification is an approach designed to combat micronutrient deficiencies by increasing the concentration and/or bioavailability of essential nutrients in plants without affecting yield and other desirable traits (Petry *et al.*, 2014). It has the potential, especially through conventional breeding to improve the nutritional status of the most vulnerable groups such as the poor and those in rural areas through the staple foods already being consumed by them thus, providing a cost-effective and sustainable, means of delivering more micronutrients and at the same time complement other intervention programs to improve micronutrient intakes (Bouis and Welch, 2010; Bouis *et al.*, 2011).

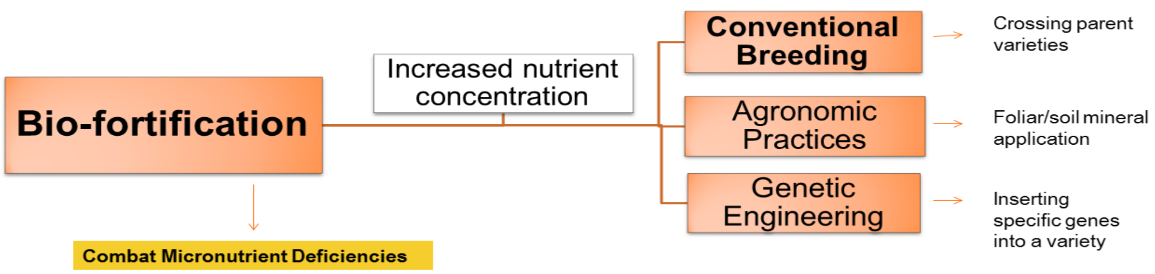

Figure 1: Bio-fortification approaches and rationale in summary

Bioavailability is the fraction of an ingested nutrient that is available for utilization in normal physiological functions and/or for storage. Iron and zinc concentrations in beans are determinants of bioavailability; that is, the higher the concentrations of iron and zinc in beans, the higher the potential for absorption of the minerals in larger amounts once ingested (Frano *et al.*, 2014). Bio-fortified foods usually have high nutrient contents; however, increased levels of nutrients are not necessarily correlated with enhanced bioavailability. This is due to the presence of dietary anti-nutritional factors that affect digestibility and bioavailability of nutrients (Gilani *et al.*, 2012; Petry *et al.*, 2012).

The nutrient content of foods, in this case beans, is usually dependent on a variety of factors such as soils in which the beans are grown. One area may not have the same nutrient composition as another area. Therefore if the soil composition is different, even when the same bean species is in question may not have the same nutrient content (Nchimbi-Msolla and Tryphone, 2010). Soils rich in nutrients (iron, phosphorus, calcium, magnesium, potassium) are likely to produce nutrient-dense crops as compared to soils poor in these nutrients. In addition, plant species, genotype, climatic conditions and agro-technical practices are other factors that affect nutrient content in foods (Glowacka *et al.*, 2015).

Iron and zinc levels in beans may vary due to various factors other than presence of dietary anti-nutritional factors, these other factors include species and variety; bio-fortification processes; and processing factors such as food preparation method, storage and cooking time, temperature, cooking method also choice of utensils used in meal preparation (Carvalho *et al.*, 2012).

1.2 Problem Statement and Study Justification

The main nutritional challenge of bio-fortification is to provide an additional amount of absorbable micronutrient that is high enough to make a useful contribution to filling the gap between current mineral intake and mineral requirements and practically addressing malnutrition (Gilani *et al.*, 2012; Petry *et al.*, 2012; Frano *et al.*, 2014).

Micronutrient malnutrition affects more than one-half of the world's population, iron and zinc being the most deficient. Iron deficiency anemia affects more than 1 billion people and an estimated 49% of the human population is at risk for inadequate zinc in their diet especially in the developing countries (Cichy *et al.*, 2009). The Joint Child Malnutrition Estimates Expanded Database on Stunting, Wasting and Overweight mapped countries with overlapping forms of stunting in children under 5, anaemia among women of reproductive age, and overweight in adult women; and highlighted Tanzania as one of the countries with all three burdens (WHO Global Health Observatory, 2019).

Bio-fortification of staple crops can therefore be a strategy to reduce and prevent micronutrient deficiencies and beans being vehicles for iron bio-fortification especially for the vulnerable groups (Brown *et al.*, 2001; Nestel *et al.*, 2006; Yun *et al.*, 2011; Petry *et al.*, 2014; Delimont *et al.*, 2017). Iron bio-fortification of beans through plant breeding has been proved to be successful especially in increasing iron content in beans (Boy *et al*, 2017); some bean lines even double the content than others. However, some studies question the bioavailability of iron when beans are used as vehicle for iron bio-fortification suggesting that increased levels of nutrients are not necessarily correlated with enhanced bioavailability due to presence of dietary anti-nutritional factors, which are often associated with inhibiting bioavailability of nutrients (Jeong and Guerinot, 2008). A study by Boy *et al* (2017) showed that increasing iron concentrations in beans, also leads to increased levels of dietary anti-nutritional factors; the iron content in a non-bio-fortified variety was 5.4 mg Fe/100g; and phytic acid was 0.98 g PA/kg iron content in an iron bio-fortified variety was 8.8 mg Fe/100g; and phytic acid was 13.20 g PA/kg.

Bio-fortified beans contain significant amounts of phytic acid, tannins and polyphenols. These dietary anti-nutritional factors strongly decrease iron and zinc bioavailability and utilization by the body. Iron has been a nutrient of concern as it is commonly inhibited. Phytic acid also affects zinc concentration by making it unavailable for absorption. In addition, iron and zinc may inhibit one another in solution. Tannin as a phenolic compound has been associated more as an inhibitory factor for non-heme iron, a form of iron obtained from plant-based sources, beans being one of them (Cichy *et al.,* 2009; Petry *et al.,* 2012; Petry *et al.,* 2014). Despite higher levels of anti-nutritional factors in bio-fortified beans, there is increased bioavailability than in common beans.

Despite that several studies acknowledge that there is increased bioavailability with bio-fortified beans, some studies raise concerns. Therefore it is important that for every new variety of bio-fortified beans that is developed, various studies be done to elucidate the outcome of bio-fortification and efficacy of consuming bio-fortified foods.

1.3 Study Objectives

1.3.1 General Objective

The main objective of this study was to determine the iron and zinc content in bio-fortified beans, and examine the effect of cooking and of anti-nutritional factors on both nutrients in bio-fortified beans.

1.3.2 Specific Objectives

- To determine mineral (iron and zinc) content in beans
- To determine the concentration of dietary anti-nutritional factors (phytic acid, tannins and polyphenols) in beans

- To determine the effect of cooking on mineral content and dietary anti-nutritional factors

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Beans as a Vehicle for Iron and Zinc Bio-fortification

The main nutrient obtained from beans is protein as well as significant amounts of minerals, for families and populations of low socio-economic status; beans serve as main source of such nutrients (Laparra *et al.,* 2009). The common bean is one of the crops targeted for iron bio-fortification because it is a major staple for over 300 million people in Africa and Latin America, thus, bio-fortified beans are a promising vehicle for increasing intakes of bioavailable iron in human populations where beans are a dietary staple as it capitalizes on the regular daily intake of a consistent and large amount of food staples (Nestel *et al.*, 2006; Tako *et al.,* 2011; Petry *et al.,* 2014). In Tanzania, about 75% of rural households depend on beans as a staple food (CIAT, 2008).

Iron (Fe) and zinc (Zn) in beans have been reported to vary depending on the enabling conditions. Some of the studies reported as follows; Fe ranges 3.5 to 9mg/100g beans (Petry *et al.,* 2010); Zn ranges from 1.78 to 3.79mg/100g (Celmeli *et al.,* 2018); Fe ranges from 4.30 to 6.81 mg/100g and Zn from 3.18 to 6.02 mg/100g (Rani and Punia, 2017) and mean Fe concentration is 5.5mg/100g (Boy *et al.,* 2017).

A study by Solomons and Schümann (2017) suggested that food fortification and particularly bio-fortification offer the greatest theoretical promise for increased iron and zinc concentrations in beans. Plant breeding strategies including bio-fortification have been reported to increase the iron concentration of common beans by 60–80% (Petry *et al.,* 2010); some have been reported to double the concentrations in non-bio-fortified varieties (Jeong and Guerinot, 2008; Petry *et al.,* 2012); some have increased concentrations of beans lines to 10mg/100g (Boy *et al.,* 2017); and some have increased concentrations 4 -10 folds more than the common varieties. These findings suggest beans as a reliable vehicle for iron bio-fortification.

According to the Codex Alimentarius Commission, for food to be labeled as a ‘source’ of a nutrient it should provide more than 15% of the dietary reference intakes (DRI) of the desired nutrient per 100g and for it to labeled as a ‘high’ source, it should contain at least double the amount as a source (FAO, 1997).

2.2 Bio-fortification Status of Beans in Tanzania

Bio-fortification of beans, alongside bio-fortification to produce Pro-Vitamin A maize in Tanzania began in 2017; two bean varieties Selian 14 and Selian 15 were released in the same year joining other countries in the region such as Rwanda, Burundi, Democratic Republic of Congo and Uganda. The Situational Analysis Report for Bio-Fortification and Bio-Fortified Crops in Tanzania (SITAN) highlighted that most of the national policies, strategies and acts of parliament do not explicitly cover bio-fortification, thus calling for increased advocacy efforts towards production and use of bio-fortified crops (Mulongo *et al.,* 2017).

2.3 Micronutrient Deficiencies

Globally, micronutrient malnutrition affects more than two billion people, mostly among resource-poor families in developing countries (Kumar *et al.,* 2018). The most vulnerable groups for micronutrient deficiencies are pregnant women, lactating women, women of reproductive age and young children, school children and adolescents and the elderly (Nestel *et al.,* 2006; Best *et al.,* 2010; Ross, 2010; Sudhagandhi *et al.,* 2011; More *et al.,* 2013; Katungwe *et al.,* 2015; Murray-Kolb *et al.,* 2017). Iron and zinc are minerals that regulate and are part of important processes in human bodies, and a deficiency of these minerals are a public health concern; iron deficiency may cause anemia while zinc deficiency may affect physical senses including smell and taste (Pereira *et al.*, 2014).

2.3.1 Iron deficiency

Iron plays several vital roles in the body, especially in the formation of hemoglobin within the red blood cells to participate in transporting oxygen from the lungs to the body tissues. It is also a transport medium for electrons within the cells, and as an integrated part of important enzyme systems in various tissues (Gupta, 2014). Iron concentration in the body tissues has to be regulated as excess iron leads to tissue damage, due to formation of free radicals. However, iron deficiency and iron deficiency anemia affects pregnancy, pregnancy outcomes and infant health; also cognitive performance especially in school children (Nestel *et al.,* 2006; Best *et al.,* 2010; Ross, 2010; Sudhagandhi *et al.,* 2011; More *et al.,* 2013; Katungwe *et al.,* 2015; Murray-Kolb *et al.,* 2017).

Iron deficiency anaemia prevalence is 58% in children and 45% in women of reproductive age in Tanzania (TDHS-MIS, 2015). The prevalence of anaemia is 32.8% in adolescent girls and women aged 15 to 49 years; and it is substantially higher in pregnant (40.1%)

than non-pregnant (32.5%) adolescent girls and women (Global Nutrition Report, 2020) Iron and zinc deficiencies in human nutrition are widespread in developing Asian and African countries (Prasad *et al.*, 2014). To prevent iron deficiency in healthy individuals, on average a daily level of intake sufficient to meet the nutrient requirements is recommended (Table 1); individuals who are already sick or deficient may require higher intake levels than the recommended (National Institute of Health, 2020).

Table 1: Recommended Dietary Allowances (RDAs) for Iron (mg)

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.27 *s	0.27 *		
7–12 months	11	11		
1–3 years	7	7		
4–8 years	10	10		
9–13 years	8	8		
14–18 years	11	15	27	10
19–50 years	8	18	27	9
51+ years	8	8		

* Adequate Intake (AI)

Source: National Institute of Health, 2020

2.3.2 Zinc deficiency

Zinc is a micronutrient that plays an important in human nutrition; other nutrients also depend on zinc for their activity. For example, about 10 % of human proteins require zinc to maintain their catalytic activity (Sida-Arreola *et al.*, 2017). Zinc deficiency can lead to a weakened immune system, an increased risk for infectious diseases and growth retardation (stunting) especially among children less than 5 years of age. Zinc deficiency also increases the risk of diarrhea and respiratory tract infections, and adversely affects pregnancy outcomes such as death and intra-uterine growth retardation (Walker *et al.*, 2009; Cichy *et al.*, 2009; Engle-Stone *et al.*, 2014; Al Hasan *et al.*, 2016). To prevent zinc deficiency in healthy individuals, on average a daily level of intake sufficient to meet the nutrient requirements is recommended (Table 2); individuals who are already sick or deficient may require higher intake levels than the recommended (National Institute of Health, 2020)

Table 2: Recommended Dietary Allowances (RDAs) for Zinc (mg)

Age	Male	Female	Pregnancy	Lactation
0–6 months	2 *	2 *		
7–12 months	3	3		
1–3 years	3	3		
4–8 years	5	5		
9–13 years	8	8		
14–18 years	11	9	12	13
19+ years	11	8	11	12

* Adequate Intake (AI)

Source: National Institute of Health, 2020

2.4 Bioavailability of Iron and Zinc from Beans

According to Petry *et al.*, (2010), studies for assessing bioavailability of iron from beans showed low iron absorption at a range of 1–3%. This suggested that, for bean bio-fortification to have a positive and significant impact on iron status in the body, it is important to increase the iron bioavailability as well (Boy *et al.*, 2017).

2.5 Dietary anti-nutritional factors in beans

Dietary anti-nutritional factors or inhibitors of nutrient absorption are crucial to plants such as legumes yet are deleterious to humans (Hamid *et al.*, 2017). These include saponins, phytic acid, plant sterols, phenolic compounds, enzyme inhibitors and lectins. They form insoluble complexes with metals reducing absorption of minerals and causing severe mineral ions deficiency in human; which can lead to detrimental effects to both humans and animal in their growth and function by impairing intake, uptake or utilization of other nutrients and feed components or by causing discomfort and stress (Bora, 2014). Anti-nutritional factors such as phytic acid; and polyphenols such as tannins are endogenous substances in the common bean (Rui *et al.,* 2016). Plants, bacteria and fungi synthesize these as a protective or defense mechanism against various unfavorable conditions. Despite the fact that these anti-nutritional factors reduce or inhibit nutrient absorption, a number of them have properties that have been shown to be of benefit to the human body such as antimicrobial, anticancer properties (Nikmaram *et al.,* 2017).

High prevalence of iron and zinc deficiencies globally is linked to anti-nutritional factors phytic acid and other iron and zinc inhibitors (Delimont *et al.,* 2017). Measures to reduce their inhibitory effects can be done through selection of beans with high iron, or low phytate and low polyphenol content, and promote intake of iron absorption enhancers in the diet (Frano *et al.,* 2014; Petry *et al.,* 2014).

2.5.1 Phytic acid

Phytates form insoluble complexes with iron and zinc and interferes with their digestibility and or absorption (Al Hasan *et al.,* 2016; Delimont *et al.,* 2017; Mihrete, 2019). The absence of intestinal phytate enzymes in human's digestive systems leads to deficiencies of these micronutrients (Prasad *et al.,* 2014; Nikmaram *et al.,* 2017). Phytates also bind to proteins causing reduced protein solubility (Pusztai, 1991). On average, the phytate content in beans is 1 - 2%, provides antioxidant effects, and may lower the risk of colon and breast cancer (Venter and Van, 2001).

2.5.2 Polyphenols

Polyphenols just as other anti-nutritional factors inhibit nutrient absorption from foods by binding and forming complexes thus rendering the nutrients unavailable for absorption. In beans polyphenols bind to and forms complexes with iron thus inhibiting iron absorption (Ma *et al.,* 2011). Despite that it is not the most significant iron and zinc inhibitor in relative comparison to phytic acid, in absence of phytic acid it becomes more dominant or more pronounced as an anti-nutritional factors in beans (Petry *et al.,* 2010). A human epidemiological study (Pérez-Jiménez *et al.*, 2010) has shown that various polyphenols have antioxidant and anti-inflammatory properties that might be preventive and/or therapeutic effects for cardiovascular disease, neurodegenerative disorders, cancer, and obesity.

2.5.3 Tannins

Tannins are complex, astringent and water soluble phenolic compounds that are known to reduce the bioavailability of nutrients in the gut, they pose some health consequences *viz.* anti-nutritional effect, reduced digestibility, mutagenic and carcinogenic effects and inducer, hepatotoxic activity and co-promoters of several diseases. Tannins affect nutrient absorption through inhibiting digestive enzymes and lowering digestibility of nutrients, especially protein and carbohydrates (Khattab and Arntfield, 2009). However, tannins may affect nutrient absorption in single meals but the effect reduces over time (Delimont *et al.*, 2017). Despite this, tannins also have the ability to precipitate with proteins and promoting resistance of the crops from microbial, insect and pest infestations.

Tannins also serve as anti-oxidants, with cardiovascular-protection and anti-cancer properties, also anti-inflammatory properties and wound healing (Polshettiwar *et al.,* 2007; Raes *et al.,* 2014; Delimont *et al.,* 2017).

2.5.4 Processes that lower concentrations of inhibitors in beans

Various methods both physical and chemical can be employed to reduce or remove anti-nutritional factors in legumes, some of these methods include; soaking, cooking, germination, fermentation, selective extraction, irradiation, and treatment with enzymes (Sharma *et al.,* 2016).

Cooking after soaking or without soaking is a method commonly employed and necessary in meal preparation of beans, boiling being the most common. studies that have assessed the effect of heat or thermal processing on anti-nutritional factors and bioavailability of minerals particularly iron and zinc, observed that cooking reduced dietary anti-nutritional factors or inhibitors and led to increased bioavailability of the nutrients iron and zinc (Khattab and Arntfield, 2009; Hefnawy, 2011).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area

All bean varieties were collected from the International Centre for Tropical Agriculture (CIAT) in collaboration with Tanzania Agricultural Research Institute (TARI) both located in Seliani, Arusha; Latitude 03º22' S and Longitude 36º37' E above the sea level, Tanzania. Nutrient analysis was conducted at Sokoine University of Agriculture (SUA). Sokoine University of Agriculture is located in Morogoro region (Latitude: -6.8514° or 6° 51' 5" south; Longitude: 37.6572° or 37° 39' 25.9" east and; Elevation: 550 meters), Tanzania. The study was conducted in a period of twelve months starting from September 2018 to August 2019.

3.2 Study Design

This study undertook a cross sectional design involving several laboratory tests on nutrient content and dietary anti-nutritional factors and the effect of cooking on both nutrient content and dietary anti-nutritional factors on the sample bean varieties.

3.3 Sample Size

The samples in this study were beans. A total number of four (4) bean varieties were used in the study; JESCA (a non-bio-fortified bean variety), Selian 13, Selian 14 and Selian 15, which are bio-fortified high-iron bean varieties. The samples were laboratory tested and analyzed in triplicates.

The samples were grown in Selian Arusha where the mean minimum temperature ranges from 12°C to 15°C and mean maximum temperature ranges from 22°C to 28°C (Massawe *et al.*, 2017). The planting time was from March 15th 2018 and the beans were harvested by July 2018. The beans were then sorted and stored in burlap bags which were placed on top of hard wooden pallets at room temperature varying a ranging averagely from 14 to 24℃ from July 2018 to April 2019 when the beans were sent to Morogoro for analysis. The soil in which the beans were grown had a pH ranging from 5.56 to 5.96; with iron at a range of 2.527 to 5.418 mg/100g; and zinc at 0.04 to 0.08mg/100g. Other traits of the bean varieties have been described Table 3.

Table 3: Traits of the bean varieties used in the study

Traits/Name	JESCA	Selian 13	Selian 14	Selian 15
Growth Habit	Bush	Bush	Climber	Climber
Seed size	Large seeded	Medium seeded	Medium seeded	Large seeded
Yield Potential	< 1750 kg/ha	< 1500 kg/ha	>2000 kg/ha	>2000 kg/ha
Maturity	75-80 days	67-75 days	90-110 days	90-110 days
Suitability	Mid-altitude areas	Mid to high-altitude areas	Mid to high-altitude areas	Mid to high-altitude areas
Cooking Time		39-50 minutes	19-40 minutes	19-40 minutes
Resistance	Moderately resistant to Angular Heat Spot. Bacterial Blight and Rust	Moderately resistant to Angular Heat Spot. Bacterial Blight and Rust	Moderately resistant to Anthracnose and Bean Virus	Moderately resistant to Anthracnose and Bacterial blight

Source: TARI Selian Centre

3.4 Sampling and Sample Collection

The sampling technique employed in this study was purposive and random sampling of the bean varieties to be included in the study. A purposive selection of bean varieties to be included and assessed in the study was done. The bean samples were collected from CIAT-Selian in Arusha. Beans were randomly selected from the top-, mid- and bottom of the sacks by a grain sampler; for each bean variety, 5 out of ten 10 sacks were sampled and placed in a dish for harmonization by swirling by hand, then 2kg of beans were weighed double-packed in plastic bags, collected together in a box and transported by bus to Morogoro within 24 hours. Upon delivery, the samples were stored under controlled temperature (4℃) for a week in the laboratory refrigerator ready for the tests.

3.5 Laboratory analysis

3.5.1 Sample preparation

Dry and raw samples (200g) from each bean variety were weighed, then blended into powder (using Professional Heavy Duty Blender; STRONGERTECH-PMC), sieved (in 1mm thickness) and packed into air-tight containers for proximate and nutrient analysis.
The same initial procedure was applied for the cooked samples where, each bean variety 200g of dry beans were weighed then boiled (using gas cooker and aluminum pan) according to the method described by Hefnawy (Hefnawy, 2011) at 100 ºC in distilled water until soft at a range of 35-50 minutes per sample for all four samples. The cooked beans were oven dried (Wagtech Laboratory Oven) at 60℃ and blended into powder and packed into air-tight containers for proximate and nutrient analysis.

Samples for mineral (Fe and Zn) analysis were prepared as per AOAC method (2005) as follows; 5g of each raw sample in powder form was weighed and placed in porcelain crucibles which were dried prior at 100℃ for 10 minutes, cooled in desiccator and weighed (W1). The sample-filled crucibles were the weighed (W2) then placed in a muffle furnace (CARBOLITE CWF 11/5 - 1100℃) at 550℃ for 8 hours, the samples were removed left to cool and reweighed (W3). The same procedure was repeated for the boiled-dried-blended samples as well. The percentage ash content which was later used in determination of iron and zinc was calculated as follow;

$$\% \text{ Ash Content} = \frac{W3 - W1}{W2 - W1} \times 100$$

3.5.1 Mineral content of beans

The content of mineral elements was determined initially using digestion with nitric-perchloric acid, and then the samples were analyzed and determined through atomic absorption spectrophotometry using an atomic absorption spectrophotometer using wave lengths of 248.3 nm for iron, and 213.9 nm for zinc (AOAC, 2005), method number 974.27 at Soil Science laboratory (AOAC, 2005).

3.5.2 Determination of dietary anti-nutritional factors

3.5.2.1 Phytic acid

Determination of phytic acid was by the principle of phytate precipitation according to the method of Wheeler and Ferrel (1971) as adopted by Makkar *et al.,* (2007); and Sharma *et al.,* (2016) which was by spectrophotometric determination of precipitation of phytate. 0.5g of each sample in both raw and cooked forms were weighed into a 125ml Erlenmeyer flask, followed by phytate extraction in 50ml of 3% TCA with occasional swirling by hand for 45minutes Ferric nitrate was used as standard with iron content of the precipitate determined colorimetrically at absorbance 480nm and the phytic acid content calculated from the value obtained

The suspension was then centrifuged (Baird & Tatlock Auto Bench Centrifuge Mark IV) then 10ml of the supernatant to a centrifuge tube followed by a rapid addition of 4ml $FeCl_3$ solution. The contents were then heated in boiling water bath. The clear supernatant was then decanted followed by washing of the precipitate twice by dispersing it in 20ml 3% TCA and heated in boiling water for 5 minutes and centrifuged. The precipitate was then re-washed and dispersed in 3ml distilled water and heated for 30 minutes followed by filtration through a retentive paper; re-washing of the precipitate with 60 to 70ml of hot distilled water; and the filtrate was discarded.

The precipitate on the filter paper was transferred and dissolved into a 100ml volumetric flask containing 40ml of hot 3.2N HNO_3. The filtrate paper was washed with several portions of distilled water and collected the washings in the same flask. Flask and contents were then cooled at room temperature and brought the volume to 100ml with distilled water. 5ml of the aliquot was transferred to another 100ml volumetric flask and diluted approximately 70ml with distilled water. 20ml of 1.5M KSCN was added to each flask and volume brought to 100ml with distilled water. Color was read immediately (within 1 minute) at 480nm using a spectrophotometer (X-ma 3000 Spectrophotometer). 2.5ml of $Fe(NO_3)_3$ solution was taken and volume was made up to 250ml in a volumetric flask. 2.5-,5-, 10-, 15-, and 20-ml aliquots of this working standard were pipetted into a series of 100ml volumetric flasks and diluted them to approximately 70ml with distilled water.

A reagent blank with each set of samples was run and the micrograms of iron present in the test were determined from the calibration curve, and calculated the phytate P as per the following equation:

Phytate P (mg/100g sample) = [Fe (μg) × 15]/ Weight of sample in g

3.5.2.2 Polyphenols

Polyphenols were analyzed in terms of total phenolic content by aqueous methanolic extract of raw and cooked beans and was determined according to the Follin-Ciocalteu reagent spectrophotometer method with the underlying principle being spectrophotometry Sharma *et al.,* 2016). Total phenolic content was calculated in the methanolic extracts, according to the Folin-Ciocalteu method with as modified by Morsy *et al.*, (2015). 1 ml of the extract was mixed with 5ml of Folin-Ciocalteu phenol reagent (diluted with water 1:10v/v) 4ml of sodium carbonate (75g/L). The tubes were agitated for 30 seconds and allowed to stand for color development. The absorbance was determined at 540nm against blank and gallic acid was used as a standard. A calibration curve of gallic acid (0-0.10 mg/ml) was prepared and tested under similar conditions. The results were expressed as mg of gallic acid equivalents

3.5.2.3 Tannins

Determination of tannin content was by the Follin-Dennis reagent titration and spectrophotometric method as described by Pearson (1976) and revised by Nair *et al.*, (2015); Sharma *et al.,* (2016); and Vanimakhal and Ezhilarasi, (2016). 100 ml of petroleum ether was added to 20g of the processed sample (both for dry-raw-blended and boiled-dried-blended) and covered with a nylon-wrap for 24 hours. The sample was then filtered with a filter paper (Whatman No. 2 retentive paper), and the filtrate was allowed to stand for 15 minutes for petroleum ether to evaporate. It was then re-extracted by soaking in 100 ml of 10% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate was collected; 25 ml of Ammonia hydroxide (NH_4OH) was added to the filtrate to precipitate the alkaloid, which was then heated to remove the residual NH_4OH in solution. 5ml of the remaining solution was taken and 20ml of ethanol was added to it and titrated with 0.1M sodium hydroxide (NaOH) using phenolphthalein as an indicator until color change to pink was observed. The reading was taken at absorbance 710nm (X-ma 3000 Spectrophotometer) and the tannin content was then calculated in %.

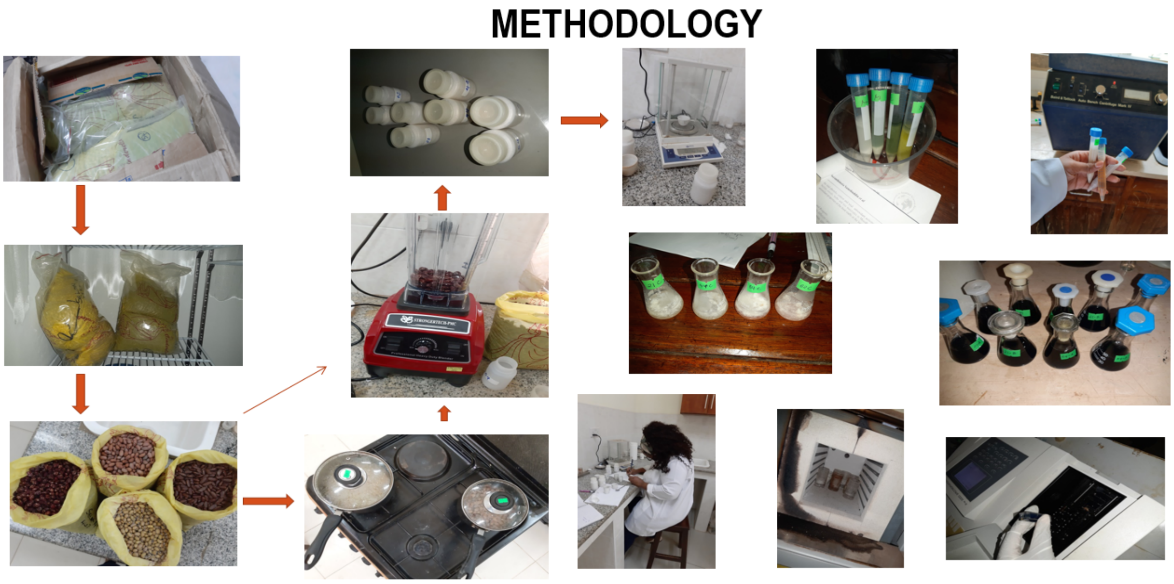

Figure 2: A summary photo of some laboratory procedures

3.6 Statistical Analysis

The Analysis of Variance method (ANOVA) was adopted in this study because it allows one to determine whether the differences between the mineral (iron and zinc) content in beans samples are simply due to random error (sampling errors) or whether there are systematic treatment effects that causes the mean in one group to differ from the mean in another. It also helps the study to ascertain whether there is a difference between the concentration of dietary anti-nutritional factors (phytic acid, tannins and polyphenols) in beans, as well as whether there is a difference between the effect of cooking on mineral content and dietary anti-nutritional factors.

Data collected was analyzed using the software Statistical Product and Service Solutions (SPSS) version 20. From the data the following analysis and test were done; analysis of variance (ANOVA). A one-way ANOVA was done, the dependent variables were the mean values of each of the treatment (bio-fortified and non-bio-fortified); and the independent variables were the different treatments. After performing a one way ANOVA, comparison of the treatment values was done using the least significance difference (LSD) test at 5% level of probability ($p<0.05$). The results were expressed as means (with standard deviations) and presented in tabular forms.

CHAPTER FOUR

4.0 RESULTS

This study included analyses of four varieties of beans; a non-high-iron bean variety (JESCA) and bio-fortified high-iron bean varieties (Selian 13, Selian 14 and Selian 15). The main objective was to determine the nutrient content in bio-fortified beans with a focus on iron and zinc and how the presence and concentration of dietary anti-nutritional factors influence iron and zinc bioavailability. These findings would then enable selection of a variety (ies) more desirable and recommend for meals as a complement to already established interventions aiming to fight malnutrition, in this case micronutrient (iron and zinc) deficiencies.

The results presented in Table 4 show the coefficients of determination (R^2) from the analysis of iron, zinc, phytic acid, polyphenols and tannins. The R^2 from all varieties were close to one (1) thus depicting a linear relationship between concentrations and absorbance.

Table 4: Coefficients of Determination of Nutrient Content

Item for Analysis	Coefficient of Determination (R^2)
Iron	0.9993
Zinc	0.9915
Phytic acid	0.9289
Polyphenols	0.9922
Tannins	0.9440

Table 5 shows results of iron and zinc content of the 4 bean varieties. JESCA, the non-bio-fortified bean variety was considered as a benchmark for comparison; Selian 14 showed a significant difference at $p \leq 0.05$ in concentrations of the nutrient zinc compared to other varieties. Higher zinc concentrations were observed in the bio-fortified bean varieties particularly Selian 14 and Selian 15 while the least concentration was observed in JESCA. The variety Selian 15 had the highest (17 mg/100g) iron content among all varieties. The difference was statistically significant ($p<0.05$). There was a significant difference between JESCA and Selian 13 and Selian 14; these varieties also exhibited a significant difference with Selian 15 at $p \leq 0.05$ which is a bio-fortified high-iron variety.

Table 5: Iron and zinc content of Beans

Bean variety	Iron (mg/100g)	Zinc (mg/100g)
JESCA	6.95 [a]	2.37 [a]
Selian 13	15.21 [b]	4.07 [a]
Selian 14	10.23 [a]	5.20 [b]
Selian 15	17.02 [b]	4.92 [a]

Values in a column with different superscripts are significantly different at $p \leq 0.05$

Table 6 presents findings from a comparative analysis between raw and cooked beans in concentrations of iron (Fe). The results show that there was a decrease of iron concentration upon cooking of the beans; the statistical analysis suggested that there was a significant difference (decrease) $p \leq 0.05$ of iron concentrations in all bean varieties upon cooking. Moreover, Figure 3 shows that JESCA had the highest percentage loss of 47% after boiling while Selian 14 had the least percentage loss of 10.4%. Selian 13 and Selian 15 had a percentage loss of 20.8% and 19.8%, respectively after boiling.

Table 6: Effect of Cooking on Iron (Fe) Content (mg/100g)

	Varieties			
Treatment	**JESCA**	**Selian 13**	**Selian 14**	**Selian 15**
Raw	6.95 ^{a}	15.21 ^{a}	10.23 ^{a}	17.02 ^{a}
Boiled (mg/100g)	3.65 ^{b}	12.04 ^{b}	9.17 ^{b}	13.65 ^{b}
Difference	3.3	3.2	1.1	3.4
% loss	**47.5**	**20.8**	**10.4**	**19.8**

Values in a column with different superscripts are significantly different at $p \leq 0.05$

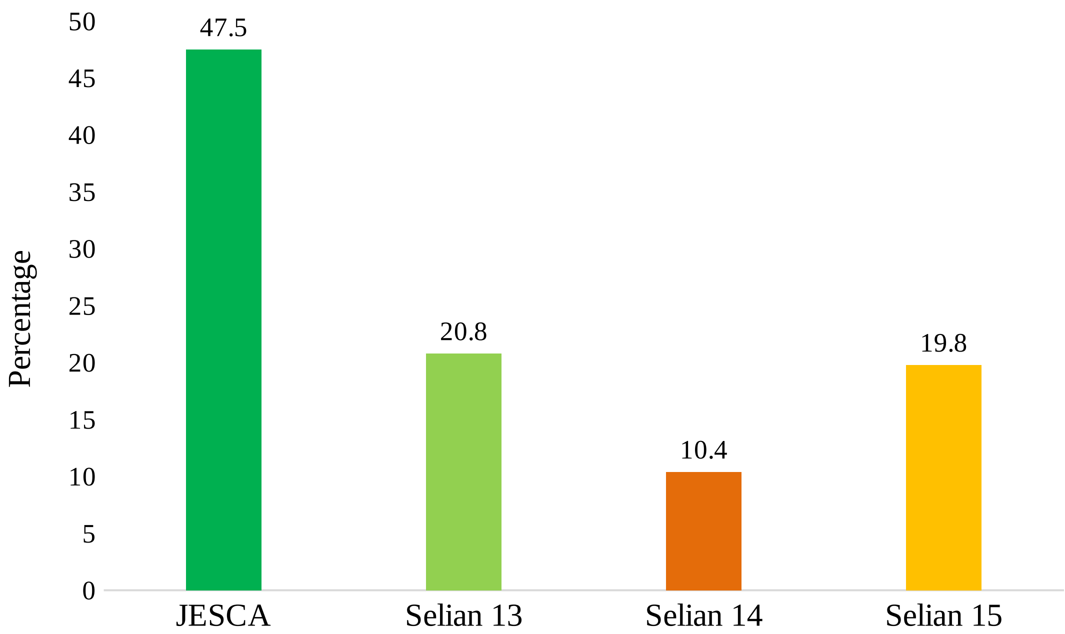

Figure 3: Losses of the nutrient iron after cooking

Table 7 presents results of the effects of cooking on zinc content in the beans. The analysis showed that there was a decrease of zinc concentration upon cooking of the beans; the statistical analysis suggested that there was a significant difference (decrease) ($p \leq 0.05$) of zinc concentrations in all bean varieties upon cooking. Further analysis as presented in Figure 4 shows that JESCA had the highest percentage loss of 81.9% after boiling while Selian 13 had the relatively least percentage loss of 40%. Selian 14 and Selian 15 had a percentage loss of 58.7% and 56.3% respectively after boiling

Table 7: Effect of Cooking on Zinc (Zn) Content (mg/100g)

	Varieties			
Treatment	**JESCA**	**Selian 13**	**Selian 14**	**Selian 15**
Raw	2.37 [a]	4.07 [a]	5.20 [a]	4.92 [a]
Boiled	0.43 [b]	2.44 [b]	2.15 [b]	2.15 [b]
Difference	1.94	1.63	3.05	2.77
% loss	81.9	40.0	58.7	56.3

Values in a column with different superscripts are significantly different at $p \leq 0.05$

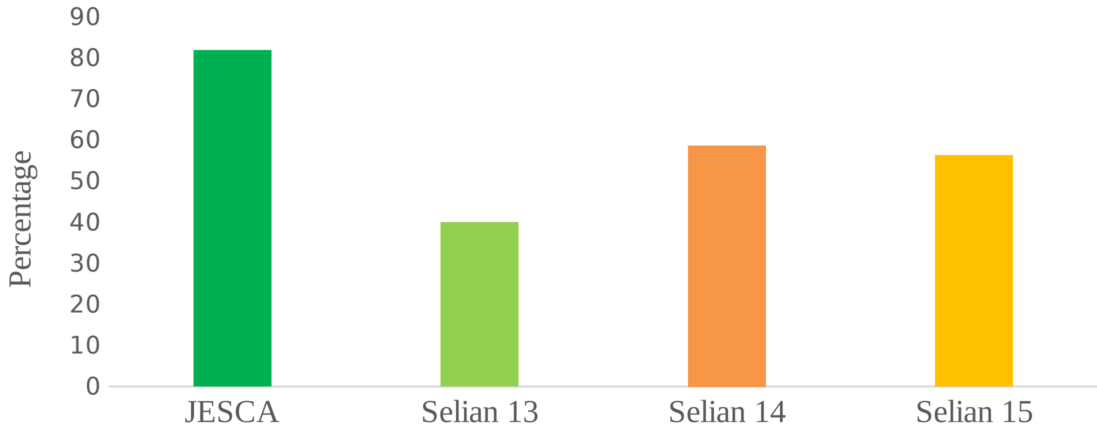

Figure 4: Losses of the nutrient zinc after cooking

Table 8 shows results from analysis of dietary anti-nutritional factors in raw beans; include findings from analysis of phytic acid content, tannin content and polyphenols in all four bean varieties. The findings from the analysis showed that there was no significant difference ($p \leq 0.05$) of the all the dietary anti-nutritional factors. This means that the levels of dietary anti-nutritional factors in all bean varieties are similar and that bio-fortification did not have a significant impact in levels of anti-nutritional factors in beans.

Table 8: Dietary Anti-nutritional Factors Content in Beans

Bean	Phytic acid (mg/100g)	Tannins (g/100g)	Polyphenols (mg/g)
JESCA	0.28 [a]	7.17 [a]	1.21 [a]
Selian 13	0.41 [a]	6.84 [a]	0.99 [a]
Selian 14	0.37 [a]	6.11 [a]	1.02 [a]
Selian 15	0.48 [a]	5.38 [a]	1.23 [a]

Values in a column with different superscripts are significantly different at $p \leq 0.05$

Table 9 presents findings from a comparative analysis between raw and cooked beans in concentrations of phytic acid. The analysis showed that there was a decrease of phytic acid content upon cooking of the beans; the statistical analysis suggested that there was a significant difference (decrease) at $p \leq 0.05$ of phytic acid content in all bean varieties upon cooking.

Table 9: Effect of Cooking on the Phytic Acid Content (mg/100g) of Beans

Treatment	JESCA	Selian 13	Selian 14	Selian 15
Raw	0.28 [a]	0.41 [a]	0.37 [a]	0.48 [a]
Boiling	0.09 [b]	0.10 [b]	0.13 [b]	0.01 [b]

Values in a column with different superscripts are significantly different at $p \leq 0.05$

Table 10 presents findings from a comparative analysis between raw and cooked beans in concentrations of polyphenols. The analysis showed that there was a decrease of polyphenols content upon cooking of the beans; the statistical analysis suggested that there was a significant difference (decrease) at $p \leq 0.05$ of phytic acid content in all bean varieties upon cooking. This suggested that cooking decreases the polyphenols content in beans thus, signifying a probability for increased bioavailability/bio-accessibility of iron and zinc from beans.

Table 10: Effect of Cooking on the Polyphenols Content (mg/g) of Beans

Treatment	JESCA	Selian 13	Selian 14	Selian 15
Raw	1.21 [a]	0.99 [a]	1.02 [a]	1.23 [a]
Boiling	0.03 [b]	0.08 [b]	0.07 [b]	0.20 [b]

Values in a column with different superscripts are significantly different at $p \leq 0.05$

Table 11 presents findings from a comparative analysis between raw and cooked beans in concentrations of tannins. The analysis showed that there was a decrease of tannic acid content upon cooking of the beans; the statistical analysis suggested that there was a significant difference (decrease) at $p \leq 0.05$ of tannic acid content in all bean varieties upon cooking.

Table 11: Effect of Cooking on the Tannic Acid Content (g/100 g) of Beans

Treatment	JESCA	Selian 13	Selian 14	Selian 15
Raw	7.17 ± 0.0^{a}	6.84 ± 0.22^{a}	6.11 ± 0.17^{a}	5.38 ± 0.23^{a}
Boiling	0.25 ± 0.00^{b}	0.26 ± 0.00^{b}	0.21 ± 0.00^{b}	0.19 ± 0.00^{b}

Values in a column with different superscripts are significantly different at $p \leq 0.05$

Table 12 presents findings from comparative analysis between dietary anti-nutritional factors on the differences in concentrations between raw and cooked forms, and percentage losses of the dietary inhibitors upon cooking. Generally, the bean varieties exhibited higher losses of all the dietary inhibitors with Selian 15 showing more desirable losses comparatively. Percentage losses of the inhibitors were as follows; phytic acid (64.86 - 97.92%), polyphenols (83.74 - 97.52%) and tannins (96.2 - 96.56%).

Table 12: Losses of dietary anti-nutritional factors in beans after cooking

Treatment		JESCA	Selian 13	Selian 14	Selian 15
Phytic acid (mg/100g)	Raw	0.28	0.41	0.37	0.48
	Boiled	0.09	0.10	0.13	0.01
	Difference	0.19	0.31	0.24	0.47
	%	**67.86**	**75.61**	**64.86**	**97.92**
Polyphenols (mg/g)	Raw	1.21	0.99	1.02	1.23
	Boiled	0.03	0.08	0.07	0.20
	Difference	1.18	0.91	0.95	1.03
	%	**97.52**	**91.92**	**93.14**	**83.74**
Tannic acid	Raw	7.17	6.84	6.11	5.38
	Boiled	0.25	0.26	0.21	0.19

(g/100g)					
	Difference	6.92	6.58	5.90	5.19
	%	**96.51**	**96.20**	**96.56**	**96.47**

CHAPTER FIVE

5.0 DISCUSSION

5.1 Iron and Zinc Content of Beans

This study observed that bio-fortification has the potential to increase content of iron and zinc in beans and that beans can serve as a vehicle towards addressing micronutrient deficiencies. A study by Solomons and Schümann (2017) suggested that food fortification and particularly bio-fortification offers the greatest theoretical promise for increased iron and zinc concentrations in beans,

5.1.1 Iron content

In this study, it was observed that there was no significant difference ($p<0.05$) in iron concentration in JESCA and Selian 14 bean varieties and in Selian 13 and Selian 15 bean varieties. Higher concentrations of iron were observed in bio-fortified varieties than in non-bio-fortified varieties. Selian 15 had the highest iron content and JESCA had the least iron content. This suggested that bio-fortification could have increased the iron content in beans. Selian 14, which is a bio-fortified variety showed an increase in iron content but the change was not statistically significant. Nevertheless, it had a higher iron content compared to the JESCA variety (a non-bio-fortified). Bio-fortification has the potential of increasing the iron concentration of common beans by 60–80% (Petry *et al.*, 2010) and therefore meeting the recommended dietary allowances (7-27mg) for iron. Selian 15 variety showed the most potential.

The cooking treatment resulted into significant differences ($p<0.05$) in iron content of the beans before and after cooking. The iron concentration in beans decreased with cooking suggesting that cooking reduced iron concentrations. This can be attributed to the cooking process and sample preparations after cooking prior to analysis; boiling resulted to leaching into the broth; but in this study it was reduced to a minimum and the whole cooked sample was oven dried. The reduction in nutrient content may be due to dried-broth loss in the aluminium foil during oven-drying; loss of sample as well as nutrients during transfer from one vessel to another; and nutrient to nutrient interactions between iron and zinc may inhibit one another when in solution. Hefnawy (2011) attributed nutrient decrease to preparation methods such as soaking; choice of cooking method such as boiling, autoclaving or microwaving; and choice of utensils and cooking pots.

In this regard to entirely conclude that bio-fortification would enhance bioavailability could be misleading. Further analysis of the post-harvest processes and cooking would offer better insights to the outcomes of the bio-fortified beans. Thus, practical measures are necessary to assess and ensure that increased mineral content translates to bio-availability of the nutrients. However, a study by Carvalho *et al.,* 2012 showed that there was an increase in iron concentrations upon cooking.

Iron retention was high for all treatments, which implies that the cooking method used in the present study did not reduce the amount of iron in the beans. The bio-fortified varieties exhibited higher iron retention (79.2-89.6%) than the non-bio-fortified JESCA (52.5%). Selian 14 showed the highest potential for iron retention after cooking.

It should be noted that inadequate dietary intake of iron and iron deficiency anemia affects maternal, infant, young children and adolescent health as well as cognitive performance of school children. It also lowers the ability of red blood cells to carry oxygen and the body to be able to generate energy, hence one's ability to concentrate and articulate issues. (More *et al.*, 2013; Katungwe *et al.*, 2015; Murray-Kolb *et al.*, 2017).

5.1.2 Zinc content

The concentration of zinc in the four bean varieties showed no significant difference ($p<0.05$). The variety Selian 14 had a higher concentration of zinc suggesting that bio-fortification may or may not have a significant impact in increasing concentration of zinc in the beans. The high-iron bean varieties demonstrated higher iron and zinc retention after cooking compared to the JESCA variety therefore justifying that bio-fortified variety can be better sources of zinc and potential to meet nutrient requirements of zinc as per WHO recommendations. Selian 14 showed to have the most potential of providing zinc in the diet. Nevertheless, it is necessary to understand the level of bioavailability of nutrients from the bio-fortified beans.

The zinc content in the JESCA variety and for each of the other bean varieties (Selian 13, Selian 14 and Selian 15), showed a significant decrease ($p<0.05$) before and after cooking. Despite the decrease in zinc content, the proportion of zinc retention was high for all varieties with an exception of JESCA variety, which is non-bio-fortified variety. Selian 13 had the highest retention of zinc after cooking. This implies that the bio-fortified varieties have the potential to retain more zinc. Bio-fortification is a cost-effective approach to addressing zinc deficiency particularly in rural areas and in poor households. Another similar study suggested that boiling may cause nutrient reduction but still have high retention to meet nutrient requirements of individuals (Pereira *et al.*, 2014). Boiling (which was followed by oven-drying) was effective in the preservation of zinc in beans. Cooking beans by boiling is the common method in Tanzania, therefore in efforts to reduce zinc deficiency this method would still be effective in providing bean meals containing adequate dietary zinc if consumed in adequate amount.

Zinc deficiency may lead to the body's weakened immune system thus increasing risks for infectious diseases such as respiratory tract infections and diarrhea; adverse pregnancy outcomes such as intra-uterine growth retardation and death; also stunting in children under the age of 5 years (Walker *et al.,* 2009; Cichy *et al.,* 2009; Engle-Stone *et al.,* 2014; Al Hasan *et al.,* 2016). Zinc is also important to maintain catalytic activity of several enzymes in the body (Sida-Arreola *et al.,* 2017).

5.2 Dietary Anti-nutritional Factors (Phytic Acid, Polyphenols and Tannins) of Beans

The concentration of dietary anti-nutritional factors (phytic acid, polyphenols and tannins) in all bean varieties was not significantly different ($p<0.05$). This suggests that bio-fortification of beans may not necessarily affect concentrations of dietary anti-nutritional factors.

Dietary anti-nutritional factors are crucial to plants, fungi and bacteria as a protective or defense mechanism against various unfavorable conditions (Bora, 2014). Notwithstanding, these anti-nutritional factors reduce or inhibit nutrient absorption, but they still benefit the human body with their antimicrobial, anti-inflammatory and anticancer properties. A study on bio-fortified beans showed that upon increasing iron concentrations in beans, levels of dietary anti-nutritional factors (phytic acid in particular) also increased (Petry *et al.,* 2014). Hurrell, (2004) suggested that for phytate to have an inhibitory effect the concentration should be about 2-10mg to inhibit iron and about 50mg to inhibit zinc intake.

5.3 Effect of Cooking on Dietary Anti-nutritional Factors (Phytic Acid, Polyphenols and Tannins) of Beans

This study has shown that losses of dietary anti-nutritional factors were very high after cooking, there was a significant ($p<0.05$) reduction of phytic acid, polyphenols and tannins by cooking in all bean varieties. Thus, cooking has a significant influence in reducing the levels of dietary anti-nutritional factor in the beans. A study by Feitosa *et al*., (2018) observed that cooking resulted in a significant reduction of total polyphenols (30%) and condensed tannins (20%).

Boiling was the cooking method that was applied in this study and it showed a significant reduction of dietary anti-nutritional factors in all bean varieties ranging from 64.9 - 97.9%. This suggests that boiling reduced the concentration and would therefore reduce the effects that dietary anti-nutritional factors would have upon ingestion and therefore enhance bioavailability of iron and zinc. Therefore, boiling is indeed effective in reduction of mineral inhibitors. This method can be applied in poor households and rural areas in Tanzania where it is already a common practice and when the bio-fortified varieties are used it would better address micronutrient deficiencies; because the method showed both higher retention of iron and zinc and higher losses of dietary inhibitors after cooking.

Other studies further projected that cooking by using a pressure cooker, autoclaving and microwaving methods showed better results and there were minimal losses in minerals and lesser cooking time compared to boiling method. Also, that several processes including soaking, thermal processing and fermentation would reduce the effect of dietary anti-nutritional factors on nutrient (mineral) absorption (Wang *et al.,* 2009; Hefnawy 2011;

Pereira *et al.,* 2014; Sharma *et al.,* 2016 and Brigide *et al*., 2019). However, the use of these other methods i.e. autoclaving and microwaving for the poor and those living in rural areas is less likely to be feasible as these methods have higher cost implications. Therefore, feasible and cost-effective methods such as boiling and optimal cooking time to reduce levels of dietary anti-nutritional factors as well as retain both iron and zinc should be developed to cater for the needs of the vulnerable populations specifically in mainstream Tanzania.

Upon inquiring on cut-off points for terming beans as high-iron particularly for bio-fortified beans employed in this study, it was highlighted that the cut-off points for iron and zinc are still in the process of being developed by Tanzania Bureau of Standards (TBS). However, as per the recommended dietary allowances (RDAs) all varieties have the potential to meet requirements at different proportions of intake. The choice of a variety should also consider traits such as yield potential, suitability, cooking time and resistance to diseases.

Smallholder farmers especially in rural areas can maximize on the benefits of bio-fortified bean varieties, for example the climber varieties have the capacity to uptake more water and nutrients from the soil while also having high yield potential as well as maximizing available land. According to Baltazari, (2014); and Massawe *et al.*, (2017), it is critical for smallholder farmers in sub-Saharan Africa such as those in Northern Tanzania, and who have limited arable land to maximize use.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has shown that beans can serve as a vehicle for bio-fortification of iron more than zinc as an intervention towards reduction of these micronutrient deficiencies. This study has also shown that cooking beans does reduce the levels of dietary anti-nutritional factors in beans which is major factor in limiting nutrient bioavailability. Cooking alone may not increase bioavailability of nutrients especially using boiling method thus, suggesting that multiple techniques should be applied in enhancing bioavailability of nutrients from beans. Whenever possible, better cooking methods should be employed to retain more nutrients during and after the cooking process.

The variety that exhibited the most desirable characteristics was Selian 15; a combination of high iron and zinc concentrations and the least concentration in dietary anti-nutritional factors. Despite that the other varieties did not exhibit the characteristics to this extent each variety exhibited a uniquely desirable characteristic such as higher zinc content in Selian 14 and higher nutrient retention in Selian 13 such that various treatments can be applied to improve their performance and capitalize on their desirable characteristics and possibly breed better varieties combining all or more of the desirable traits. Therefore bio-fortified beans would serve an effective strategy in addressing micronutrient deficiencies especially in populations with low socio-economic status.

6.2 Recommendations

Based on the findings of this study, the following are recommended;

i. Promotion of bio-fortified bean varieties and the improvement of high-iron bean varieties in addressing iron and zinc deficiencies targeting the most vulnerable groups.
ii. Research on and product development of the bean varieties through application of various techniques and in combinations that would maximize nutrient bioavailability and absorption from the beans.
iii. Further research to assess bioavailability of iron and zinc; use of animal and/or human studies to get a clear picture under appropriate conditions through which nutrient absorption occurs.
iv. Use of these beans as a source of iron should be accompanied with storage, and preparation information to offer intended benefits.

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