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Effect of different bran, germ and endosperm composition of white and brown sorghum varieties on iron-binding polyphenols and phytate: Implications to iron bioavailability during fermentation

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Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been duely acknowledged.

Signature: - …… **Date:-……**

The thesis has been approved for submission by:

Name of supervisors	**Signature**	**Date**
Prof. Negussie Reta	……	……
Dr. Kaleab Baye	……	……

Dedication

This work is dedicated to God, who enabled me to start and finish this journey. I just want to say thank you Lord.

Acknowledgment

First, I would like to express my heartfelt appreciation to my advisor **Dr. Kaleab Baye** for believing in me and being my advisor and for his constant and relentless engagement in my work. He was always there for me, being appreciative -a brother and a friend. I can tell you that you were and will continue to be a great mentor. I just want to say thank you. The lessons that I took from you will stay with me forever.

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Abbreviations/acronyms

AA	Ascorbic acid
AAS	Atomic absorption spectrometer
ADF	Acid detergent fiber
ANOVA	Analysis of Variance
CE	Catechin equivalent
DHS	Demographic and Health Survey
DM	Dry matter
DMF	Dimethylformamide
-dpH/dtmax	Maximal rate of pH change in time
DY	Decortication yield
EHNRI	Ethiopian Health and Nutrition Institute
ER	Extraction rate
ESIP	Ethiopian Sorghum Improvement Program
FAS	Ferric ammonium sulfate
FW	Fresh weight
GDP	Gross domestic product
GI	Gastro-intestinal tract
Hb	Hemoglobin
HCl	Hydrochloric acid
HCN	Hydrogen cyanide

HE	Higher extraction
HEFD	Higher extraction rate fermented dough
HPLC	Higher performance liquid chromatography
IBPC	Iron-binding phenolic compounds
IDA	Iron deficiency anemia
IE	Intermediate extraction rate
IEFD	Intermediate extraction rate fermented dough
LE	Lower extraction rate
LEFD	Lower extraction rate fermented dough
LSD	Least significance difference
MIE	Meko intermediate extraction rate
MHE	Meko higher extraction rate
MLE	Meko lower extraction rate
MV-HCl	Modified vanillin-hydrochloric acid test for tannin determination
PPO	Polyphenol oxidase
RBV	Relative biological value
SHE	Seredo higher extraction rate
SIE	Seredo intermediate extraction rate
SL	Seredo lower extraction rate
SPSS	Statistical Product and Service Solutions
TA	Tannic acid

TADD	Tangential Abrasive Dehulling Device
TKW	Thousand kernel weight
UNICEF	United Nations Children's Fund
UV-Vis/NIR	Ultra-violet-near infra-red spectrophotometer
WHO	World Health Organization

ABSRACT

The improvement of sorghum varieties for beneficial agricultural traits might affect their nutritional and processing properties. The nutritional profile of agriculturally improved varieties (low tannin and high tannin) and the effect of traditional decortication and *injera* fermentation on nutritional characteristics and iron bioavailability were investigated. Low- (Meko) and high-tannin (Seredo) sorghum varieties were collected and characterized for their grain characteristics and nutritional profile. Household decortication was characterized and its effect on nutrient retention and mineral chelating agents was evaluated. Further experiments were conducted by extending and shortening of the decortication time and its effect on nutritional profile was investigated. The effect of fermentation on mineral chelating compounds was evaluated. Iron bioavailability was estimated using phytate to iron molar ratio and Hallberg and Hulthén's algorithm. Seredo has slightly higher pericarp thickness than Meko, but had lower seed size and nearly the same germ proportion. The iron-binding polyphenols content of Seredo was six to nine times higher than that of Meko ($P< 0.05$). Decortication had numerous effects on nutrient retention. Significant losses in iron-binding polyphenols were observed for both varieties ($P< 0.05$). However, the losses of iron-binding polyphenols were affected by extraction rates. Seredo had higher loss of phytate and tannin than Meko. Decortication led to iron losses of 27.3% in Meko and 23.7% in Seredo. Despite the loss of chelating compounds; the phytate to iron molar ratio was above 1. Further reduction in chelating compounds was observed during fermentation. Fermentation led to significant losses of iron-binding polyphenols for Seredo ($P< 0.05$) but not for Meko. Losses of tannin and phytate were also observed. However, the observed phytate degradation was not enough to improve the phytate to iron molar ratio to values that are likely to improve iron bioavailability (phytate/Fe< 1). Nevertheless, iron bioavailability estimated using Hallberg and Hulthén's algorithm predicted two times more fractional iron absorption in Meko than in Seredo. The present study has shown that retention of nutrients during decortication was dependent on variety. Furthermore, the degradation of phytate achieved during fermentation did not result in significant improvements in iron bioavailability estimates. However, combining decortication and fermentation showed the possibility of reducing the iron-binding phenolics content of the high tannin variety to the extent of the low tannin one. This suggests the need for improving the efficiency of phytate and polyphenols degradation during fermentation of dough for *injera* preparation.

Key words: Sorghum; traditional decortication; fermentation; iron; phytate; iron-binding polyphenols; bioavailability

Chapter 1 – *Introduction*

1. Introduction

1.1. Background

The major crops consumed by a large proportion of the world's population include wheat, maize, rice, barley and sorghum. Sorghum is the fifth most important crop in the world and originated in Africa (Taylor, 2003). The world annual sorghum production is over 60 million tones, of which Africa produces about 20 million tons (Taylor, 2003). This makes sorghum, quantitatively the second most important cereal grain in Africa after maize. Its production takes places across the continent, with the northern African countries of Nigeria, Sudan, Ethiopia and Burkina Faso accounting for nearly 70% of Africa's production. In Ethiopia, sorghum with teff, wheat, maize and barley accounts for about three-quarters of total area cultivated, 29% of agricultural GDP in 2005/2006 (14% of total GDP) and 64% of calories consumed (Taffesse et al., 2011). It is uniquely adapted to Africa's climate, being both drought resistant and able to withstand periods of water-logging (Taylor, 2003).

Sorghum is a staple food in most parts of Africa and thus one of the main supplies of energy, protein, vitamin, and minerals. It contains comparable levels of starch and other major nutrients as compared to other cereals. However, relative to other cereals, sorghum has abundant amount of polyphenolic compounds, which can reach as much as 10% of the dry matter in some sorghum *(Sorghum bicolor)* cultivars (Bravo, 1998). However, the total polyphenol content in sorghum is affected by genetic and environmental factors, such as plant color, thickness of the pericarp, and growth conditions (Svensson et al., 2010). The grain color varies from white colored grains with low polyphenol contents to red ones rich in polyphenols (Dykes and Rooney, 2006). The deoxyanthocyanidins, a specific type of polyphenol, is responsible for the color of the grain

(Svensson et al., 2010). Higher polyphenol content is associated with higher resistance to biotic and abiotic stress, as polyphenols are secondary metabolites serving as defense mechanisms of plants. This association also explains the close correlation between phenolic contents and antioxidant and antimicrobial activities (Svensson et al., 2010).

Polyphenols, particularly tannins are located primarily in the testa layer of the grain (Bravo, 1998, Svensson et al., 2010). Some of the phenolic compounds in sorghum form large complexes with iron through their carboxylic and hydroxyl groups in the intestinal lumen, hence making iron unavailable for absorption (Bravo, 1998). For instance, polyphenols in black tea and coffee strongly inhibited Fe absorption from composite meals, with coffee having about half the inhibitory effect of tea. In addition, red wine and cocoa, as well as various vegetables with high polyphenol content have been found to inhibit iron absorption (Richard F. Hurrell, 1999).

Iron deficiency is the most common and widespread nutritional disorder in the world. It affects a large number of children and women in both developing and developed countries. Around two billion people or about 30% of the world's population are anemic and about half of the associated burden is due to iron deficiency, often exacerbated by infectious diseases in resource poor areas. According to the most recent DHS (2012) data, more than 44% of Ethiopian children are anemic. Some 21% have mild anemia, 20% have moderate anemia, and three percent have severe anemia. The prevalence is highest among children of 9-11 months of age (73%). Anemia in children is associated with impaired mental and physical development and as well as increased morbidity and mortality (DHS, 2012, UNICEF. et al., 2001, WHO, 2012).

Nutritional iron deficiency occurs when the diet supplies insufficient amount of bioavailable iron to meet the body's requirements for growth, pregnancy, and for the replacement of iron lost from

the body through the gastrointestinal tract, skin, urine, and menstruation (Lynch, 2011). The underlying factor is poverty as this limits dietary diversity. Moreover, the agricultural revolution that resulted in displacement of animal foods rich in bioavailable iron by plant-based diets containing high amounts of mineral absorption inhibitors such as phytate and phenolic compounds have played their share in the widespread of nutritional iron deficiency, especially in the life stages where iron requirements are specially high (growth and pregnancy) (Benoist et al., 2008).

1.2. Statement of the Problem

Several studies have reported the advantages and disadvantages of sorghum's polyphenols and phytates in general, to human health (Bravo, 1998, Towo et al., 2011). The negative impact of these compounds on health is mainly associated with their strong binding/chelation of essential nutrients that form insoluble complexes that inhibit both the accessibility to digestive enzymes and intestinal absorption (Bravo, 1998). There are numerous types of poyphenols, and not all of them bind dietary iron. The iron-binding property of polyphenols is attributed to the galloyl and catechol groups of phenolic compounds (Bravo, 1998, Pascal C. Agbangnan D. and Sohounhloue, 2012). Phytates and fibers are also known to bind iron thereby decreasing their bioavailability (SHAFIQUE et al., 2006).

However, different traditional food processing techniques can decrease the amount of polyphenols and phytates and thus may have an impact on the nutritional value of sorghum. Such processes include decortication, soaking, malting (germination), fermentation, etc. However, the effect of microbial fermentation on polyphenols has been less consistent as some studies have shown increases (Sripriya et al., 1997, Kayodé et al., 2007) while other showed decrease in

polyphenols contents (Svensson et al., 2010). Such variations are likely to be explained by the fact that decortication parameters were uncontrolled in these studies. This is unfortunate, since the level of decortication may influence substrate (polyphenols) availability for enzymatic degradation by polyphenol oxidases/tannases and thus determine the final content. On the other hand, degradation of phytates due to fermentation, although to varying extent, has been consistently reported. For instance, 95% of the initial phytate content was reduced in West African beer production particularly in the process of germination, mashing, boiling and fermentation (Kayodé et al., 2007), Furthermore the combination of enzymatic (polyphenol oxidase) treatment with other processing methods such as soaking, germination and fermentation has shown to enhance the degradation of polyphenols and phytate compounds (Towo et al., 2011), probably because processing increased accessibility of the polyphenols and phytates for enzymatic degradation

Furthermore, decortication of grains has also been shown to have a significant effect on the contents of nutrient absorption inhibitors. Both traditional and abrasive decortication significantly reduced phytate content in sorghum (Hama et al., 2011). Abrasive decortication for five minutes reduced the tannin content of the sorghum variety-Seredo by 82% (Yetneberk et al., 2005) and a study in pearl millet suggested decortication as a relevant process to reduce the quantity of total polyphenols, iron–binding polyphenols and phytates (Lestienne et al., 2007). However, there is lack of information regarding the effect of traditional sorghum decortication on absorption inhibitors like iron-binding polyphenols and phytates. In addition to this the grain seed color (brown sorghums with a pigmented testa contains significant levels of tannins other than white or red pericap colored sorghums) (Awika and Rooney, 2004) and the nutrients are unequally distributed in the different fractions of the grain (Hama et al., 2011, Hama et al., 2012), thus it

may also have an effect on the extent and amount of reduction of these nutrients which in turn have an implication on the bioavailability.

Currently, the Ethiopian Sorghum Improvement Program (ESIP) conducts research on local varieties and accessions from the world sorghum collection for improvement of sorghum in Ethiopia. The factors requiring consideration in sorghum improvement include: yield increases, early maturity, resistance to yield limiting biotic and abiotic factors and end use quality traits (YETNEBERK, 2004). Beyond improving those agricultural traits, it is important to take into account the nutritional composition of the crops especially the micronutrient composition and the amount that remain in the grains after standard processing, as well as their bioavailability due to the low mineral phytoavailability of the areas in which the crops are grown (Hama et al., 2012).

Furthermore, knowledge on how such new breeds are affected by traditional processing is very important. Previous attempts to estimate nutrient losses during decortication were based on data obtained on laboratory decortication using Tangential Abrasive Dehulling Device (TADD), which have limitations in reproducing in-field decortication (Hama et al., 2011). Such data on nutrient losses will be very useful as recommendations on the optimum level of decortication for a better retention of iron as well as removal of chelating agents can be formulated. Such practical recommendations based on widely applied household food processes are likely to be accepted by the general public and thus contribute to the alleviation of nutritional deficiencies through integrating with intervention strategies such as dietary modification, which is sustainable without external support and has an ability to combat multiple micronutrient deficiencies simultaneously. This approach enables to increase the bioavailability of the nutrients, while not increasing the cost, preparation or cooking time and also maintaining the cultural acceptability to the community. In addition to this, it will not change the type and quantities of food items commonly

consumed, but based on the existing food consumption patterns, and be adapted from local food processing procedures and recipes (Gibson et al., 2000).

1.3. Objectives

1.3.1. General objective

The overall aim of this research was to study the effect of different components of sorghum grains (white and brown) subjected to traditional decortication on iron-binding phenolics, phytates and polyphenol oxidase activity, and understand its implications to iron bioavailability. Furthermore, to examine the effect of fermentation of the two sorghum varieties that have different bran, germ and endosperm composition on the above mentioned compounds. To this end, the amount of iron that can be absorbed from the different varieties and treatments was predicted by making use of phytate: iron molar ratio and iron absorption algorithm.

1.3.2. Specific objectives

- To determine the difference in the amount of iron-binding polyphenols, phytates, protein and lipids between sorghum cultivars (white and brown).
- To study the effect of different germ, bran, and endosperm composition achieved during traditional decortication on iron-binding polyphenols, phytate content and polyphenol oxidase activity.
- To predict the extent of iron absorption of the two sorghum varieties due to traditional decortication.
- To show the effect of sorghum *injera* fermentation on iron-binding polyphenols, phytates, and to predict iron absorption using phytate to iron molar ratio and Hallberg and Hulthén's algorithm.

This MSc thesis is organized as follows:-

Chapter two presents literature review that includes a brief description on the role of iron in humans with the prevalence and causes of its deficiency. The different forms of iron, with more emphasis given to non-heme iron were reviewed. Then the role of sorghum as a staple and nutrient source was reviewed. Dietary factors affecting iron absorption and how they are affected by processing conditions were reviewed. Finally, the different methods used for the measurement of iron bioavailability were reviewed.

Chapter three gives a description of the experimental design, and the materials and methods used and also indicated the type of statistical analysis used in the study.

Chapter four presents the results and discussion of the study. Characterization of the two sorghum varieties (high and low tannin) was presented. The effect of processing (decortication and fermentation) on mineral chelating agents and nutrient retention was shown. The implication of decortication and fermentation on iron bioavailability was shown using phytate to iron molar ratio and Hallberg and Hulthén's algorithm. Finally, the limitations of the study were indicated.

Chapter five presents the conclusion and recommendations.

Chapter 2 – *Literature review*

2. Literature Review

2.1. Iron deficiency

Iron plays an essential role in many metabolic processes as a cofactor including oxygen transport, respiration, amino acid, vitamin A and sulfite metabolism and various other redox reactions (Lynch, 1997). Its deficiency arises when the physiological requirement can't be met by iron absorption from the diet (Zimmermann and Hurrell, 2007). Iron deficiency is the most widespread and prevalent nutritional deficiency in the world (Charlton and Bothwell, 1983). Iron deficiency is characterized by a drop in serum ferritin below 12. If this negative balance persists, iron tissue availability is compromised (iron deficient erythropoiesis). At this stage, an early progressive rise in the concentration of serum transferrin receptors occur; followed by an increase in free erythrocyte protoporphyrin, a decrease in transferrin saturation (ratio of serum iron to total iron binding capacity) and the slow fall in hemoglobin begins. If the iron deficit continues, the last stage becomes evident when Hb falls below –2 SD (120 g/lt in women and 130 g/lt in men (WHO, 2002)) for a given population, which is referred as iron deficiency anemia (IDA) (Olivares et al., 1999).

IDA sometimes co-exists with other conditions such as, protein/energy malnutrition, vitamin A deficiency, folate deficiency and infections in developing countries. In addition parasitic infestation and haemoglobinopathies often co-exist with iron deficiency in tropical regions (Olivares et al., 1999). The main cause if IDA is low bioavailability of iron in populations consuming monotonous plant-based diets with little meat (Zimmermann and Hurrell, 2007).

There are two forms of dietary iron in the diet with respect to the mechanism of absorption; heme iron (derived from hemoglobin and myoglobin) and non-heme iron (derived mainly from cereals,

fruits and vegetables). The absorption of these two kinds of iron is influenced differently by dietary factors. Heme iron forms a relatively minor part of iron intake, even in diets with a high meat content it accounts for only 10-15% of the total iron intake (Hallberg, 1981). Diets in developing countries usually contain negligible amounts of heme iron (Hallberg, 1981). In addition, the average absorption of heme iron in meat containing meals is about 25%, calcium being the only dietary factor that negatively influence its absorption (Olivares et al., 1999). Heme iron is the best absorbed form of iron; it will be freed from the porphyrin ring after being taken up from the lumen into the mucosal cell (Charlton and Bothwell, 1983). Dietary iron in all the other chemical forms found in food is available for absorption depending on the extent to which it ionizes within the lumen (Charlton and Bothwell, 1983).

However, non heme iron from plant based diets is poorly absorbed because dietary factors, such as the amount of polyphenols, phytate and calcium (Killip et al., 2000) and also the iron status of the individual's dictate the amount absorbed from a particular diet, in the later case more iron will be absorbed in iron deficient subjects than in iron replete subjects. This is due to the body's mechanism to maintain iron homeostasis; due to the high erythropoietic drive normally occurs to compensate the loss at the time (Lonnerdal and Kelleher, 2007).

Furthermore, iron from fortificants are also found in foods (Hallberg, 1981, Olivares et al., 1999). There have been different forms of iron used during fortification, which differs in bioavailability. For instance, ferrous sulfate and ferrous fumarate are considered to have good bioavailability while that of elemental (reduced) iron powders is believed to be lower (Bovell-Benjamin et al., 1999). In contrast, elemental iron considered as safer than ferrous sulfate and fumarate as it doesn't accelerate rancidity (Bovell-Benjamin et al., 1999). Several studies are underway to

investigate the bioavailability and stability of different forms of iron in order to find the best iron to use as a fortificant (Bovell-Benjamin et al., 1999).

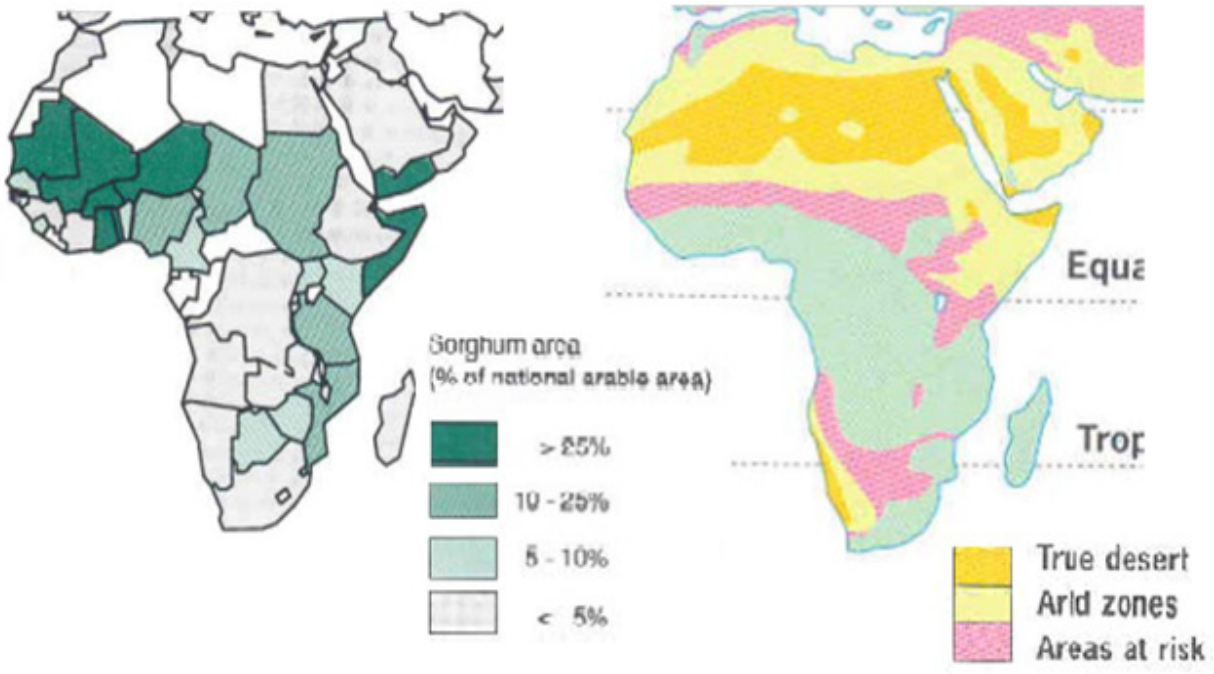
Additionally, a significant amount of extrinsic iron found in diets of developing countries (Harvey et al., 2000), which referred as contaminant iron. The soruces of contaminant iron are soil, dust and water; metal fragments from milling; leaching from iron or steel pots during processing, storage or cooking (Harvey et al., 2000). The contaminant irons are majorly in ferric hydroxide and ferric oxide form (Harvey et al., 2000). It has been evident that, contaminant iron contributes significantly to the iron intake (Hallberg and Björn-Rasmussen, 1981). However, the impact of contaminated iron on the iron status is controversial (Harvey et al., 2000). This suggests the need for further investigation.

Non-heme iron is majorly found in cereals and legumes (Gillooly et al., 1984). Among cereals sorghum is a major food crop in Africa (fifth important staple) (Doggett, 1988) and represents half of the total cereal production on the continent and a major source of protein and energy for the population (Belton and Taylor, 2004).

2.2. **Sorghum** (*Sorghum bicolor (L) Moench*)

Sorghum ranks fifth among the most important cereal crops in the world following wheat, maize and barley (House, 1995) and also Africa produces one-third of world's annual production. It is generally cultivated at subsistence level and consumed as food by humans, especially in the areas of arid and semi-arid tropics (Cothren et al., 2000, PO, 1982) where the annual rainfall is in the range of 0-300 mm and 300-600 mm, respectively (Sidahmed, 1996). Surprisingly, sorghum is also an important crop in East Africa where overall there is good rainfall. In fact sorghum is not

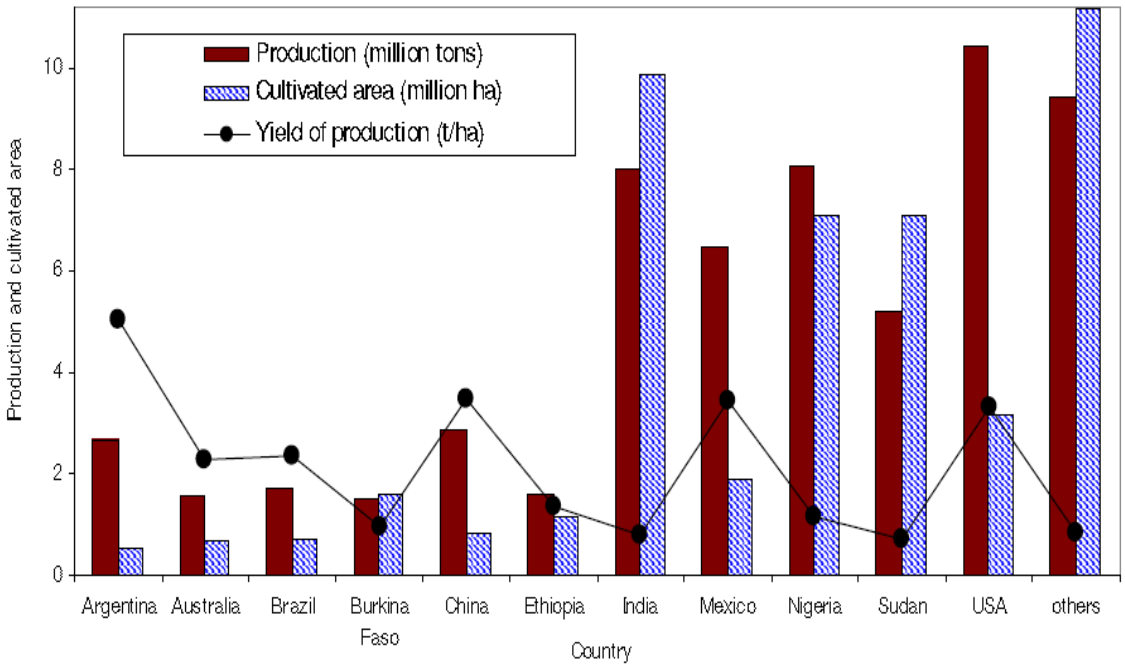
only drought-resistant, it can also withstand periods of water-logging (Taylor, 2003). Thus, it contributes significantly to the nutritional livelihood of impoverished populations of the world.



Source: (Taylor, 2003)

Figure 2.1 Relative importance of sorghum in African countries and the continent's arid zones

Sorghum is mainly eaten as porridge, fermented and unfermented breads, leavened and unleavened bread, snacks, non-alcoholic beverages and sorghum beer and malt (Murty and Kumar, 1995). In Ethiopian context, about 80% of the annually produced sorghum is used for *injera* making, 10% goes to home-brewed beverages and the rest of the sorghum grain produced goes for making porridge (genfo), unleavened bread (quitta), boiled whole grain (nifro), roasted grain (kolo), and animal feed (Gebrekidan and GebreHiwot, 1982).



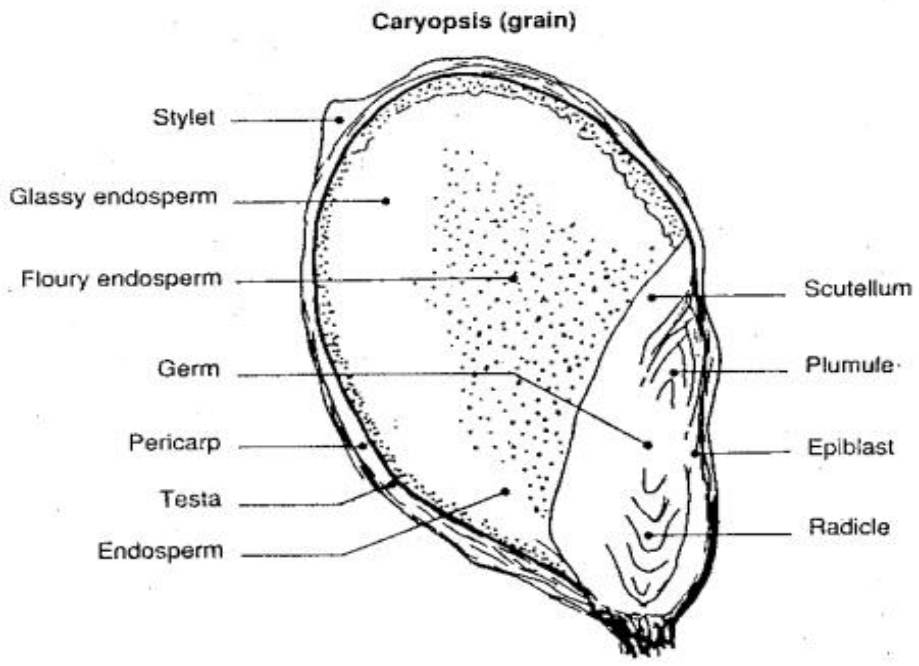
Source: (Dicko et al., 2006)

Figure 2.2 Annual sorghum production and cultivated area throughout the world

2.2.1. Anatomy of sorghum grain

The sorghum kernel is composed of three main parts the outer covering (pericarp), the storage tissue (endosperm) and the embryo (germ) (Rooney and Miller, 1982). The endosperm makes up 84.2%, the germ 9.4% and the pericarp 6.5% of the grain depending on the kernel size (Dahlberg et al., 2004). The sorghum kernel is called a caryopsis because the ovary wall dries and adheres strongly to the mature ovule. The pericarp originates from the ovary wall, which divided into the epicarp, the mesocarp, the cross cell layer and the tube cell layer. The epicarp is the outermost layer of the kernel and is divided into the epidermis containing pigments, and the hypodermis. The mesocarp is the middle part of the pericarp and may vary in thickness from thin (translucent) without starch granules to thick (chalky) with starch granules. The endocarp is the innermost part of the pericarp containing the cross and tube cells.

The endosperm consists of the aleurone layer, peripheral, horny (corneous) and floury portions (Rooney and Miller, 1982). The peripheral endosperm has starch granules embedded in a dense matrix of protein bodies, which makes the protein poorly available for hydrolysis. The corneous endosperm is located beneath the peripheral endosperm and is often called hard, vitreous or horny.



Source: (Dicko et al., 2006)

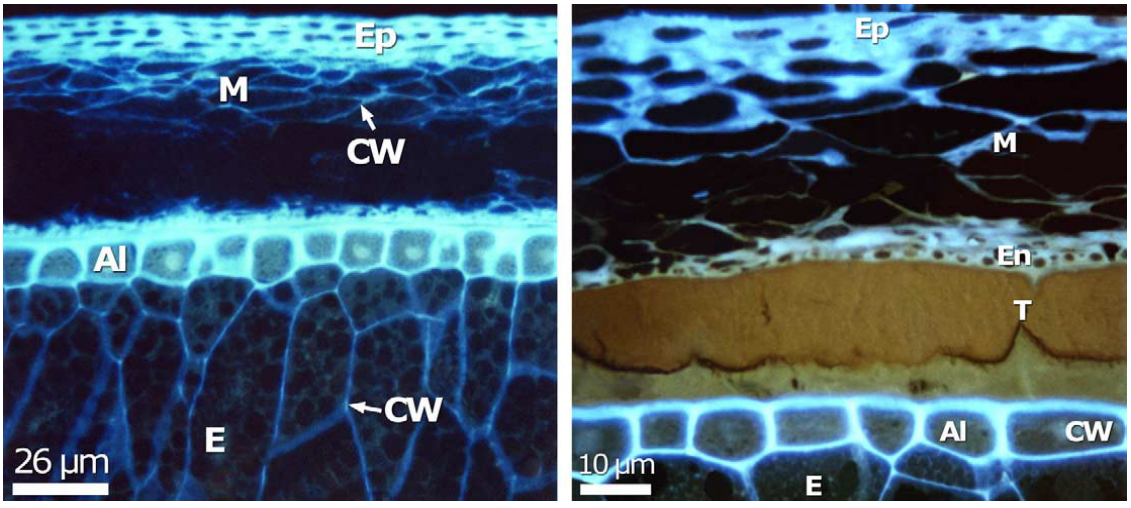
Figure 2.3 Structure of sorghum grain

The starch granules in this part of the endosperm are angular in shape with depressions where protein bodies were located. This part of the endosperm has strong starch-protein bonds and the starch granules often break easily rather than pull from the protein matrix (Rooney and Miller, 1982). The floury endosperm is located in the inner most part of the kernel, and is composed mainly of starch with a smaller amount of protein bodies than the corneous endosperm (Rooney and Miller, 1982). The relative proportion of corneous to floury endosperm within a sorghum kernel is often referred to as endosperm texture. The texture can be determined by visual

examination of kernels cut longitudinally (Rooney and Miller, 1982). A rating of 1 can be assigned to a kernel which is predominantly floury (Rooney and Miller, 1982). Endosperm texture is important during processing. Sorghums with a corneous endosperm texture have higher milling yields because the pericarp is more readily separated from the intact endosperm (Rooney and Miller, 1982). Sorghums with corneous endosperm are also more resistant to insect attack during storage than those with a floury endosperm.

The embryo or germ is composed of two main parts, the embryonic axis and the scutellum (Rooney and Miller, 1982). The scutellum contains oil globules, protein bodies and a few starch granules (Rooney and Miller, 1982). Therefore, embryo plays a major role in moisture uptake and mold susceptibility of the sorghum kernel. Moreover, sorghum grain contains condensed tannins when there is the presence of pigmented testa (Awika and Rooney, 2004). The pigmented testa found just beneath the cross and tube cell layers (Rooney and Miller, 1982). The presence or absence of the testa is controlled by the complementary B_1 and B_2 genes with the testa present when both the B_1 and B_2 genes are dominant (B_1-B_2-). Some sorghum lines contain a partial testa that is found at certain places around the kernel. Testa thickness varies among sorghum genotypes and within the individual kernels with the thickest part at the crown and the thinnest area over the embryo. The color of the testa can also vary among sorghum lines, which is controlled by the Tp gene (Dykes and Rooney, 2006). The testa color is purple when Tp is homozygous recessive (tptp) and brown when it is dominant (Tp) (Dykes and Rooney, 2006). The testa develops from the inner integument which has a definite cellular structure. However, as the kernel matures and the endosperm expands, the cellular configuration usually gives way to a continuous layer (Rooney and Miller, 1982).

Sorghums have been classified into groups I, II and III based on the presence or absence of a pigmented testa (Rooney and Miller, 1982). Group I sorghums don't have a pigmented testa; group II have a testa (B_1-B_2ss) and group III have a pigmented testa and a spreader gene (B_1, B_2SS). The group one sorghums don't contain significant levels of tannin shown by low values of protein precipitation, Vanillin-HCl (This method might give false positives for sorghums genetically have no tannin as a results it has to be used with caution (Awika and Rooney, 2004)) and anthocyanin production assays.. Group II sorghum tannins are extractable in acidified methanol but not methanol alone. The typical high-tannin sorghums classified as group III sorghums, contain methanol-extractable tannins as well as group II type tannins extractable only in acidified methanol (Rooney and Miller, 1982). Sorghum grains has been classified based on their whole grain tannin content as follows: low tannin sorghums ≤ 0.25%, medium tannin sorghums 0.26-0.5%, high tannin sorghums 0.51-0.75% and very high tannin sorghums ≥ 0.75% of tannin (Dicko et al., 2002).



Source: (Awika and Rooney, 2004).

Figure 2.4 Fluorescence photomicrograph of sorghum bran cross-section, showing structural differences between a non-tannin sorghum without a testa (left) and a tannin sorghum with a pigmented testa (right). Al, aleurone layer; CW, cell wall; E, endosperm; En, endocarp; Ep, epicarp; M, mesocarp; T, pigmented testa

2.2.2. Nutritional compositions of sorghum

Sorghum is similar in chemical composition to corn (*Zea mays*) (Léder, 2004), where starch is the main reserve polysaccharide and the principal source of carbohydrate (Dicko et al., 2006). Furthermore, sorghum is gluten free, which makes it good choice for celiac patients (Taylor et al., 2006). It has a good proportion of macro and micro-nutrients, however there are also chelating compounds that can hamper mineral absorption (Hama et al., 2011). Sorghum contains substantial levels of a wide range of phenolic compounds (Awika and Rooney, 2004).

Table 2.1 Proximate composition of sorghum grain

Macro-components (g/100 g FW)		**Essential amino-acids**		**Vitamins (mg/100 g DM)**		**Minerals (mg/100 g DM)**	
Carbohydrates	65-80	Leu	832	Vitamin A	21 RE*	Ca	21
Starch	60-75	Ile	215	Thiamin	0.35	Cl	57
Amylose	12-22	Met/Cys	190	Riboflavin	0.14	Cu	1.8
Amylopectin	45-55	Lys	126	Niacin	2.8	I	0.029
Non starch	2-7	Phe/Tyr	567	Pyridoxine	0.5	Fe	5.7
Low M_w carbohydrates	2-4	Thr	189	Biotin	0.007	Mg	140
Proteins	7-15	Trp	63-187	Pantothenat	1	P	368
α-kafirins	4-8	Val	313	Vitamin C	<0.001	K	220
β-kafirins	0.2-0.5	Arg	500			Na	19
γ-kafirins	0.7-1.6	His	200			Zn	2.5
Other proteins	2-5						
Fat	1.5-6						
Ash	1-4						
Moisture	6-12						

*-retinol equivalent, FW-fresh weight, DM-dry matter

Source: (Dicko et al., 2006)

- **Macronutrients**

In sorghum the majority of the carbohydrate is starch, while other forms such as soluble sugar, pentosans, cellulose, hemicelluloses are low (Léder, 2004). Regular endosperm sorghum types contain 23-30% amylase, but waxy varieties contain less than 5% amylase. Sorghum is also a good source of fiber, the insoluble accounts for 86.2%, which can decrease the transit time and prevent gastrointestinal problems (Léder, 2004). In general starch exists inside the endosperm of cereals bounded in a protein matrix, which is particularly strong in sorghum and corn, thus have low starch digestibility (Rooney and Pflugfelder, 1986). The primary reason is the resistance of the hard peripheral endosperm layer to digestive action (Rooney and Pflugfelder, 1986). Other factors such as tannins inhibit the activity of α-amylase (Alonso et al., 2000) likewise phytates also reduce the starch digestibility(Schlemmer et al., 2009).

The protein in sorghum ranged from 7-15% (FAO, 1995, Beta et al., 1995). This variation is due to the genotype, and water availability, temperature, soil fertility and environmental conditions during grain development (Dendy, 1995). Using the solubility-based classification, sorghum proteins have been divided into albumin, globulins, kafirins (aqueous alcohol-soluble prolamins), cross-linked kafirins and glutelins. The kafirins fraction comprises about 50-70% of the proteins in sorghum (Dicko et al., 2006), thus making the protein of poor nutritional quality (Anglani, 1998). Furthermore, processes such as fermentation and germination increases protein digestibility (Taylor and Taylor, 2002), whereas cooking decreases it (Axtell et al., 1981).

The fat in sorghum grain (mainly localized in the germ) is rich in polyunsaturated fatty acids (Glew et al., 1997). The fatty acid composition of sorghum includes linoleic acid (49%), oleic acid (31%), palmitic (14%), linolenic (2.7%) and stearic (2.1%), which have similar content to that of corn fat, but it is more unsaturated (Dicko et al., 2006).

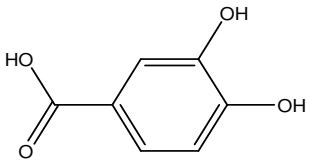
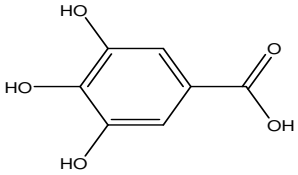
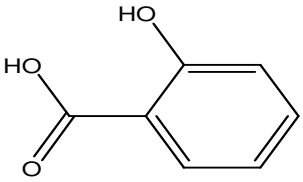
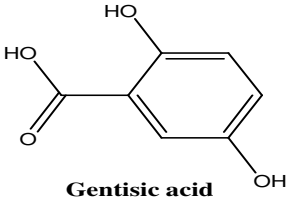
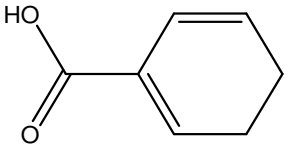
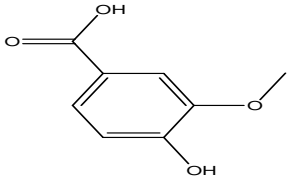
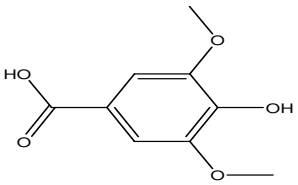
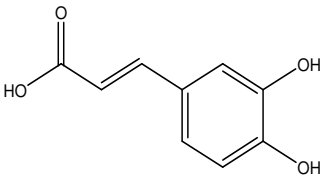
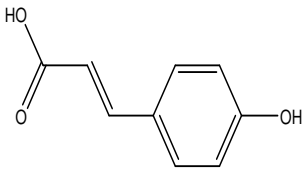
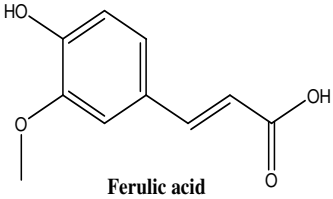
- **Micronutrient**

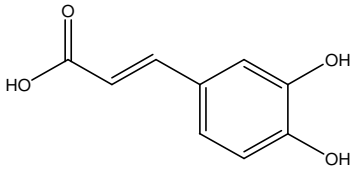
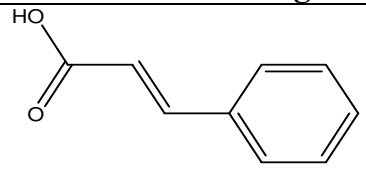
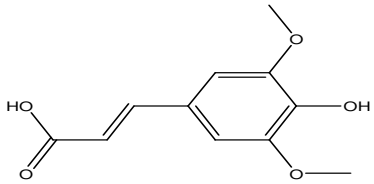
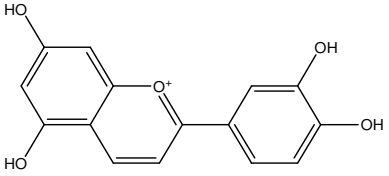
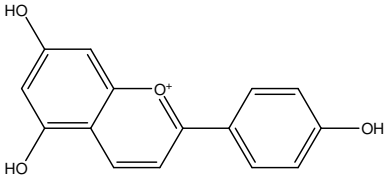
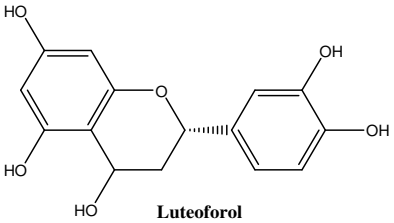
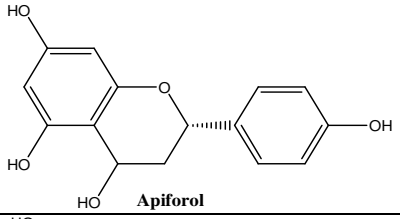
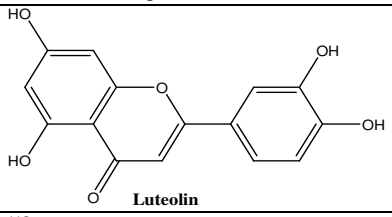
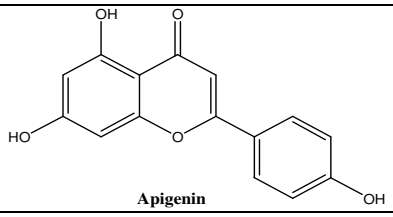
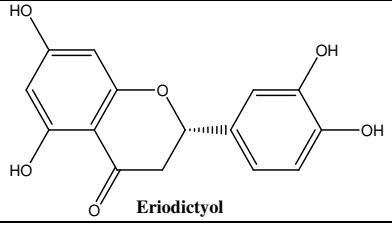
Sorghum is a good source of vitamins, notably the B vitamins (thiamin, riboflavin, pyridoxine), which are localized in the aleurone layer and germ, and the lipid-soluble vitamins A, D, E and K (Dicko et al., 2006). It has been found that processes such as decortication decreases the amount of thiamine, riboflavin and niacin by about 50% in the flour (Léder, 2004). Besides, sorghum is a good source of more than 20 minerals of which iron and zinc are the most important in terms of public health significance (Dicko et al., 2006). However, sorghum has considerable amounts of absorption inhibitors such as polyphenols and phytates.

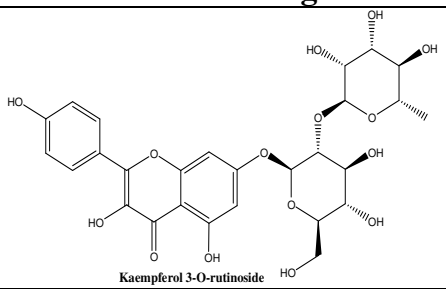
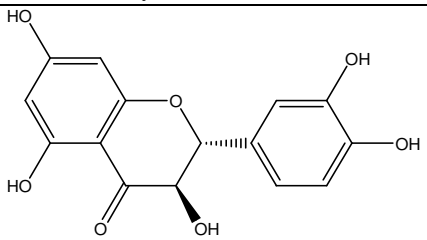
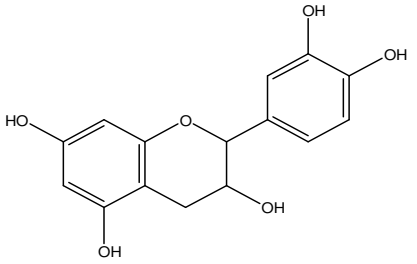
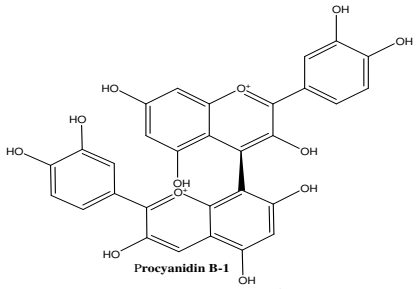
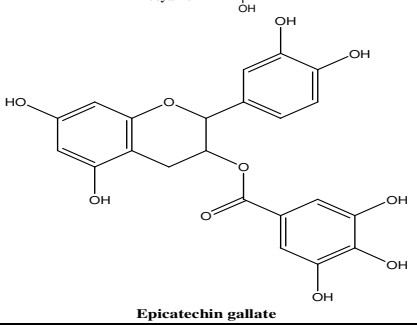
- **Absorption inhibitors**

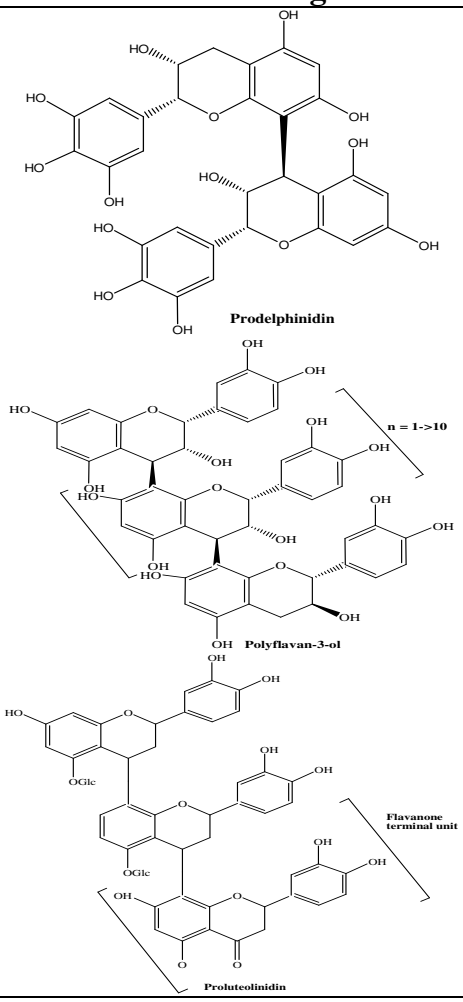
Sorghum has considerable amount of phenolic compounds, which fall into two major categories known as phenolic acids and flavonoids (**Table 2.2**). The amount and kind of phenolic compounds in the grain are dependent on both genetics and environmental conditions (Awika and Rooney, 2004). In general sorghums with a pigmented testa have a high amount of polyphenols (Awika and Rooney, 2004). This hampers the absorption of iron. However, this property is only limited to those phenolic compounds bearing catechol and galloyl groups (Brune et al., 1991). **Table 2.2** presents the type of polyphenols found so far in sorghum and their classification based on iron-binding property.

Table 2.2 Classification of polyphenols in sorghum by iron-binding properties*

Types of polyphenols	Iron-binding	Not iron-binding
Phenolic acids		
Hydroxybenzoic acids	 **Protocatechuic acid**  **Gallic acid**  **Salicylic acid**  **Gentisic acid**	 **p-Hydrobenzoic acid**  **Vanillic acid**  **Syringic acid**
Hydroxycinammic acids	 **Caffeic acid**	 **Coumaric acid**  **Ferulic acid**

Types of polyphenols	Iron-binding	Not iron-binding
Hydroxycinammic acids	 **Caffeic acid**	 **Cinnamic acid**  **Sinapic acid**
Flavonoids		
Anthocyanins	 **Luteolinidin**	 **Apigeninidin**
Flavanols	 **Luteoforol**  **Apiforol**	
Flavones	 **Luteolin**	 **Apigenin**
Flavanones	 **Eriodictyol**	

Types of polyphenols	Iron-binding	Not iron-binding
Flavonols	 Kaempferol 3-O-rutinoside	
Dihydroflavonols	 Taxofolin	
Proanthocyanidin monomers/dimmers	 Catechin  Procyanidin B-1  Epicatechin gallate	

Types of polyphenols	Iron-binding	Not iron-binding
Proanthocyanidin polymers		

*Based on catechol and galloyl groups

Furthermore, sorghum has a considerable amount of phytates, which is in the range of 0.25-3.5 g/100 g (Idris et al., 2005, Mohammed et al., 2011, Hama et al., 2011, Schlemmer et al., 2009). This variability is due to the different methods employed for phytate analysis. The low phytate content may due to the low recovery of phytates with some methods. However modern HPLC techniques are more sensitive and result in good recovery. In sorghum, phytate content can reach as high as 3.5 g/100 g.

2.3. Dietary factors affecting iron absorption

There are different factors in diet that inhibits or enhance the absorption of iron from the diet; especially non-heme iron. The most important inhibitors of iron absorption are phytate, polyphenols and calcium (Hallberg and Hulthén, 2000). Polyphenols and phytate form insoluble iron complexes, which are unavailable for absorption in the gastrointestinal tract (Olivares et al., 1999). However, the inhibitory effect of calcium is mainly through delaying the uptake of iron into the intestinal mucosal cells or by influencing further transfer of iron from these cells into the circulation (Hallberg et al., 1991). The amount should be above 200 mg in the diet in order to reduce iron absorption(Hallberg and Hulthén, 2000). On the other hand ascorbic acid acts as a promoter of iron absorption through forming soluble complexes with iron that remains stable over a wide pH range, thus prevents the formation of insoluble complexes with phytate and polyphenols (Lynch, 1997, Teucher et al., 2004). Other organic acids such as lactic, citric, malic and tartaric acids also have similar effect (Charlton and Bothwell, 1983). Furthermore, meat also improves iron bioavailability, which is related to muscle protein (Hurrell et al., 2006).

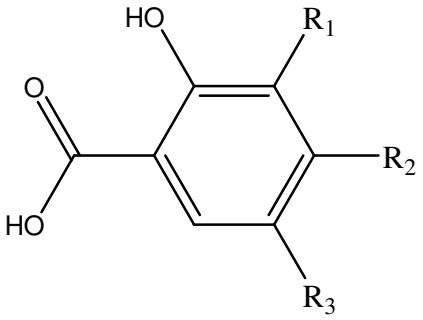
2.3.1. Phenolic compounds

Phenolic compounds or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom, with more than 8000 phenolic structures currently known. Polyphenols are products of the secondary metabolism of plants. Most polyphenols arise from phenylalanine or tyrosine after being deaminated to cinnamic acids, which will enter the phenylpropanoid pathway (Castellano et al., 2012) so as to produce a large number of secondary metabolites such as plant pigments, molecules with antioxidant, antimutagenic, antiviral, antibacterial, algicidal, antifungal, insecticidal, estrogenic and keratolytic activities that may

serve to protect the organism from competing ones in their biological environment (Herrmann, 1995, Castellano et al., 2012).

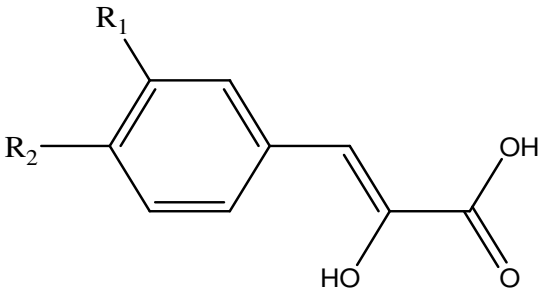
Natural polyphenols can range from simple molecules like phenolic acids to highly polymerized compounds, such as tannins. The classification of polyphenols is based on the number of phenolic rings and the structural elements that link these rings. The main groups are phenolic acids with subclasses derived from hydroxybenzoic acids and hydroxycinnamic acid, flavonoids, stilbenes, and lignans and the polymeric lignins (Han et al., 2007).

Hydroxybenzoic acid

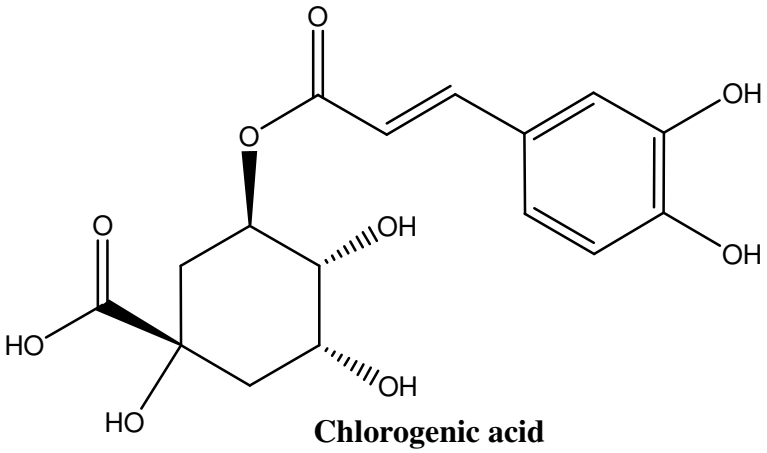


R_1= R_2 = OH, R_3 = H; Protocatechuic acid
R_1 = R_2 = R_3 = OH; Gallic acid

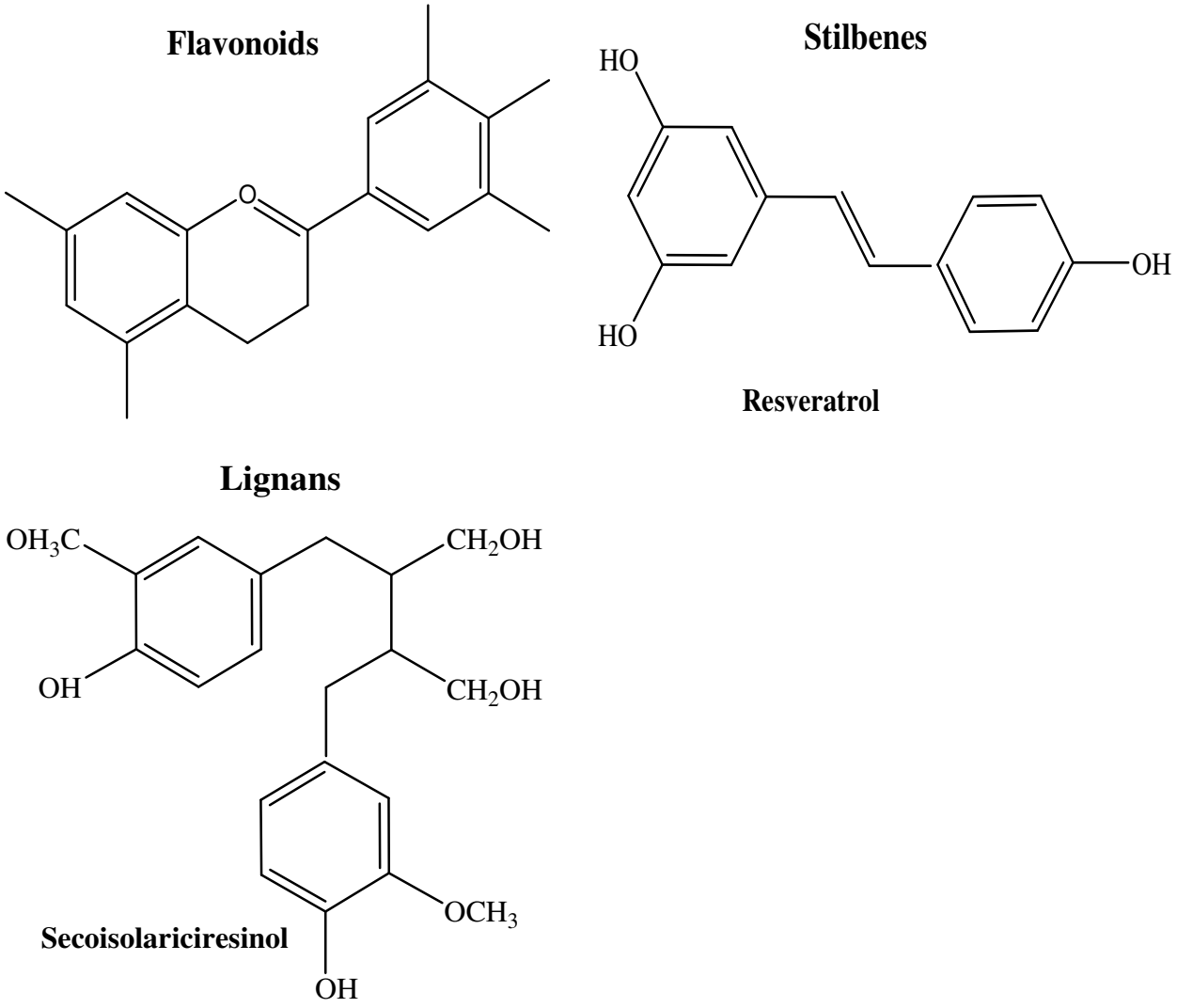
Hydroxycinnamic acid



R_1 = OH; Coumaric acid
R_1 = R_2 = OH; Caffeic acid
R_1 = OCH_3, R_2 = OH; Ferulic acid



Chlorogenic acid



Source: (Manach et al., 2004)

Figure 2.5 Chemical structures of polyphenols

Polyphenols occur primarily in conjugated form with one or more sugar residues linked to hydroxyl groups. However, direct linkage of the sugar unit to an aromatic carbon atom also exists (Bravo, 1998). These compounds have a health benefits such as prevention of cardiovascular diseases, cancers, osteoporosis and suggests a role in prevention of neurodegenerative diseases and diabetes mellitus (Scalbert et al., 2005).

Polyphenols, especially high molecular weight ones, are also known inhibitors of dietary proteins, endogenous proteins in the intestinal tract such as digestive enzymes, which in turn

reduces the absorption of other macronutrients like starches, lipids. Inhibition of amylolytic enzymes and the subsequent reduction of dietary carbohydrate hydrolysis can decrease the postprandial glycemic response (Bravo, 1998).

In addition, phenolic compounds also inhibit the absorption of minerals such as iron and zinc. The iron binding property of polyphenols is attributed to phenolic compounds with two or three adjacent hydroxyl groups, namely galloyl (gallic acid) and catechol groups (catechin), respectively (Brune et al., 1991). Monomeric flavonoids in green and herb teas (catechins), phenolic acids in coffee (chlorogenic acid), polymerized products in black tea and cocoa and wine polyphenols have been shown to reduce iron bioavailability (Bravo, 1998).

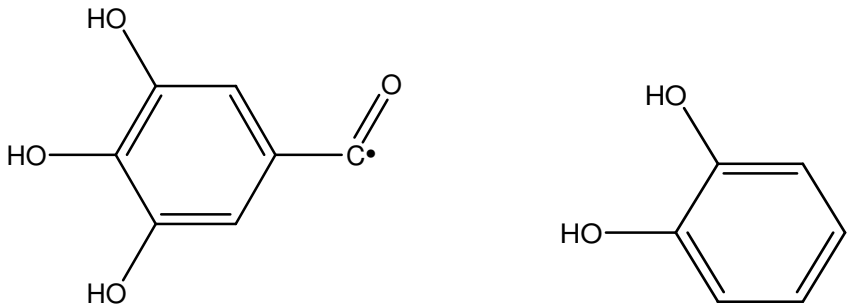
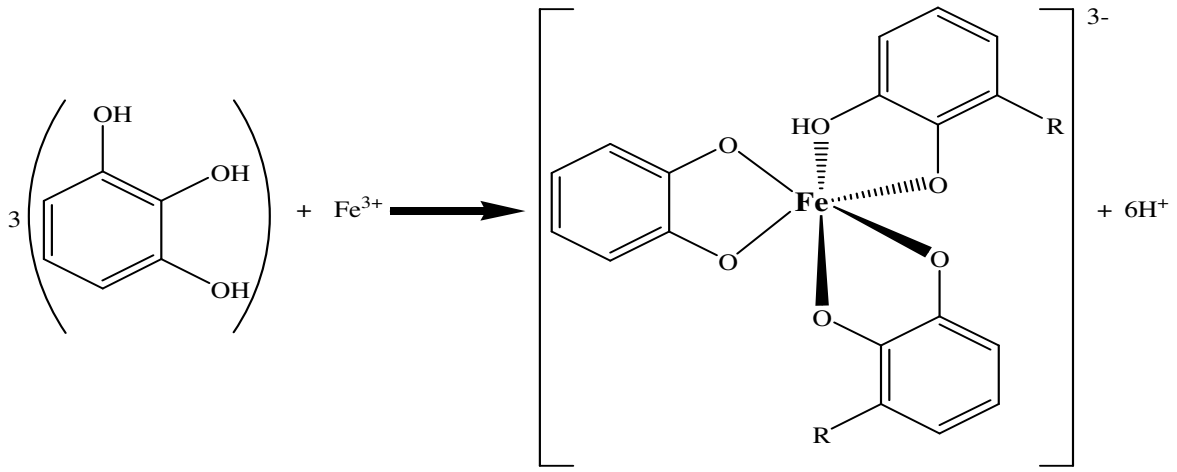


Figure 2.6 Catechol and galloyl groups responsible for iron-binding property

Iron-binding polyphenols bind ferric ion (Fe^{3+}) and render it insoluble in the intestinal lumen (Bravo, 1998). The gallol and catechol groups become deprotonated to form complexes with iron (Fe^{2+} and Fe^{3+}). In principle polyphenols with catechol and gallol groups always bind iron in 3:1 fashion. The more stable complex is the one formed with Fe^{3+} rather than Fe^{2+} (Perron and Brumaghim, 2009). Consequently, the unstable Fe^{2+}-polyphenol complex rapidly oxidizes in the presence of O_2 to give Fe^{3+}-polyphenol complexes, a process called auto-oxidation (Chvátalová et al., 2008). Auto-oxidation has negative nutritional consequence as ferrous (Fe^{2+}) is more readily absorbed than ferric (Fe^{3+}) (Moore et al., 1944). In general the rate of auto-oxidation is faster in gallate complexes than catecholate complexes (Perron and Brumaghim, 2009).



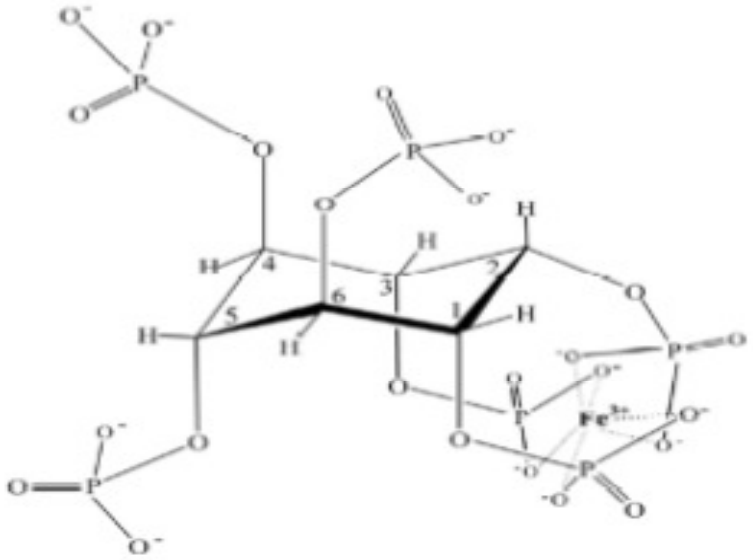
Source: (Perron and Brumaghim, 2009)

Figure 2.7 Iron-polyphenol complexes

2.3.2. Phytate

Phytic acid (myo-onositol 1,2,3,5/4,6-hexakis [dihydrogen phosphate]) is a common constituent of most mature cereals grains and some vegetables and fruits representing 60-90% of total phosphorous in plants (Lehrfeld, 1989). Phytate represents a complex class of naturally occurring compounds that can significantly influence the functional and nutritional properties of foods (Maga, 1982), which is made up of an inositol ring with six phosphate ester groups and associated with salts such as magnesium, calcium or potassium phytate. It is the principal storage form of phosphorus in cereals, legumes and oleaginous seeds. The content in cereals varies from 0.06 to 2.22%, with unrefined cereals and legumes containing the highest amount (Lopez et al., 2002). In most cereals, phytate is concentrated in the bran (aleurone layer), which accounts to 90% of the phytin and the other 10% in the embryo (Gibson et al., 2010). On the contrary, in maize most of the phytin is found in the germ and only a small fraction is found in the aleurone (Gibson et al., 2010, Lopez et al., 2002). It is also shown that grains of different varieties have different localization of phytate. Excessive amounts of phytic acid forms insoluble complexes

with Cu^{2+}, Zn^{2+}, Fe^{3+} and Ca^{2+} at physiological pH and consequently, reduces the bioavailability of these minerals (Lehrfeld, 1989).



Source: (Schlemmer et al., 2009)

Figure 2.8 Molecular structure of ferric-phytate: iron (Fe^{3+}-ion)

The iron-binding property of phytate is dependent on the phosphate groups; basically one mole of phytate can theoretically bind up to six mole of iron (Davidsson et al., 1997). Phytate forms insoluble complexes with both ferric and ferrous iron and inhibits iron absorption in a dose dependent manner. The effect is pronounced even at lower concentration of phytate. It has been demonstrated that the amount of total inositol phosphates (phytate-P) in a bread based meal containing approximately 0.05 mg Fe/g should be reduced to below 0.13 mg/g (a molar ratio of phytate and iron of 0.84) to achieve an improvement in iron absorption (Brune et al., 1992). Others suggest a reduction in molar ratio below 0.4 for a significant improvement in iron bioavailability (Hurrell, 2004).

Different processing methods can improve the bioavailability. In general processing such as soaking, germination, cooking and fermentation dephosphorylate the higher inositol phosphates

to the lower ones such as, tri, tetra and penta inositol phosphates (Han et al., 1994). The lower inositol phosphates have different affinity for metals. For instance, inositol phosphates with less than five phosphate groups (IP-1 to IP-4) do not form insoluble complexes with zinc, whereas inositol phosphates with less than three phosphate groups do not chelate iron (Gibson et al., 2010).

2.3.3. Organic acids

The most potent promoter of iron absorption is ascorbic acid, this is due to its reducing effect either through preventing the formation of insoluble ferric complexes or forming soluble complexes with ferric ions, which preserve the iron solubility on the more alkaline duodenal pH (Hallberg, 1981, Charlton and Bothwell, 1983). Other organic acids, such as lactic acid, citric, malic and tartaric acids with the exception of oxalic acid have a similar effect. Their presence in vegetables such as broccoli, beetroot, pumpkin, cauliflower and cabbage is responsible for the high bioavailability of the iron in such foods (Charlton and Bothwell, 1983).

2.4. Processing methods to enhance the bioavailability of plant-based diets

There are several traditional food processing and preparation methods that can be used at the household level to enhance the bioavailability of micronutrients in plant-based diets. These includes thermal processing-cooking, decortication, soaking, fermentation and germination/malting (Hotz and Gibson, 2007, Gibson and Ferguson, 1998).

2.4.1. Thermal processing

Thermal processing methods are known to improve the bioavailability of micronutrients. It has been shown that thermal processing such as boiling significantly reduced the tannin content in high tannin sorghum varieties (Mitaru et al., 1984) and also boiling for 30 min and roasting for

10 min resulted in 73% and 17% reduction of polyphenols, respectively. This is due to the leaching of phenolic compounds into the water (Barroga et al., 1985). Thermal processing needs to be optimal as it also causes the leaching of water soluble vitamins (Sood and Malhotra, 2002). In contrast, the effect of thermal processing on phytate degradation is inconsistent, and depends on the plant species, temperature and pH (Gibson et al., 2006). Some studies suggest that home thermal processing do not degrade phytate sufficiently enough to improve iron absorption unless the initial phytate content reduced by other processing methods before cooking (Hurrell et al., 2002). This is due to the gentler heat treatment used (Hurrell et al., 2002). Another study reported moderate losses of phytate and improved mineral bioavailability in maize chapattis (Khan et al., 1991).

2.4.2. Soaking and germination

Soaking has been advocated as a practical, non-enzymic, household method to reduce the phytic acid (Gibson and Ferguson, 1998) as well as the phenolic content of certain cereals and legumes (Towo et al., 2011). The effect on the phytic acid is primarily due to enzymatic hydrolysis of higher inositol phosphates to lower inositol and inorganic phosphate, induced by the enhanced activity of endogenous cereal phytase (myo-inositol hexaphosphate phosphohydrolase) (Perlas and Gibson, 2002), while controlling the conditions as the activity depends on the pH and temperature of the medium. It is important as it might trigger optimal endogenous phytase activity for grain that have low activity such as maize and sorghum (Gibson and Ferguson, 1998). Secondly, leaching of phytic acid content of some cereals and legumes into the soaking medium is responsible for the decrease. This is due to the presence of phytate as water soluble salt of sodium or potassium salts (Perlas and Gibson, 2002).

Furthermore, soaking of whole grains in water and alkali significantly reduced the phenolic compound. Some studies show that, the tannin contents in sorghum were reduced by 27-43% during steeping of the grains for 48 hrs (NWASIKE, 1989). This is due to leaching of phenolic compounds into the soaking medium. Further reduction of polyphenols is also possible if soaking is followed by germination; a reduction of up to 61 and 33% was possible at 96 and 24 hrs of germination (Obizoba and Atii, 1991) and also a study on mug beans sprout shows ~36% decrease in polyphenols after 48 hrs of germination. When germination was followed by fermentation, especially after longer fermentation time an increase of polyphenols occurred. (Barroga et al., 1985). This can be explained by the increase in protein in sprouting owing to the release of much greater amino acids for synthesis of protein due to breakdown of tannin-protein complexes, which will be higher around 96hrs (Obizoba and Atii, 1991). In contrast germination increases the concentration of hydrogen cyanide (HCN), which is normally present in low concentration in grains (Obizoba and Atii, 1991, NWASIKE, 1989). This is due to increased inherent enzymatic activity activated during sprouting of seeds (Obizoba and Atii, 1991). Alkali treatment also shows a significant reduction of tannins and phytates without compromising the germinability and organoleptic properties of the grain (Ochanda S.O, 2010).

2.4.3. Decortication

Decortication of sorghum and millets is a wide spread practice in sub-Saharan Africa. This allows the removal of the bran from the starchy endosperm of grains for human consumption (Hama et al., 2011). During sorghum decortication, the primary objective is to remove the pericarp and associated pigments, tannins (if present) and the germ (Yetneberk et al., 2005). There are three types of decortication known as traditional decortication (hand-pounding), mechanical decortication and abrasion with Tangential Abrasive Dehulling Device (TADD).

These have different mechanism and serve for different purposes (Hama et al., 2011). During traditional decortication, the shock between the pestle and the grains, generating strong interactive forces and resulting in the breaking of large pieces of hull (Yetneberk et al., 2005). On the other hand mechanical decorticator uses friction between the grains and the rotor of the equipment in order to remove the bran and germ from the endosperm. The other type of decorticator is based on abrasion with TADD in laboratory scale to successively remove the different grain fractions (Oomah et al., 1981). It has been used to improve the sorghum *injera* quality by Yetneberk et al. (2005), to define the amount of decortication needed to improve the color and yield of sorghum(Aboubacar et al., 2006) and also to fractionate sorghum grains to separate wax-rich fractions (Lochte-Watson et al., 2000)

Decortication affects the nutritional profile of cereals; this includes macro and micro-nutrients and also the absorption inhibitors. Some studies showed the effect of decortication on nutritional profile of sorghum and millet cultivars. For instance, during decortication of millets using abrasive decorticator the loss of ADF fiber, iron, iron-binding polyphenols and phytase activity was observed during the removal of the pericarp due to their localization on the outer part of the grain (Lestienne et al., 2007). Protein, lipid and zinc contents were not changed to a great extent following decortication as these compounds were uniformly distributed in millet grains. Furthermore, the phytate content of the millets remained high after decortication, this suggests that insignificant amount found in the germ and endosperm of the grain (Lestienne et al., 2007). In contrast a reduction of ~70% of phytate has been observed in sorghum varieties at 50% dry matter loss (Hama et al., 2011). This suggests that the localization of nutrients in the grain dictates the extent of their loss. Even though decortication reduces chelating compounds there is also higher loss of minerals such as iron and zinc. It has been shown that nearly 70% of phytates

has been decreased at a cost of 80% of the iron (Hama et al., 2012).. These losses of chelating compounds at a cost of minerals have negative consequence on bioavailability. Consequently, either optimizing the process for retention of the micronutrients while reducing the content of absorption inhibitors or including processes such as fermentation is necessary to increase the bioavailability of micronutrients.

2.4.4. Fermentation

Food fermentation is practiced by humans all over the world (Scott and Sullivan, 2008). Fermentation as a food preservation technique can be traced back for thousands of years. For instance, art of cheese-making was developed 8000 years ago in Iraq, at a time when plants and animals were just being domesticated (Paul Ross et al., 2002). Later, alcoholic fermentations involved in winemaking, and brewing was developed (Soomro et al., 2002). In general, three types of fermentation processes are used, which includes alcoholic, lactic acid and alkali fermentations (Scott and Sullivan, 2008, Mensah, 1997). Lactic acid fermentation is a common technique used to prepare and preserve different types of dishes including cereals, soybeans, fruits, vegetable and dairy products. Fermentation can increase the palatability and nutritional value (Rhee et al., 2011).

In cereals, either lactic acid bacteria or yeasts are used through spontaneous fermentation by activating the naturally occurring microbes in the milled grains (Poutanen et al., 2009). It has been shown that fermentation has a potential to significantly reduce the amount of chelating compounds in cereals (Svensson et al., 2010). Several studies report the decrease in the amount of phytates and polyphenols during fermentation (El Hag et al., 2002, El Khalifa and El Tinay, 1994, Idris et al., 2005). In contrast, some studies reported the increase in total polyphenols after fermentation. For instance, the amount of polyphenols in pearl millet decreased during

germination but increased during subsequent fermentation. This is may be due to the hydrolysis of high molecular weight phenols to the lower ones (Sripriya et al., 1997) or the release of phenols from complexes with other constituents by the action of enzymes.

2.5. Measurement of iron bioavailability

Among the different methods available for measuring mineral bioavailability, in-vivo, in-vitro and mathematical models based on phytate to iron molar ratio and algorithm are the most commonly used.

2.5.1. In-vivo methods

There are different kinds of in-vivo iron bioavailability measurements, which include balance studies, hemoglobin repletion test in rats, human efficacy test and isotopic techniques.

- **Balance studies**

Balance studies are based on measuring the whole-body retention of iron. Mass balance can quantify the whole-body retention of the minerals from a controlled diet containing the food or foods of interest (King et al., 2000). It is done by measuring the iron absorption indirectly by calculating the difference between oral input and fecal outputs after oral administration of a known amount of iron and a periodical collection of a fecal output. The drawback of using this method is that it is very laborious and expensive and also has the disadvantage of being imprecise (Rossander et al., 1992).

- **Hemoglobin repletion tests in rats**

Hemoglobin repletion tests in rats were developed to resemble human iron absorption studies as closely as possible. In this assay different groups of male anemic rats are fed diets fortified with either the iron compound or a control iron, which Hb repletion relative to the control is measured.

The iron compound (including the control iron) is fed to the groups of rats and the increase in Hb is measured after 14 days. The Relative Biological Value (RBV) of the particular iron compound is obtained by comparing the plotted slope between the iron concentration and the Hb against the same slope obtained from the control iron. It has been suggested as a useful technique to assess the RBV of different iron compounds (Swain et al., 2003). However, studies shows that dietary inhibitors and enhancers of non-heme iron absorption had different effects in humans and in rats (Reddy and Cook, 1991). As a result, this assay cannot be considered as a suitable model for studying the effect of different dietary factors on human iron absorption. In order for the method to be used the same standardized meal without absorption inhibitors and enhancers must be used.

- **Human efficacy**

Human efficacy method is quite similar to the hemoglobin repletion test in rats but is more complicate and used in human efficacy trials. It measures the efficacy of a specific iron compound in increasing the iron status of a group in comparison to a control group. An efficacy trial is performed under strictly controlled conditions. A well-designed human efficacy trial is characterized by more randomization, double-blinded placebo-control and a strict compliance control. Iron status parameters, such as hemoglobin, serum ferritin and soluble transferrin receptor concentrations are measured at baseline and post-trial. It is combined with effectiveness trial to give an outcome in real life situations through incorporating the distribution systems, compliance and other biological factors, which makes it cumbersome and expensive methodology to be used as a screening tools (INACG, 2004).

- **Isotopic technique**

Isotopic technique is a preferred method for measuring iron bioavailability in humans. It is done through administration of oral tracers, which can be intrinsically or extrinsically labeled to the food. An intrinsic tracer is one that has been incorporated into the food during growth of the plant or animal; an extrinsic tracer is added to the food in small amounts as inorganic salts before feeding. When an extrinsic tracer is used, it is assumed that the label equilibrates with all pools of iron in the food and is absorbed to the same extent and at the same rate as the intrinsic element in the food. An extrinsic iron tracer is the preferred method for administering the oral tracer, because it is less expensive and easier to prepare than an intrinsic label. The tracers can be incorporated as radioactive (iron-55 and iron-59) or stable isotopes (iron-54, -57 and -58) (Gislason et al., 1992, Boza et al., 1995). The later is preferable due to the absence of a radiation dose, which makes it suitable in studies involving children's and pregnant women (Parr and Fjeld, 1994).

2.5.2. In-vitro methods

- **HCl-extractability**

HCl-extractability is a relatively old method used to measure the bioavailability of iron from a diet. It is done through measuring the solubility in dilute hydrochloric acid (HCl) as a way of simulating the gastrointestinal environment (Forbes et al., 1989). However, the limitation of this method is that it does not consider the pH changes and the enzymes involved in the GI tract during absorption.

- **In-vitro solubility/dialysability**

In-vitro solubility/dialysability better mimic the iron absorption taking place in human GI tract. The method involves simulated gastrointestinal digestion followed by measurement of soluble, low molecular weight iron. A mixture of foods are homogenized and exposed to pepsin at pH 2 further pH adjustment is done using dialysis. The digestion continues till the addition of pancreatin and bile salts. The digested mixture diffuses across a 6 to 800 molecular weight cutoff semi-permeable membrane as an indicator of available iron (Miller et al., 1981). Studies often suggest that for this model to be effective, it has to be combined with culture of human intestinal epithelial cells (Yun et al., 2004).

- **Caco-2 cells**

Caco-2 cells are derived from human colon adenocarcinoma, they exhibit many features of small intestinal cells, including the influence from dietary factors on iron absorption (Han et al., 1994). In this model, there is a formation of ferritin by Caco-2 cells exposed to digested food serves as an indicator of bioavailable iron. An increase in cell ferritin is unequivocal evidence that iron has entered the cell because cells produce ferritin in response to increases in intracellular iron. In contrast, an increase in the apparent cellular content of a radio iron tracer could represent surface-bound iron as well as intracellular iron (Yun et al., 2004).

2.5.3. Mathematical models

- **Phytate to iron molar ratio**

Determining the molar ratio of phytate to iron in foods and diet is one of the methods to measure bioavailability. As phytate known to inhibit the absorption of iron even present in small amount

in foods. The phytate to iron molar ratio should be >1 and preferably below 0.4 in cereal based foods in order to increase the absorption of iron (Hurrell, 2004).

- **Algorithms**

There has been different version of algorithms developed for estimating iron absorption. The current algorithm developed by (Hallberg and Hulthén, 2000) takes into account the different inhibitors and enhancers of iron absorption. The formula is the product of the basal factor 22.1(it represents the absorption of iron from a meal containing no known inhibitors and enhancers of iron absorption) multiplied by one or more of the eight factors present in each meal such as phytate factor, ascorbic acid factor, polyphenols factor (tannic acid), calcium factor, egg factor and the alcohol factor. Besides considering individual factors; it also considers the interaction between factors during absorption, for instance the presence of phytates or polyphenols can enhance the positive impact of ascorbic acid. The value obtained in a meal at an iron status corresponding to reference dose absorption of 40% in fasting state or iron-deplete subjects. Studies suggest that it correlates well with human absorption studies (Hallberg and Hulthén, 2000).

Chapter 3 – *Materials and Methods*

3. Materials and Methods

3.1. Location of the study

The study has been conducted in two different locations. Traditional decortication was done at Harerge region (Hirna), which is located at lowland part of Ethiopia and well known for its sorghum production. The biochemical analyses were conducted at Center for Food Science and Nutrition of Addis Ababa University and Ethiopian Health and Nutrition Institute (EHNRI) laboratories.

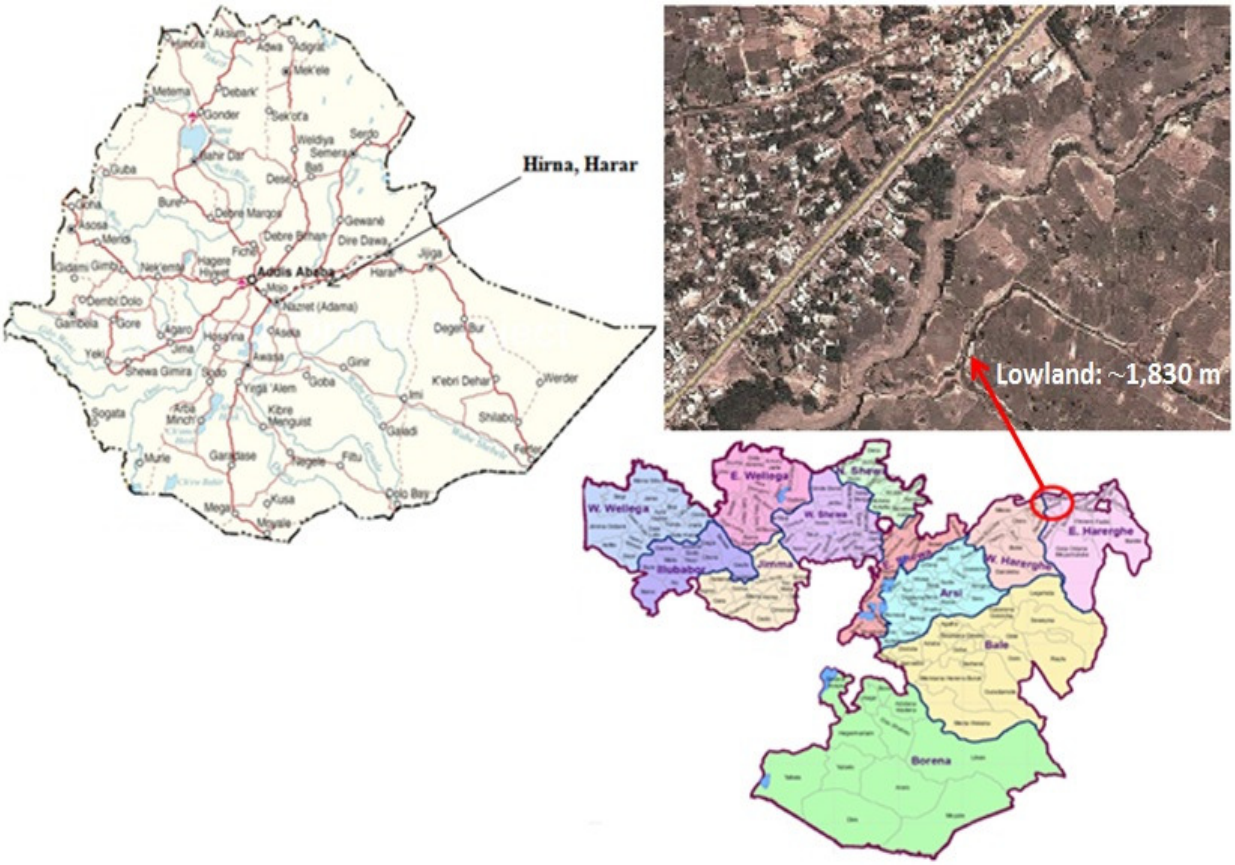


Figure 3.1 Ethiopian map showing the study site "Hirna, Harar"

3.2. Chemicals and reagents

All chemical and reagents used in laboratory analyses were analytical grade.

3.3. Sorghum cultivar collection

Two sorghum cultivars were used in this study. Meko (white) and Seredo (brown) were collected from Ethiopian Sorghum Improvement Program (ESIP) at Melkassa Agricultural Research Center, Nazret Ethiopia.

3.4. Experimental design

The general objective of this thesis was to examine the effect of traditional decortication and fermentation on iron-binding phenolics, phytates, polyphenol oxidase activity and understand its implication to iron bioavailability. The iron, calcium, iron-binding phenolics, tannin, polyphenol oxidase activity and phytate content were determined for each variety before and after decortication and fermentation, in order to determine how much the grains were affected due to these treatments. Furthermore, iron bioavailability was predicted using phytate to iron molar ratio and algorithm (sorghum *injera*). In addition, the moisture content of the grains at each levels of decortication were determined to calculate the extraction rate and decortication yield, this permitted description of the grain fraction remaining after decortication. All analyses were done in triplicate.

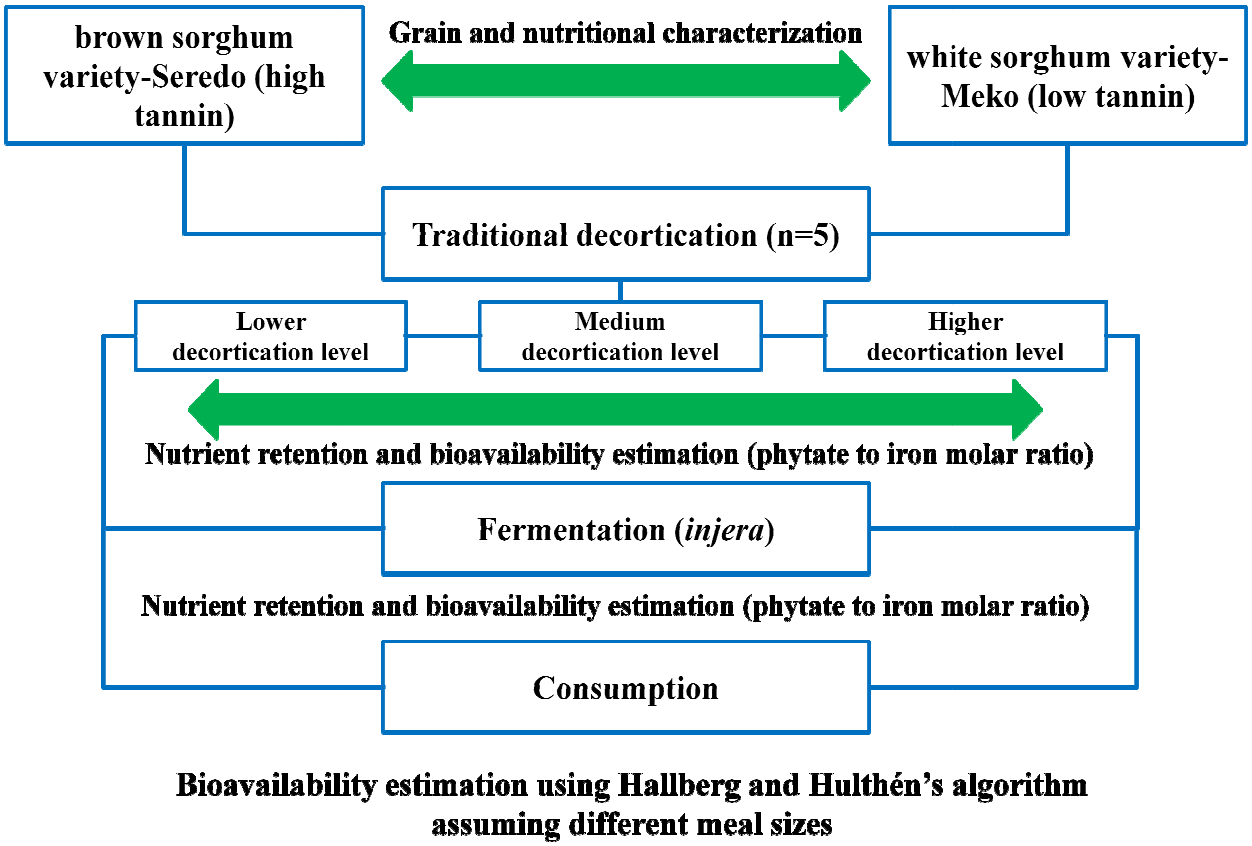


Figure 3.2 General experimental design of the study

3.5. Sample preparation and traditional decortication

The grains of both cultivars were cleaned manually to remove husk, damaged grains, stones, dust, light materials, glumes, stalks, undersized and immature grains and other extraneous materials. Besides, the samples were winnowed and hand sorted to remove additional impurities. The two cleaned sorghum varieties (Seredo and Meko) were subjected to traditional decortication using wooden mortar and pestle. Five women were selected based on their skills of traditional decortication. The women used the same wooden mortar and pestle and used their own know-how in deciding the amount of water added for the initial conditioning of the grains to facilitate decortication, as well as the length and the intensity of the decortication. Parameters such as, amount of water added, time of decortication and decortication rate (pounding/min) were

determined. This was used for the determination of mean extraction rate of decorticated grains as practiced. Once the average length of the decortication process was determined (1:45 min) further experiments were done at 1 and 3 min, designated as lower and higher levels.

About 2 g of sample was taken for measurement of DM content and the rest of the decorticated grains were oven-dried, milled, and sieved before further analysis. The milled samples were packed in airtight polythene plastic bags until further analysis.

3.5.1 Calculation of decortication parameters

Extraction rates of grain samples were calculated after each decortication step as the proportion of the weight of decorticated grains to the initial weight of the grains (g/kg). Decortication yield (DY%) is calculated similarly but on dry matter basis (DM), and DM loss were calculated as (100-DY) according to Hama et al. (2011), Lestienne et al. (2007).

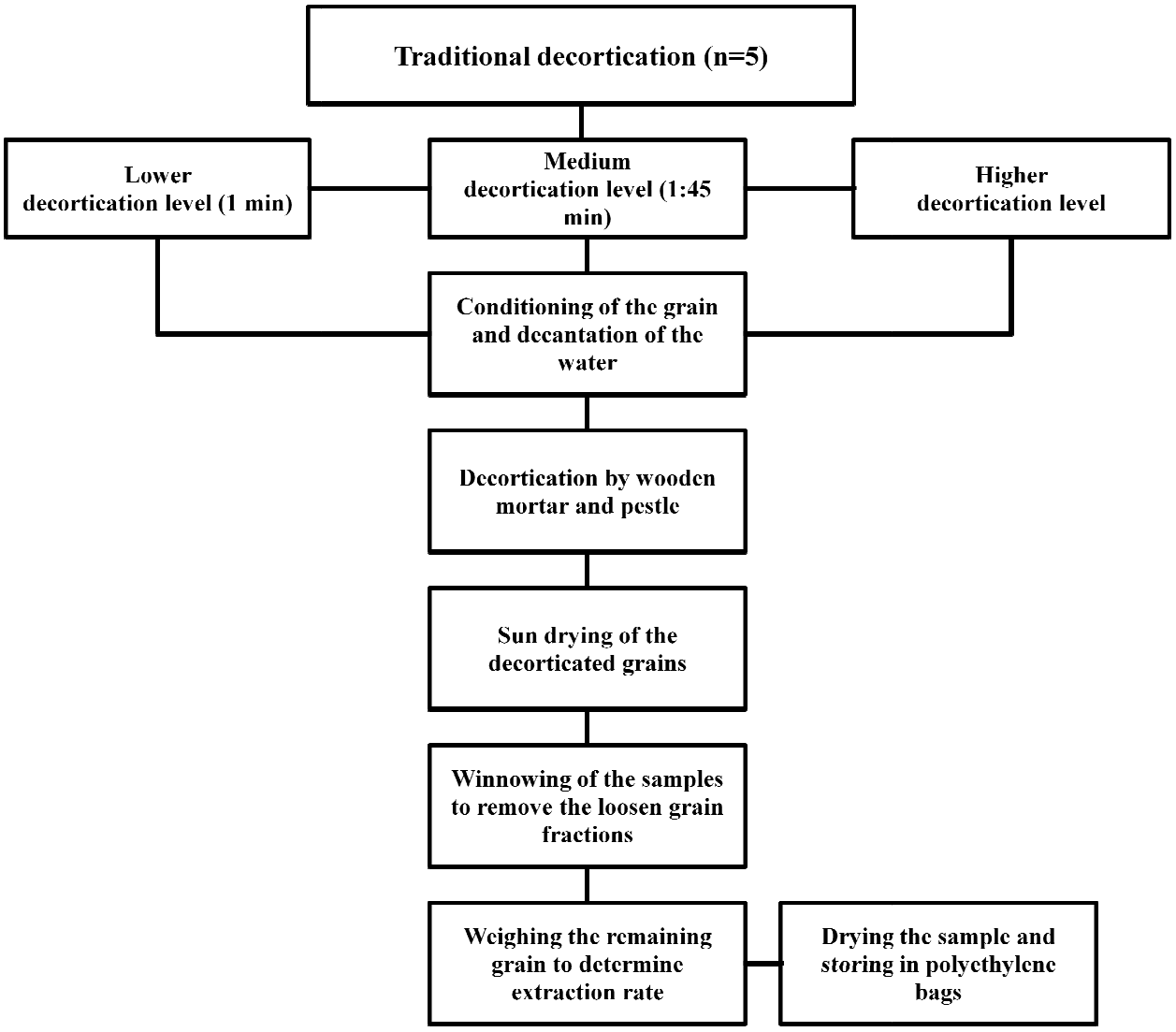
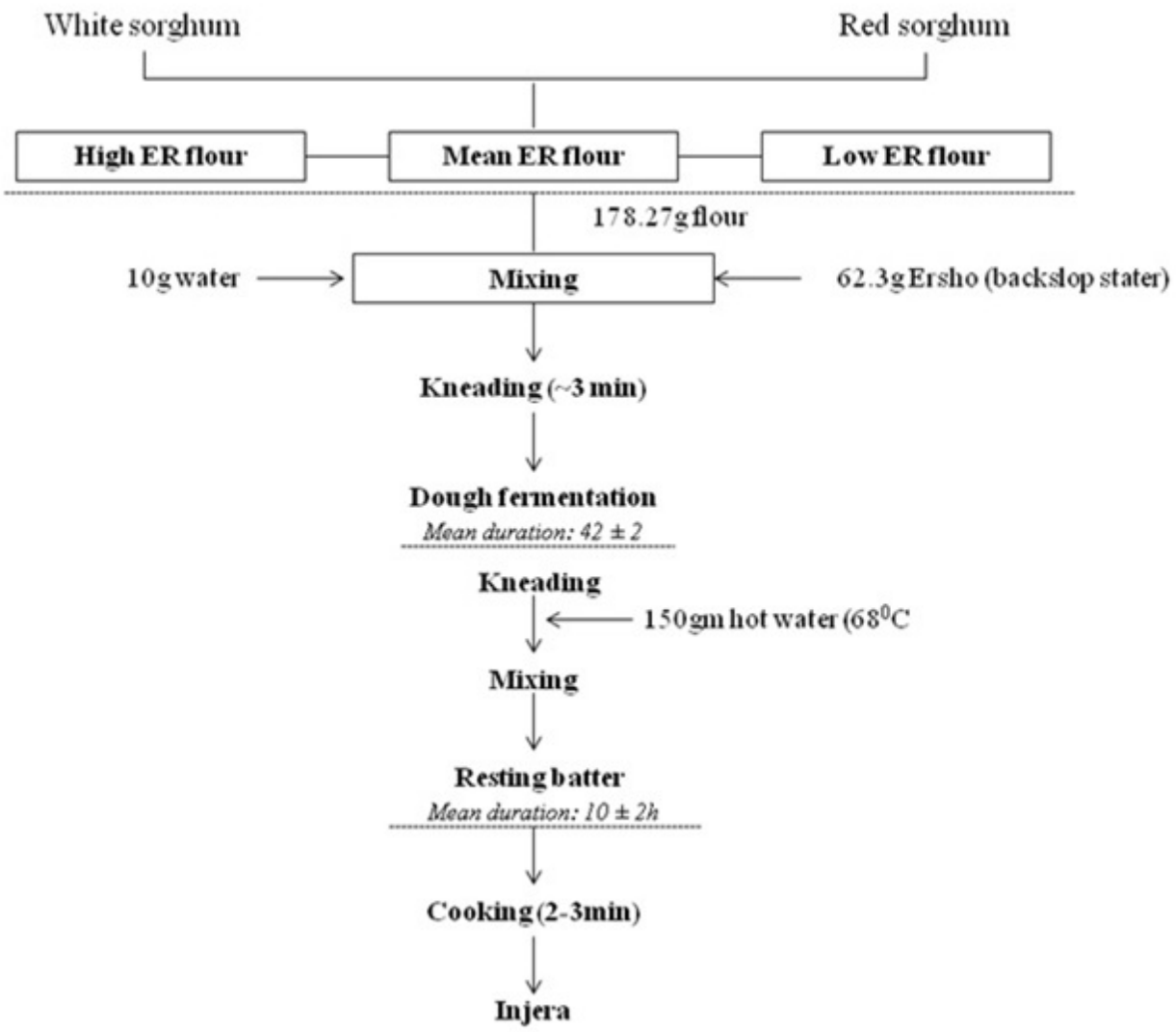


Figure 3.3 Traditional decortication process

3.6. Fermentation for preparation of *injera*

The cleaned sorghum grains were milled into fine flour using laboratory miller. A starter culture was taken from previous fermentation. The sorghum flour was then mixed with the starter and was allowed to ferment for 42 hrs. Baking of *injera* was done on a hot plate (steam bake) in a thin layer on a covered clay griddle 2-3 min.



Source: (Baye et al., 2012)

Figure 3.4 Sorghum *injera* preparation

3.7. Analytical methods

3.7.1. Grain characterization

The diameter and length of 20 different undamaged grains was measured with a caliper rule (PRIESSER, IP54). Thousand kernel weight (TKW) was measured by determination of the weight of one thousand undamaged grains by using analytical balance (Adventurer, OHAUS, China).

3.7.2. Dry matter (DM) analysis

Empty dishes and their lids were dried using drying oven for an hour at 105^0C, transferred to the desiccators and cooled for some time and weighed. After that one gram of sample was transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven till constant weight (AOAC, 1999). The % DM was calculated as follow:-

Calculation: $$\% \text{ DM} = 100 - \frac{(M_{initial} - M_{dried})}{M_{initial}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

3.7.3 pH determination

The pH of the fermented dough was determined every 2 hrs interval by using a pH meter (Mp511, China) for the first eight hours of fermentation and the other measurements were taken during 24, 32 and 42 hrs in triplicate. The pH meter was calibrated using pH 4.0, 7.0 and 10.01 buffers.

3.7.4. ADF fiber analysis

Acid detergent fiber (ADF) was measured gravimetrically by measuring the insoluble residue after chemical and enzymatic solubilization of elements not falling within the category of dietary fiber. The residue corresponded to the indigestible carbohydrate (cellulose, lignin). Weight loss was measured after incineration residue, provides organic carbohydrate fraction without the mineral. This fraction corresponds to insoluble fiber (cellulose and lignin) (Van Soest et al., 1991).

3.7.5. Crude fat analysis

Crude fat was determined by using soxhlet method. The solvent builds up in the extraction chamber for 5-10 min and completely surrounds the sample and then siphons back to the boiling flask. Fat content was measured by weight loss of the sample or by weight of the fat removed.

Ten gram of sample was weighed into an extraction thimble and covered with absorbent cotton. Then 150 ml solvent (petroleum ether) (Sigma-Aldrich, USA) was added to a pre-weighed cup. Both the thimble and the cup were attached to the extraction unit (Barnstead electro-thermal, UK). The sample was subjected to the extraction with solvent for 30 min followed by rinsing for 1.5 hrs. Then, the solvent was evaporated from the cup to the condensing column. Extracted fat in the cup was placed in an oven (Cintex precison, India) at 110°C for 1 hr and after cooling the crude fat was calculated using following formula (AOAC, 1998):

$$\text{Crude fat (\%)} = (\text{Extracted fat} / \text{Sample weight}) \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

3.7.6. Crude protein analysis

Protein (N×6.25) was determined by Kjeldahl method. In this method, proteins and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. Then, the total organic nitrogen was converted to ammonium sulfate. The digest is neutralized with alkali and distilled into a boric acid solution. The borate anions formed are titrated with standardized HCl, and are converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food (AOAC, 2000).

Addition of reagent: 0.6 g of sample was weighed in a tector tube and placed in the tecator rack. Then 6 ml of acid mixture was added by using pipette carefully and mixed with the sample immediately. Then 3.5 ml of hydrogen peroxide was added step by step till the reaction stops. As

soon as the reaction has ceased, the tube was hand-shaken for a few minutes and was put back into the rack. Then, 3 g of catalyst (K_2SO_4 with a mixture of selenium 100 to 0.5 ratio) was added and the mixture was allowed to stand for 5-15 min before digestion.

Digestion: The tube in the rack was lowered into the digester in the fume hood at 370^0C. The exhaust manifold was located on top of tubes and the digestion was continued until clear solution appeared (3 hrs) in the fume hood.

The total content of nitrogen in the samples was determined after titration using 0.1N HCl (Sigma-Aldrich, USA) using automatic nitrogen determination apparatus (Gerhardt vapodest, Germany). The total protein was calculated using the following formula:-

$$\text{g N in the sample} = V \times N \times 14$$

$$\text{g nitrogen / 100 g sample} = \text{g of nitrogen} \times 100/\text{g sample} \quad \text{..... (3)}$$

$$\text{Total nitrogen (\%)} = [(V - V_b) \times N \times 14)]/ W$$

$$\text{Crude protein (\%)} = \text{total nitrogen (\%)} \times 6.25$$

Where: **V** = volume of sulfuric acid consumed to neutralized the sample

V_b= the volume of acid consumed to neutralize the blank

N × 14 = normality of the acid × Eq. wt of nitrogen

6.25 = conversion factor from total nitrogen to crude protein

3.7.7. Total ash and mineral (iron and calcium) determination

Total ash content was determined after the removal of organic material by dry ashing. The crucibles were washed with 10% HCl (Sigma-Aldrich, USA) and glass wares with 10% nitric acid before starting the analysis. Then dried in oven (105^0C for 30 min) and cooled in desiccators for 30 min. One gram of sample weighed and charred on hot plate to avoid spattering of the sample. The charred samples were put in furnace (55^0C for 30 min) and were allowed to cool.

Five drops of water was added, and then the samples were charred. The charred samples were put in the furnace for another 30 min. Then, five drops of nitric acid and 3 drops of water were added to further remove organic compounds. The samples were put in the furnace for another 2 hrs, then cooled in a dedicator and weighed. Total ash was determined by using the following formula:-

$$\text{Total ash (\%)} = \frac{\text{wt. of ashed sample}}{\text{wt. of fresh sample}} \quad \text{..... (4)}$$

The ash was digested with 10 ml of 6N HCl to wet it completely and dried on a low temperature on hot plate. Then, 10 ml of 3N HCl was added and the crucible was heated on the hot plate until the solution boils. Then it was allowed to cool and then was filtered through a filter paper into a graduated flask (50 ml). The crucibles were washed nearly three times with deionized water and the washings were filtered into the flask. The filter paper was washed thoroughly and washings were collected in the flask. Then, 2.5 ml/50 ml of solution was added in all samples to minimize the interference of phosphates on calcium atomization. The content of the flask was cooled and diluted to the mark (50 ml) with deionized water. The blank sample was prepared by taking the same amount of reagents. The samples solutions were transferred to polyethylene bottle. The iron and calcium content was determined by Atomic Absorption Spectrophotometer (AAS) after calibrating with iron and calcium standards (Mohammed et al., 2011). The following formula was used to express the amount of iron and calcium in mg/100g.

Calculation:

$$\text{mg/100 g of metal content} = [(C_s - C_b)*V]/[10*W] \text{…….. (5)}$$

Where, C_s: concentration of sample in ppm

C_b: concentration of blank in ppm

V: volume (ml) of extract

W: weight (g) of samples

3.7.8. Analysis of iron -binding polyphenols

About 0.5 g of sample was extracted using 10 ml of 50% dimethylformamide (DMF) solution. The samples were incubated for 16 hrs under shaking (160 rpm) at 28^0C. The samples were then filtered using whatman GF/A filter paper and 0.5 ml of filtrate was transferred into two test tubes. Two milliliter of reagent (with ferric ammonium sulfate (FAS)) was added to 0.5 ml of filtrate and sample blank was prepared by adding 2 ml of reagent (without FAS). Finally, the absorbance of the sample and sample blank were determined using UV-Vis/NIR spectrophotometer (Lambda 950, Perkin Elmer, UK) at 680 and 578 nm for catechol and galloyl groups, respectively (Brune et al., 1991).

Solution preparations: - Na acetate buffer (0.1M pH 4.4) was prepared by mixing 335 ml of 0.1M Na acetate and 665 ml of 0.1 M acetic acid until the pH reached 4.4. 1M HCl was prepared by mixing 85 ml of 37% HCl (Sigma, USA) to one liter of distilled water. 50% DMF was prepared by mixing 70 ml of Na acetate buffer pH 4.4 and 70 ml of DMF. Arabic gum (1%) solution has been prepared by dissolving 0.5 g of arabic gum in 50 ml of distilled water. Urea solution (50%) was prepared by dissolving 200 g of urea in 400 ml of 0.1M Na acetate buffer at pH 4.4. FAS solution (5%) was prepared by dissolving 1 g of $FeNH_4(SO_4)_2$ in 20 ml of 1M HCl. Reagent (FAS) was prepared by mixing 267 ml of urea solution, 30 ml of 1% gum arabic and 3 ml of 5% FAS. The reagent without FAS was prepared by mixing 90 ml of 50% urea solution and 10 ml of 1% arabic gum.

Standard preparation: - catechin solution was prepared by dissolving 50 mg of catechin in 50 ml of 0.1M Na acetate buffer (pH 4.4). After that a serious of standard solutions was prepared by mixing 0, 20, 50, 100 and 150 µl of catechin solution with water till the final volume reached 0.5 ml. Likewise, a solution of tannic acid was prepared by dissolving 50 mg of tannic acid in 100 ml of 0.1M acetate buffer (pH 4.4), then a serious of standard solutions was prepared by mixing 0, 50, 100, 200 and 300 µl of tannic acid with distilled water till a final volume of 0.5 ml. Finally, 2 ml of reagent with FAS was added to each of the standard solutions and the absorbance was read using UV-Vis spectrophotometer (Lambda 950, Perkin Elmer, UK) at a wavelength of 578 and 680 nm.

3.7.9. Determination of tannin content

Modified vanillin-hydrochloric acid (MV-HCl) method was used. The method is based on 1% HCl in methanol extraction of condensed tannin and determination using vanillin reagent. The extraction was done by weighing 1 g of sample in a screw cap test tube and adding 10ml 1% HCl in methanol to the tube containing sample. After this the tube was put on mechanical shaker for 24 hrs at room temperature and centrifuged at 1000× g for 5 min. Then, 1 ml of the supernatant was taken and mixed with 5 ml of vanillin-HCl reagent in another test tube. The mixture was put at rest for 20 min in order for the reactions to complete. Then, the absorbance of the sample and blank (1 ml of supernatant with 1% HCl in methanol) were read at 500 nm using a UV-Vis spectrophotometer.

Standard solution preparation: About 40 mg D-Catechin was weighed and dissolved in 100 ml of 1% HCl in methanol (stock solution). A serious of (0, 0.2, 0.4, 0.6, 0.8 and 1 ml) of stock solution was taken in a test tube. The volume of each tube was adjusted to 1 ml with 1% HCl in methanol and 5 ml of vanillin-HCl reagent was added in each tube. The reaction was completed

in 20 min and the absorbance was read at 500 nm. The calibration curve (absorbance Vs concentration) was plotted using excel and the slope and intercept were calculated after adjusting for the blank zero (Maxson and Rooney, 1972, Burns, 1971).

Calculation:

$$\text{Tannin in mg/g} = \frac{[(A_s - A_b) - \text{Intercept}]*10}{\text{Slope x d x w}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Where:

A_s= Sample absorbance

A_b= Blank absorbance

d = Density of solution (0.791 g/ml)

W = Weight of sample in g

3.7.10. Determination of phytate content

About 0.5 g of dried sample was extracted with 10 ml of 0.2N HCl for 1 hr at an ambient temperature using a mechanical shaker (400 rpm). The extracted sample was centrifuged for 30 min at 3000 rpm and the clear supernatant was used for phytate determination. 2 ml of Wade reagent was added to 3 ml of supernatant sample solution that was homogenized and centrifuged for 10 min at 3000 rpm. The absorbance of the solution was measured at 500 nm using UV-Vis spectrophotometer. The phytate content was calculated from the difference between the absorbance of the blank (3 ml of 0.2N HCl + 2 ml of Wade reagent) and that of assayed sample. The amount of phytic acid was calculated using phytic acid standard curve and the result was expressed as phytic acid in mg/100 g DM.

Standard solution preparation: A series of standard solution was prepared containing 0, 4, 9, 18, 27, 36 µg/g phytic acid in 0.2N HCl. Then, 3 ml of each standard was pipetted into 15 ml

centrifuge tubes and 3 ml 0.2N HCl (blank). Then, 2 ml of Wade reagent was added to each tube, and the solution was mixed on a vortex mixer for 5 sec. The mixture was then centrifuged for 10 min at 3000 rpm and the supernatant read at 500 nm The calibration curve (absorbance Vs concentration) and the slope and intercept were calculated (Latta and Eskin, 1980, Vaintraub and Lapteva, 1988) as follows:

Calculation:

$$\text{Phytic acid in } \mu g/g = \frac{[(A_b - A_s) - \text{Intercept}] * 10}{\text{Slope} \times w \times 3} \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Where:

A_s= Sample absorbance

A_b= Blank absorbance

W= Weight of sample

3.7.11. Polyphenol oxidase activity assay

PPO activity was measured indirectly by determining the decrease in iron-binding polyphenols (catehcol and galloyls). One gram (1 g) of sample was incubated for 1 hr in a phosphate buffer (sodium phosphate) with constant shaking. The pH was set at 6.5, as this is the optimum condition for the activity of PPO's (Towo et al., 2006). The samples were then freeze dried and the amount of catechol and galloyl was determined (Matuschek et al., 2001, Brune et al., 1991). PPO activity was expressed as µmole of catechol and galloyl/min/g DM.

3.7.12. Prediction of iron absorption using algorithm

Iron absorption was predicted using the Hallberg & Hulten's algorithm (Hallberg and Hulthén, 2000). Analyzed values of phytate, calcium and tannic acid were used. Ascorbic acid (AA) values of the two sorghum varieties were taken from the Ethiopian food composition table.

The following formula was used:

$$\% \ absorption = 22.1 \times f_1 \times f_2 \times f_3 \times f_4 \quad \ldots\ldots\ldots\ldots\ldots (8)$$

* Note: - 22.1 is the absorption of iron from a wheat roll containing no known inhibitors or enhancers of iron absorption and adjusted to a reference dose absorption of 40%

$$f_1 = 10^{[-0.30*\log(1+\text{phytate-P})]}$$

$$f_2 = [1+0.01A + \log \text{phytate-P} + 1]*0.01*10^{(0.8875*\log(AA+1)}$$

$$f_3 = 10^{[0.4515-0.715*\log \text{tannic acid}]}$$

$$f_4 = 0.4081 + [0.6059/(1+10^{-(2.022-\log(Ca+1))*2.919)}]$$

The algorithm uses base 10 logarithms; phytate-P, AA, TA (tannic acid) and Ca were expressed in mg/meal. Factors f_1, f_2, f_3 and f_4 of this algorithm respectively adjust for the effects of phytic acid, AA, TA and Ca on iron absorption and include interactions between these components.

3.7.13. Phytate: Fe molar ratio

The mole of phytate and minerals were determined by dividing the weight of phytate and iron with its atomic weight (phytate: 660 g/mol and Fe: 56 g/mol). The molar ratio between phytate and iron was obtained after dividing the mole of phytate with the mole of minerals (Norhaizan and AW, 2009).

3.8. Data analysis

All results were presented as mean values ± standard deviation. Differences in the mean values of nutrients were determined by analysis of variance (ANOVA). Significance levels were obtained using LSD and Duncan tests and student's t-test was used to compare inter-variety differences. Differences were considered statistically significant for $P< 0.05$. All analyses were done in triplicate. SPSS version 20 was used for statistical analyses.

Chapter 4 – *Results and discussion*

4. Results and discussion

4.1. Characterization of sorghum varieties

The thousand kernel weight (TKW) of Seredo was slightly lower (35.1 g) than Meko (39.3 g) (**Table 4.1**). The TKW is in the range of previously reported values by Awika et al. (2005). However, the TKW of Seredo was higher than what was reported by Yetneberk et al. (2005). This may be due to difference in growing conditions. For instance, water stress may reduce seed filling and thus affect the final seed size (De Souza et al., 1997). The length to mean diameter ratio of Meko and Seredo was 1.1 which suggest a more rounded shape (Hama et al., 2011, Mwasaru et al., 1988).

The ADF fiber content of Seredo (9.16% DM) was significantly higher than that of Meko (8.06% DM). The ADF to TKW ratio of Meko was lower than Seredo suggesting that the latter had a thicker pericap. Such an inverse relationship between seed size and pericap thickness has already been previously reported (Abdelrahman et al., 1984). Furthermore, Yetneberk et al. (2005) have also reported that Seredo has a thick pericap and soft endosperm texture.

Despite a smaller seed size, Seredo had higher amount of protein and nearly the same amount of lipids than Meko. Given that proteins are mainly located in the endosperm of grains, Seredo is expected to have a higher floury endosperm fraction. On the other hand, the germ fraction of the grains is expected to be similar. This is in-line with previous reports stating that the amount of germ is not proportional to the size of the grain (Hama et al., 2012).

The total ash content of the two sorghum varieties ranged from 1.6-1.8%, which is comparable to previously reported values (1.37-1.75%) by Mohammed et al. (2011) and Tizazu et al. (2009).

Slightly higher ash content was observed for Seredo than Meko due to its thick pericarp. However, Meko had higher total iron content than Seredo (6.12 Vs 4.93 mg/100 g) but no significant difference was observed in their calcium content. This finding is also in-line with previous reports (FAO, 1995, Glew et al., 1997, Vietmeyer, 1996).

Table 4.1 Characteristic of sorghum varieties

Sorghum variety	Seredo	Meko
Kernel characteristics		
Thousand kernel weight (g)	35.1	39.3
Mean diameter (mm)	4.18	4.4
Mean length (mm)	4.63	4.9
Pericarp thickness	0.26 ± 0.02^{a}	0.21 ± 0.003^{b}
Nutrient composition		
Crude protein (% DM)	12.29 ± 0.05^{a}	11.12 ± 0.07^{b}
Crude fat (% DM)	3.30 ± 0.10^{a}	3.17 ± 0.15^{a}
Total Ash (% DM)	1.75 ± 0.02^{a}	1.56 ± 0.05^{b}
Catechol (g eq catechin/100 g DM)	2.35 ± 0.03^{a}	0.26 ± 0.06^{b}
Galloyl (g eq tannin/100 g DM)	1.46 ± 0.04^{a}	0.25 ± 0.04^{b}
Phytate (mg/100 g DM)	294.2 ± 10.8^{a}	345.3 ± 15.8^{b}
Tannin (g/100 g *CE DM)	1.48 ± 0.03^{a}	0.69 ± 0.01^{b}
Iron (mg/100 g DM)	4.93 ± 0.15^{a}	6.12 ± 0.13^{b}
Calcium (mg/100 g DM)	25.54 ± 1.10^{a}	24.55 ± 1.2^{a}
ADF fiber (% DM)	9.16 ± 0.58^{a}	8.06 ± 0.12^{b}

Different superscript letters in the same row correspond to significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations; *CE, data expressed as catechin equivalent; DM, dry matter.

As expected, the high tannin sorghum variety (Seredo) had six to nine times higher contents of iron-binding phenolic compounds (IBPC) (galloyl and catechols) **(Table 4.1)**. The amount of

tannin in Seredo was 1.48 g/100 g *CE DM, which is in the range of previously reported values for high tannin sorghums (Jambunathan and Mertz, 1973, Awika, 2000). The tannin content in the low-tannin variety (Meko) was 0.6 g/100 g *CE DM, which is also in agreement with (El Khalifa and El Tinay, 1994).

The two sorghum varieties had phytate contents in the range of 250-350 mg/100 g DM which is in-line with previous results (Mohammed et al., 2011, Idris et al., 2005). Meko had higher phytate content than Seredo (345.3 and 294.2 mg/100 g, respectively).

During traditional decortication, not only contents in mineral absorption inhibitors such as phytates and IBPC, but also the contents in minerals and proximate parameters can be affected. However, given that high tannin sorghum varieties have a thicker testa than the low-tannin ones (Awika and Rooney, 2004), the influence of decortication on the nutritional composition, in particular on the contents in IBPC may be different. Therefore, the effect of traditional decortication of one high-tannin (Seredo) and one low-tannin (Meko) variety was evaluated.

4.2. Decortication parameters

The household traditional decortication of sorghum grains revealed that the process spanned on average 1-2 min. The decortication was conducted using wooden mortar and pestle on conditioned grains. Conditioning was performed in order to facilitate removal of bran during decortication. The mean number of poundings per min was 73 ± 5. Decortication resulted in flours with 93-105% extraction rate, which is higher than what is often observed in household traditional decortication (Hama et al., 2011). Nearly 10% increase in moisture was observed due to moisture absorption of grains. Therefore, decortication yield (extraction rate on dry-matter basis) being corrected for the added water was more reliable than extraction rate. The

decortication yield for the two sorghum varieties (corrected extraction rate) was ~93% which corresponds to high extraction rate flour.

Decortication time was realistically extended (higher) and shortened (lower) in order to evaluate its application on nutrient composition of grains. The lower decortication time was set at 1 min while the higher was set at 3 min. The corrected extraction rate for the shorter time span decortication ranged from 92-98%, whereas it averaged 88% for the higher time span decortication. There was no major difference between the two sorghum varieties in their decortication characteristics.

Table 4.2 Decortication characteristics of sorghum varieties

	Decortication time (min)					
	Seredo			**Meko**		
Decortication characteristics	**1 min**	**1:45 min**	**3 min**	**1 min**	**1:45 min**	**3 min**
Extraction rate (%)	105.2 ± 3.0	99.7 ± 2.1	93.4 ± 0.7	105.2 ± 3.1	99.7 ± 2.1	93.4 ± 0.7
Dry matter (%)	80.7 ± 0.9	82.7 ± 0.9	83.4 ± 0.5	82.4 ± 0.5	83.4 ± 0.7	83.7 ± 0.2
Decortication yield (%)	95.4 ± 2.9	92.7 ± 2.8	87.6 ± 0.2	96.5 ± 3.5	92.7 ± 1.6	87.1 ± 0.7
Decortication time (min)	1 ± 0.0	1.45 ± 0.4	3 ± 0.0	1 ± 0.0	1.45 ± 0.4	3 ± 0.0
Dry matter loss (%)	4.6 ± 2.9	7.3 ± 2.8	12.4 ± 0.2	5.4 ± 1.4	7.3 ± 1.6	13.4 ± 0.0
Number of pounding per min	74 ± 7	73 ± 5	66 ± 3	74 ± 7	73 ± 5	65.8 ± 6

Data are expressed as mean ± standard deviations.

4.3. Effect of traditional decortication on nutrient contents

4.3.1. Effect of decortication on grain proximate composition (protein, fat and ADF fiber) as histological markers

The maximum loss in ADF was 19% and 13% for Meko and Seredo, respectively **(Table 4.3)**. Given that ADF fiber corresponds to plant cell wall constituents (cellulose and lignin) the ADF loss implies that decortication affected the cell wall components of the peripheral parts (pericarp and aleurone) of the grains. However, the two sorghum varieties are not equally affected since they exhibit morphologic differences. Due to thicker testa in high-tannin varieties, the cell walls in Seredo might have been less accessible than in Meko.

Lipid loss in Seredo (26%) was higher than in Meko (7%). Since lipids are mainly located in the germ (Glew et al., 1997). The higher lipid loss in Seredo implies greater germ removal. Given that Seredo has a softer endosperm than Meko, the germ might be more accessible once the pericarp is removed.

Table 4.3 Proximate composition and decortication losses of sorghum during traditional decortication

Extraction rates	ADF fiber (% DM)	Crude fat (% DM)	Crude protein (% DM)
Seredo			
Whole grain	9.16 ± 0.6^{a}	3.67 ± 0.1^{a}	12.29 ± 0.1^{a}
Higher (95%)	$8.76 \pm 0.1(4.4\%)^{ab}$	$3.25 \pm 0.1(11.4\%)^{b}$	$12.18 \pm 0.2(0.9\%)^{ab}$
Intermediate (93%)	$8.21 \pm 0.3(10.4\%)^{b}$	$3.25 \pm 0.01(11.4\%)^{b}$	$12.15 \pm 0.02(1.4\%)^{ab}$
Lower (88%)	$7.93 \pm 0.3(13.4\%)^{bc}$	$2.7 \pm 0.06(26.4\%)^{c}$	$12.02 \pm 0.1(2.2\%)^{b}$
Meko			
Whole grain	8.06 ± 0.1^{d}	3.53 ± 0.2^{d}	11.12 ± 0.1^{c}
Higher (97%)	$7.12 \pm 0.1(11.7\%)^{de}$	$3.41 \pm 0.05(3.4\%)^{de}$	$11.05 \pm 0.1(0.6\%)^{c}$
Intermediate (93%)	$7.09 \pm 0.2(12\%)^{de}$	$3.36 \pm 0.03(4.8\%)^{de}$	$10.81 \pm 0.01(2.8\%)^{d}$
Lower (87%)	$6.52 \pm 1.1(19.1\%)^{e}$	$3.30 \pm 0.01(6.5\%)^{e}$	$10.58 \pm 0.2(4.9\%)^{e}$

Different superscript letters in the same column and variety correspond to significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations (% loss due to decortication).

Proteins are mainly localized in the endosperm of the grain (~80%) (YOUNGS, 1977), but ~ 4% can be found in the pericarp. Therefore, the 2-5% loss of protein may only be associated to the

removal of the pericarp. Furthermore, the DM loss which did not exceed 13% may also confirm that the endosperm was minimally affected by the decortication process. A moderate level of decortication, enabling the removal of mineral absorption inhibitors while preserving the endosperm has been recently recommended (Hama et al., 2011). The decortication level in this study is in conformity with this recommendation and thus their effect on mineral retention as well as removal of absorption inhibitors is of interest.

4.3.2. Effect of decortication on mineral content

The total ash loss was in the range of 24-28%. This loss in ash content was associated with iron and calcium losses that were in the range of 24-27% and 38-43%, respectively. The higher mineral loss in Meko than in Seredo is in agreement with the higher fiber loss for this variety. This suggests that bran removal is positively correlated with mineral loss. This is in-line with reported values for similar extraction rates (Hama et al., 2011). Such minimal mineral losses are beneficial provided that it is associated with important losses in absorption inhibitors. Therefore, the effect of decortication on mineral absorption inhibitors was evaluated.

Table 4.4 Mineral content of the two sorghum varieties of traditionally decorticated sorghums

Extraction rates	Total Ash (% DM)	Iron (mg/100 g DM)	Calcium (mg/100 g DM)
Seredo			
Whole grain	1.75 ± 0.02[a]	4.93 ± 0.15[a]	25.54 ± 1.1[a]
Higher (95%)	1.6 ± 0.03(8.6%)[a]	4.84 ± 0.22(1.8%)[a]	23.37 ± 0.37(8.5%)[b]
Intermediate (93%)	1.84 ± 0.03(↑5.1%)[b]	5.11 ± 0.04(↑3.7%)[a]	20.95 ± 0.45(18%)[c]
Lower (88%)	1.51 ± 0.06(13.7%)[c]	3.76 ± 0.13(23.7%)[b]	14.67 ± 0.26(42.6%)[d]
Meko			
Whole grain	1.56 ± 0.05[d]	6.12 ± 0.13[c]	24.55 ± 1.20[e]
Higher (97%)	1.46 ± 0.04(6.4%)[e]	4.49 ± 0.21(26.6%)[d]	21.85 ± 0.30(11%)[f]
Intermediate (93%)	1.29 ± 0.02(17.3%)[f]	4.41 ± 0.21(27.9)[d]	18.2 ± 0.42(25.9%)[g]
Lower (87%)	1.21 ± 0.0(22.4%)[g]	4.44 ± 0.22(27.5%)[d]	15.24 ± 0.28(37.9%0[h]

Different superscript letters in the same column and variety correspond to significant difference (*P< 0.05*); Data are expressed as mean ± standard deviations (% loss due to decortication); DM, dry matter.

4.3.3. Effect of decortication on mineral absorption inhibitors

Seredo had several fold higher catechol and galloyl contents than that of Meko (*P< 0.05*). This is expected given the high-tannin content of Seredo. Decortication significantly reduced the catechol and galloyl groups (*P< 0.05*) which is in-line with previous reports (Lestienne et al., 2007). About 30-50% loss in galloyls and catechols was observed as a result of the decortication.

However, the decreases in iron-binding polyphenols were not homogenous for all decortication levels. For Seredo, catechol levels decreased along with the intensity of the decortication (*P< 0.05*) whereas galloyls decreased to a certain extent (WG to ME) and then remained constant (ME to LE). This may suggest that galloyls are mainly localized in the outer layer of the grain (Lestienne et al., 2007). On the other hand, no difference in both galloyl and catechol contents was observed for Meko. This is not surprising given the low amount of polyphenols found in this variety.

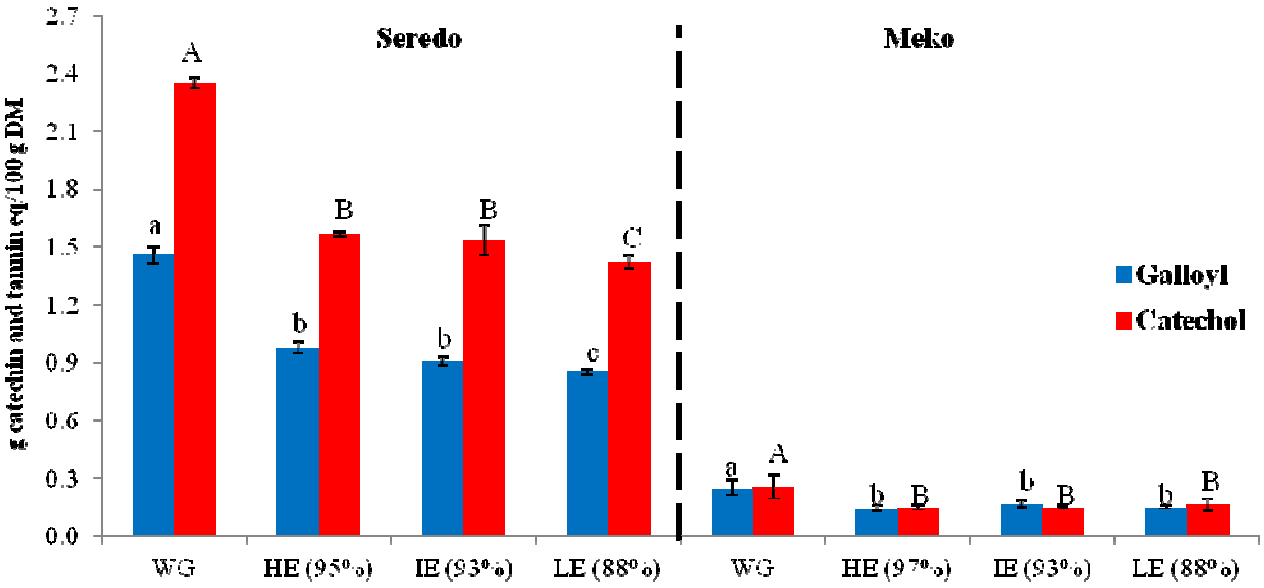


Figure 4.1 Iron-binding phenolics content of Seredo and Meko whole grain and decorticated grain; Error bars represent the standard deviation of means; Different superscript letters for the same variety represents statistically significant difference (*P< 0.05*); WG, whole grain; HE, higher extraction rate; IE, intermediate extraction rate; LE, lower extraction rate; DM, dry matter;.

The amount of tannin decreased with extraction rate in both varieties. The decrease in tannin content was in the range of 5-35 and 15-30% in Seredo and Meko, respectively. Despite the lower loss of ADF, Seredo had slightly higher loss of tannins compared to Meko. This implies that most of the tannins in Seredo were localized in the outer layer of the grain (Dlamini et al., 2007).

Table 4.5 Tannin content of traditionally decorticated sorghums

Extraction rates	Tannin (g/100 g *CE DM)
Seredo	
Whole grain	1.48 ± 0.03[a]
Higher (95%)	1.38 ± 0.03(7%)[b]
Intermediate (93%)	1.30 ± 0.04(12%)[c]
Lower (88%)	0.96 ± 0.05(35%)[d]
Meko	
Whole grain	0.69 ± 0.01[a]
Higher (95%)	0.50 ± 0.01(28%)[b]
Intermediate (93%)	0.63 ± 0.02(9%)[c]
Lower (88%)	0.57 ± 0.02(17%)[d]

Different superscript letters in the same column and variety correspond to significant difference (*P< 0.05*); Data are expressed as mean ± standard deviations (% loss due to decortication); *CE, expressed as catechin equivalent; DM, dry matter.

Phytate loss in Seredo and Meko was 15.3 and 9.5%, respectively. The higher loss in phytates and lipids along with the lower loss of ADF in Seredo than in Meko suggests that a significant portion of phytate resides in the germ of the grain (Hama et al., 2011, Lestienne et al., 2007).

Table 4.6 Phytate content of traditionally decorticated sorghums

Extraction rates	Phytate (mg/100 g DM)
Seredo	
Whole grain	294.23 ± 10.78[a]
Higher (95%)	284.73 ± 14.11(3.2%)[a]
Intermediate (93%)	274.5 ± 14.81(6.7%)[a]
Lower (88%)	249.23 ± 6.24(15.3%)[b]
Meko	
Whole grain	345.33 ± 15.77[c]
Higher (97%)	339.47 ± 3.17(1.7%)[c]
Intermediate (93%)	341 ± 5.55(1.3%)[c]
Lower (87%)	312.4 ± 5.50(9.5%)[d]

Different superscript letters in the same column and variety correspond to significant difference (*P< 0.05*); Data are expressed as mean ± standard deviations (% loss due to decortication); DM dry matter.

Given that decortication of sorghum grain allowed significant decreases in iron-binding polyphenols, tannin and phytates, iron bioavailability is likely to be improved. Such changes in bioavailability can be monitored using phytate/iron molar ratio.

4.3.4. Phytate/iron molar ratio

In cereal-based products containing little mineral absorption enhancers, phytate to iron molar ratios should be below 1, and preferably below 0.4 in order to significantly improve iron absorption (Hurrell, 2004). Phytate to iron molar ratio for the two sorghum varieties was above 1, irrespective of the extent of decortication. In fact, decortication increased the phytate to iron molar ratio. Such increases have also been reported during decortication in the range of high extraction flour (Hama et al., 2012). This suggests that during minimal decortication of grains, more iron and less phytate is lost. This may be due to the localization of iron in the outer layer (pericarp) of the grain, while the localization of phytate being in the inner part of the grain (germ).

Table 4.7 Phytate to iron molar ratio of traditionally decorticated sorghums

Extraction rates	Phytate: iron molar ratio
Seredo	
Whole grain	5.07 ± 0.19^{a}
Higher (95%)	4.99 ± 0.25^{a}
Intermediate (93%)	4.56 ± 0.25^{b}
Lower (88%)	5.64 ± 0.14^{c}
Meko	
Whole grain	4.79 ± 0.22^{d}
Higher (97%)	6.43 ± 0.06^{e}
Intermediate (93%)	6.33 ± 0.12^{e}
Lower (87%)	5.97 ± 0.12^{f}

Different superscript letters in the same column and variety correspond to significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations.

Some studies have shown that when extending the decortication intensity of sorghum grains lower phytate/iron molar ratios can be reached. Nevertheless, the phytate/iron molar ratio was above 1 and over 80% of the iron was lost (Hama et al., 2012). This implies that further extending the decortication time may not be beneficial, especially since this may decrease the energy density of the flour (Hama et al., 2012). In contrast, fermentation may decrease the contents of mineral absorption inhibitors (i.e. phytates) while not affecting the iron content. Thus, fermentation may be preferable than extending the decortication intensity. Consequently, the potential effect of fermentation on flours with different extraction rate was evaluated.

4.4. Effect of sorghum *injera* fermentation on nutrient contents

pH kinetics

The same fermentation pattern was observed for the different sorghum varieties irrespective of the flour extraction rate. The initial pH was ~5.6 and was reduced to a minimum value of 3.8 and 3.4 for Seredo and Meko, respectively.

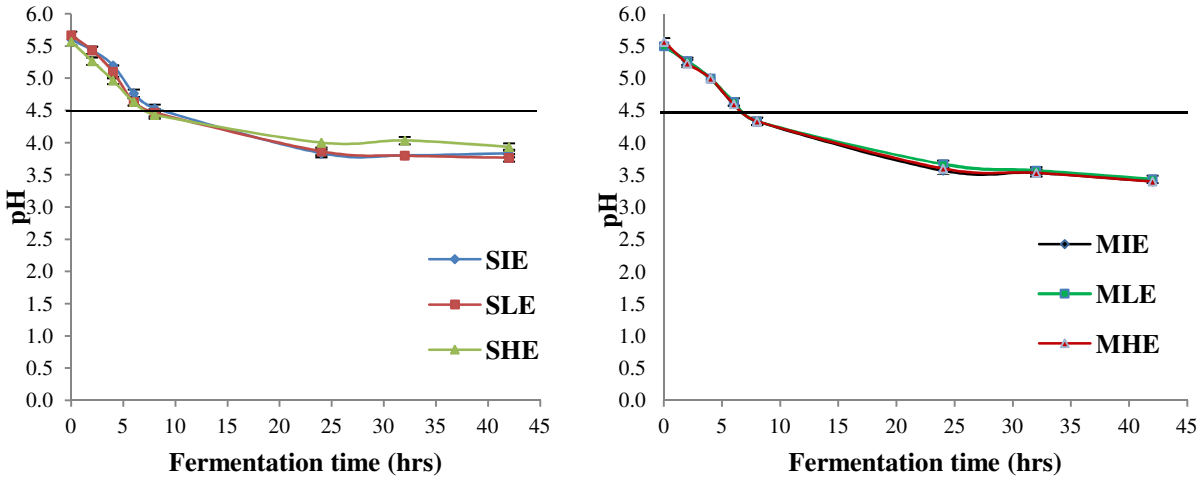


Figure 4.2 Changes in pH during Seredo- and Meko-sorghum dough fermentation for *injera* making; Error bars represent the standard deviation of means; SIE, Seredo intermediate extraction rate; SL, Seredo lower extraction rate; SHE, Seredo higher extraction rate; MIE, Meko intermediate extraction rate; MLE, Meko lower extraction rate; MHE, Meko higher extraction rate.

The maximal rate of pH ($-dpH/dt_{max}$) change was not affected by extraction rate in the case of Meko where as it was found decreased in low extraction Seredo flour fermented dough (**Table 4.8**).

Table 4.8 Kinetic parameters of pH change of fermented dough for injera making

Flour extraction rates	Initial pH	$-dpH/dt_{max}$	Time (hrs) to reach $-dpH/dt_{max}$	Time (hrs) to reach pH 4.5	Final pH
Seredo					
Higher (95%)	5.7 ± 0.06^{a}	0.30 ± 0.05^{ab}	8.0	7.67 ± 0.75^{a}	3.8 ± 0.06^{a}
Intermediate (93%)	5.6 ± 0.00^{ab}	0.35 ± 0.05^{b}	8.0	8.5 ± 0.81^{a}	3.8 ± 0.06^{ab}
Lower (88%)	5.6 ± 0.06^{b}	0.22 ± 0.03^{a}	8.0	7.5 ± 0.64^{a}	3.9 ± 0.06^{b}
Meko					
Higher (97%)	5.5 ± 0.00^{c}	0.33 ± 0.03^{c}	8.0	6.6 ± 0.21^{b}	3.4 ± 0.06^{c}
Intermediate (93%)	5.5 ± 0.00^{c}	0.38 ± 0.03^{c}	8.0	6.6 ± 0.21^{b}	3.4 ± 0.00^{c}
Lower (87%)	5.6 ± 0.06^{c}	0.37 ± 0.03^{c}	8.0	6.6 ± 0.29^{b}	3.4 ± 0.00^{c}

Different superscript letters in the same column and variety correspond to significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations.

Effect of *injera* processing on mineral absorption inhibitors

The iron-binding phenolics contents of Seredo was significantly reduced by fermentation to prepare *injera* ($P< 0.05$), whereas that of Meko remained the same. Fermentation resulted in 35-40% loss, with additional losses during decortication 60-64% reductions in IBP was observed. Fermentation did not result in significant difference between different extraction rate flours.

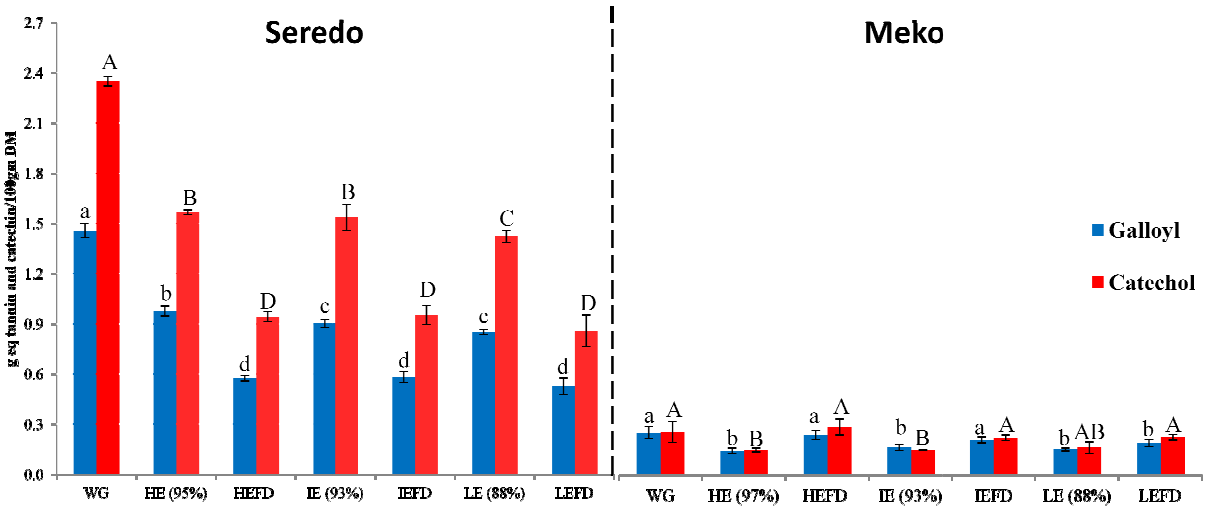


Figure 4.3 Iron-binding phenolics content of Seredo and Meko whole grain and fermented dough; Error bars represent the standard deviation of means; Different superscript letters for the same variety represents statistically significant difference (*P< 0.05*); WG, whole grain; HE, higher extraction rate flour; HEFD, higher extraction rate fermented dough; IE, intermediate extraction rate; IEFD, intermediate extraction rate fermented dough; LE, lower extraction rate; LEFD, lower extraction rate fermented dough; DM, dry matter.

- **Polyphenol oxidase activity (PPO)**

Seredo had significantly higher PPO activity than Meko (**Figure 4.4**). Sorghum varieties containing high polyphenol contents are known to have high PPO activity (Dicko et al., 2002). The whole grains of the two sorghum varieties had significantly higher PPO activities than the decorticated ones (*P< 0.05*). This suggests that PPO along with polyphenols is located in the outer layer of the grain (Demeke et al., 2001).

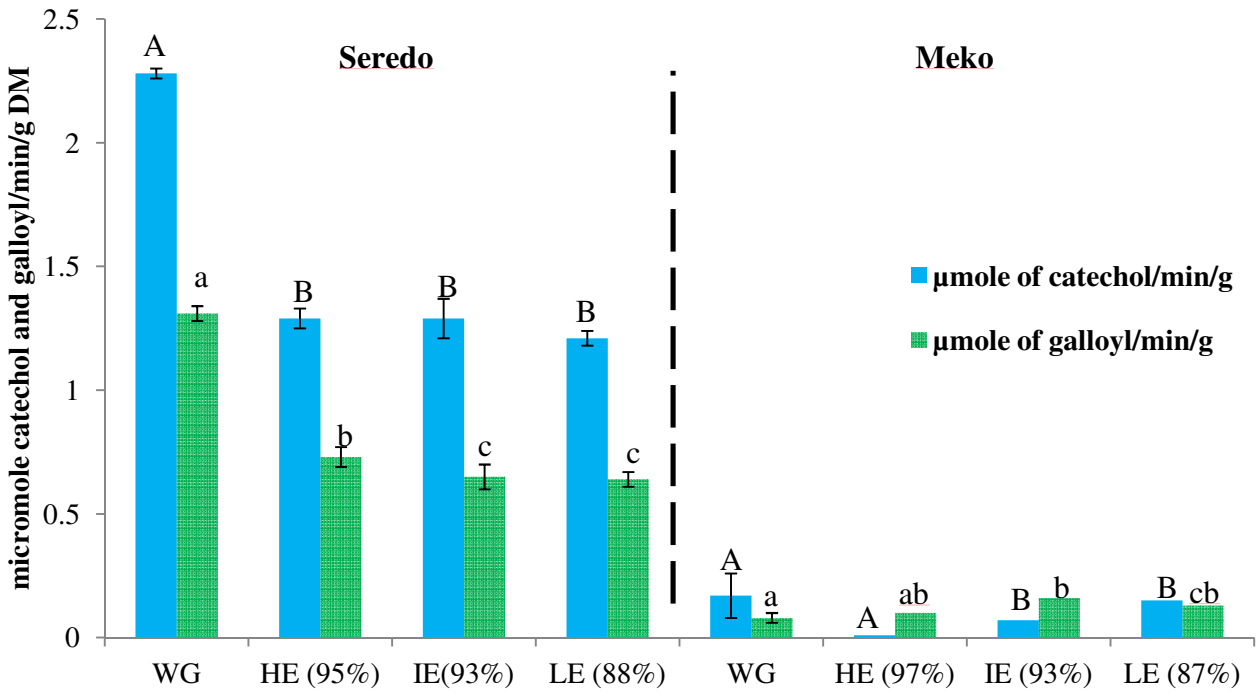


Figure 4.4 Polyphenol oxidase activity of Seredo and Meko whole and decorticated grain flours; Error bars represent the standard deviation of means; Different superscript letters for the same variety represents statistically significant difference (*P< 0.05*); WG, whole grain; HE, higher extraction rate; IE, intermediate extraction rate; LE, lower extraction rate; DM, dry matter.

For an indirect evaluation of endogenous PPO activity, flours (whole and decorticated grains) were incubated at pH conditions favorable for PPO activity. The PPO activity decreased with the decrease in extraction rate for Seredo, while it increases in the case of Meko (**Figure 4.4**). This difference may be due to difference in PPOs localization and warrants further investigation.

The tannin content was significantly decreased during fermentation for *injera* processing for both sorghum varieties (*P< 0.05*). Although the % loss in tannin is higher in Meko, quantity-wise higher loss of tannins was observed in Seredo, given its higher initial tannin content (**Table 4.9**). The decrease in tannin due to fermentation has consistently been reported (Idris et al., 2005, Osman, 2004).

Table 4.9 Tannin content of fermented sorghum dough

Extraction rates	Tannin (g/100 g *CE DM)		% loss due to fermentation
	Flour	Fermented dough/batter	
Seredo			
Whole grain	1.48 ± 0.03[a]		
Higher (97%)	1.38 ± 0.03[b]	1.06 ± 0.03[b]	23
Intermediate (93%)	1.30 ± 0.04[c]	0.99 ± 0.02[c]	24
Lower (87%)	0.96 ± 0.05[d]	0.79 ± 0.02[d]	18
Meko			
Whole grain	0.69 ± 0.01[a]		
Higher (97%)	0.50 ± 0.01[b]	0.47 ± 0.00[b]	6
Intermediate (93%)	0.63 ± 0.02[c]	0.48 ± 0.01[b]	24
Lower (87%)	0.57 ± 0.02[d]	0.41 ± 0.00[c]	28

Different superscripts in the same column for the same variety represent statistically significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations; *CE, expressed as catechin equivalent; DM, dry matter.

Similarly, decreases in phytate concentration have been observed during *injera* processing (**Table 4.10**). The decrease in phytate content during fermentation ranged from 11-17% for Seredo, while the decrease was in the range of 23-29% for Meko. The slightly lower loss of phytate in Seredo may be attributed to the inhibitory effect of tannins on enzymatic hydrolysis of phytate (Svanberg et al., 1993).

Table 4.10 Phytate content during of fermented sorghum dough

Extraction rates	Phytate (mg/100 g DM)		% loss due to fermentation
	Flour	Fermented dough/batter	
Seredo			
Whole grain	294.23 ± 10.78[a]		
Higher (95%)	284.73 ± 14.11[a]	253.1 ± 3.48[b]	11
Intermediate (93%)	274.5 ± 14.81[a]	244.5 ± 1.35[c]	11
Lower (88%)	249.23 ± 6.24[b]	207.3 ± 4.56[d]	17
Meko			
Whole grain	345.33 ± 15.77[c]		
Higher (97%)	339.47 ± 3.17[c]	262.0 ± 5.19[f]	23
Intermediate (93%)	341 ± 5.55[c]	246.2 ± 4.96[g]	28
Lower (87%)	312.4 ± 5.50[d]	221.2 ± 5.28[h]	29

Different superscript letters in the same column correspond to significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations; DM, dry matter.

The decrease of phytate due to fermentation is reported elsewhere (Mohammed et al., 2011, Idris et al., 2005). In general, the reduction of phytate may be induced by phytase activity produced by fermenting microorganisms (Andlid et al., 2004, Kerovuo and Tynkkynen, 2000) or activation of endogenous phytase activity by the fermentation process, through providing optimum pH 4.5-5.0 (Svanberg and Lorri, 1997).

4.5. Estimation of iron bioavailability

Unlike decortication, fermentation was not associated with mineral loss (**Table 4.11**). This along with the observed phytate degradation may thus improve the bioavailability of minerals.

Table 4.11 Mineral content of fermented sorghum dough

	Minerals		
Extraction rates	**Total Ash (% DM)**	**Iron (mg/100 g DM)**	**Calcium (mg/100 g DM)**
Seredo			
Whole grain	1.75 ± 0.02^{a}	4.93 ± 0.15^{a}	25.54 ± 1.1^{a}
Higher (95%)	1.76 ± 0.6^{a}	4.14 ± 0.18^{b}	23.93 ± 0.03^{a}
Intermediate (93%)	1.82 ± 0.02^{a}	4.68 ± 0.16^{a}	24.49 ± 0.37^{a}
Lower (88%)	1.45 ± 0.04^{b}	3.40 ± 0.13^{c}	17.98 ± 0.54^{b}
Meko			
Whole grain	1.56 ± 0.05^{c}	6.12 ± 0.13^{d}	24.55 ± 1.20^{cd}
Higher (97%)	1.50 ± 0.06^{c}	4.45 ± 0.11^{e}	23.07 ± 0.63^{c}
Intermediate (93%)	1.36 ± 0.05^{de}	5.27 ± 0.24^{f}	22.71 ± 0.37^{d}
Lower (87%)	1.33 ± 0.06^{e}	5.12 ± 0.21^{f}	17.95 ± 0.45^{e}

Different superscript letters in the same column correspond to significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations; DM, dry matter.

4.5.1. Changes in phytate/iron molar ratio during fermentation

In the fermented dough, the lowest phytate to iron molar ratio was obtained in higher (95%) and lower (87%) extraction rate flour of Seredo and Meko, respectively. The phytate to iron molar ratio was not significantly affected by fermentation. This might be due to the low phytase activity of sorghum cultivars, which resulted in minimal loss of phytate during fermentation.

Table 4.12 Phytate to iron molar ratio of fermented sorghum dough

	Phytate: iron molar ratio	
Extraction rates	**Flour**	**Fermented dough/batter**
Seredo		
Whole grain	5.1 ± 0.19^{a}	
Higher (95%)	5.0 ± 0.25^{a}	4.2 ± 0.01^{b}
Intermediate (93%)	4.6 ± 0.25^{b}	4.4 ± 0.03^{b}
Lower (88%)	5.6 ± 0.14^{c}	5.2 ± 0.11^{a}
Meko		
Whole grain	4.8 ± 0.22^{d}	
Higher (97%)	6.4 ± 0.06^{e}	5.0 ± 0.06^{b}
Intermediate (93%)	6.3 ± 0.12^{e}	4.0 ± 0.08^{c}
Lower (87%)	6.0 ± 0.12^{f}	3.7 ± 0.09^{d}

Different superscript letters in the same column and variety correspond to significant difference ($P< 0.05$); Data are expressed as mean ± standard deviations.

In all cases the phytate to iron molar ratio exceeded the critical value (< 1) above which improvement in iron bioavailability can be expected. This suggests that the bioavailability of iron from these sorghums is low. However, not only the presence of phytates but also polyphenols and to some extent calcium can influence iron bioavailability. Given that significant reduction in IBP was observed for Seredo how this can affect iron bioavailability needs to be investigated.

4.5.2. Iron absorption prediction using Hallberg & Hulten's algorithm

Iron absorption was predicted by assuming three different meals sizes, which were adapted from Baye et al. (2012) and Hallberg and Hulthén (2000). The children diet were assumed 40 g and two adult meal sizes were used; the minimum and maximum being 120 and 200 g, respectively. Given the low IBP contents and the higher phytate degradation in Meko compared to Seredo, ~ 2 times more iron was predicted to be bioavailable (**Table 4.13**). However, the estimated

percentage absorption did not exceed 3%. Furthermore, no significant differences were observed between *injera* made from flours of different extraction rates.

Table 4.13 percent absorption of iron from different meal size of *injera*

	Meal size (g FW)		
		Adult diet	
	Children diet	**Minimum**	**Maximum**
Extraction rates	**40**	**120**	**200**
Seredo			
Higher ER *injera* (95%)	1.2	0.4	0.2
Intermediate ER *injera* (93%)	1.3	0.4	0.3
Lower ER *injera* (88%)	1.2	0.4	0.3
Meko			
Higher ER *injera* (97%)	2.2	0.7	0.4
Intermediate ER *injera* (93%)	2.4	0.8	0.5
Lower ER *injera* (87%)	2.7	0.9	0.5

ER, extraction rate; FW, fresh weigh

4.6. Limitations of the study

Some limitations to consider when interpreting the present findings are:

The extraction rates used in this study are in the range of high extraction rate, thus the effect of decortication on nutrient composition of the sorghums was minimal. Extending the level of decortication might have permitted better insight on the localization and distribution of nutrients within the grains.

Furthermore, the use of abrasive decorticator would have permitted a much better description of the localization of nutrients; however, no abrasive decorticator was at our disposition during the study.

Elsewhere, phytate was analyzed using a less sensitive colorimetric method using Wade reagent. This method has been criticized for its low recovery; hence caution in interpreting phytate to iron molar ratios is required.

Furthermore, the ascorbic acid values for prediction of iron absorption using algorithm was obtained from a secondary data (Ethiopian food composition table-EHNRI).

Finally, the main aim of the study was to see the implication of processing (decortication and fermentation) on iron bioavailability. Due to this small sample size (white and brown sorghum) were used. This suggests the need for further investigation by including different sorghum varieties.

Chapter 4 – *Conclusion and recommendations*

5. Conclusion and recommendations

5.1. Conclusion

The two sorghum varieties (Seredo and Meko) have different pericarp thickness and seed size with nearly the same germ proportion. Seredo had relatively less iron than Meko, but had higher tannin and iron-binding phenolic compounds (galloyl and catechol) contents. Decortication led to significant loss of iron as well as chelating compounds (IBP, tannins and phytates). Nutrient retention during decortication was depended on the sorghum variety. Despite loss of mineral chelating compounds, phytate to iron molar ratio did not predict any improvement in bioavailability. This suggested that the associated loss of iron during decortication has offset any potential improvement in iron bioavailability that might have resulted from the loss of chelating compounds. This implies the need for processing that can decrease mineral chelating agents with minimal effect on minerals such as fermentation may be more beneficial.

Fermentation of different extraction rate flours into *injera* resulted further reduction of chelating compounds (IBP, tannin and phytates) but not to the extent needed to improve iron bioavailability. Phytate to iron molar ratio of the fermentation were still above 1. Given the important difference in polyphenols between the two sorghum varieties, bioavailability was further estimated using Hallberg and Hulthén's algorithm that take into account the negative effects of polyphenols on iron bioavailability. The algorithm predicted absorption of Meko (low tannin) was twice that of Seredo (high tannin). This difference in bioavailability between varieties was not detected when using molar ratio. The use of molar ratios in samples containing significant amount of polyphenols may be inadequate.

5.2. Recommendation

This study showed the possibility of reducing the iron-binding phenolic compounds to the extent of low tannin varieties through combined processing (decortication and fermentation). Further reduction of IBPC can be obtained through modifying the processing condition for optimum PPO activity. On the other hand, the minimal loss of phytate observed during fermentation suggests the need for improving the efficiency of phytate degradation during fermentation. This can be done either through addition of cereals with higher phytase activity such as wheat, rye and barley or optimizing microbial enzyme production through providing optimum environment for maximum activity and also isolation of microbes with high phytase as well as polyphenol oxidase activity is also beneficial.

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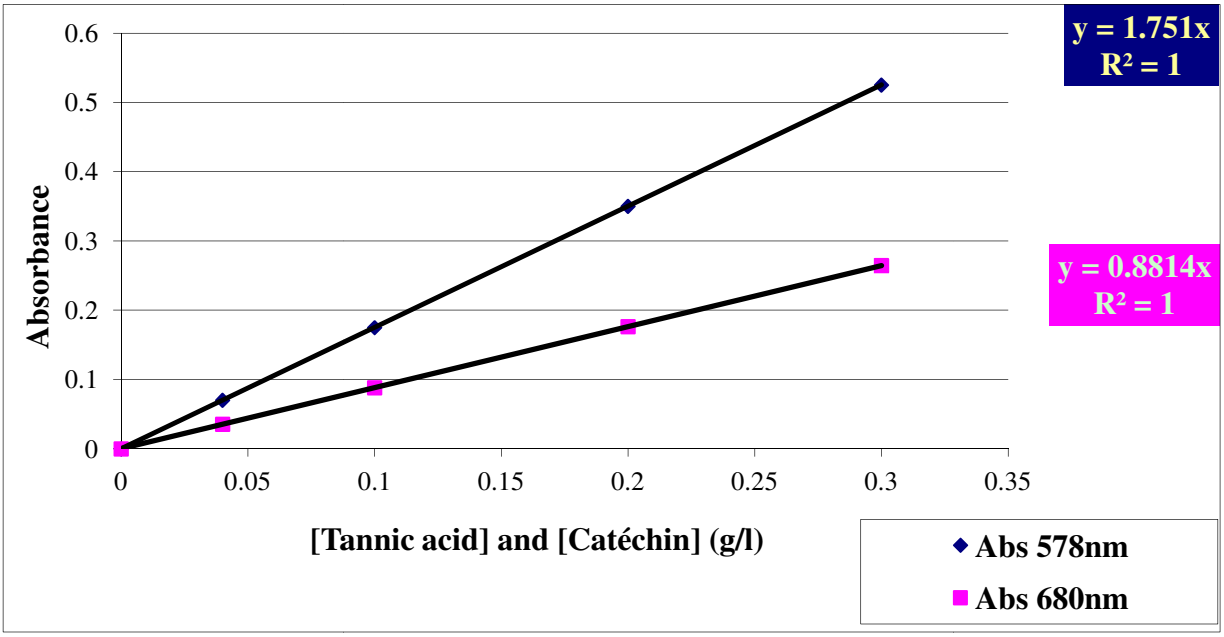
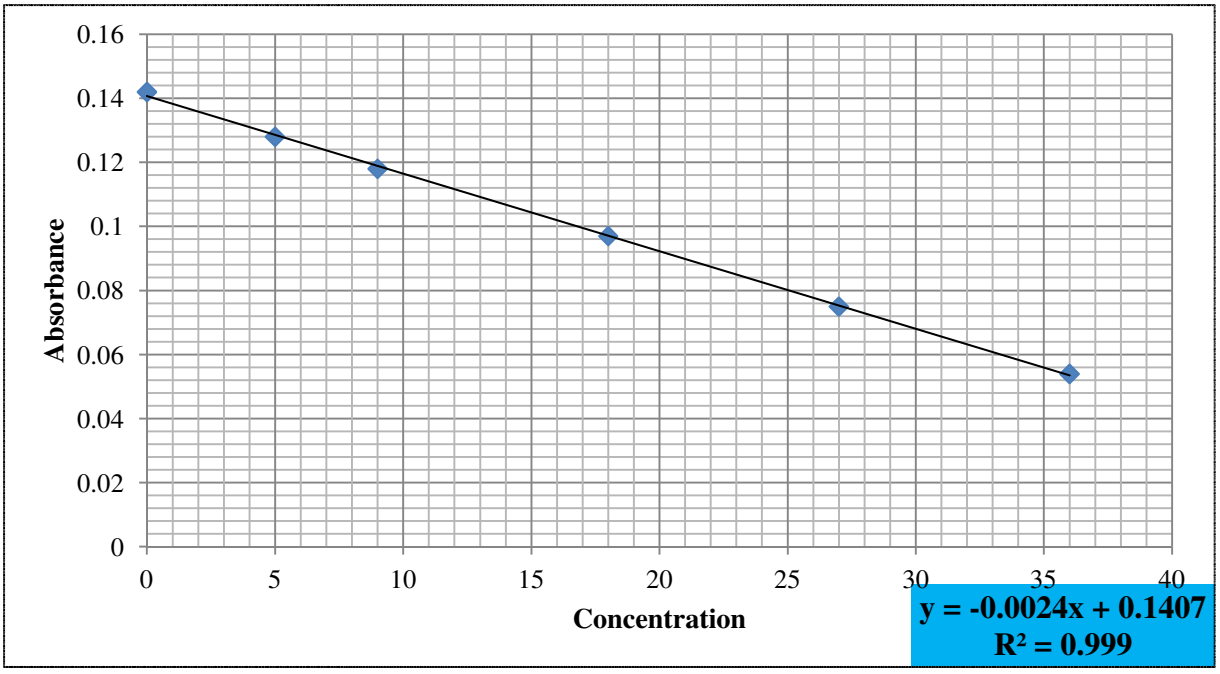
Annex A: Standard curve for iron-binding phenolic compounds (catechol and galloyl) determination



Annex B: Standard curve for phytate determination



Annex C: Standard curve for tannin determination