DETERMINATION OF ZINC, IRON, RESISTANT STARCH, PHYTIC ACID AND THEIR EFFECT ON RHEOLOGICAL AND ORGANOLEPTIC PROPERTIES FOR SELECTED KENYAN WHEAT VARIETIES

KARIITHI SAMUEL NGURE

A Thesis submitted to the Graduate School in Partial Fulfillment for the Requirements of the Award of Master of Science Degree in Biochemistry and Molecular Biology of Egerton University

EGERTON UNIVERSITY

SEPTEMBER, 2016

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and it has not been presented for examination in any institution.

Signature..... Date.....

Kariithi Samuel Ngure

SM14/3660/13

Recommendation

This thesis has been submitted for examination with our approval as supervisors:

Signature..... Date……..

Dr. Stephen A. Indieka

Department of Biochemistry and Molecular Biology

Egerton University

Signature..... Date……..

Dr. Peter N. Njau

Highland Agri-consult Services Limited

Njoro

ACKNOWLEDGEMENTS

I thank the Almighty God for His unending love and mercies which saw me through my study period and during the trying times of the project. I wish to express my gratitude to the administrative, academic staff of Egerton University and staff at the Biochemistry and Molecular Biology department for allowing me to pursue my studies at the institution and for their support during my study period. To my supervisors Dr. Stephen Indieka and Dr. Peter Njau, I am greatly indebted to them for their patience, guidance, criticisms and unwavering support during this whole process of Laboratory work and thesis writing. Their presence and availability for consultations and provision of materials all in the process of making this study a success is humbly and greatly appreciated. I wish to extend my gratitude to the following institutions for providing financial support and facilities for this project; Durable Rust Resistance in Wheat (DRRW), International Atomic Energy Agency (IAEA) and the Kenya Agricultural and Livestock Research Organization (KALRO) at the Food Crops Research Centre (FCRI), Njoro. My gratitude is extended to Mr. John Ndung'u and staff members of Cereal chemistry laboratory for their guidance and support during this time. I also extend my gratitude to Mr. John Kamundia for assistance on data analysis. To my coursemates; Philip, Edwin, Justice, Tabitha, Velma and Mercy a big 'thank you' for the encouragement they gave me when times were tough. To my loving parents Paul Kariithi and Margret Kariithi and siblings brothers Peter and James, Nancy and Terry I owe them a lot for being patient with me during this period. Their prayers, material and moral support have greatly contributed to who I am today.

DEDICATION

This work is dedicated to my loving parents, Mr. Paul Kariithi and Margret Kariithi who have set a good example of love, sheer hard work, discipline and dedication to education that have brought me this far. The work is also dedicated to my siblings Peter Mugo, Nancy Wanjiru, James Chege and Teresiah Njoki for their unwavering support, encouragement and prayers throughout the study period.

ABSTRACT

Micronutrient deficiency of zinc and iron is a major problem worldwide, especially in the developing countries that heavily rely on cereal rich diets for sustenance. Wheat is a popular staple cereal crop containing substantial levels of micronutrients and could be used as a tool for alleviating their deficiencies. The study reported herein focused on determining the concentration of zinc, iron, resistant starch (RS) and phytic acid in 9 newly released wheat varieties and their effect on rheological and organoleptic properties of dough and bread, respectively. The varieties were planted in three agroecological zones namely Eldoret, Mau-Narok and Naivasha in a randomized complete block design after which whole meal flour was analysed for zinc, iron, resistant starch and phytic acid levels. Zinc and iron concentrations were determined by digesting flour samples using the khejdal method and their absorbance read at 213.86 and 248.33 nm, respectively using the Atomic Absorption Spectrophotometer (AAS). RS was determined using megazyme assay kit following manufacturers protocol, while phytic acid was determined using a modified protocol previously used on rice mutants. The proximate composition of whole meal flour was determined using the Near Infrared Spectrophotometer (NIR), while rheological properties were determined using the Farinograph and Chopin Alveograph machines using procedures adapted from method 54-21 and 54-30A, of the approved methods of the American Association of Cereal Chemists. Results obtained showed that genotype and environment greatly influenced levels of micronutrient analysed ranging between 26 to 91 ppm and 111 to 305 ppm for zinc and iron, respectively. Naivasha recorded the highest concentration for zinc while Eldoret had the highest concentrations for iron. The levels of phytic acid ranged from 2.66 to 5.05µg/g, with Naivasha recording the highest concentrations among the three sites. Resistant starch results ranged from 0.37 to 6.03g/100g with the highest levels recorded in Eldoret while the lowest were in Naivasha. Rheological properties of whole meal flour showed increased water absorption for all the varieties across the three sites which ranged from 72 to 80%, while the resistance to elasticity (P) and resistance to extensibility (L) values ranged from 21 to 79mm and 16 to 51mm, respectively. Organoleptic analysis revealed that varieties from Eldoret were much preferred for bread baking compared to the Mau-Narok and Narok, while correlation analysis revealed no major effect of micronutrients on either the rheological or organoleptic properties. The information on nutritional quality of the nine wheat varieties provides an insight on the potential use these varieties in addressing micronutrient deficiencies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

AMG	Amylo Glucosidase
DCT1	Divalent Cation Transporter
DMA	Deoxymugineic Acid
DMT1	Divalent Metal Transporter
GOPOD	Glucose Oxidase/Peroxidase
HCP-1	Heme Carrier Protein 1
HRG-1	Heme Response Gene 1
IAA	Indole Acetic Acid
IP6	Inositol Hexaphosphate
KOH	Potassium Hydroxide
LSD	Least Significant Difference
NA	Nicotine Amine
NAAT	Nicotine Amine Amino Transferase
NAS	Nicotine Amine Synthase
RCBD	Randomized Complete Block Design
RS	Resistant Starch
SAM	S-Adenosyl Methionine
Ug99	Uganda 1999
ZIP	Zinc regulated transporters in the Iron regulated transporter like Protein
ZnT	Zinc Transporters

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Wheat (*Titricum aestivum*) is a major staple food crop in many parts of the world in terms of cultivated area and food source. This contributes 28 % of the world's edible dry matter and up to 60 % of the daily calorie intake in many developing countries (Gill *et al*., 2004). In Kenya, wheat is the second most important cereal commodity after maize in terms of calories consumed and its consumption has grown steadily at a rate of 4% annually from 1960-2011. This increase in consumption has been associated with urbanization and general increase in incomes (Monroy *et al*., 2013). Knowledge of the nutritional value of wheat is extremely important because it is one of the key crop species being extensively grown as staple food. Wheat kernel is usually ground into flour or semolina and forms the basic ingredients for the baking industry. The major product consumed is bread (raised or flat bread) and flour associated products such as spaghetti, lasagna, macaroni among others.

Malnutrition is considered to be among the most serious global challenges, with over three billion people currently suffering from micronutrient deficiency (Bouis, 2003; Welch and Graham, 2004; Philip and Broadley, 2009). This global crisis in nutritional health is the result of dysfunctional food systems that do not consistently supply enough of these essential nutrients to meet the nutritional requirements of high-risk groups. The availability of sufficient amounts of mineral nutrients in the human diet depends primarily on their composition in food crops (Sands *et al*., 2009; Cakmak *et al*., 2010), particularly staple food crops such as cereal grains. Therefore, enhancement of grain mineral nutrients (biofortification), either conventionally (breeding) or biogenetically (transgenics), is considered the most promising and cost-effective approach for alleviating malnutrition and related health problems (Buois 2007; Cakmak, 2008).

In the developing world cereals such as wheat, rice and maize with inherently low zinc and iron concentrations in their grains, form an important source of calories (Cakmak, 2008). Therefore genetic bio-fortification and selecting genotypes with high accumulation efficiency of Zn and Fe in their endosperm could go a long way in reducing micronutrient deficiency and related problems in the developing world (Velu *et al*., 2013). The bioavailability of nutrients of interest is another

concern, since they are highly reduced by chelating agents. Among these agents is phytic acid (myo inositol hexa-phosphoric acid, IP6), which is the major phosphorus storage compound of most seeds and cereal grains; accounting for more than 70% of the total phosphorus. Phytic acid has a strong ability to chelate multivalent metal ions, especially zinc, calcium and iron. Their binding can result in very insoluble salts, hence affecting bioavailability of these mineral nutrients (Zhou and Erdman, 1995). Therefore there is need for development of wheat varieties with a low phytic acid content in order to improve Zn and Fe bioavailability.

Mineral absorption is greatly enhanced by the presence of Resistant Starch (RS) in the diet. Resistant starch is sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals. It has also been classified as part of dietary fiber with physiological effects that are beneficial to consumers. In wheat, starch constitutes 65-75% of the grain dry weight, with amylose contributing between 20-30% of the total starch and the remaining 70-80% is amylopectin (Cornel, 2003). Resistant starch improves and enhances micronutrient absorption, bowel health (reduces colorectal cancer, ulcerative colitis and inflammatory bowel disease), increase satiety and reduced caloric intake, and an adjunct to oral rehydration therapies (treatment of cholera and chronic diarrhea) (Nugent, 2005; Fuentes-Zaragoza *et al*., 2010). Resistant starch is degraded slowly resulting in decreased postprandial glucose and insulin responses, which is beneficial for people with diabetes and hyperlipidemia (Wood *et al.,* 2000). The Resistant Starch fermented in the colon also produces many short-chain fatty acids (SCFA) that are helpful in preventing colonic diseases (Ferguson *et al*., 2000; Tharanathan and Mahadevamma, 2003).

Dough rheology is the science of deformation and how materials respond to applied stress or strain (Mirsaeedghazi *et al.,* 2008). These properties are evaluated using rheometers such as Farinogh and Alveograph machines. Dough rheology is highly influenced by protein physical and chemical interactions (Shiau and Yeh, 2001), with increasing gliadin/ glutenin ratio increasing dough resistance to extensibility (Angioloni and Rosa, 2005). Rheological measurements aid in process control and process design and tells us how dough behaves under a given set of conditions and are used to predict end product quality such as mixing behaviour, sheeting and baking performance (Dobraszczyk, 2004a).

Organoleptic properties are the characteristics of food, water and other substances a consumer experiences through their senses such as sight, taste, smell and even touch. Organoleptic

analysis is an essential aspect in the food industry as it allows manufacturers to identify and respond to consumer preferences (Saha *et al.*, 2009). The information gained helps manufacturers to improve their products increasing competition in the industry which leads to better quality products (Ponte *et al.*, 2004).

1.2 Statement of the problem

Micro nutrient deficiency especially of Zn and Fe is a major problem affecting billions of people worldwide, particularly young children and pregnant women. The problem is exacerbated by low accumulation of these micronutrients in most popular staple cereal crops such as wheat. The presence of metal ion chelators such as phytic acid in the diet reduces bioavailability of these nutrient elements further compounding the problem. Wheat is a popular staple cereal and contains substantial amount of resistant starch. High quantities of resistant starch enhance absorption of mineral nutrient elements as it increases the time and provide large surface area for their absorption and it therefore offers a platform to address the problem of micronutrient bioavailability and deficiency simultaneously. However to develop wheat varieties with increased levels of Zn and Fe, reduced phytic acid and high resistant starch, researchers should first have adequate and accurate information on the existing wheat varieties. This information is crucial because it will facilitate breeders to select varieties to be improved and in the process reduce time required for breeding wheat varieties with the desired agronomic traits and high nutrition qualities.

1.3 Objectives

1.3.1 General objectives

To evaluate Iron, Zinc, phytic acid concentrations and the content of resistant starch in nine selected wheat varieties across sites and their influence on end use properties particularly baking.

1.3.2 Specific objectives

1. To determine iron, zinc, phytic acid and resistant starch concentrations in selected Kenyan wheat varieties across the three agroecological zone.
2. To evaluate the effects of zinc, iron, phytic acid and resistant starch content on the quality of whole meal flour for bread baking.
3. To evaluate the effect of iron, zinc, phytic acid and resistant starch on the organoleptic properties of baked bread.

1.4 Hypotheses.

1. H0: Iron, zinc, phytic acid and resistant starch concentrations differ significantly across the environments and varieties.
2. H0: There exists a positive correlation between high concentrations of zinc, iron, phytic acid and resistant starch and improved rheological properties.
3. H0: High iron, zinc, phytic acid and resistant starch concentrations improves the organoleptic properties of the bread.

1.5 Justification

Wheat offers an attractive opportunity to address the problem of micronutrient deficiency in particular Zn and Fe that affects large number of people from different social-economic backgrounds. This is because it is a popular staple food crop worldwide and consumed in many forms such as bread, chapati, mandazi, cake, and biscuit among others. Furthermore it also contains promising quantities of resistant starch that is known to enhance absorption of metal ions. Although development of new wheat varieties has for a long time focused mainly on improving yields and resistance or tolerance to pest and diseases, however they do not or rarely incorporate improving nutritional qualities of the new varieties. Currently there is no information on the nutritional qualities of wheat varieties released in Kenya. Availability of nutrition information will help breeders to select and prioritize varieties that need improvement to have the desired nutrition qualities apart from possessing best baking properties, disease resistance among other desired properties or to be selected for biofortification programmes. A promising and sustainable means of improving Fe and Zn nutrition is biofortification which may entail engineering of staple crops to accumulate additional nutrients in the edible parts. While improved nutritional quality is desirable, there is need to determine their effect on the rheological and organoleptic properties. Rheological and organoleptic properties determine the end quality and impact greatly the preferences and choice of whole meal bread and bakery products made for consumers. Thus information generated will provide fundamental knowledge on the status of the nutritional quality of the 9 released rust resistant wheat varieties that is useful for Kenyan wheat breeders and bakers too.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wheat

Wheat is the most important stable food crop for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crops (Shewry, 2009). It is a grass plant that grows up to a height of two or three feet and produces edible seeds known as a kernels for human consumption. There are two major classes of wheat the spring and winter wheat. Winter wheat is planted in September and harvested the following summer. Spring wheat is planted in April or May and is harvested in August or September.

2.1.1 Properties, uses and value addition of wheat

The wheat kernel is ground to flour (polished or whole meal) which forms a good source of protein, starch, minerals, B-group vitamins and dietary fiber (Kumar *et al*., 2011). It is mixed with water to form the principal ingredient widely used in the baking industry due to presence of gluten proteins. Gluten is composed of glutenin and gliadin subunits that gives dough its elastic and viscous nature (Jingyuan *et al.,* 2007). Wheat flour is used to prepare bread, biscuits, confectionary products, noodles and vital wheat gluten or seitan. It is also used as animal feed, ethanol production, brewing wheat beer, wheat based raw material for cosmetics, wheat protein in meat substitutes and to make wheat straw composites (Kumar *et al*., 2011).

Wheat germ contains oil and glycerides which have been used as skin conditioning agents for decades and in the manufacture of lipstick and moisturizers. Wheat gluten is also used in the manufacture of makeup, hair and skin conditioners due to its binding effect (Neil, 2012). Wheat flour is also used together with gluten free materials to produce composite flour such as cassava/wheat flour, wheat/pumpkin flour, wheat/maize/sweetpotato flour among others (Igbabul *et al*., 2014; Shittu *et al*., 2013; Shittu *et al*., 2008).

2.1.2 Wheat breeding and challenges in Kenya

Wheat is ranked as the second most important cereal crop in Kenya but its production has been threaten by low yields (Mahagayu *et al*., 2007). The main growing districts in Kenya are mainly Nakuru, Narok, Uasin Gishu, Trans-Nzoia and Nyandarua. This low yields are brought about by diseases and unfavorable weather conditions. Marker Assisted Selection (MAS) is a

technique that has been developed and used to select wheat lines and varieties with desired characteristics such as resistance to diseases, drought tolerance among others. Mutation breeding has also been used in wheat breeding to develop new varieties with desired properties within a short period of time.

Wheat breeding in Kenya has for a long time focused on developing and releasing varieties that are resistant to diseases and tolerant to drought. On the other hand little has been done in terms of developing varieties with superior nutrition qualities, such as ability to accumulate sufficient micronutrients in their grain such as zinc and iron (WHO, 2010), reduced anti-nutritive compounds such as phytic acid and increased resistant starch. Zinc and iron deficiencies are associated with dysfunctional food systems that avail very little for absorption. In Kenya diets are composed mainly of cereals such as wheat which contain phytic acid a compound known to inhibit zinc and iron absorption. There is therefore need for breeding wheat varieties that can accumulate high levels of these micronutrients which would improve their absorption into the body.

2.2 Zinc

Zinc is an essential microelement required for growth of animals, human and in plants. It is a vital nutrient in plants because it is required for various enzymatic reactions, metabolic processes, and oxidation- reduction reactions (Hafeez *et al*., 2013). Zinc plays three main roles in living organisms mainly catalytical, regulatory and structural roles; catalytically, nearly 300 different enzymes depend on zinc for their ability to mediate vital chemical reactions (King, 2011). These zinc dependent enzymes, are found in all known classes of enzymes. Regulatory role of zinc occurs through gene expression, where zinc finger proteins act as transcription factors, whereas structurally, zinc plays an important role in the structure of proteins and cell membrane (Debjit *et al*., 2010).

2.2.1 Role and source of zinc in humans

Zinc plays an important role in the human body by boosting the immune system, healing and protecting of the skin, stimulating taste and smell, boosting brain activity and maintenance of normal testosterone levels (Debjit *et al*., 2010). Zn shortens the period for common cold when administered not later than 24hrs after infection (Hulisz, 2004). Most zinc is obtained from either animal or plant products with meat having a higher percentage (40-60%), pulses about 20-40%

and dairy products about 10-30% (Jerome, 2007). Its bioavailability in most common foods typically ranges from 10-30%.

Zinc deficiency is one of the most prevalent risk factor for nutrient-related diseases, and is a leading contributor to the global burden of anemia (Jerome, 2007). A diet consisting of a high proportion of cereal-based foods with low Zn content is considered as one of the major reasons for the widespread occurrence of Zn deficiency in humans, especially in the developing countries (Biesalski, 2013). Depending on their dietary habits, young children as well as pregnant women are at highest risk of zinc deficiency, which may occur throughout their life span. Populations in the developing countries generally consume limited animal products and are highly dependent on plants or cereal meals which may contain high nutrient bioavailability inhibitors, therefore predisposing them to increased risk of zinc deficiency.

Zinc deficiency is associated with primary T-cell lymphocyte immune system dysfunction leading to failure to terminate incipient malignancies, viral and fungal infections and frequent opportunistic infections such as common cold (Prasad *et al*., 2012), due to inability to protect cell membranes from viruses, toxins, complement, and venoms. Deficiency also results in skin allergies, asthma and chronic diarrhea, abnormal neurosensory changes, poor appetite particularly in the young and aged, fertility problems including hypogonads, failure of sexual maturity, benign prostatitis in men, and menstrual cramping and bloating in women, birth defects; growth failure (dwarfism) growth retardation; premature aging (Maret and Sandstead, 2006). It has also been demonstrated that Zn deficiency can lead to vision problems with its supplementation preventing 25% of age related macular degeneration (Prassad, 2009).

In humans, zinc absorption occurs in the gastrointestinal tract and in particular the small intestines. In humans Zrt and Irt-like Protein genes (ZIP genes) are responsible for Zn uptake (Wang *et al*., 2002). This genes lead to expression of specific transporters (ZnT) which span the gut epithelial membrane, which complexes with Zn^{2+} internalizing them into the body system (Wang and Bing, 2010).

2.2.2 Role and source of zinc in plants

Zn plays very important roles in plant metabolism influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions, synthesis of cytochromes and those involved in carbohydrate metabolism (Sayed *et al*., 2013). The regulation, maintenance and

expression of genes required for environmental stress tolerance in plants are also Zn dependent (Cakmak, 2000). Zinc is required as a cofactor in the synthesis of tryptophan which is a precursor for synthesis of auxins such as Indole Acetic Acid (IAA) (Brennan, 2005; Alloway, 2004). IAA induces gametogenesis, embryogenesis, cell plasticity, geotropism and seedling growth in higher plants (Ottoline, 2010; Yunde, 2010).

Zinc deficiency in plants results in various abnormalities which are manifested as the plant grows. Its deficiency generally causes a decrease in plant growth and yield (Yasushiro *et al*., 2011). The most common symptoms of Zn deficiency include: stunted growth, shortened internodes and petioles, and small malformed leaves (little leaf) which results in the "rosette" symptom in the early growth stages of dicotyledons and "fan shaped" stems in monocotyledons (Peck *et al*., 2010). Zinc deficiency may cause large reductions in crop quality and yield. For example wheat and barley show significant decreases in growth and grain yield under Zn-deficient conditions in the field (McDonald *et al*., 2001). In marginal deficiency, crop quality and yield may be reduced because of hidden Zn deficiency without obvious symptoms (Alloway, 2004).

Plants absorb zinc primarily from the soil where it exists as mineral ions, Zn organic matter, Zn adsorbed phosphates and Zn adsorbed iron oxyhydroxides (Sarret *et al*., 2004). In low zinc soils, uptake is enhanced by the exudation of low molecular weight compounds called phytosiderophores mostly malate and mugineic acid. The phytosiderophores which are negatively charged are released to the rhizosphere where they bind to the positively charged zinc ions forming a complex which through a carrier mediated transport are internalized into the plant cells and transported to different cells (Arnold *et al*., 2010; Widodo *et al*., 2010; Suzuki *et al*., 2006). Mugineic acid is synthesized from S-adenosyl methionine (SAM). Three molecules of SAM are combined to form one molecule nicotineamine (NA) a reaction catalyzed by nicotineamine synthase (NAS). NA is then converted to 3-ketoacid by nicotineamine aminotransferase (NAAT). Deoxymugineic acid synthase then acts on the keto form to produce 2-deoxymugineic acid (Fig. 2.1) (Bashir *et al*., 2006; Bashir and Nishizawa, 2006).

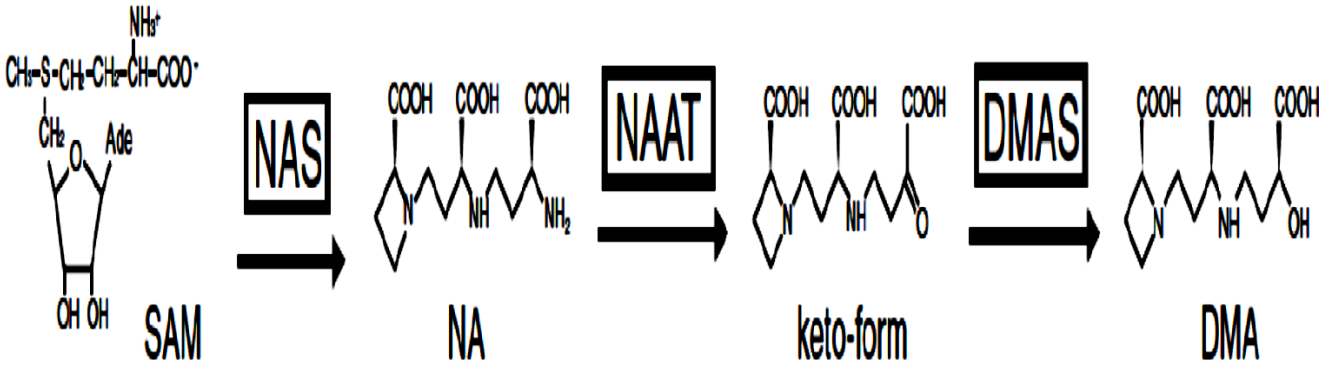

Figure 2.1: Deoxymugineic acid synthesis from S-Adenosyl-l-Methionine (Source: Yasushiro *et al.*, 2011).

Zinc transport also occurs through cation transporters such as Zinc and iron regulated transporter protein (ZIP). In seeds Zn is localized in different areas for example in rice zinc is localized in the embryo, endosperm and the aleruone layer, although it is high in the embryo (Takahashi *et al.*, 2009). However during germination, this changes and concentrations in the endosperm decreases while in the embryo it increases especially in the radicle and leaf primordium. The relocation of zinc occurs as it is required to activate respiration enzymes in the two tissues since their cells are rapidly growing and dividing, and with time, Zn concentrations increase in the scutellum and the vascular bundle of the scutellum (Bashir *et al*., 2010). At this point zinc activates proteinases and other enzymes leading to break down of stored proteins and starch required for feeding, germination and growth (Takahashi *et al*., 2009).

2.3 Iron

Iron is an essential nutrient for every living organism because it is required in a number of biological processes that serve to maintain life (Nathalie, 2013). However, there are notable exceptions particularly in bacterial species belonging to the *Lactobacillus* and *Bacillu*s families that can sustain life without iron (Crichton *et al*., 2001).

2.3.1 Role and source of iron in humans

Iron is a key element in hemoglobin and myoglobin which facilitate binding and transport of oxygen in the blood and muscle tissues of vertebrates. It is located at the centre of protoporphyrin IX molecule forming the heme molecule involved in oxygen transport (Fig. 2.2).

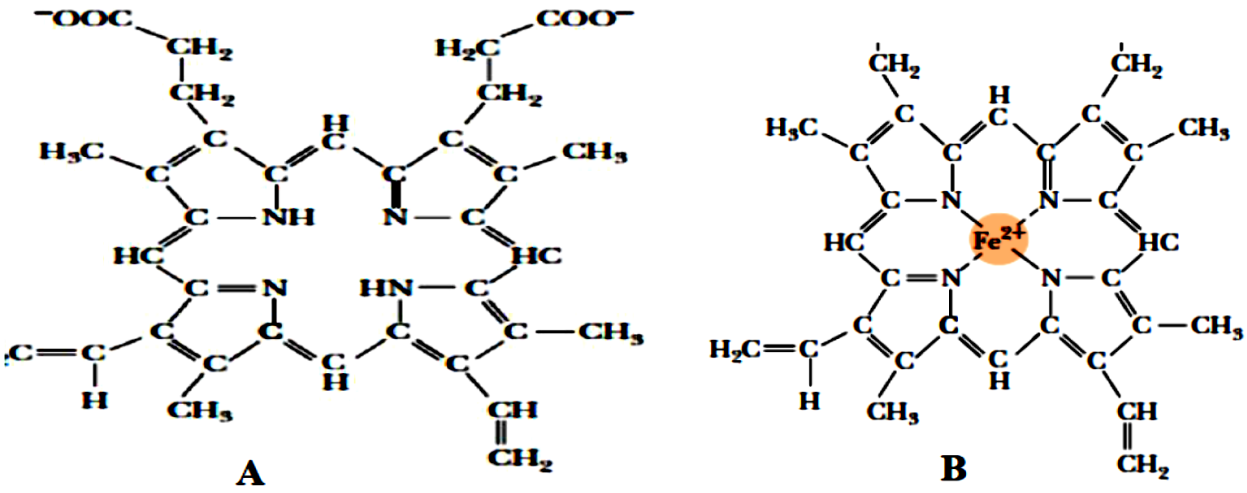

Figure 2.2: Iron forms part of the heme molecule and it is involved in the binding and transport of oxygen. **A-** Structure of protoporphyrin IX molecule. **B-** Structure of heme molecule i.e. Fe2+complexed to protoporphyrin IX molecule, (Source: David and Michael, 2004).

Under normal conditions, iron lies at the centre of the protoporphyrin molecule and is bonded to the four pyrrole nitrogen atoms usually in the ferrous state (Fe^{2+}). The iron still has two additional planes at which hemoglobin binds with oxygen ready for transport. Iron forms part of the cytochrome p450 family of enzymes (Yogesh *et al*., 2013). For humans Fe is obtained from two main forms which are heme iron, mostly found in animal meat (5-10%) and non-heme iron (including ferric oxides and salts, ferritin and lactoferrin) which is predominant in all diets (legumes, vegetables and fruits) and comprise upto 90- 95% of total iron consumed (Beard and Han, 2009). Although heme iron constitutes a lesser percentage, it is the most widely available for absorption in the duodenum (Sharp *et al*., 2007).

Globally, Fe deficiency is the most prevalent nutritional deficiency affecting approximately 1.62 billion people, or about 25% of the world's population as of 2009 (Bruno *et al*., 2009). The main symptom of Fe deficiency in humans is anemia, which can be as a result of poor iron intake, chronic blood loss, or impaired absorption (Goldberg *et al*., 2013). Fe deficiency causes major health problems and if it occurs during pregnancy it can lead to impaired physical and cognitive development of the infant. In adults it causes acute fatigue leading to reduced work productivity. Deficiency due to poor Fe intake arises because Fe present in the diet cannot be

absorbed in its stored form (Nielson *et al*., 2013). Deficiency is more pronounced in people who primarily consume cereals; because the negatively charged phosphate groups of phytate present in cereals complexes the Fe making it unavailable for absorption (Barbara *et al.,* 2013).

Absorption of Fe occurs in the proximal small intestine and is mediated by specialized epithelial cells called duodenal enterocytes. It is absorbed from the diet either as inorganic iron (iron salts or chelates) or as a part of heme, which is usually released after digestion of hemoglobin and myoglobin in dietary meat (Vashchenko and Ross, 2013). The non-heme iron which is not bioavailable has to be reduced into a more readily absorbed ferrous state. Reduction of ferric form into ferrous form is aided by a number of reducing agents such as ascorbic acid, histidine and cysteine amino acids. In the highly acidic environment, iron is absorbed into the cells via a Divalent Metal Transporter 1 (DMT1), also known as Divalent Cation Transporter 1 (DCT1) (Sharp *et al*., 2007).

The heme iron is absorbed into the body through two main carriers called; Heme Carrier Proteins (HCP-1) and the Heme Response Gene (HRG-1). HCP-1 is expressed in the small intestines while HRG-1 is expressed in the small intestines, brain, heart and kidney (Rajagopal *et al*., 2008; Shayeghi *et al*, 2005). For Fe to be released, heme is degraded by heme oxygenases releasing Fe which is then absorbed into the body (Ganna *et al*., 2013).

2.3.2 Role and source of iron in plants

Iron is crucial to plants since it not only participates in respiration and cell division but also in the synthesis of chlorophyll and in electron transport chain during photosynthesis. In legumes iron is required in a greater amount for nodule formation than for host plant growth (Weria *et al*., 2013). In addition it is also required as a cofactor for nitrogenase enzyme involved in the fixation of atmospheric nitrogen. The importance of Fe in biological nitrogen fixation is well demonstrated in *Herbaspirillum seropedicae*, a bacterium involved in nitrogen fixation, was found to be inefficient in low Fe concentrations (Federico, 2006). Iron in plants is also involved in the donation and accepting of electrons and thus serving as an important cofactor for a number of metalloenzymes implicated in respiration and photosynthesis (Erin *et al*., 2003). Fe is also a key element in various redox reactions of respiration, photosynthesis and reduction of nitrates and sulphates (Yellamandha *et al*., 2002). Iron forms part of ferrodoxin molecule in photosystem II of photosynthesis where it is linked to cysteine residues leading to a 4FE-4S cluster that accepts electrons from photosystem I. ferrodoxin then transports the electrons to ferrodoxin-Nicotinamide

Adenine Dinucleotide Phosphate ($NADP^{+)}$ reductase, which using the electrons, a molecule of Nicotinamide Adenine Dinucleotide Phosphate Oxidase (NADPH) is regenerated (Fig. 2.3).

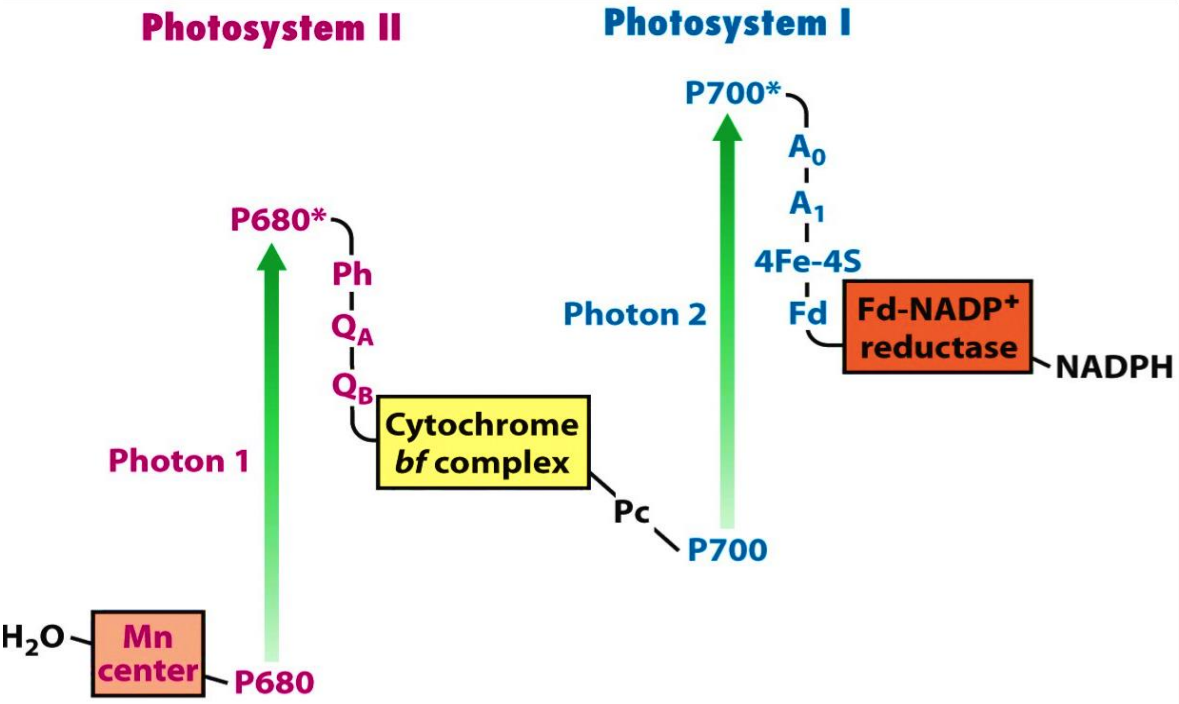

Figure 2.3: Iron forms part of the ferrodoxin molecule which transports electrons through ferrodoxin-NADP+ reductase to generate a molecule of NADPH (Source: Jerremy *et al.*, 2002).

Iron deficiency in plants is characterized by symptoms such as iron chlorosis, which usually appear on the youngest and newest leaves. The area between the leaf veins becomes pale yellow or white, a condition referred to as interveinal chlorosis (Hamdollah, 2011). Fe deficiency in plants leads to reduced photosynthetic pigment this is because iron is part of the chlorophyll molecules in plants. In fruits it impairs quality and yield and ultimately leads to tree death (Fernandez *et al*., 2006; Alvarez-Fernandez *et al*., 2003). Tree death is usually as a result of inefficient and low chlorophyll amounts leading to reduced photosynthesis which may ultimately lead to plant death.

Iron deficiency in pea plants causes reduced plant growth and the reduced growth rate is dependent on its concentration and availability (Nenova, 2006). Reduced Fe leads to decreased photosynthetic pigments and few healthy photosystems (Jeong *et al*., 2008), and with inefficient

photosystems, the photosynthesis rates are reduced greatly leading to inadequate carbon compounds (carbohydrates and proteins) required for plant growth.

Plants acquire Fe mainly from the rhizosphere and though Fe is the most abundant metal in the earth's crust, its availability to plants is very low and is dictated by soil redox potential and pH (Joe *et al*., 2009). In soils that are highly aerobic and at a high pH, Fe is oxidized to insoluble forms of ferric oxides. There are two strategies that plants use for uptake of Fe. Strategy 1, also known as acidification/reduction mechanism involves enhancing Fe solubility prior to uptake. In this strategy, plants secrete protons into the rhizosphere to lower the pH of the soil and thus increase the solubility of Fe^{3+}. The Fe^{3+} is then reduced further to Fe(II) by root ferric chelate reductase thereby enhancing Fe solubility; since Fe(II) is more soluble than Fe^{3+} (Conte and Walker, 2011). The second approach known as chelation-based strategy, it involves the use of metal chelators and specific transporters for Fe uptake. In this strategy, mugineic acid phytosiderophores are secreted to the rhizosphere and complex with iron molecules to form ferric-phytosiderophores complexes. These complexes are then internalized through iron specific carriers spanning the outer membrane of cells that are highly specific for the complexes (Currie *et al*., 2001).

2.4 Phytic acid

Phytic acid also known as inositol hexakisphosphate, IP6, or phytate when in salt form is an organic acid extracted from cereals. Phytic acid is used as an acidulant for pH adjustment in the food and beverage industry. The most outstanding feature of phytic acid is its strong metal chelation function (Arun, 2011), it strongly adsorbs monovalent and divalent metal ions such as iron, zinc, calcium and magnesium reducing their absorption potential by humans and animals (Fig. 2.4). Zn^{2+} is the essential mineral element most adversely affected by phytate (Lonnerdal, 2002; Lopez *et al*., 2002). Phytate is found in relatively high amounts in plant foods, particularly in cereals and legumes (Schlemmer *et al*., 2009).

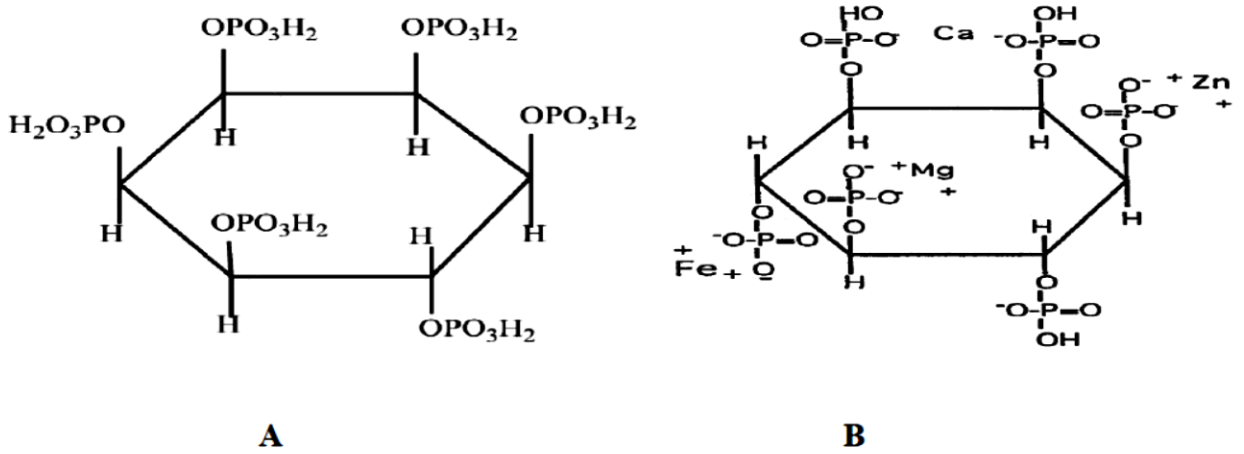

Figure 2.4: A- Chemical structure of phytic acid **B-** Structure of phytic acid complexed to metal ions, Zn2+, Fe2+, Ca2+ and Mg+ (Source: Arun Shunmugam, 2011).

During seed development, phytic acid is deposited as mixed phytate salts of potassium, magnesium, calcium, zinc and iron in globoids; hence, several different cations may be associated with each phytate molecule. In seeds the reserve of phytic acid is degraded by phytase, this releases bound mineral ions required for proper germination and plant growth (Raboy, 2000). On the other hand monogastric animals including humans lack phytase enzyme, therefore they are unable to degrade phytates; hence the bound mineral ions become unavailable for absorption (Bregitzer and Raboy, 2006).

Phytic acid is synthesized via two metabolic pathways which are lipid dependent and lipid independent pathways. In the lipid independent pathway glucose-6-phosphate is isomerized to 1-L-myo-inositol-1-phosphate (MIP) by myo-inositol-1-phosphate synthase (MIPS) (Loewus *et al*., 2000). Further steps in the biosynthesis of phytic acid are the step-wise phosphorylations of MI-1-P by phosphoinositol kinases. In the lipid dependent pathway, phytic acid biosynthesis starts from free myo-inositol through phosphatidyl inositol phosphorylations occurring at the membrane bound intermediate levels (Stevenson-Paulik *et al*., 2002). Mostly, lipid independent pathway has been suggested to be functional in higher plants, whereas MIPS is considered to be catalyzing the first committed step for this biosynthesis and myo-inositol-kinase (MIK) facilitates a salvage mechanism for free myo-inositol to be recycled into a pool of MI-1-P (Loewus and Murthy, 2000). Action of MIK allows the synthesis of phytic acid from free myo-inositol, though little is known

about the regulation, localization and role of MIK. Biosynthesis of phytic acid is illustrated below (Fig. 2.5).

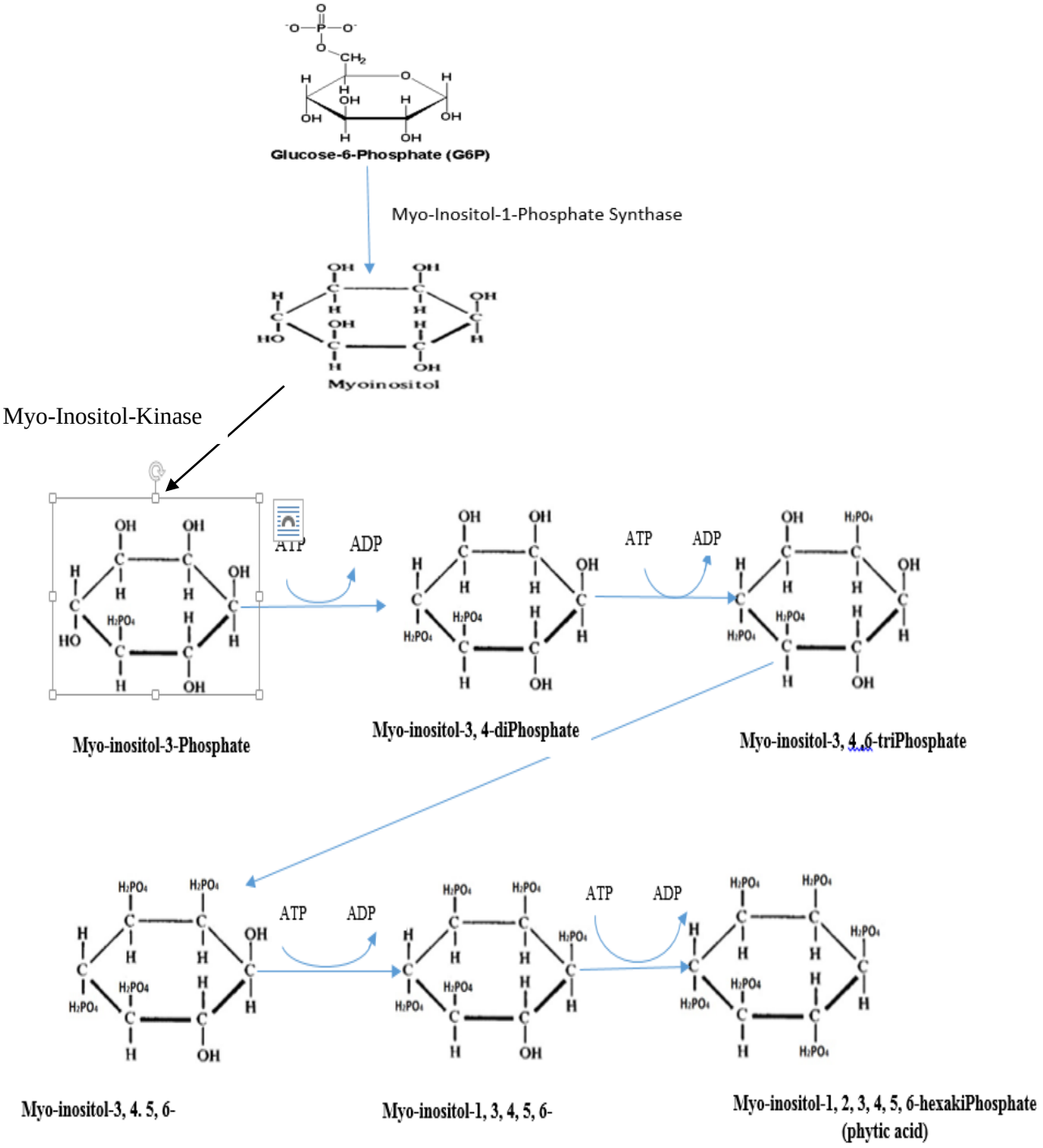

Figure 2.5: Biosynthetic pathway for phytic acid from glucose-6-phosphate (Source: Caspi *et al.*, 2010).

2.5 Resistant starch

The starch that cannot be degraded by amylase enzymes is known as resistant starch (RS). It passes unabsorbed through the small intestine and enters the large intestine, where it is the substrate for bacterial fermentation, producing short chain fatty. According to Leszczynski (2004), resistant starch (RS), is categorized into four groups (RS1, RS2, RS3 and RS 4).

The first group, (**RS 1)** includes starch present in plant cells with undamaged cell walls, typical of partially milled cereal grains. It is unavailable to amylolytic enzymes, since the gastrointestinal tract lacks enzymes capable of degrading cellulose, hemicelluloses, lignins, and other constituents of plant cell walls. Therefore, such starch together with fragments of plant tissue passes the small intestine undigested. RS1 is measured chemically as the difference between the glucose released through enzyme digestion of a boiled homogenized food sample and that from an un-boiled, non-homogenized food sample. In raw starch granules, starch is tightly packed in a radial pattern and is relatively dehydrated. This compact structure limits the accessibility of digestive enzymes, various amylases, and accounts for the resistant nature of RS1.

Group two (**RS 2)** starch exists in granule form especially in potato and banana, and it has high amylose content. It is the main type of resistant starch in raw foods. This type of starch exists as large granules which are not easily degraded by amylase enzymes due to a limited surface area for amylase activity.

The third group (**RS 3)** is retrograded starch, i.e. spontaneously or artificially-precipitated from starch paste, occurring in the form of water-insoluble semi-crystalline structures. As a result of retrogradation, more thermostable structures are formed by amylose rather than by amylopectin. The amount of resistant starch produced this way increases along with the increasing amylose content of starch.

The final group **(RS 4)** is resistant starch that is chemically or physically-modified mainly through thermal treatment. Chemically modified starch could be through acetylating and the degree of resistance increases with increased substitution of the hydroxyl groups with other functional groups (Hoover and Zhou, 2003).

Since RS is not easily absorbed into the body, it has been suggested that it may play a role in prevention of colonic cancer. This is because the butyric acid formed through RS fermentation prevents growth of malignant cells in the body and it has been shown to prevent proliferation of tumor cells *in vitro*, by arresting the G1 phase of cell cycle.

Foods containing high levels of RS are thought to reduce the rate of digestion and they release glucose slowly into the blood stream (Sajilata *et al*., 2006). This means that the body then results to the utilization of stored body fats to obtain the much needed energy (Nugent, 2005). It has also been used as a probiotic to encourage growth of beneficial microorganism such as bifidobacterium (Brown *et al*., 1996). The most important benefit of RS is the ability to enhance the absorption of mineral ions (Zn^+, Fe^+, Mg^+, and Ca^+) in the small intestines (Lopez *et al*., 1998). The non-digestible RS remains in the lumen longer and delays gastric emptying which provides more time for mineral absorption.

2.6 Dough rheology

In the baking industry wheat is the preferred and most commonly raw material because of its unique rheological properties which imparts desirable positive effects on the baking quality (Svec and Hruskova, 2010). The rheological properties of dough are an important basis for the process of production of quality bakery products (Moreira *et al.,* 2010). For expanding the range of bakery and pastry products, various recipes for product enrichment with fibre, especially β-glucan, proteins, vitamins, micronutrients and other nutrients for a healthier diet have been developed (Martins *et al.,* 2011). Dough rheology is evaluated using rheometers (machines) which makes it possible to record the mechanical changes resulting from mixing, heating and also simulate the mechanical work as well as the heat conditions that might be expected during the baking process (Rosell *et al.,* 2007). During the baking process, flour compounds are subjected to mechanical work and heat treatment that promote changes in their rheological properties (Bollain and Collar, 2004).

2.6.1 Effects of zinc and iron on whole meal dough rheology

Although the viscoelastic properties of wheat flour are mainly influenced by the quality and quantity of gluten (Neelam and Khatar, 2014), there is need to evaluate the relationship of microelements on dough rheology. This is due to an increase in desire to consume mineral rich and healthy food products. In a study on effect of mineral fortification on dough rheology, water absorption, dough development time, dough stability and the viscoelastic properties were highly influenced by zinc and iron fortification (Saeed *et al.,* 2008). This was as a result of the fortificants absorbing more water and complexing with protein present which increased the dough stability and dough development time.

2.6.2 Effects of resistant starch and phytic acid on whole meal dough rheology

High resistant starch increases water absorption during whole meal dough formation and decreases dough elasticity (Hung *et al.,* 2007). This is because increase in resistant starch reduces the protein component which is responsible for dough elasticity. High resistant starch molecules in the dough combines with gluten proteins, forming discontinuous, irregular matrix around starch granules which reduces dough elasticity (Gomez *et al.,* 2003).

Phytic acid is usually found in close association with wheat bran thus an increase in bran particles increases levels of phytic acid (Katina *et al.,* 2006). High bran content in a sample increases water absorption, dough development time but reduces the viscoelastic nature of dough. In a study conducted by Mosharafa *et al.,* (2009), hydrothermal treatment of bran containing flours softened the bran particles, reduced phytic acid content and improved the rheological properties of whole meal dough. However there exists no direct effect of phytic acid on dough rheology.

2.7 Organoleptic properties

Organoleptic analysis is done on bakery products based on various hedonic measurements which are fundamental in comprehending the relationship of the chemical senses to food preference and selection (Juyun *et al.,* 2009). This measurements are classified based on the degree of pleasure sensations or thrills such as pleasant or unpleasant. With increased consumer awareness, there has been need to consume bread with superior nutrition quality thus the need to prepare whole meal bread and related confectionaries (Ndife *et al.,* 2006). Whole meal bread contains functional ingredients such as minerals (Zn, Fe, Mg, and Ca among others), fiber, essential amino acids and phytochemicals which are located in the bran and germ of the whole grain (Jideani *et al.,* 2009). Since sensorial fulfilment is a major factor influencing the consumption of bakery products, it then becomes a necessity to carry out organoleptic evaluation to classify consumer preferences and know which areas to improve on.

2.7.1 Effect of zinc and iron on organoleptic properties of whole meal flour

Previous organoleptic analysis of zinc and iron fortified products have revealed that the fortified products are well liked generally (Aaron *et al.,* 2011). In that study, the fortified bread products though indistinguishable from non-fortified wheat bread scored highly especially for taste, colour appearance and general acceptability. In a study to evaluate acceptability of zinc and

iron fortified bread in Peru, it was concluded that the products were well liked though products fortified with zinc or iron oxide scored lowly compared to those fortified with zinc or iron only (Lopez *et al.,* 2002).

2.7.2 Effect of resistant starch and phytic acid on organoleptic properties of whole meal flour

High resistant starch flours bake bread with a deep brown colour due to an increase in glucose residues. The free glucose residues react with the proteins present resulting in brown coloured products (Sangnark and Noomhorn, 2004). The appearance, taste, aroma and general acceptability of bread fortified with resistant starch did not differ from the control (Abdoulaye *et al.,* 2013). In a study conducted by Ihsan *et al.,* (2003), different fermentation times of wheat flour reduced the levels of phytic acid and this improved the sensory attributes such as taste, colour, texture and general acceptability. This was in line with a study conducted by Salim *et al.,* (2007), who upon treating dough with sour dough bacteria discovered that levels of phytic acid decreased which led to improved bread shape, crust colour, taste, aroma and texture.

2.8 Selected Kenyan wheat varieties

The following 9 wheat varieties namely, Kenya Wren, Kenya Tai, Kenya Korongo, Kenya Sunbird, Kenya Hawk 12, Kenya Kingbird, Eagle 10, Robin and Njoro BW2 were used in this study. Selection of these wheat varieties was based on the fact that they were released recently. In all the cultivars listed, six were released in the year 2012 and two in the year 2011. Variety Njoro BWII was released in 2001 and was used as the control because it is high yielding and popular among wheat farmers. The characteristics of the selected wheat varieties are indicated in Table 2.1.

Table 2.1: Recently released Kenyan wheat varieties and their characteristics.

Variety	**Year of release**	**Description**	**Yield** (tonnes/hectare)
Robin	2011	It is a red, hard grain resistant to stem rust especially Ug99 strain and is classified as a bread wheat variety that exhibits spring growth habits.	8.1

Eagle 10	2011	It is a red, hard grain resistant to stem rust especially Ug99 which exhibits spring growth habits and matures in 100-110 days.	6.5
Kenya Tai	2012	This is a red, hard grain which is resistant to stem and yellow rust and exhibits spring growth characteristics. This bread wheat variety matures in 100-110 days.	6.5
Kenya Sunbird	2012	This is a red, hard grain is resistant to stem and yellow rust. This bread wheat variety matures in 100-110 days.	6.5
Kenya Wren	2012	This is a variety which has a red, hard grain that is resistance to stem rust strain Ug99. It is a bread wheat variety that matures in 120-130 days and has spring growth characteristics.	8.5
Kenya Korongo	2012	Also known as KSRR-VIII, it is a white hard grain with spring growth habits. It is resistant to stem rust strain Ug99 and this bread wheat variety matures in 120-130 days.	8.5
Kenya Hawk 12	2012	A bread wheat variety with a hard, red grain and spring growth habits. It is resistant to stem rust strain Ug99 and matures in 120-130 days.	8.5
Kenya Kingbird	2012	This is bread wheat variety with a white, hard grain that is resistant to stem rust strain Ug99. It exhibits spring growth characteristics and matures in 90-110 days.	6.0
Njoro BWII	2001	A bread wheat variety with a red, hard grain, resistant to sprouting and is popular among farmers in the highlands. It exhibits spring growth characteristics.	

CHAPTER 3

EVALUATION OF GRAIN ZINC, IRON, PHYTIC ACID AND RESISTANT STARCH

3.1 Introduction

Human beings get their nutritional requirements through consumption of animal products and edible plants. The nutrition quality of edible plant parts is highly affected by both soil composition and environmental factors, which also greatly influence nutrient uptake and storage in plants (Marschner, 2012). Environmental changes especially in temperature and precipitation, affect plant physiological processes which in turn influence nutrient uptake (Olesen *et al.,* 2011). The effect of soil composition is known to influence variations of micro-elements composition such as Zn and Fe in kernels of cereals such as wheat (Abrar *et al.,* 2010). The variations have mainly been attributed to soil types, farm practices and types of fertilizers applied (Banziger and Long, 2000). Similar to the micronutrients Zn and Fe, the anti-nutritive elements such as phytates in cereal crops are also influenced by soil composition and environmental factors. In addition the concentration of anti-nutritive phytates in plants varies depending on the stage of maturity and genotype. For instance, it is well demonstrated that high temperatures and water-deficit conditions lead to increased phytic acid content in the wheat grains (Gordana *et al*., 2015; Singh *et al*. 2012).

As discussed earlier in section 2.2.2 and 2.3.2 of this thesis, majority of cereal based diets especially maize and wheat, are inherently low on levels of Zn and Fe and the little present is lost during milling and polishing (Polleti *et al.,* 2004). Most of the seed Zn and Fe is located in the embryo and aleurone layer whereas the endosperm is very low in Zn concentration (Ozturk *et al*., 2006). Although Zn and Fe are important micronutrients in wheat, there are variations in their concentrations and therefore there is need to bio-fortify wheat to improve their levels.

Bio-fortification of cereal crops has been achieved through over expression of the transcription factor *NAM-B1* (Uauy *et al*., 2006). The *NAM-B1* gene was initially used to increase protein levels but is being used to increase Zn and Fe levels in cereals (Distelfeld *et al.,* 2007). Wheat varieties with improved levels of these micronutrients could be used to address Fe and Zn malnutrition, which still affects over 25% of the global population (Philippa *et al*., 2014).

Wheat also contains substantial amounts of resistant starch which has been found to improve the absorption of micronutrients in the gut. It is classified as a prebiotic which is the non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth

and/or activity of one or a limited number of bacteria in the gastrointestinal tract and thereby exert a health-promoting effect (Schrezenmeir and de Vrese, 2001). Resistant starch has been used as a nutrition approach to reduce the number of people who are at risk of developing type II diabetes (Mindy *et al.,* 2013). In a study conducted in rodents fermentation of Resistant Starch resulted in what appears to be a healthier gut (Michael *et al.,* 2015) which was demonstrated by increased amounts of short chain fatty acids, positive change in the microbiota, and increased gene expression for gene products involved in normal healthy proliferation and apoptosis of potential cancer cells. Additionally, consumption of resistant starch has been associated with reduced abdominal fat and improved insulin sensitivity.

Phytic acid (PA) is the main storage form of phosphorous in seeds accounting for 60% to 90% of the total seed phosphorous and 1.5% of the seed dry weight (Bohn *et al.,* 2008). The negatively charged phosphates in PA strongly bind to metallic cations (such as K^+, Mg^+, Mn^+, Fe^+, Ca^+, and Zn^+) to form a mixed salt called phytin or phytate. Metal cation-phytate complexes can be formed in 2 ways: simple phytate-mineral complexes or fibre-phytate-mineral complexes. The stability and solubility of the metal cation-phytate complexes depend on the individual cation, the phytate-to-cation molar ratio, pH, and presence of other compounds in the solution (Greiner *et al.,* 2006). In seeds phytate is predominantly found in the protein bodies of embryo and aleurone layers, where there is a high deposition of minerals as well (Steadman *et al.,* 2001). The chelation of iron and zinc with PA has a strong negative effect on absorption of these minerals in humans and other monogastric animals that largely lack the phytase enzyme, which is required to degrade phytate.

The objective of this study was to evaluate the concentrations of the microelements (zinc and iron), phytic acid and resistant starch for nine wheat varieties grown in 3 different wheat growing regions of Kenya, namely Eldoret, Mau-Narok and Naivasha.

3.2 Materials and methods

3.2.1 Selection of the study sites

Wheat farming requires moderate rainfall ranging between 500mm to 1270mm and temperatures ranging between 15^{o}C to 20^{o}C for at least three months to enable its maturity. Towards the end of its growing period, warm dry sunny spell are required to enhance ripening and

harvesting of wheat. Its farming is preferably practiced in high altitudes ranging from 1500-2900 metres above sea level as this reduces severity and incidences of diseases and requires fairly level land or gentle slopes as this allows for mechanization.

The project study sites Eldoret, Mau-Narok and Naivasha (Fig. 3.1), were selected based on the above characteristics and the fact that farmers in these areas have been associated for a long time with both large and small scale wheat farming.

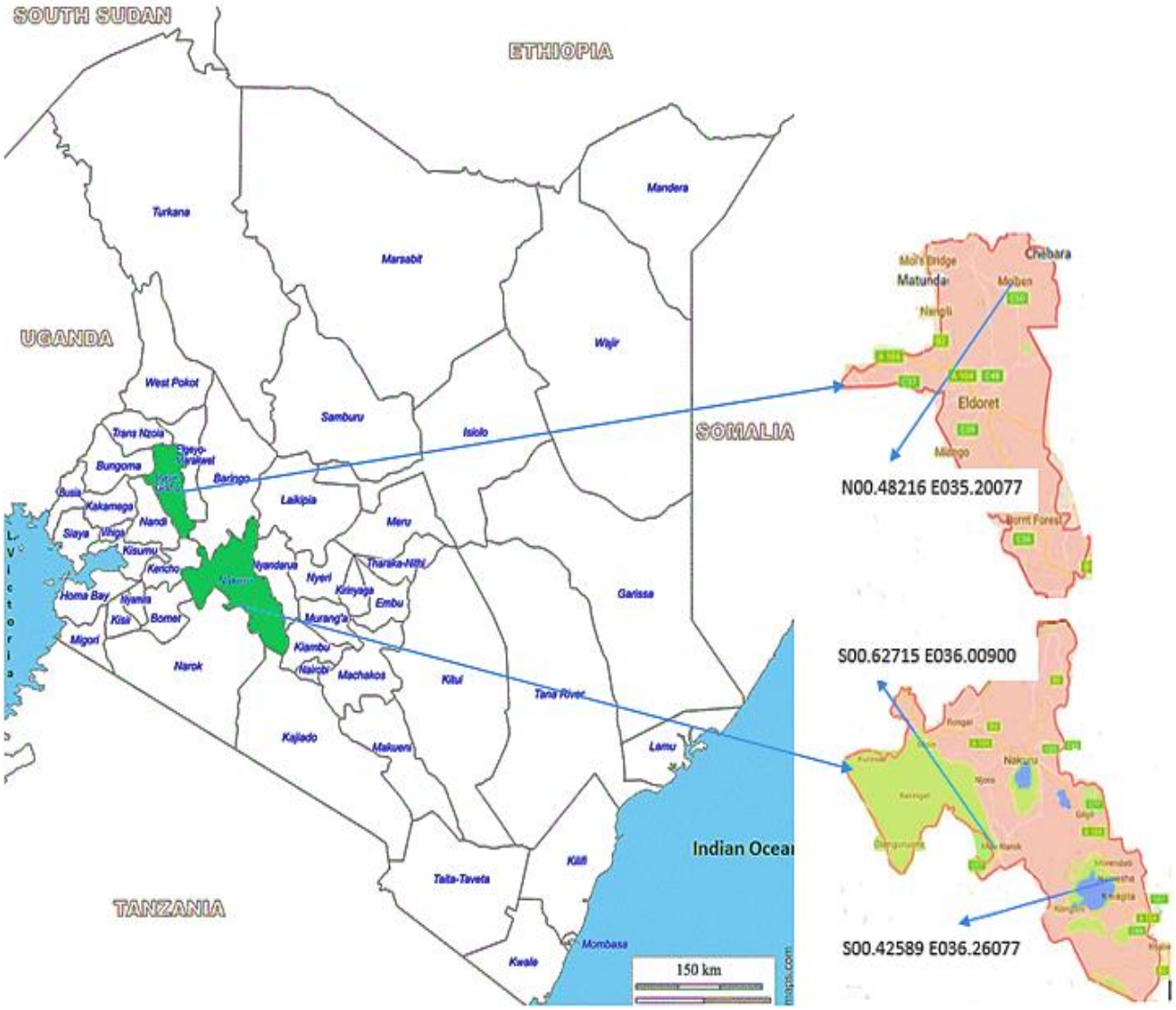

Figure 3.1: Physical location of the two counties in Kenya associated with wheat growing. One experimental site was located in Eldoret, Uasin Gishu County and two sites in Naivasha and Mau-Narok located in Nakuru County.

Mau-Narok experimental site was located at the highest altitude at 2830 m above sea level among the three experimental sites and at latitude $0^0$36S and longitude $36^0$0E. Soil in Mau-Narok are well drained, dark reddish brown with an acidic humic top soil and are classified as andosols (Volcanic soils). The pH for Mau-Narok is more acidic and ranges from 4.8-5.6 with Zn concentrations ranging between 25 ppm, while Fe concentrations are in the range of 100ppm. Naivasha was the lowest among the three sites and was laid at an altitude of 1884 m and latitude 0º43'S and longitude 36º26'E. Soils in Naivasha are a complex of well drained, deep dark brown to grayish brown friable and are classified as cambisols. The soil is characterized by high organic matter, yellowish brown to brown top soil. The pH of Naivasha borders between slightly acidic to almost neutral and ranges from 6.2 to 7.4, with Zn concentrations ranging between 25-65ppm while Fe concentrations were in the range of 110ppm. The site in Eldoret was located at an altitude of 2073m and latitude 0°31'N and longitude 35°17'E. Soil in Eldoret is slightly acid with a pH of 5.4-6.0 and the iron concentrations are in the range of 20ppm with Zn concentrations being around 5 ppm. It is characterized by dark brown to reddish brown loamy soils which are well drained and classified as volcanic soils.

3.2.2 Plant materials and experimental design

Nine wheat varieties namely Robin, Eagle 10, Kenya Tai, Kenya Sunbird, Kenya Wren, Kenya Korongo, Kenya Hawk 12, Kenya Kingbird and Njoro II were all obtained from the Kenya Agricultural and Livestock Research Organization, Food Crops Research Centre located at Njoro. The variety Njoro BWII was used as the control due to its superior baking properties especially high protein and gluten levels. The seeds prior to planting were coated with copper oxychloride ($Cu_2(OH)_3Cl$) a broad spectrum fungicide that controls both fungal and bacterial diseases. To control weeds after the wheat seedlings had emerged, the experimental plots were sprayed with 'Thunder herbicide' manufactured by Agri-Star, with imazethapyr and glyphosate as the active agents. The herbicide is a post emergent control measure that kills a wide variety of broadleaf weeds, grasses, and sedges. Thunder herbicide was supplemented with 'Buctril MC' manufactured by Bayer crop Science Company, a herbicide with bromoxynil octanoate and ethyl hexyl ester as the active agents that also acts on broad leaf weeds. The wheat varieties were planted in a Completely Randomized Block Design (RCBD). In each of the three study sites Eldoret, Mau-Narok and Naivasha, land was prepared and divided into three blocks of equal dimensions 6 m length and 1.5 m width, and each block subdivided into nine equal plots that were separated by a

path of 1m and a path of ½ m between the blocks (Fig. 3.2). The land was treated with Di-Ammonium Phosphate (DAP) fertilizer at the rate of 50 Kg/ ha before planting. The varieties were then allocated randomly on the plots within the three blocks, to give rise to three replicates plots for each variety, using the using a random number table (Fig. 3.2). No irrigation was undertaken during planting and growing of the wheat crop in the three study sites.

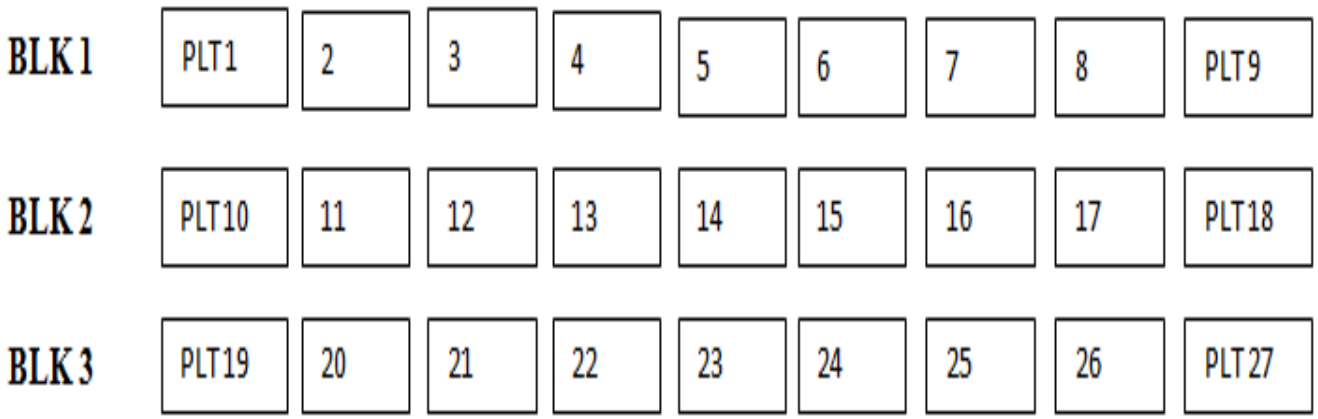

Figure 3.2: Randomized Complete Block Design (RCBD) experimental design showing how the 9 wheat varieties were planted in the 3 study sites. Legend BLK1-BLK3 represents Block 1 to Block 3 and PLT1-PLT27 represents Plot 1-Plot 27. Robin-PLT 1, 12 and 20, Eagle10-PLT 2, 18 and 26, K.Tai- PLT 3,15 and 23, K.Sunbird-PLT 4, 13 and 25, K.Wren- PLT 5,16 and 22, K.Korongo- PLT 6, 14 and 27, K.Hawk12-PLT 7, 17 and 24, K.Kingbird-PLT 8, 9 and 21, Njoro BWII- PLT 9, 10 and 19.

3.2.3 Crop harvesting and sample preparation

After the varieties had matured, the dry wheat heads were harvested in each of the study sites and harvest from each plot were placed separately in clean well labeled bags and transported to the laboratory for further processing. The grains were threshed and winnowed to remove any chaff present and then dried further to reduce the moisture levels of the kernels to about 12-13% which was confirmed using the Near Infrared (NIR) spectrophotometer grain analyzer machine. At this moisture level, the grains were safe for long term storage and still remained viable to be used as seed in future.

3.2.4 Zinc and Iron analysis

Ten grams of sample were obtained from each of the bags and separately milled using a chromium ball mill (Retsch mill model, MM 400) whose milling compartment was coated with teflon. After each milling round, the compartment was thoroughly wiped clean using wet cloth to avoid cross contamination of the next sample. The resulting whole meal flour was then stored in dry clean brown envelopes. For analysis of Zn and Fe content in the kernels, 0.3g of the finely ground flour sample was weighed and placed in a dry clean glass digestion tube and 4 mls of the digestion mixture (selenium-sulphuric acid mixture) was then added and heated to 200^{o}C in a block digester. After cooling 3 successive portions of 1 ml of hydrogen peroxide was added, with at least 10 seconds intervals between each addition. The tubes were then returned to the block digester and temperatures adjusted to 330^{o} C. After cooling the digest transferred into a 100ml volumetric flask and filled up to the mark with de-ionized water and analyzed for iron and zinc at 248.33nm and 213.86nm using the Atomic Absorption Spectrophotometer, (Shimadzu Model AA-6300, Tokyo-Japan). Standards at concentrations of 2.5, 5.0, 7.5 and 10 ppm were prepared from a standard stock solution of 1000ppm and their absorbance determined. The stock solutions were prepared from salts of zinc nitrate [$Zn(NO_3)_2$] and [$Fe(NO_3)_3$] for zinc and iron standards, respectively. The results were then used to construct a calibration curve with absorbance against concentration whose trend line had an R^2 value of 0.999 (Fig 3.3). After reading the absorbance for the samples for both zinc and iron, the actual concentrations were determined by equating the corresponding absorbance in the equations (Y=0.004X for iron and Y=0.34X for zinc) where Y=absorbance and X=actual concentration.

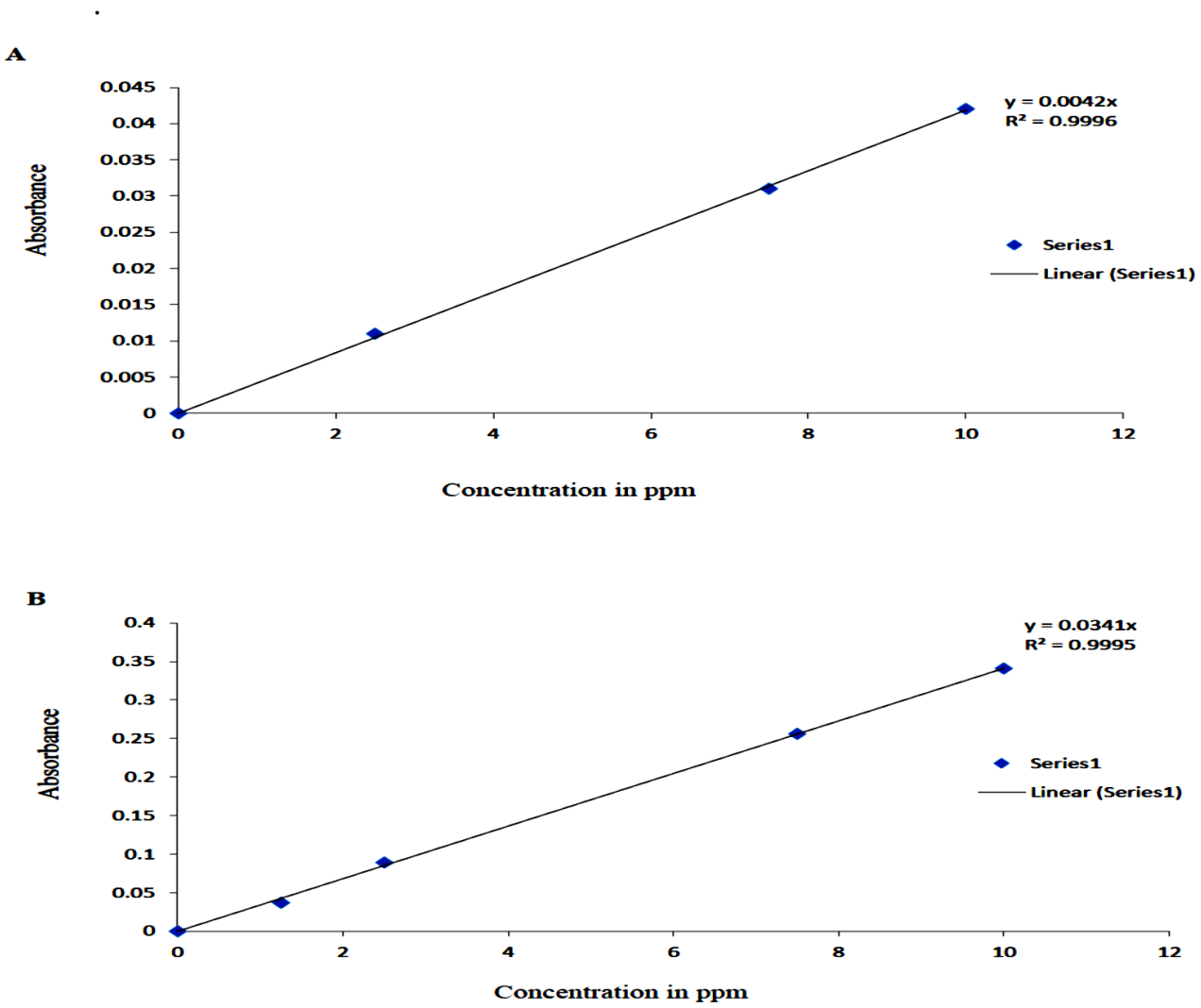

Figure 3.3: Trace element standard curves **A-** Standard curve for Iron **B-** Standard curve for Zinc.

3.2.5 Determination of phytic acid content

From the whole meal flour stored in brown envelope, 1 g of the flour was weighed and transferred into a 1.5 ml microfuge tubes to which 1 ml of 0.4M hydrochloric acid was added and incubated at 4^{o}C for 12 hours to extract the phytic acid present in the samples. The samples and prepared standards were then vortexed briefly and 20µl aliquot transferred into microtitre plates and supplemented with 180 µl of Chen's reagent (1 volume 6N H_2SO_4, 1 volume 2.5% ammonium molybdate; 1 volume 10% ascorbic acid, 2 volumes H_20). Addition of Chen's reagent in a sample containing phytic acid induces the formation of phospho-molybdate compound which has a blue coloration and the intensity of colour formed is dependent on phytic acid concentration in the

sample. The assay was then allowed to develop up to 2 hours at room temperature after addition of Chen's reagent. An image of the microtitre plates was then taken after 1 and 2 hours and comparison was made between colour intensity of the samples and the standards. The standards were prepared by dissolving 0.174 grams of di-Potassium hydrogen phosphate (K_2HPO_4) in 1 liter distilled water to give a concentration of 1Mm K_2HPO_4. Eight standards were then prepared in order of increasing concentration by pipetting 15, 30, 45, 60, 75, 105 and 120µl and supplementing it with 100 µl of Chen's reagent, 10 µl of 0.4 M HCl and distilled water in order of reducing volumes (Table 3.1). The protocol used for determining phytic acid content in wheat was adopted from a study conducted on rice mutants with low phytate (Raboy *et al.,* 2000).

Table 3.1: Preparation of phytic acid control standards. Reagents and volumes used in standard constitution.

Standard	µL of 1mM K_2HPO_4	µL of 0.4M HCl	µL of $_dH2O$	µL of Chen's reagent	Standard (µg)
1	15	10	175	100	2.61
2	30	10	160	100	5.22
3	45	10	145	100	7.83
4	60	10	130	100	10.44
5	75	10	115	100	13.05

3.2.6 Resistant starch determination

Resistant starch was analyzed using the megazyme resistant starch assay procedure (11/02 AOAC Method 2002.02, AACC Method 32-40). Grain samples (50 seeds per variety) were ground and passed through a 1.0mm sieve. The flour was then transferred into a plastic jar and mixed thoroughly by shaking and inverting. A sample of 100 mg was then weighed, placed in a test tube and then 4 Ml of pancreatic α-amylase with amyloglucosidase (AMG) was immediately added to each tube. The mixture was incubated in a shaking water bath at 37°C for 16 h during which non-resistant starch was solubilised and hydrolysed to D-glucose by the combined action of the two enzymes. The reaction was terminated by addition of an equal volume of ethanol, and the RS recovered as a pellet upon centrifugation at 3000 revolutions per minute (rpm). The pellet was washed twice by re-suspending it in 50% ethanol v/v (50% ethanol dissolved in 50% distilled water), followed by centrifugation and decanting of the supernatant. The decanted supernatant was

set aside and was later assayed for soluble glucose. The RS in the pellet was dissolved in 2 M KOH by vigorously stirring on an ice water bath using a magnetic stirrer. This solution was later neutralised with 8ml of 1.2M sodium acetate buffer and the starch quantitatively hydrolysed to glucose with amyloglucosidase (AMG). D-Glucose was then measured with glucose oxidase/peroxidase reagent (GOPOD). Upon addition of the reagent, the sample solution developed a reddish colour (Fig. 3.4) whose intensity depended on the concentration of the hydrolyzed glucose. Absorbance of the colour intensity was measured at 510 nm using a UV/ Visible light spectrophotometer. The absorbance was then converted to actual RS content using a formula provided with the assay kit (Appendix 2). The values obtained for Resistant Starch and soluble glucose were summed up to give total starch levels.

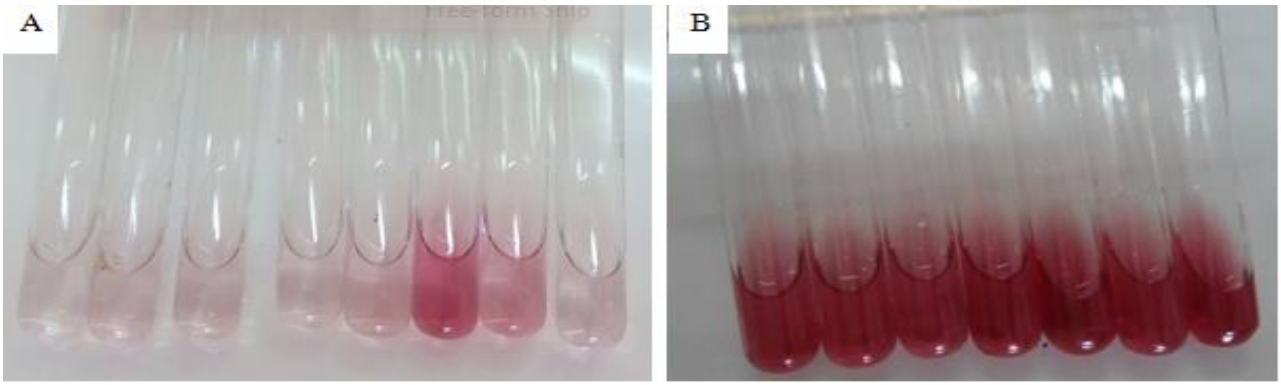

Figure 3.4: Resistant starch and soluble glucose assay. **A-** Colour development for resistant starch assay after addition of GOPOD reagent. Samples with higher concentrations of RS had intense colour. **B-** Colour development for soluble glucose assay in wheat samples.

3.3 Data analysis

All data was collected in triplicates per variety for each experimental site and data was then subjected to Analysis of variance (ANOVA) to determine content differences for Zn and Fe, phytic acid and resistant starch among the varieties at $P<0.05$ level of significance. Differences between the means were then ranked by Fisher's least significant difference (LSD) test. ANOVA and LSD were done using SAS software Version 9.1.3 (SAS Institute, Inc., 2004). The means were further analysed using MINITAB software to cluster the varieties with highest similarity of the nutritional qualities. The software uses neighbour joining algorithm to cluster means into different groups. Using this algorithm, means that those closest to each other distance-wise in respect to a central point are grouped together to form a distinct node or cluster.

3.4 Results

3.4.1 Influence of variety and site on seed Fe and Zn content

Seed Fe concentrations ranged between 111 and 305 ppm irrespective for the varieties and Fe concentration was significantly ($p<0.05$) influenced by cropping site and variety × site interaction (Appendix 3). For varieties grown in the Eldoret site, Fe concentrations ranged between 138 to 305ppm, while those grown in Mau-Narok and Naivasha ranged between 111 to 222ppm (Fig 3.5a). Overall the highest Fe concentrations were recorded in the varieties K.Korongo and K.Hawk 12 for the crop grown in Eldoret. In addition, the control variety Njoro BWII generally recoded the lowest Fe concentration for crop grown in the Naivasha and Eldoret sites, while K.Wren and K.Korongo recorded the lowest concentrations for the crop grown in Mau-Narok site (Fig. 3.5a).

Varietal analysis for zinc indicated levels ranging between 26 to 91 ppm among the varieties (Fig. 3.4b). The Zn concentrations were significantly ($p<0.05$) influenced by variety site and variety × cropping site (Appendix 4). For the Naivasha crop, the Zn concentrations ranged between 32 to 91ppm, while for Eldoret and Mau-Narok crop it ranged between 26 to 88ppm. Generally the highest concentrations of Zn were obtained on the control variety Njoro BWII in wheat crop grown in Naivasha and Eldoret. On the flip-side, varieties K.Wren and K.Korongo had the lowest Zn concentration across all the three study sites (Fig. 3.5b). However relatively higher concentration of Zn were generally obtained on varieties Eagle10 and Robin across all the three study sites, when compared to the test varieties unlike the control variety Njoro BWII (Fig. 3.5b). Overall, highest concentrations were recorded in the control Njoro BWII, Eagle10, Robin and K.Kingbird whereas lowest concentrations were recorded in K.Wren and K.Korongo (Fig. 3.5b).

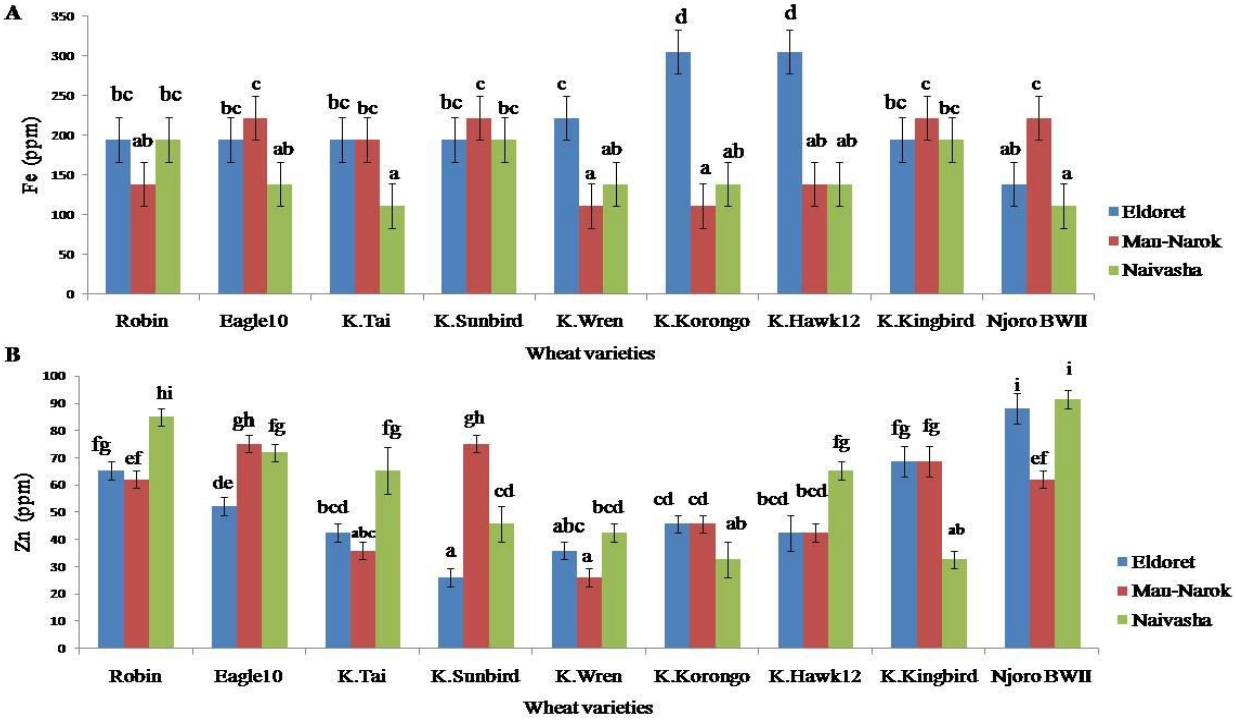

Figure 3.5: Micro element concentrations in 9 Kenyan wheat grown in 3 study sites (Eldoret, Mau-Narok and Naivasha). **A-** Fe concentration in whole meal flour and **B-** Zn concentrations in whole meal flour.

3.4.2 Variations of resistant starch, glucose and total starch content.

Resistant starch, glucose and total starch concentrations ranged between 0.37 and 6.00g per 100g, 20 and 33g per 100g and 22 and 37g per 100g, respectively (Table 3.2). The ANOVA revealed that RS, soluble glucose and total starch were significantly ($p<0.05$) influenced by variety, site and, variety × site (Appendix 5).

For wheat crop grown in Eldoret site, analysis of RS indicated that highest concentration were obtained on K.Hawk 12, K.Tai and K.Wren, with 6.03, 5.90 and 4.66 g per 100g, respectively. Further analysis of the means revealed that the three varieties had significantly ($p<0.05$) higher concentration of RS starch when compared to the control Njoro BWII which had RS concentration of 2.01g per 100g. However for Naivasha and Mau-Narok sites, significantly ($p<0.05$) higher concentration of RS were obtained on the control variety when compared to the 8 varieties tested (Table 3.2). Conversely, highest concentration of glucose for wheat crop grown in Eldoret site was in K.Sunbird and the levels were significantly ($p<0.05$) higher than that obtained for Njoro BWII variety. For Naivasha and Mau-Narok sites varieties with higher glucose

concentrations were not significantly different when compared to the control variety, except for K.Kingbird in Mau-Narok site (Table 3.2). For total starch, highest concentration for the crop grown in Eldoret was obtained on K.Sunbird, and K.Hawk 12, the starch concentration in these two varieties were significantly ($p<0.05$) higher than in the control variety. For Naivasha and Mau-Narok sites, however the varieties with higher total starch concentrations were not significantly ($p<0.05$) different when compared to the control variety, except for K.Kingbird in Mau-Narok site (Table 3.2).

Table 3.2: Determination of resistant starch, glucose and total starch concentration in 9 Kenyan wheat-bread varieties grown in three sites located in Eldoret, Mau-Narok and Naivasha.

	Resistant starch			**Glucose**			**Starch**		
Variety	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha
Robin	1.17^{efgh}	1.47^{h}	0.37^{a}	26.16^{defgh}	24.88^{bcde}	27.92^{efghi}	27.33^{cde}	26.35^{cd}	28.29^{defg}
Eagle 10	1.42^{gh}	1.16^{efgh}	0.45^{ab}	31.73^{kl}	29.11^{ghijk}	26.09^{defg}	33.14^{hij}	30.26^{efgh}	26.54^{cd}
K.Tai	5.90^{m}	2.65^{jk}	1.30^{fgh}	21.91^{ab}	28.38^{ghij}	29.07^{ghijk}	27.81^{cdef}	31.03^{ghi}	30.38^{efgh}
K.Sunbird	1.47^{h}	0.48^{abc}	0.37^{a}	32.91^{l}	20.16^{a}	33.09^{l}	34.38^{jkl}	20.65^{a}	33.46^{ijk}
K.Wren	4.66^{l}	0.83^{bcde}	0.98^{defg}	22.43^{abc}	30.34^{ijkl}	25.28^{cdef}	27.09^{cd}	31.17^{ghi}	26.26^{cd}
K.Korongo	2.27^{ij}	0.78^{abcde}	0.68^{abcd}	28.36^{fghij}	21.86^{ab}	26.03^{defg}	30.63^{fghi}	22.64^{ab}	26.72^{cd}
K.Hawk12	6.03^{m}	0.91^{bcdef}	0.68^{abcd}	31.10^{jkl}	21.97^{ab}	24.15^{bcd}	37.13^{l}	22.88^{ab}	24.84^{bc}
K.Kingbird	2.35^{ij}	2.98^{k}	0.92^{cdef}	22.90^{abc}	33.41^{l}	30.96^{ijkl}	25.25^{bcd}	36.39^{kl}	31.88^{hij}
Njoro BWII	2.01^{i}	2.04^{i}	2.01^{i}	29.30^{ijk}	29.77^{ijk}	29.24^{hijk}	31.31^{ghij}	31.81^{hij}	31.25^{ghi}

Means for the same parameter followed by same letter are not significantly ($p<0.05$) different as revealed by Fisher LSD test. All values are in g per100g of whole meal flour.

3.4.3 Influence of site and wheat genotype on phytic acid concentrations

Similar to RS, glucose and total starch concentration reported for the 9 varieties in section 3.3.2, site, variety and interaction (site × variety) significantly ($p<0.05$) influenced the level of phytic acid in wheat varieties (Appendix 6). Irrespective of wheat variety, the level of phytic acid concentration for the crop grown in the Eldoret site ranged between 2.66 ppm to 4.79 ppm, whereas

for Mau-Narok and Naivasha sites the range was 2.66 to 5.05 ppm. Generally highest phytic acid content was observed in the wheat crop grown in Naivasha experimental site except for K.Korongo variety, while those grown in Mau-Narok site had lowest content, except for the control variety Njoro BWII (Fig. 3.6). For Eldoret site highest phytic acid content was obtained on K.Sunbird, K.Kingbird and Njoro BWII. None the less, a general trend was observed where highest phytic acid was obtained with Njoro BWII variety across all study sites, while the lowest phytic acid values were obtained with K.Wren, Eagle10 and Robin varieties (Fig. 3.6).

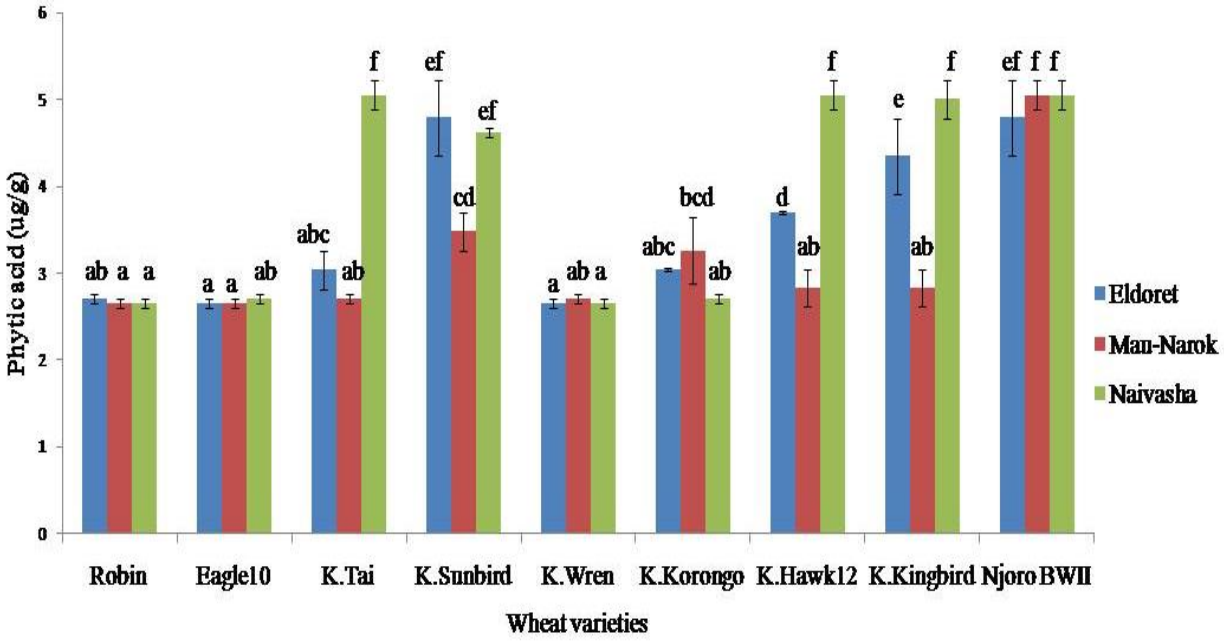

Figure 3.6: Phytic acid concentration in Kenyan bread wheat varieties grown in Eldoret, Mau-Narok and Naivasha.

3.4.4 Overall nutrition quality analysis

Cluster analysis of the varieties based on the means of all the nutritional qualities analyzed, namely phytic acid, resistant starch, iron, zinc, glucose and total starch, revealed three distinct clusters, A, B and C (Fig. 3.7). Cluster A was composed of varieties K.Sunbird and K.Kingbird, cluster B was composed of K.Tai and K.Hawk12 while cluster C was composed of Robin, K.Wren and K. Korongo. The levels of similarity were 64%, 62 % and 58 % for cluster A, B and C, respectively. The varieties Eagle 10 and the control Njoro BWII did not cluster with any of the varieties used in this study.

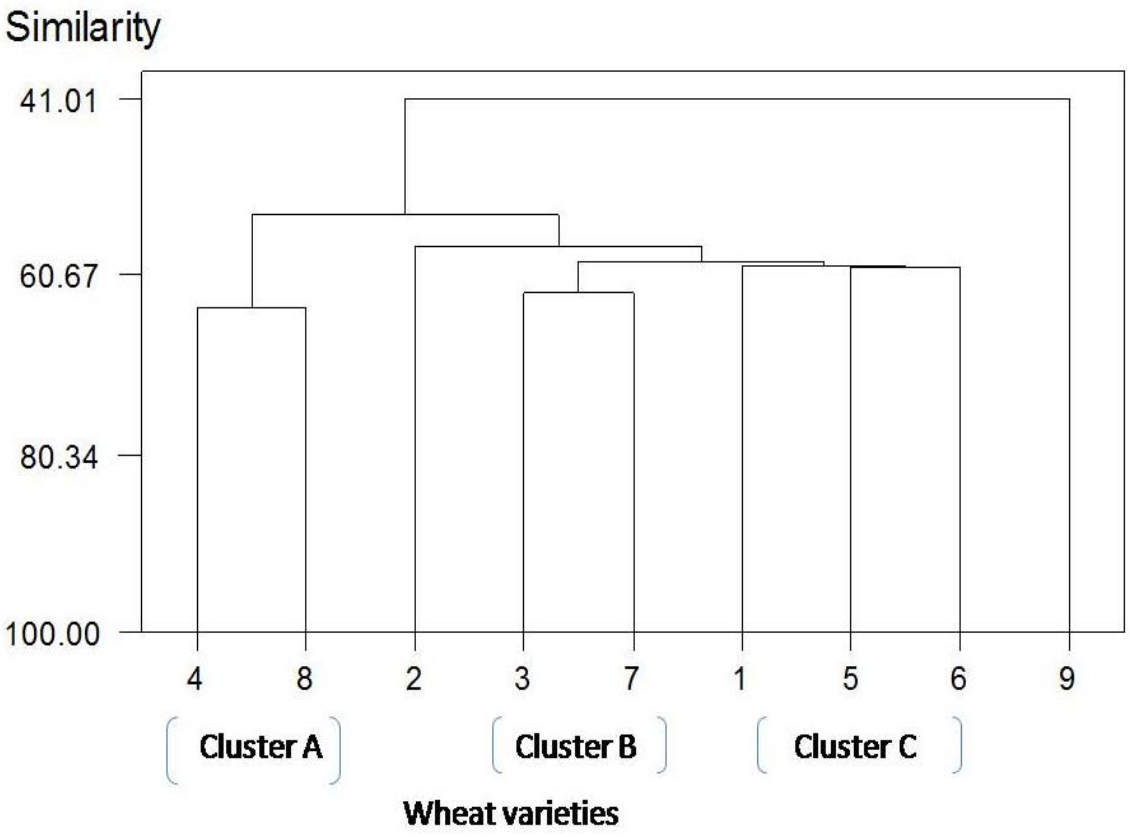

Figure 3.7: Dendogram showing differential clustering of Kenyan bread wheat varieties based on their nutritional qualities. Legend 1-9 represents variety 1 to variety 9: 1-Robin, 2-Eagle10, 3-K.Tai, 4-K.Sunbird, 5-K.Wren, 6-K.Korongo, 7-K.Hawk12, 8-K.Kingbird and 9-Njoro BWII.

3.5 Discussions

3.5.1 Environmental and genotypic effect on seed Fe and Zn concentrations

Micronutrient concentrations especially of Fe and Zn in most cereals are relatively low. Preliminary studies with wild relatives and landraces of wheat have demonstrated that considerable variation exists in grain Zn and Fe concentration (Genc *et al*., 2005; Gomez-Becerra *et al*., 2010a, b). The targets for Fe and Zn biofortification in wheat grain are around 60 and 40ppm, respectively (Ortiz-Monasterio *et al*., 2007). Suggestions put forward are that current grain Zn and Fe concentrations should be increased by at least 10 and 25ppm in order to have a measureable biological impact on human health, (Chatzav *et al.,* 2010; Ortiz-Monasterio *et al.,* 2007). With regard to Fe concentrations, the 9 wheat varieties were all above the target with the lowest

concentrations being almost 2 fold more than the target while the highest was 5 times more whereas for varietal Zn concentrations, 4 of the varieties registered higher levels across the 3 sites some even 2 fold more than the target. Fe and Zn concentrations are highly influenced by soil conditions especially the soil *p*H and soil microelement composition. Varieties grown in areas with high *p*H and low Fe concentrations such as Eldoret accumulated more Fe in their kernels compared to the varieties grown in soils with adequate Fe composition and slightly lower *p*H such as Mau-Narok and Naivasha. Uptake of Fe and Zn usually occurs through direct transport using ZRT-, IRT-like proteins (ZIPs) or via secretion of phytosiderophores (PS) which chelate Fe cations and are subsequently taken up by yellow stripe like (YSL) transporters (Sperotto *et al*., 2012). Monocots plants such as wheat prefer the chelation strategy for Fe uptake. Among the chelators is nicotineamine (NA) and in Fe deficient soils, plants over express the nicotine amine synthase (NA) gene producing more NA which binds with the iron present in the soil and transports it into the plant sinks increasing their concentration. In a study conducted on transgenic soy beans which were genetically engineered to over-express the NA synthase gene, the plants accumulated 2 to 4 fold more Fe compared to the non-transgenic plant (Tomoko *et al*., 2014).

Kernel (seeds) with more than 20ppm of zinc are classified as Zn sufficient while those with less than 10ppm are deficient (Bhupinder *et al*., 2005). Concentrations recorded in all the test varieties across the 3 sites revealed that all were zinc sufficient. Comparison between Zn concentration per cropping site and the grain concentration revealed that crops grown in sites with high levels maintained the same high levels in their grains such as in Mau-Narok and Naivasha whereas, there was an increased concentration of Zn in grain grown in Eldoret site whose soil concentration for the same was less. This observation might be attributed to enhanced release of phytosiderophores especially 2'-Deoxy-mugineic acid in wheat grown in soil deficient/ low in zinc, hence increased uptake of zinc and its ultimate storage in grains (Dotaniya *et al*; 2013a). Once released phytosiderophores chelate Zn ions in the soil and transport them to various tissues first through the xylem and the phloem in the later stages of maturity.

Varietal differences in grain iron and zinc concentrations in cultivated wheat has also been attributed, in part to allelic variation at a chromosomal locus that promotes early senescence and remobilization of protein, iron and zinc from senescing leaves to seeds (Distelfeld *et al*., 2007). This may explain the reason why some varieties recorded overall higher levels irrespective of site. During senescence, cytoplasmic components such as organelles in the leaves are gradually

dismantled and degraded and their components such as zinc and iron translocated to the sinks especially grains where they form a reservoir for future generations (Mathieu *et al*., 2014).

3.5.2 Varietal and environmental variation in resistant starch, soluble glucose and total starch concentrations.

RS, soluble glucose and starch content in cereals vary depending mostly on site and genotype however for RS, environmental variation is difficult to predict and control compared to genetic variation (Birt *et al*., 2013). Genetic variation is due to allelic variation in the starch biosynthetic genes as is the case in commercial maize varieties, which exhibit little variation in resistant starch levels, (Pollack *et al*., 2011; Rohlfing *et al*., 2010). This is in line with variations observed in the study genotypes where some recorded up to 5 fold more RS than others when grown in the same cropping site. Wheat varieties contain the same synthetic genes for RS but some genotypes are able to over express them more than in the other varieties leading to enhanced RS levels. Environmental effect particularly rainfall and temperature affects RS levels by influencing enzymes involved in its synthesis (Keeling *et al*., 1994). Eldoret and Mau-Narok sites experience much higher rainfall and cool temperatures and this may have favored the enzyme activities involved in enhancing RS synthesis leading to higher concentrations in these sites whereas hot weather conditions with low rainfall experienced in Naivasha could have affected the enzyme activities leading to low RS levels recorded.

The starch content in wheat constitutes 65 to 75% of the grain dry weight, with amylose contributing 20 to 30 while amylopectin contributes the remaining 70 to 80%. The amylose portion is positively correlated with RS levels (Shrestha *et al.,* 2010). The amylose portion is usually degraded slowly by enzymes which have to remove the terminal glucosyl residues each at a time compared to the amylopectin portion which is easily degraded due to its branching. The results for starch for the nine Kenyan wheat varieties were below previously reported values by almost half. The low levels might have been contributed by size of grain and also presence of bran particles of whole meal flour used which might have reduced percentage content of starch. The amount of soluble glucose was highly correlated where starch content with varieties having more starch also recording high levels of soluble glucose. Variety interacting with site conditions has been shown to influence starch levels (Maryke *et al*., 2007). This trend was observed in K.Korongo and K.Hawk12 when grown in Eldoret site compared to the other 2 sites such that a variety may contain

high levels when grown in one location, the levels are not certain and may differ when the same variety is grown under different environmental conditions.

3.5.3 Variations in seed phytic acid concentrations.

Phytic acid content in plant seeds and grains ranges from 0.5-5% and reaches upto 90% in dormant seeds (Loewus, 2002). In flours, concentrations of 3.77ppm, 2.96ppm and 8.50ppm has been reported for hand-made refined flours, factory refined flours and for the whole grain flours, respectively (Febles *et al*., 2002). Depending on the amount of plant derived foods in diets and level of processing, daily intake can be as high as 4500 mg (Reddy, 2002). The mean daily intake of phytate is estimated to be 200-2000 mg for vegetarians and in developing countries it, is about 150-1400 mg.

Phytic acid results obtained in this study for whole meal flour were all below 6ppm irrespective of site or variety and were below previously reported values. The results also indicated that site and varietal factors had significant influence on phytic acid content in wheat grains, but site appears to be a predominant factor in influencing the phytic acid content. Similar results have been observed on studies done on barley and rice which concluded environmental effect as the main contributor to phytic acid content in the grains (Fei *et al.,* 2007; Liu *et al*., 2005b). The effect of site and especially the prevailing weather conditions such as temperature and rainfall have a great effect on phytic acid content. High temperatures and low rainfall increases the anti-nutrients such as phytic acid in cereals (Sondeep *et al*., 2012). This was observed in Naivasha cropping site which experiences lower rainfall and high temperatures than the other two cropping sites, while Mau-Narok which experiences low temperatures and high rainfall recorded lowest phytic acid levels. Temperature and rainfall has a great impact on phytic acid synthesis, which starts with the rate limiting step involving the enzyme *myo*-inositol-3-phosphate synthase (MIPS). This enzyme is involved in the conversion of D-glucose to myo-inositol phosphate a product which undergoes various phosphorylations step to give rise to phytic acid. MIPS activity is highly affected by temperature and *p*H with its optimum temperature in plants being 35^{o}C while the *p*H is around 7.0 to 7.5 (Chhetri *et al*., 2006). The high levels of phytic acid content for varieties grown in Naivasha may be attributed to the high temperatures (25 to 29 ^{o}C) experienced in the area throughout the year.

Also associated with levels of phytate in seed, is the amount of phosphorus concentration in the soil. Seeds from locations with high soil phosphorous have enhanced levels of phosphorous

and this may influence seed phytate content (Kim *et al*., 2002). Crops require phosphorous in large amounts and once internalized, it is redistributed to various plant cells while some of the portion is stored in form of phytates. While each cropping site was treated with the same amount of fertilizer (DAP), soils conditions in Naivasha especially *p*H may have favoured phosphate uptake by the varieties as compared to the other sites. Mobility of phosphorous is enhanced at *p*H range of 6.0 to 7.0 since at this range, the inorganic phosphate (P*i*) is free compared to lower ranges observed in Eldoret and Mau-Narok which provides an environment for binding of inorganic phosphorous to metal ions making it unavailable for plant uptake (Rastija *et al*., 2014).

3.5.4 Overall nutritional quality

The clusters obtained revealed that the control Njoro BWII and Eagle 10 did not cluster with any of the other varieties. Njoro BWII and Eagle 10 are the oldest among the other test varieties used in the experiment and the results obtained suggests their nutritional content based on Fe, Zn, RS, glucose and starch levels differ significantly from all the others. The two exhibit spring growth conditions and are resistant to sprouting. K.Sunbird and K.Kingbird formed their own distinct clusters and it could be as a result of growth conditions and maturity time which for both was between 100 to 110 days while K.Tai and K.Hawk12 while Robin, K.Wren and K.Korongo formed the other sub-cluster. In a study conducted on phytic acid in rice and pearl millet, varieties which matured early and were grown in areas which experienced high rainfall had less of the anti-nutrient due to inadequate time to concentrate it in the grain and leaching to deeper levels of phosphorous inaccessible to plant roots (Pelig-Ba, 2009). In this study, genotypes used are spring varieties which do well in moderate temperatures. The varieties which clustered together have similar growth characteristics as described (Table 1) especially the maturity time and yields. This observation suggests that maturity duration for wheat varieties greatly influences the levels of the evaluated parameters.

3.6 Conclusions

The phytic acid levels at <6 ppm for all the nine varieties were in a range lower and safe for human consumption compared to the estimated daily consumption of around 150-2000 mg. While effect of environment was quite evident on the levels of phytic acid, the levels did not differ greatly thus in relation to concentrations of phytic acid for the nine varieties, it is safe to grow them in the three environments.

Resistant starch levels differed significantly among the varieties and across the sites while the interaction between site and variety was also evident. For higher RS concentrations, Eldoret should be the preferred site for the wheat varieties followed by Mau-Narok while for mineral elements concentrations, Njoro BWII and Robin should be the most ideal varieties when concentrating on zinc nutrition as they recorded the highest levels overall. K.Sunbird and K.Kingbird would be the overall ideal varieties if the target was increased iron levels.

Overall site analysis for the nutrients analysed indicate that Eldoret is the environment which would give the preferred nutrition values since all the parameters were in the desired range such as high RS, high iron concentrations and low phytic acid levels with the only drawback being low levels of zinc micronutrient. Mau-Narok site recorded moderate nutritional values while Naivasha would not provide desired nutritional quality except for Zn concentrations.

3.7 References

Abrar H., Hans L., Ramune K. and Eva J. (2010). Mineral composition of organically grown wheat genotypes: contribution to daily minerals intake. *International Journal of Environmental Research and Public Health*, **7:** 3442-3456.

Banziger M. and Long J. (2000). The potential for increasing the iron and zinc density of maize through plant breeding. *The Food Nutritional Bulletin*, **21:**397-400.

Bhupinder S., Senthil K.A.N., Singh B.K. and Usha K. (2005). Improving zinc efficiency of cereals under zinc deficiency. *Journal of Current Science*, **88(1):**36-44.

Birt D.F., Terri B., Suzanne H., Jay-Lin J., James H., Li L., John M., Samuel M., Gregory J.P., Matthew R., Kevin S., Paul S. and Elizabeth M. (2013). Resistant Starch: Promise for Improving Human Health. *Journal of Advances in Nutrition*, **4:** 587-601.

Bohn L., Meyer A.S., Rasmussen S.K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science*, **9:**165-191.

Chatzav M., Peleg Z., Ozturk L., Yazici A., Fahima T., Cakmak I. and Saranga Y. (2010). Genetic diversity of grain nutrients in wild emmer wheat: potential for wheat improvement. *Annals of Botany*, **105 (7):** 1211-1220.

Chhetri. D.R., Mukherjee A. K. and Adhikari J. (2006). *Myo*-inositol content in pteridophytes, the isolation and characterization of L-*myo*-inositol-1-phosphate synthase from *Diplopterygium glaucum. Brazillian Journal of Plant Physiology*, **18(2):**291-298.

Distelfeld A., Cakmak I., Peleg Z., Ozturk L., Yazici A.M. Budak, H., et al. (2007). Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Journal of Plant Physiology*, **129**:635-643.

Dotaniya ML, Meena HM, Lata M, Kumar K (2013a). Role of phytosiderophores in iron uptake by plants. *Journal of Agricultural Science Digest*, **33(1):** 73-76.

Febles C.I., Arias, A., Hardisson A., Rodrıguez-Alvare, C. and Sierra A. (2002). Phytic acid level in wheat flours. *Journal of Cereal Science*, **36:**19-23.

Fei D., Junmei W., Saihua Z., Zhenzhen X. and Guoping Z. (2007). Genotypic and environmental variation in phytic acid content and its relation to protein content and malt quality in barley. *Journal of Food Chemistry*, **105:**606-611.

Genc Y., Humphries J.M., Lyons G.H. and Graham R.D. (2005). Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. *Journal of Trace Elements and Medical Biology*, **18:**319-324

Gomez-Becerra H.F., Erdem H., Yazici A., Tutus Y., Torun B., Ozturk L. and Cakmak I. (2010a). Grain concentrations of protein and mineral nutrients in a large collection of spelt wheat grown under different environments. *Journal of Cereal Science*, **52:** 342-349.

Gomez-Becerra H.F., Yazici A., Ozturk L., Budak H., Peleg Z., Morgounov A., Fahima T., Saranga Y. and Cakmak I. (2010b). Genetic variation and environmental stability of grain mineral nutrient concentrations in *Triticum dicoccoides* under five environments. *Euphytica*, **171:**39-52.

Gordana B., Vesna D., Dejan D., Miroslav Z., Desimir K., Sladana Z., Srbislav D., and Gordana S. (2015). Genotype and Environment interaction for antioxidants and phytic acid contents in bread and durum wheat as influenced by climate. *Chilean Journal of Agricultural Research*, **75(2):**139-146.

Greiner R., Konietzny U. and Jany K.D. (2006). Phytate -An undesirable constituent of plant-based foods? *Journal of Ernaehrungsmed*, **8:**18-28

Keeling P.L., Banisadr R., Barone L., Wasserman B.P.and Singletary G.W. (1994). Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. *Australian Journal of Plant Physiology*, **21:**807-827.

Kim J.C., Mulla B.P., Selle P.H and Pluske J.R. (2002). Levels of total phosphorus and phytate in three varieties of Western Australian wheat in response to growing region and growing season. *Australian Journal of Agricultural Research*, **53:**1361-1366.

Liu Z. H., Cheng F. M. and Zhang G.P. (2005b). Grain phytic acid content in japonica rice as affected by cultivar and environment and its relation to protein content. *Journal of Food Chemistry*, **89:**49-52.

Loewus, F.A. (2002). Biosynthesis of phytate in food grains and seeds. In: Reddy N.R., Sather, and S.K Eds). *Food Phytate*. CRC Press, Boca Raton Florida, 53-61.

Marschner P (2012). Marschner's Mineral Nutrition of Higher Plants, 3rd Edition. Elsevier Publishers, Oxford UK.

Maryke T., Labuschagne N.G. and Garry O. (2007). The influence of environment on starch content and amylose to amylopectin ratio in wheat. *Journal of Starch*, **59:**234-238.

Mathieu P., Celine M., Kohki Y. and Sebastien T. (2014). Autophagy as a possible mechanism for micronutrient remobilization from leaves to seeds. *Frontiers in Plant Science*; **5(11):** 1-8.

Michael J.K., June Z., Maren H., Christine P., Holiday A.D., Diana B.C., and Roy J.M. (2015). Role of resistant starch in improving gut health, adiposity and insulin resistance. *Journal of Advances in Nutrition*, **6:**198-205.

Mindy P., Maziarz M.S, RD L.D. (2013).Role of Fructans and Resistant Starch in Diabetes Care. *Journal of Diabetes Spectrum*, **26(1):**35-39.

Ortiz-Monasterio J.I., Palacios-Rojas E., Meng E., Pixley K., Trethowan R. and Pena R.J. (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science*, **46:**293-307.

Ozturk L., Yazici M.A., Yucel C., Torun A., Cekic C., Bagci A., Ozkan H., Braun H.J., Sayers Z. and Cakmak I. (2006). Concentration and localization of zinc during seed development and germination in wheat. *Journal of plant physiology*, **128:**144-152.

Pelig-Ba K.B. (2009). Assessment of phytic acid levels in some local cereal grains in two districts in the upper east region of Ghana. *Pakistan Journal of Nutrition*, **8 (10):** 1540-1547.

Philippa B., James M.C., Janneke B., Anthony J.M., Dale S. and Cristobal U. (2014). Biofortification of wheat grain with iron and zinc: integrating novel genomic resources and knowledge from model crops. *Journal of plant science*, **5:53** doi: 10.3389/fpls.2014.00053.

Poletti S., Gruissem W. and Sautter C. (2004). The nutritional fortification of cereals. *Journal of Current Opinions in Biotechnology*, **15:**1-4.

Pollak L.M., Scott M.P. and Duvick S.A. (2011). Resistant starch and starch thermal characteristics in exotic corn lines grown in temperate and tropical environments. *Journal of Cereal Chemists*, **88:**435-440.

Rastija M., Jurica Jovic., Dario Iljkic., Vlado Kovacevic and Domagoj R. (2014). Response of winter wheat to ameliorative phosphorus fertilization. *49th Croatian and 9th International Symposium on Agriculture*, Dubrovnik Croatia, 412-415.

Reddy N.R. (2002). Occurrence, distribution, content and dietary intake of phytate. In: Reddy, N.R. and Sathe S.K. (Eds). *Food phytates*. CRC Press, Boca Raton Florida, 25-51.

Rohlfing K.A., Pollak L.M. and White P.J. (2010). Exotic corn lines with increased resistant starch and impact on starch thermal characteristics. *Journal of Cereal Chemists*, **87:**190-193.

Schrezenmeir J. and de Vrese M. (2001). Probiotics, prebiotics and synbiotics: Approaching a definition. *American Journal of Clinical Nutrition*, **73:** 2361S-2364S.

Shrestha A.K., Lopez-Rubio A., Blazek J., Gilbert E.P. and Gidley M.J. (2010). Enzyme resistance and structural organization in extruded high amylose maize starch. *Journal of Carbohydrate Polymers*, **80:**699-710.

Singh S., Gupta A.K. and Kaur N. (2012). Influence of drought and sowing time on protein composition, anti-nutrients and mineral contents of wheat. *The Scientific World Journal Article* 485751. doi:10.1100/2012/485751.

Sondeep S., Anil K.G. and Narinder K. (2012). Influence of drought and sowing time on protein composition, anti-nutrients, and mineral contents of wheat. *The Scientific World Journal*, doi:10.1100/2012/485751.

Sperotto R.A., Ricachenevsky F.K., Waldow V.D. and Fett J.P. (2012). Iron biofortification in rice: it's a long way to the top. *Journal of Plant Science*, **190:**24-39.

Steadman K.J., Burgoon M.S., Lewis B.A., Edwardson S.E. and Obendorf R.L. (2001). Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. *Journal of Science Food and Agriculture* **81:**1094-1100.

Tomoko N., Suyoen K., Yusuke K., Michiko T., Hiromi N. and Naoko K. N. (2014). Enhanced levels of nicotineamine promote iron accumulation and tolerance to calcareous soil in soybean. *Journal of Bioscience, Biotechnology and Biochemistry*, **78(10):**1677-1684.

Uauy C., Distelfeld A., Fahima T., Blechl A., and Dubcovsky J. (2006). A NAC gene regulating senescence improves grain protein, zinc and iron content in wheat. *Journal of Science*, **314:**1298-1301.

Welch R.M. and Graham R.D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, **55:**353-364.

CHAPTER 4

ASSESMENT OF RHEOLOGICAL PROPERTIES OF WHOLE MEAL FLOUR

4.1 Introduction

Bread is an important staple food and is one of the most widely consumed bakery products. It is described as a fermented confectionary product produced mainly from wheat flour, water, yeast and salt by a series of rheological processes involving mixing, kneading, proofing, shaping and baking (Abdelghafor *et al.,* 2011; Banu *et al.,* 2012). Dough rheology is defined as the study of how dough deforms and flows when force is applied (Muhammad *et al.,* 2013). Dough rheology is carried out on flour and is the major factor that is used to evaluate the quality of wheat flour and helps determine the end use of different wheat varieties and also in their classification. The wheat kernel is made up of different parts namely germ, pericarp layers (outer and inner), seed coat, aleurone layer and starchy endosperm. The objective of milling is to separate the starchy endosperm from the kernel, and to ground it into flour. The aleurone layer, pericarp layer and seed coat form the bran. Rheological properties are mostly investigated on highly processed white flours devoid of the bran and the germ layers as compared to whole meal wheat flour.

The physicochemical and rheological properties of flour differ significantly among wheat varieties and they have far reaching effects on the end use quality of wheat (Stathopoulos *et al.,* 2008). The quality of the end product depends upon the quality of wheat grain and wheat suitable for one particular use may have certain properties that are totally unsatisfactory for other uses (Anjum *et al.,* 2008). Wheat flours from various classes and cultivars display great diversity in their functional properties especially the resistance to elasticity, resistance to extensibility, water absorption and dough stability. These variations in functional properties of wheat cultivars are attributed largely to their gluten quality and quantity (Rao *et al.*, 2000).

The main rheological properties are evaluated mainly with the with Farinograph and Chopin alveograph machines. The Farinograph machine evaluates water absorption, mixing tolerance index, dough stability and Dough Development Time (DDT). In this test, water hydrates the flour components to the desired level to form dough of maximum consistency which is neither tough nor watery (Fu *et al.,* 2008). Stability is defined as the time taken from start of dough formation to the time when dough start weakening, while DDT is described as a measure of how long it takes for the given dough to attain maximum consistency before weakening of the dough

starts. The mixing tolerance index is described as a measure of the angle in degrees formed by the ascending and descending curves at the apex, located in the center of the curve (Singh *et al.*, 2003). The Chopin alveograph evaluates the resistance to elasticity and resistance to extensibility offered by wheat flour of varying quality. Evaluation of rheological properties of whole meal flour is crucial since the flour has substantial amounts of nutrients especially the micronutrients which are lost when the bran is removed (Kunkulberga *et al.*, 2007). In the process of milling white flour, many important nutrients including dietary fibres are lost, because these components are mainly located in bran and germ (Dewettinck *et al.*, 2008). This chapter therefore focused on evaluation of rheological properties of whole meal flour prepared from 9 wheat varieties grown in the three study sites described in section 3.2.1 and 3.2.2.

4.2 Materials and methods

4.2.1 Sample harvesting, preparation and milling

Wheat seeds for the nine bread wheat varieties were obtained from samples that were harvested, as described in section 3.2.3 and before analysis, they were cleaned using sieves of different mesh sizes to get rid of the broken grains, weeds, stones and every other chaff. After cleaning, one kilogram of each sample was placed in clean plastic jar awaiting milling. Milling of the samples was done using a Perten laboratory bench mill model 3100 to give rise to whole meal flour. The miller is designed in such a way that it grinds the wheat kernel without separating the starchy endosperm from the embryo and testa which gives high flour extraction rates of above 80%. After each sample had been milled, the machine was blown using compressed air to remove any remaining sample to avoid cross contamination.

4.2.2 Proximate composition of whole meal flour

The proximate flour composition was determined using the Near Infrared Spectrophotometer (NIR). About 5 grams of flour was packed in a flour cuvette and placed in the NIR measuring cell for analysis of flour protein, moisture, gluten and ash levels. A beam of light from the machine was then passed through the sample with a certain fraction absorbed by the sample depending on the parameter to be tested and the quantity of the parameter of interest. Three samples were analysed per parameter and each parameter was read three times and the average taken.

4.2.3 Water absorption of flour

The water absorption of the flour was determined based on the procedure adapted from method 54-21.02, AMAACCI (1999) 11th edition. A flour sample on a basis of 14% moisture was weighed and placed into a mixing bowl. Warm water (at 30° C) from a burette was then added to the flour and mixed to form dough. As the dough was mixed the Farinograph recorded the curve on a graph paper. The amount of water added affected the position of the curve on the paper with less water increasing dough consistency and moving the curve upward. From the graph obtained, results for dough development time, mixing tolerance index and stability were determined and analysed.

4.2.4 Dough strength and extensibility

Dough strength and extensibility was determined using a procedure adapted from method 54-30.02, AMAACCI (1999) 11th edition. The dough strength of each flour sample was determined using the Chopin-Alveograph machine Model number 1484. Saline water at room temperature, at a concentration of 12.5% was added to 60g of whole meal flour, mixed for eight minutes then rolled into a flat sheet of dough. The flat sheet of dough was cut into three circular pieces of dough and incubated at 25^{0}C for 35 minutes. The pieces of dough were then blown using air pressure until it burst. The force required for blowing and breaking the bubble of dough was recorded in a graph and from the graph, dough strength, area under the curve, resistance to elasticity (P), resistance to extensibility (L) and the ratio of resistance to elasticity (P) to resistance to extensibility (L) were determined.

4.2.5 Bread weight and volume

Whole meal bread was baked using the straight dough method adapted from method 10-09.01, AACCI (1999) 11th edition. The ingredients used were 100g whole meal wheat flour, 3g shortening, 3g milk powder, 0.03g malt, 4g sugar, 2g salt, 2g yeast and water based on the results obtained using the Farinograph machine. After baking whole meal bread weight was determined and after cooling, the bread volume was determined by the rapeseed displacement method adapted from method 10-05.01 AACCI (1999) 11th edition. In this method, a dummy loaf of 400cc is used to calibrate the machine using rapeseeds and the amount of displaced seeds is equivalent to the loaf volume. The test loaf is then placed on top of the dummy loaf and the amount of displaced seeds is determined as the volume of the loaf.

4.3 Data analysis

Values obtained for proximate composition, rheological properties, bread volume and weight were subjected to ANOVA analysis ($p<0.05$) using SAS software and the means separated by LSD ($p<0.05$). The means were further analysed using MINITAB software as described in section 3.2.7.

4.4 Results

4.4.1 Proximate composition of whole meal flour.

The protein and gluten composition for whole meal flour was significantly ($p<0.05$) influenced by variety and interaction of variety × cropping site (Appendix 7 and 8). The highest protein and gluten levels were recorded in crop grown in Naivasha, particularly K.Sunbird K.Korongo and K.Kingbird, while low levels were obtained for crop grown in Mau-Narok except for the varieties Robin and the control Njoro BWII (Table 4.1). Varieties grown in Eldoret recorded mixed results with some varieties having high protein levels but low gluten levels as opposed to varieties grown in Mau-Narok and Naivasha sites, whose protein and gluten were highly correlated i.e. high protein and gluten levels (Table 4.1). Overall, the control Njoro BWII and Robin varieties recorded high protein and gluten levels while lower protein and gluten levels were recorded in K.Hawk12 (Table 4.1).

Table 4.1: Protein and gluten concentrations in whole meal flour from Kenyan bread wheat varieties grown in Eldoret, Mau-Narok and Naivasha experimental sites.

	Protein (%)			Gluten (%)		
Varieties	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha
Robin	14.03^{k}	13.90^{k}	13.57^{i}	19.47^{i}	19.60^{i}	20.83^{k}
Eagle 10	13.73^{j}	10.37^{a}	13.97^{k}	18.87^{h}	11.27^{c}	20.43^{j}
K.Tai	13.43^{hi}	11.80^{d}	13.37^{h}	8.97^{b}	14.97^{e}	21.57^{l}
K.Sunbird	13.47^{hi}	10.83^{b}	14.27^{l}	15.53^{f}	14.73^{e}	23.07^{n}
K.Wren	13.37^{h}	10.87^{b}	13.73^{j}	13.70^{d}	11.20^{c}	20.30^{j}
K.Korongo	12.87^{g}	11.43^{c}	14.53^{m}	22.23^{m}	7.80^{a}	23.40^{n}
K.Hawk12	12.13^{e}	10.27^{a}	13.47^{hi}	15.03^{e}	11.50^{c}	19.53^{i}
K.Kingbird	12.50^{f}	11.47^{c}	14.20^{l}	21.03^{k}	17.70^{g}	24.23^{o}
Njoro BWII	14.03^{k}	14.03^{k}	14.03^{k}	19.47^{i}	19.47^{i}	19.47^{i}

For each parameter (protein or gluten), the means followed by same letter are not significantly ($p<0.05$) different as revealed by Fisher LSD test.

The moisture content for whole meal flour was influenced significantly ($p<0.05$) by variety, variety × cropping site (Appendix 9). The values for moisture and ash levels ranged from 10.33 to 12.87 and 1.13 to 1.33%, respectively (Table 4.2). The control variety Njoro BWII recorded overall the highest moisture content and lowest ash content while, K.Wren and K.Tai recorded lowest moisture content except for samples from Naivasha (Table 4.2).

Table 4.2: Ash and moisture content of whole meal flour from Kenyan bread wheat varieties grown in Eldoret, Mau-Narok and Naivasha.

	Moisture (%)			Ash (%)		
Varieties	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha
Robin	12.87^{k}	12.77^{jk}	12.73^{jk}	1.23^{ab}	1.17^{a}	1.23^{ab}
Eagle 10	11.93^{ef}	12.00^{efg}	12.80^{k}	1.23^{ab}	1.23^{a}	1.17^{ab}
K.Tai	11.20^{c}	11.90^{e}	12.77^{jk}	1.27^{a}	1.27^{a}	1.27^{a}
K.Sunbird	12.43^{i}	11.37^{d}	12.77^{jk}	1.33^{a}	1.27^{a}	1.23^{ab}
K.Wren	11.90^{e}	11.03^{b}	12.63^{j}	1.13^{b}	1.27^{a}	1.27^{a}
K.Korongo	12.23^{h}	10.33^{a}	12.80^{k}	1.27^{a}	1.17^{a}	1.13^{b}
K.Hawk12	12.13^{gh}	11.43^{d}	12.77^{jk}	1.33^{a}	1.23^{a}	1.17^{ab}
K.Kingbird	12.07^{fg}	11.87^{e}	12.63^{j}	1.27^{a}	1.23^{a}	1.27^{a}
Njoro BWII	12.87^{k}	12.87^{k}	12.87^{k}	1.13^{b}	1.17^{a}	1.17^{ab}

Means followed by same letter for each parameter investigated are not significantly ($p<0.05$) different as revealed by Fisher LSD test.

4.4.2 Determination of Water Absorption, tolerance, Dough Development Time and stability of whole meal flour

The Water Absorption (WA), Mixing Tolerance Index (MTI), Dough Development Time (DDT) and stability were significantly ($p<0.05$) influenced by site, variety and variety × cropping site (Appendix 10, 11, 12 and 13). The crop grown in Naivasha recorded high WA results compared to Eldoret and Mau-Narok, with varieties Robin, Eagle 10 and K.Kingbird recording highest WA across the three study sites whereas K.Wren and K.Hawk12 recorded lowest levels (Fig. 4.1a). However for MTI, the crop grown in Naivasha recorded higher values whereas lowest MTI values were recorded for crop grown in Eldoret (Fig. 4.1b).

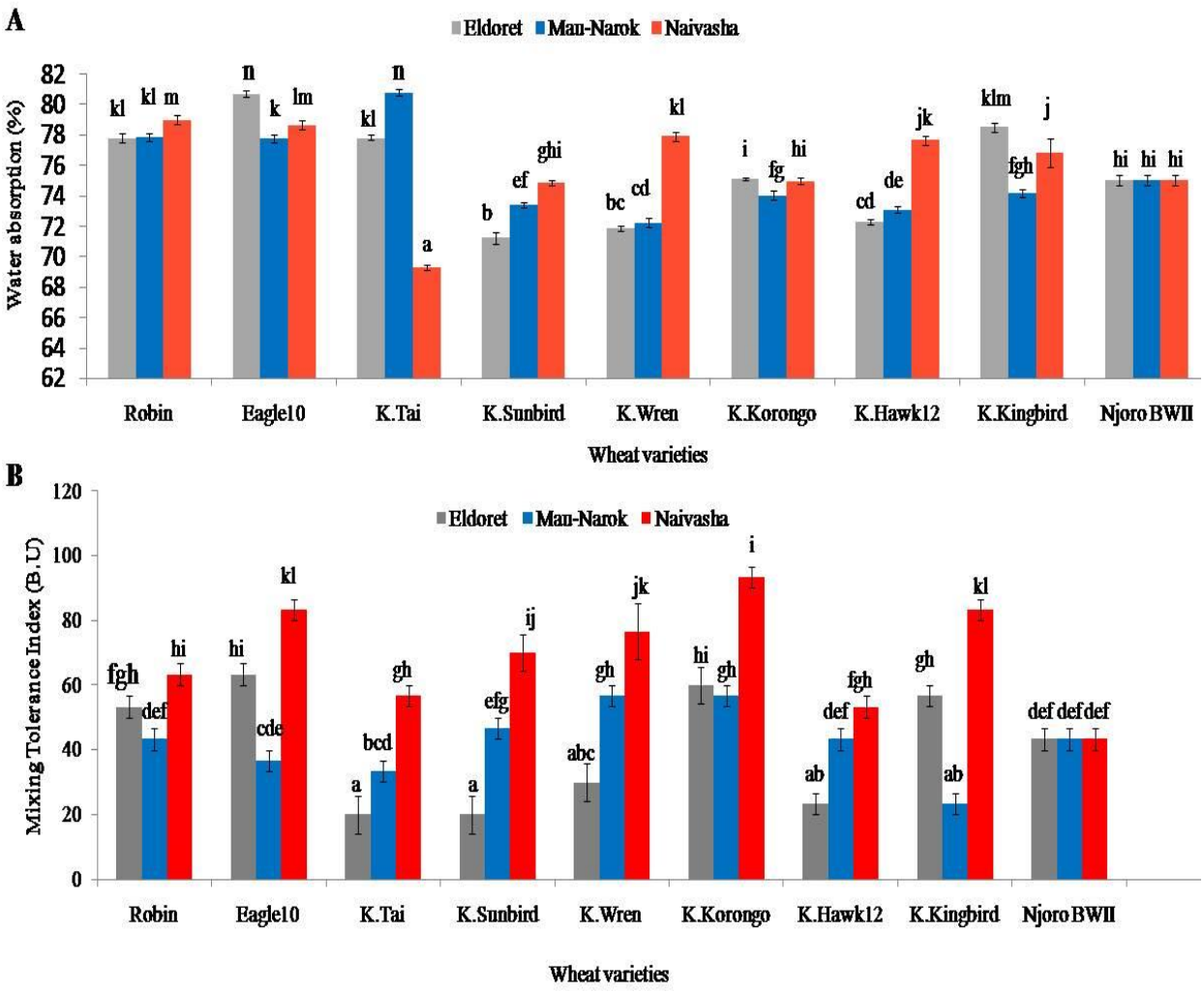

Figure 4.1: Rheological properties of whole meal flour. **A-** Water absorption for whole meal flour from Kenyan bread wheat varieties. **B-** MTI for whole meal flour of Kenyan wheat varieties grown in Eldoret, Mau-Narok and Naivasha.

DDT results varied across the 3 study sites irrespective of variety grown though varieties Robin, the control Njoro BWII and K.Korongo recorded almost the same values (Fig. 4.2a). Exceptionally high DDT was recorded for varieties grown in Eldoret especially for K.Tai and K.Sunbird, while low DDT was recorded for crop grown in Mau-Narok except in K.Kingbird (Fig. 4.2a). Analysis of dough stability revealed that K.Tai, K.Hawk12 and the control Njoro BWII recorded almost the same value while the rest posted varied results (Fig. 4.2b). Remarkably high

values were recorded in Robin, Eagle 10 and K.Wren when grown in Mau-Narok and in K.Sunbird for Eldoret site (Fig. 4.2b).

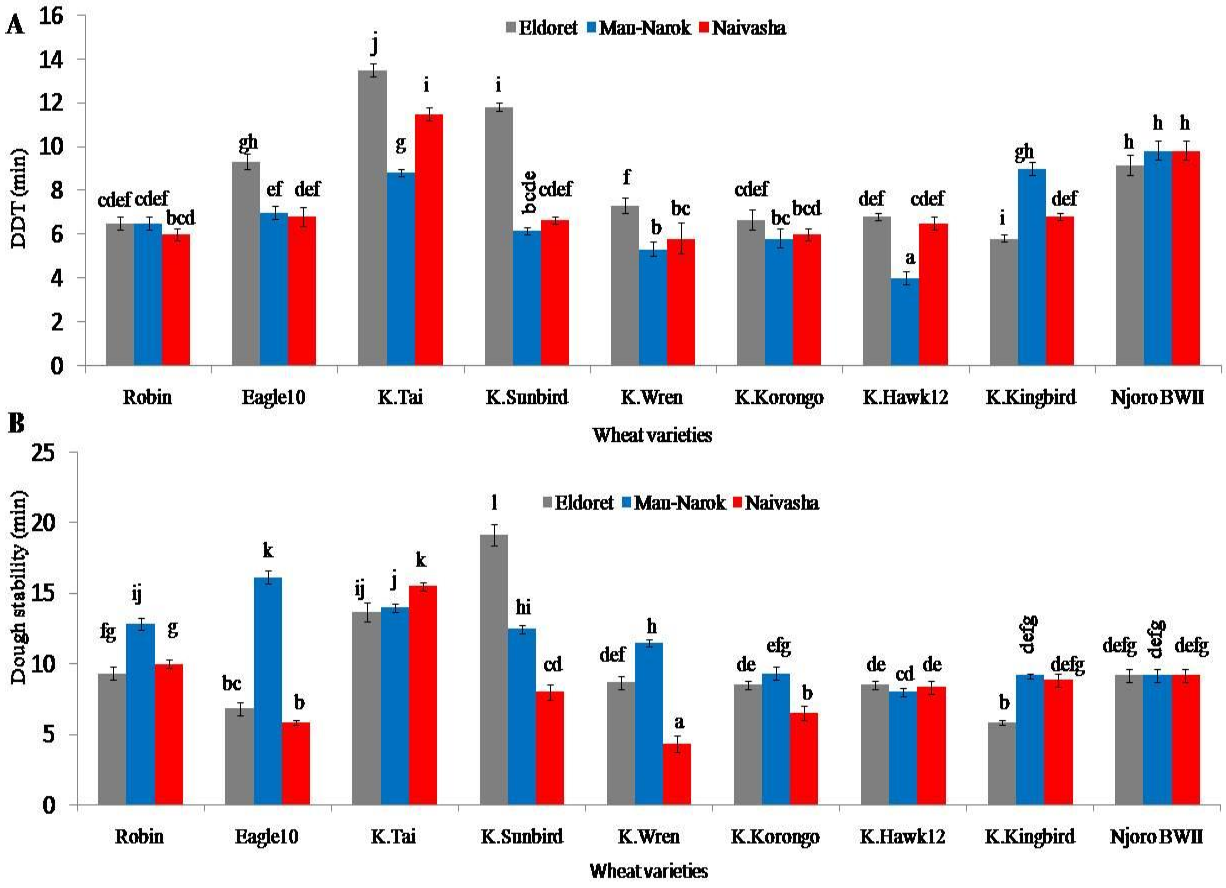

Figure 4.2: Rheological properties of whole meal flour. **A-** DDT of whole meal flour. **B-** Stability of whole meal flour of Kenyan wheat varieties grown in Eldoret, Mau-Narok and Naivasha.

4.4.3 Assessment of resistance to elasticity (P), resistance to extensibility (L) and P/L ratio of whole meal flour

ANOVA results for resistance to extension (L), resistance to elasticity (P) and P/L ratio were significantly ($p<0.05$) influenced by variety, variety × cropping site (Appendix 14). In Eldoret and Mau-Narok, varieties K.Tai, K.Hawk12 and K.Sunbird recorded the highest L values while K.Korongo and Robin recorded the lowest values in the same sites. P values for varieties Eagle 10, K.Sunbird and K.Sunbird were exceptionally high when the crop was grown in Mau-Narok and low for K.Wren and K.Korongo when crop was grown in Eldoret (Table 4.3). Results

for P/L ratio were higher for varieties grown in Naivasha compared to the other 2 study sites with K.Tai and Eagle10 posting noticeably higher values than other varieties in Naivasha and Mau-Narok respectively (Table 4.3).

Table 4.3: Resistance to elasticity (P), resistance to extensibility (L) and P/L ratio of whole meal flour from Kenyan bread wheat varieties grown in Eldoret, Mau-Narok and Naivasha.

	Length (L)			Height (P)			P/L ratio		
Variety	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha
Robin	29.67^{bc}	18.67^{g}	39.67^{a}	45.33^{b}	40.00^{e}	42.33^{c}	1.53^{bc}	2.15^{b}	1.07^{g}
Eagle 10	24.67^{d}	30.00^{d}	16.00^{e}	42.00^{c}	79.33^{a}	39.00^{d}	1.71^{ab}	2.65^{a}	2.44^{b}
K.Tai	43.33^{a}	51.00^{a}	22.67^{d}	37.00^{d}	55.33^{d}	66.67^{a}	0.86^{e}	1.08	2.95^{a}
K.Sunbird	30.00^{bc}	42.33^{b}	22.67^{d}	41.00^{c}	66.67^{c}	49.00^{b}	1.37^{c}	1.58^{c}	2.23^{d}
K.Wren	31.33^{b}	26.67^{e}	21.00^{d}	23.33^{f}	23.67^{g}	27.67^{e}	0.75^{e}	0.89^{f}	1.33^{f}
K.Korongo	19.67^{e}	20.33^{fg}	21.33^{d}	21.67^{f}	26.67^{g}	48.67^{b}	1.11^{d}	1.32^{d}	2.29^{cd}
K.Hawk12	43.00^{a}	22.00^{f}	21.33^{d}	29.33^{e}	57.67^{d}	51.33^{b}	0.68^{e}	2.63^{a}	2.41^{bc}
K.Kingbird	28.67^{bc}	34.33^{c}	33.00^{b}	51.00^{a}	70.67^{b}	41.67^{dc}	1.78^{a}	2.06^{b}	1.26^{f}
Njoro BWII	28.50^{c}	29.00^{de}	28.50^{c}	50.33^{a}	32.67^{f}	50.33^{b}	1.78^{a}	1.13^{e}	1.78^{e}

Means in the same column for each parameter followed by same letter are not significantly ($p<0.05$) different as revealed by Fisher LSD test.

4.4.4 Assessment of whole meal bread weight and volume from Kenyan wheat varieties

Analysis of whole meal bread weight and volume was significantly ($p<0.05$) influenced by variety, site and variety × cropping site (Appendix 15). Bread from varieties grown in Eldoret had more weight and volume compared to those baked from crop grown in Mau-Narok (Fig. 4.3a & b). Comparison of bread weight results across the 3 sites showed that those baked from varieties grown in Eldoret recorded higher weights except for K.Tai and the control Njoro BWII, while for samples grown in Mau-Narok and Naivasha, K.Tai and K.Wren respectively recorded more weight compared to the same varieties in other sites (Fig. 4.3a). Comparison of bread volume results across the 3 sites showed that those baked from samples grown in Mau-Narok and Naivasha recorded almost the same volumes which were lower compared to volumes for the same varieties when grown in Eldoret (Fig. 4.3b).

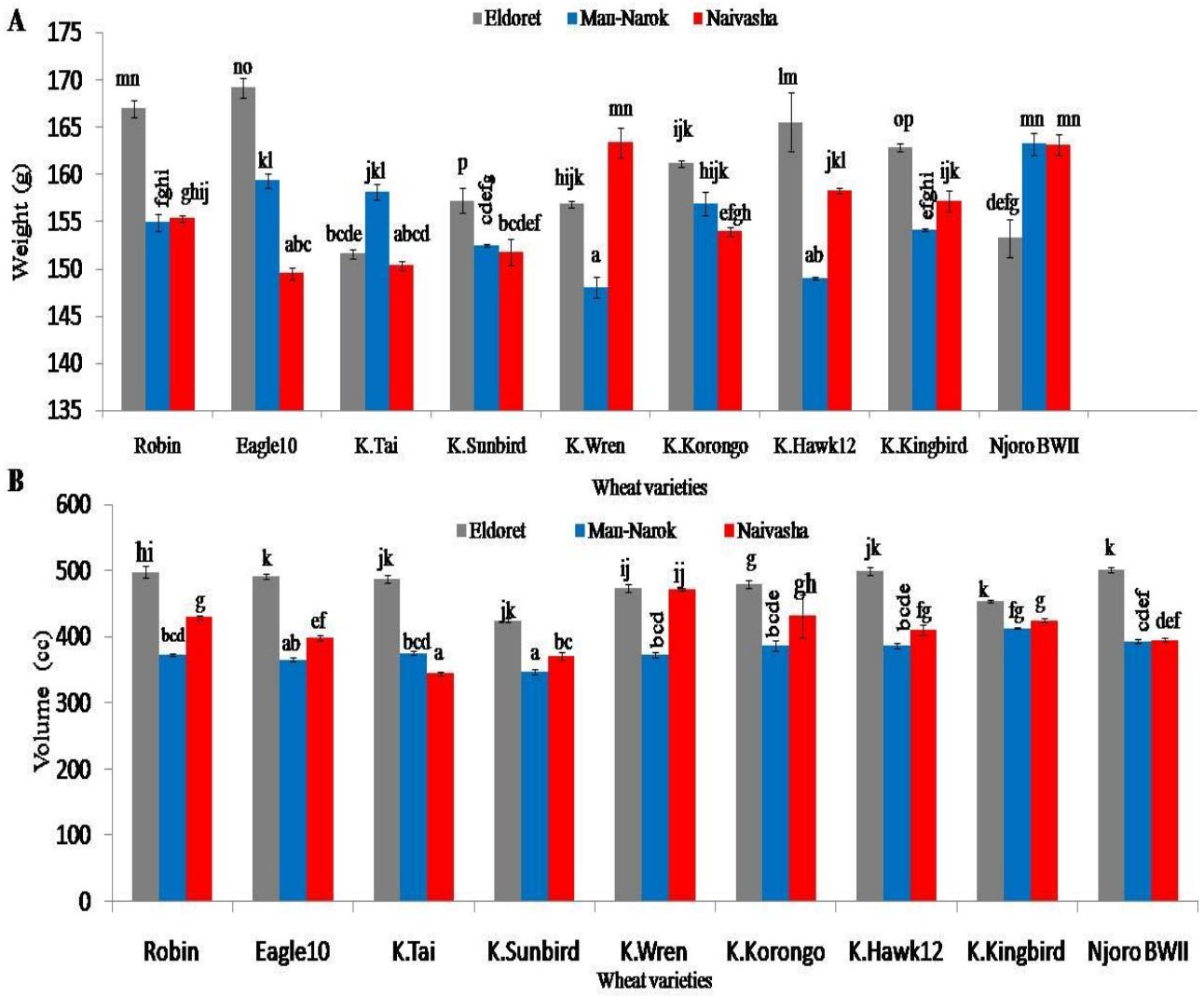

Figure 4.3: Properties of whole meal bread. **A-** Weight of whole meal bread of Kenyan wheat varieties. **B-** Volume of whole meal bread from Kenyan wheat varieties grown in Eldoret, Mau-Narok and Naivasha.

4.4.5 Overall analysis of rheological properties.

Rheological properties clustering revealed that the 9 wheat varieties were grouped into three distinct groups i.e. A, B and C (Fig. 4.4). Robin and Njoro BWII formed cluster A while K.Wren and K.Korongo formed cluster B both at a similarity level of about 62%. The third distinct cluster C was comprised of K.Tai and K.Sunbird at a similarity level of about 58%. In all the three sites, water absorption (WA) was high while the resistance to elasticity and extensibility was highly reduced and was most probably due to presence of bran particles.

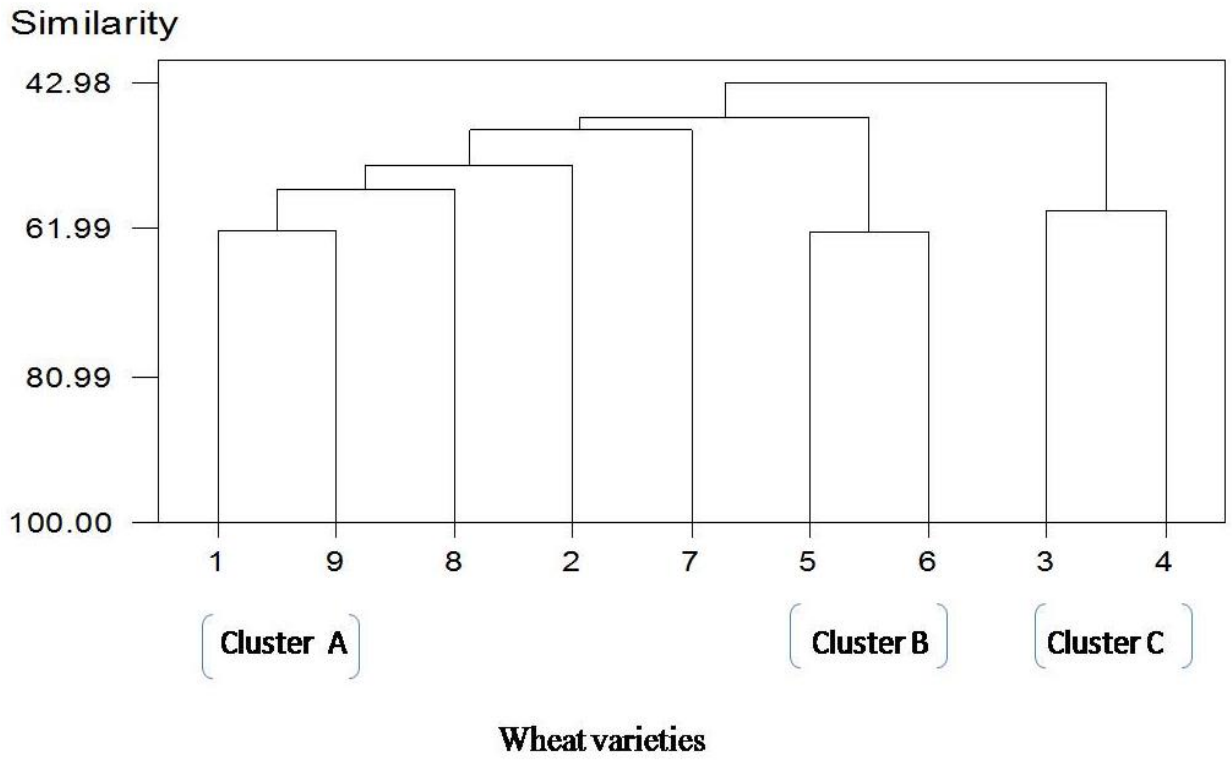

Figure 4.4: Dendogram showing differential clustering of Kenyan wheat varieties based on their rheological properties. Legend 1-9 represents variety 1 to variety 9. 1- Robin, 2- Eagle 10, 3-K.Tai, 4- K.Sunbird, 5- K.Wren, 6- K.Korongo, 7- K.Hawk12, 8- K.Kingbird and 9- Njoro BWII.

4.5 Discussions

4.5.1 Variation in proximate composition of wheat grain.

Protein and gluten levels are highly influenced by the genotype, environment and interaction between genotype × environment (Williams *et al*., 2008). Gluten is highly correlated with protein levels where high protein levels usually indicate high gluten levels and thus factors affecting protein levels have a direct influence on gluten levels (Zhao *et al*., 2009). Wheat varieties grown in dry areas are reported to have high protein levels of up to 15% compared to varieties grown in cooler temperatures (Saleem *et al*., 2015). Results obtained for protein and gluten levels show that varieties grown in Naivasha generally recorded higher protein levels compared to the same varieties in Mau-Narok and Eldoret; this is in line with the report by Saleem, *et al.* (2015).

Naivasha is an area that generally experiences higher temperatures and low rainfall compared to the Eldoret and Mau-Narok. In a study conducted by Mladenov *et al*., (2001), the effects of interaction between genotype × environment on protein levels were significant though in most cases much lower compared to the effects of genotype or environment. In this study, the results obtained indicated that genotypes with higher protein levels maintained the same across the 3 study sites. Similarly those with lower levels followed a similar trend, although environmental effect was clearly evident especially for Naivasha and Mau-Narok which recorded the highest and lowest protein levels, respectively. The genotype effect on grain protein levels was evident in the control Njoro BWII and Robin which recorded high levels, while K.Hawk12 recorded lower levels; irrespective of site the crop was grown. Genotypic effect on gluten levels was evident in the control Njoro BWII, K.Kingbird and Robin varieties which recorded higher levels, while K.Sunbird, K.Wren and K.Hawk12 recorded lower protein levels across the 3 study sites.

Proximate composition of wheat grains especially protein and gluten may be affected by yield of the genotype (John and Olson, 2012). In their report, the yield of wheat grown in dry areas is usually low hence their composition is usually concentrated in the few grains available leading to higher protein levels. From the results obtained, very high yielding varieties such as K.Hawk12 and K.Korongo tended to have lower protein levels in the kernel, whereas low yielding varieties like K.Kingbird had higher protein levels which may be associated to dilution effect of nitrogen. Nitrogen component is a major constituent of amino acids thus influences levels of proteins. High uptake of nitrogen by low yielding varieties ensures that it is channeled to protein synthesis in the few grains available leading to high protein concentration while in high yielding varieties, it will be distributed evenly leading to reduced protein content per grain (Triboi *et al*., 2006).

High moisture levels provide a suitable environment for microorganisms which act on the flour causing it to go bad. Moisture levels of above 14.5% attract mould, bacteria and insects and cause deterioration of flour samples (Kalnina *et al*., 2015). Moisture levels recorded for the samples across the three study sites were within a safe range though the crop grown in Naivasha recorded slightly higher levels than in the other two sites. Moisture content on a 14% basis has been used as a conversion factor for rheological tests in which the results are affected by moisture and is also a measure of profitability in the milling industry and should not exceed 15% (Keran *et al*., 2009).

Ash levels define the thoroughness of bran separation from the starchy endosperm and also from the germ. According to a study conducted by Craig *et al*., (2009) ash levels in wheat though not useful in baking, they can be used as an indicator of mill efficiency and also predict levels of microelements in the kernel. Results obtained from the 3 sites show fairly constant ash levels indicating that for all the varieties contained high levels of micro elements.

4.5.2 Rheological properties of whole meal flour

Wholemeal flour absorbs high amounts of water for them to be hydrated to the desired level for baking. Water absorption for whole meal flour increases with the amount of whole meal flour added (Kalnina *et al*., 2015). WA for the varieties was enhanced irrespective of site with highest level being 80% while the lowest was at 69%. The high WA level observed in whole meal flour is usually as a result of the inclusion of a higher amount of bran which contains increased levels of pentosans and these pentosans require more water for them to be hydrated (Sanz-Penella *et al*., 2008).

From results obtained for DDT, varieties with high protein levels and high quality gluten recorded high DDT values while low protein varieties recorded low values. This is because dough with low protein levels and low gluten cannot withstand prolonged mixing and kneading as opposed to those with high protein and gluten levels. On closer inspection, varieties which had high protein levels also recorded high DDT times. The presence of bran in whole meal flours, increases the level of proteins which require more mixing time for water molecules to hydrate the flour components to the desired and the required consistency level. While high DDT is one measure and indicator of strong good quality dough, moderate DDTs are much preferred since one is able to mix large volumes of dough within the given time and still get good quality end products.

Results obtained for dough stability show that Mau-Narok whole meal flour had the highest stability while flour from varieties grown in Naivasha generally recorded low stability times. Dough with more stability is highly preferred since it is able to withstand vigorous mechanical processes in the baking process without weakening and one still produces good quality products in the end. From a baking perspective, dough with very high stability require more mixing time, more mixing energy (high electrical cost) and leads to long production time and high cost of production, as such dough with moderate stability that will still give high quality products is much preferred.

MTI is measured by the position of the curve five minutes after the dough starts weakening and is a measure of the rate of dough weakening or softening and together with the other farinograph parameters is used in predicting the quality of end product to expect. Flours with MTI's values of ≤30 Brabender Units (BU) are preferred as they indicate flours that don't weaken easily. Flour from wheat crop grown in Naivasha recorded the highest MTI values while for the crop grown in Mau-Narok and Eldoret, moderate values were recorded. High levels of MTIs are associated with flours that weaken easily while low levels are associated with flours that are strong. In general, flours from Naivasha had the lowest stabilities and also highest MTI values indicating their weakness in terms of baking properties. Flours with high MTIs give low quality end products since they cannot withstand the harsh conditions in the baking process such as vigorous and thorough mixing and the high baking temperatures used.

4.5.3 Resistance to extensibility (L), resistance to elasticity (P) and P/L ratio

The height and length of the alveogram gives the measure of resistance to elasticity and resistance to extension and is an indicator of gluten quality. Gluten which is composed of glutenins and gliadins is a non-functional/storage protein in wheat and is highly responsible for dough formation, extensibility and elasticity (Belderok *et al*, 2000). Upon correct mixing, high quality gluten assumes the properties of an elastic material that is capable of stretching under pressure until its elastic limit is exceeded. The elastic limit is usually exceeded when the dough bubble ruptures. The major gluten component responsible for elasticity is the glutenin which is part of the seed storage protein and is water insoluble (Belderok *et al.*, 2000). However like any elastic material, the glutenin matrix also has its elastic limit which when exceeded, leads to dough rupture. Results obtained for P showed that overall, higher values across the 3 sites were recorded in K.Tai, K.Hawk12 and K.Kingbird, whereas K.wren and K.Korongo recorded lower values irrespective of site. This matrix when continuous gives rise to high P value but when interrupted, the values become low. Genotypes with high protein and good quality gluten have elastic doughs while some varieties with low protein and low quality of the glutenin component have low P values. Varieties with low quality glutenin, their elasticity for whole meal flour is disadvantaged due to presence of germ and bran particles which weaken the matrix quickly, and reduce the dough elastic limit thresh hold.

In whole meal flour, bran and germ particles integrate into the dough matrix disrupting the continuity of the gluten protein network especially the gliadin component which results in weaker and less firm dough (Manthey and Schorno, 2002). Koehler *et al*. (2010) in their study on temperature effect on gluten protein discovered that gliadin molecules reduce their stiffness and increase the extensibility of the gluten phase resulting in higher values of L. However in study reported herein, due to presence of differently sized bran and germ particles which interrupted the protein matrix, low L values were observed for all the varieties grown in the three locations.

P/L values give the ratio of resistance to elasticity to resistance to extensibility. Very high ratios indicate that the dough is too elastic while low values are an indicator of how extensible the dough is. For Naivasha and Mau-Narok crop, dough from all the varieties was highly elastic but not highly extensible, while Eldoret varieties were elastic and extensible almost to the same magnitude. As a result bread baked from these varieties would be compact and dense.

4.5.4 Whole meal bread volume and weight

Apart from Eldoret, the breads made from varieties grown in Mau-Narok and Naivasha had low volumes and high bread weights. Products formed from whole meal flour are usually denser compared to those from processed flours. This is because very little air spaces are able to integrate into the dough making it more compact and dense which leads to high bread weights. The volume of bread largely depends on how elastic the dough is and how much it extends. Whole meal breads have lower bread volumes since presence of bran and germ particles decrease and interfere with the quality of gluten rendering it less elastic and extensible.

4.5.5 Overall rheological properties

From the dendogram obtained on rheological properties for all the 9 varieties, Robin and the control Njoro BWII, K.Wren and K.Korongo, K.Tai and K.Sunbird formed 3 distinct clusters while the rest did not cluster with any other. Robin and Njoro BWII are among the highly preferred bread wheat varieties and due to their high protein and gluten characteristics. Their clustering confirms that they share most of the preferred rheological properties. K.Tai and K.Sunbird, and K.Wren and K.Korongo exhibit similar growth conditions, yields and maturity time, respectively. The similar growth characteristics impact almost the same effect on their grain proximate composition which has a direct influence on their rheological properties thus the clustering.

4.6 Conclusions

From the results obtained for the rheological properties of whole meal dough, it is evident that these properties are highly affected by the variety and site. Influence of site could most probably be due to differences in varietal proximate composition especially on protein and gluten levels which have a direct impact on the rheological properties. With this information on rheological properties, wheat breeders are in a better position to choose which varieties to recommend for industrial baking and also which varieties to improve. Information on site gives both farmers and breeders an option on which site will give them superior and preferred qualities which offer better baking properties.

4.7 References

American Association of Cereal Chemists International (AACCI) (1999). Approved Methods of Analysis, 11th edition. Method 54-21.02. Rheological behaviour of flour by Farinograph. Approved November 3rd. AACC International, St. Paul, MN, U.S.A

American Association of Cereal Chemists International (AACCI). Approved Methods of Analysis, 10th edition. Method 54-30.02. Alveograph method for soft and hard wheat flour. Approved November 3rd. AACC International, St. Paul, MN, U.S.A

American Association of Cereal Chemists International (AACCI) (1999). Approved Methods of Analysis, 11th Ed. Method 10-05.01. Measurement of volume by rapeseed displacement. Approved November 3rd. AACC International, St. Paul, MN, U.S.A

American Association of Cereal Chemists International (AACCI) (1999). Approved Methods of Analysis, 11th Ed. Method 10-09.01. Basic straight dough bread baking method- Long fermentation. Approved November 3rd. AACC International, St. Paul, MN, U.S.A.

Abdelghafor R.F., Mustapha A.I., Ibrahim A.M.H. and Krishnan P.G. (2011). Quality of bread from composite flour of sorghum and hard white winter wheat. *Advanced Journal of Food Science and Technology*, **3(1):**9-15.

Anjum F.M., Ahmad I., Butt M.S., Arshad M.U. and Pasha I. (2008). Improvement in end use quality of spring wheat varieties grown in different eras. *Journal of Food Chemistry*, **106(2):**482-486.

Banu I., Georgeta S., Violeta S.I. and Luliana A. (2012). Effect of the addition of wheat bran stream on dough rheology and bread quality. *Journal of Food Technology*, **36(1):**39-42.

Belderok B., Mesdag J., Donner D.A. (2000). Bread making quality of wheat: A century of breeding in Europe. Kluwer Academic Publisher: Dordrecht, the Netherlands, pp. 30–31

Craig F.M., Shuobi L., King G.E., Doug A.E., John W.B. and Andrew S.R. (2009). A comprehensive genotype and environment assessment of wheat grain ash content in Oregon and Washington: Analysis of variation. *Journal of cereal chemistry*, **86(3):**307-312.

Dewettinck K., Van Bockstaele F., Kuhne B., Van de Walle D., Courtens T.M. and Gellynck X. (2008). Nutritional value of bread: influence of processing, food interaction and consumer perception. *Journal of Cereal Science,* **48:**243–257.

Fu L., Tian J., Sun C., and Li C. (2008). RVA and farinograph properties study on blends of resistant starch and wheat flour. *Journal of Agricultural Science of China,* **7(7):**812-822.

Jones, C. and Olson R.K. (2012). Practices to increase wheat grain protein. Montana State University Extension. EBO206. Http: // landresources.montana.edu/soil fertility/ PDF by format/ publication.

Kalnina S., Rakcejeva T., Kunkulberga D. and Galoburda R. (2015). Rheological properties of whole wheat and whole triticale flour blends for pasta production. *Journal of Agronomy Research* **13(4):**948-955.

Keran H., Salkic M., Odobasic A., Jasic M., Ahmetovic N. and Sestan I. (2009). The importance of determination of some physical-chemical properties of wheat and flour. *Journal of Agricultural Conspectus Science,* **74(3):**197-200.

Koehler P., Kieffer R. and Wieser H. (2010). Effect of hydrostatic pressure and temperature on the chemical and functional properties of wheat gluten III: Studies on gluten films. *Journal of Cereal Science,* **51**: 140-145.

Kunkulberga D., Klava D., Ruza A., Zirvite I. and Riekstina A. (2007). Development of whole grain products. In**:** Proceedings of 4 International congresses "Flour-Bread'07". *6th Croatian congress of cereal technologists, Croatia,* p. 300-305.

Manthey F.A. and Schorno A.L. (2002) Physical and cooking quality of spaghetti made from whole wheat durum. *Journal* of *Cereal Chemistry,* **79**:504-510.

Mladenov N., Przulj N., Hristov N., Djuric V and Milovanovic M. (2001). Cultivar by environment interactions for wheat quality traits in semi-arid conditions. *Journal of Cereal Chemistry*, ***78*(3):**363-367.

Muhammad R.A., Aamir S., Shahzad H., Muhammad A.S., Moaazam R.K. and Muhammad S. (2013). A comprehensive review on wheat flour dough rheology. *Pakistan Journal of Food Science,* 23(2): 105-123.

Rao V.K., Mulvaney S.J. and Dexter J.E. (2000). Rheological characterisation of long and short mixing flours based on stress-relaxation. *Journal of Cereal Science*, **31(2):**159-171.

Saleem N., Ahmad M., Wani S.A.., Vashnavi R. and Dar Z.A. (2015). Genotype environment interaction and stability analysis in Wheat (*Triticum aestivum L*.) for protein and gluten contents. *Journal of Scientific Research and Essays*, **10 (7):**260-265.

Sanz-Penella J.M., Collas C. and Haros M. (2008). Effect of wheat bran and enzyme addition on dough functional performance and phytic acid levels in bread. *Journal of Cereal Science*, **48**:715-721.

Singh N., Bajaj I.K., Singh R.P. and Gujral H.S. (2003). Effect of different additives on mixograph and breadmaking properties of Indian wheat flour. *Journal of Food Engineering*, **56(1):**89-95.

Stathopoulos C.E., Tsiami A.A., David S.J. and Dobraszczyk B.J. (2008). Effect of heat on rheology, surface hydrophobicity and molecular weight distribution of glutens extracted from flours with different bread-making quality. *Journal of Cereal Science*, **47(2):**134-143.

Triboi E., Martre P., Girousse C., Ravel C. and Triboi-Blondel A.M. (2006). Unraveling environmental and genetic relationships between grain yield and nitrogen concentration for wheat. *European Journal of Agronomy*, **25 (2):** 108-118.

Zhao C.X., He M.R., Wang Z.L., Wang Y.F. and Lin Q. (2009): Effects of different water availability at post anthesis stage on grain nutrition and quality in strong-gluten winter wheat. *Comptes Rendus Biologies*, **332**: 759–764.

CHAPTER FIVE

ORGANOLEPTIC PROPERTIES OF BREAD PREPARED USING WHOLEMEAL WHEAT FLOUR

5.1 Introduction

Wheat flour is the main raw material that is processed in different forms to be used in baking. Highly processed white flour that lacks bran is popular with bread making at commercial scale and for home baking. However, white flour has reduced nutrients and fibre since bran and germ have been removed. The current trends in nutrition encourage the consumption of healthy foods in terms of micronutrients and fibers obtained in our daily diets so as to alleviate deficiencies and gastro intestinal disorders being experienced today. Hence with this in mind, foods should not be evaluated only on their safety and nutritional value but also on its sensorial and its rheological value (Muler and Steinhart, 2007).

Whole meal bread is a good source of carbohydrates and micronutrients nutrients such as vitamins and minerals and it provides the simplest pleasures of daily living (Kent, 2002). The use of white flour derived from the processing of whole wheat grain, though aimed at improving the aesthetic value of white bread, has led to drastic reduction in the nutritional density and fibre content when compared to bread made from whole grain cereals (Maneju *et al*., 2011). The consumers' awareness of the need to eat healthy functional foods has led to the increase in consumption of wholemeal flour products (Ndife and Abbo, 2009).

When it comes to commercial production of bread, sensory evaluation is mostly relied to give feedback of consumer preference on the end product produced and its ranking when compared to others (Crina *et al*., 2012). The most analyzed features are usually taste, aroma, colour of crumb and crust, uniformity of bake, bread shape and general acceptability. The sensorial quality of food products plays an important role in the choice of food. Hedonic testing is often used to determine consumers' attitude towards the food by determining the degree of acceptance of a new product or improving the existing food product

This chapter therefore focuses on examining the organoleptic qualities of bread made from wholemeal flour, prepared from the 9 selected Kenyan wheat varieties grown in 3 Kenyan sites.

5.2 Materials and methods

5.2.1 Preparation of whole meal flour per sample.

Wheat samples (1Kg each) were obtained from the harvested batch described in section 3.2.3. The varieties were separately milled to give wholemeal wheat flour whose extraction rate for all samples was above 75%. Dough was prepared using wholemeal flour for the nine different varieties then subjected to baking and later organoleptic analysis was done on the baked breads.

5.2.2 Baking

Baking was done using the straight dough method, where a definite amount of sample flour (100 g) was weighed (determined by the flour moisture content), mixed with milk powder (3 g), sugar (4 g), malt and baker's yeast (1.5 g). The correct amount of water determined based on the farinograph water absorption data, as reported in section 4.4.2, was then added to each sample and mixed thoroughly. The resulting dough was then placed in a bread bowl and incubated in the fermentation unit for 105 minutes. After first rising, dough underwent first punching to reduce air bubbles and fermented for another 50 minutes. The dough underwent second punching and was placed in bread pan and placed again in the fermentation unit for 25 minutes. After the dough rose while in the bread pan it was baked at 205^0C for 25 minutes and its weight and volume determined after cooling.

5.2.3 Panelist training

During organoleptic analysis, various hedonic scales are used to give scores to each parameter and training for the participants is usually carried out prior to the analysis. Thirty evaluators composed of both male and female were randomly chosen and taken through a demonstration on the parameters that are mostly evaluated and how the scores are assigned. They were then given five different loaves of bread with differing characteristics for evaluation. The loaves were composed of whole bread for evaluating the external characteristics and a sliced loaf for internal and flavour evaluation. They were then given the sample score sheets (Appendix 16) for evaluation. After tasting each loaf, the evaluators were provided with clean drinking water to rinse off the taste of the previous sample before proceeding to the next.

5.2.4 Sensory evaluation

Sensory evaluation was done on the baked breads with the following parameters being examined: loaf symmetry, crust colour, aroma, taste and general acceptability. Evaluation was

done on a 5 point hedonic scale where 1=very bad, 2=bad 3=moderate 4=good and 5=very good as indicate in the score sheet (Appendix 17). The evaluation focused on external loaf characteristics mainly loaf shape and crust colour, flavor properties mainly taste and aroma; internal loaf characteristics mainly crumb colour and crumb softness and the general acceptability.

5.3 Data analysis

Results obtained for the organoleptic analysis were subjected to ANOVA analysis and means separated using LSD ($p<0.05$). The means obtained were further analysed using MINITAB software which clustered them into similarity groups. To evaluate the correlation between the nutritional quality and organoleptic properties, results for iron, zinc, phytic acid and resistant starch in chapter 3, were subjected to Pearson's correlation analysis together with the means for organoleptic properties.

5.4 Results

5.4.1 External loaf characteristics

Bread baked from whole meal flour produced loaves that differed in shape (Fig 5.1). Bread baked from wheat varieties with high quality gluten especially those grown in Eldoret and Mau-Narok, produced well shaped breads as opposed to those baked from varieties with low quality gluten such as those from Naivasha. Whole meal bread baked from Naivasha had lower scores for crust colour when compared to the scores for the loaves baked from varieties grown in Mau-Narok and Eldoret.

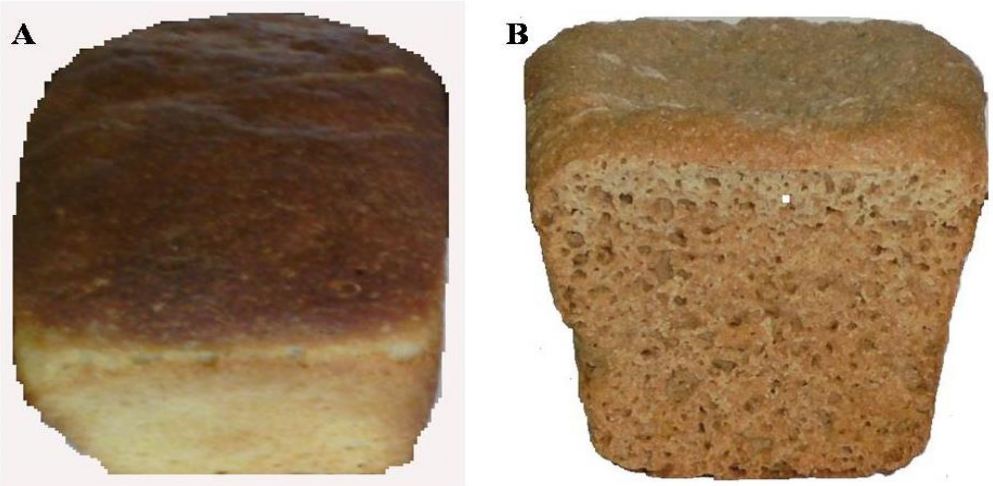

Figure 5.1: Breads baked using whole meal wheat flour. **A-** Well shaped bread baked from K.Kingbird variety grown in Eldoret. **B-** Bread from K.Kingbird with a collapsed crust due to low quality gluten characteristic of varieties grown in Naivasha.

Bread shape and crust colour scores, ranged between 2.2 to 4.3 and 2.80 to 4.10, respectively across the 3 study sites (Fig. 5.2a & 5.12b) and were significantly ($p<0.05$) influenced by variety, site and site × variety for bread shape (Appendix 17) and site for crust colour (Appendix 18). Bread baked from varieties grown in Eldoret had highest scores except for K.Wren and K.Korongo, while those from Mau-Narok recorded lowest scores except for the control Njoro BWII. Bread baked from varieties grown in Naivasha recorded moderate scores with the highest score obtained for K.Tai while K.Korongo recorded the lowest at 2.40 (Fig. 5.2a). Varietal comparison across the sites showed that the control Njoro BWII posted higher scores compared to the rest while K.Wren and K.Korongo posted lower scores. Analysis per site revealed that varieties grown in Eldoret had the most preferred loaves while Mau-Narok had the least preferred based on shape (Fig. 5.2a).

Analysis of whole meal bread crust colour revealed that all the varieties recorded desirable crust colour regardless of site. However exceptional values were recorded for K.Hawk12 and K.Kingbird for crop grown in Mau-Narok while the lowest score was recorded for bread baked using K.Wren flour for crop grown in Eldoret (Fig. 5.2b). Overall crusts of bread baked using flours from varieties grown in Naivasha and Mau-Narok scored higher values compared to those grown in Eldoret (Fig. 5.2b).

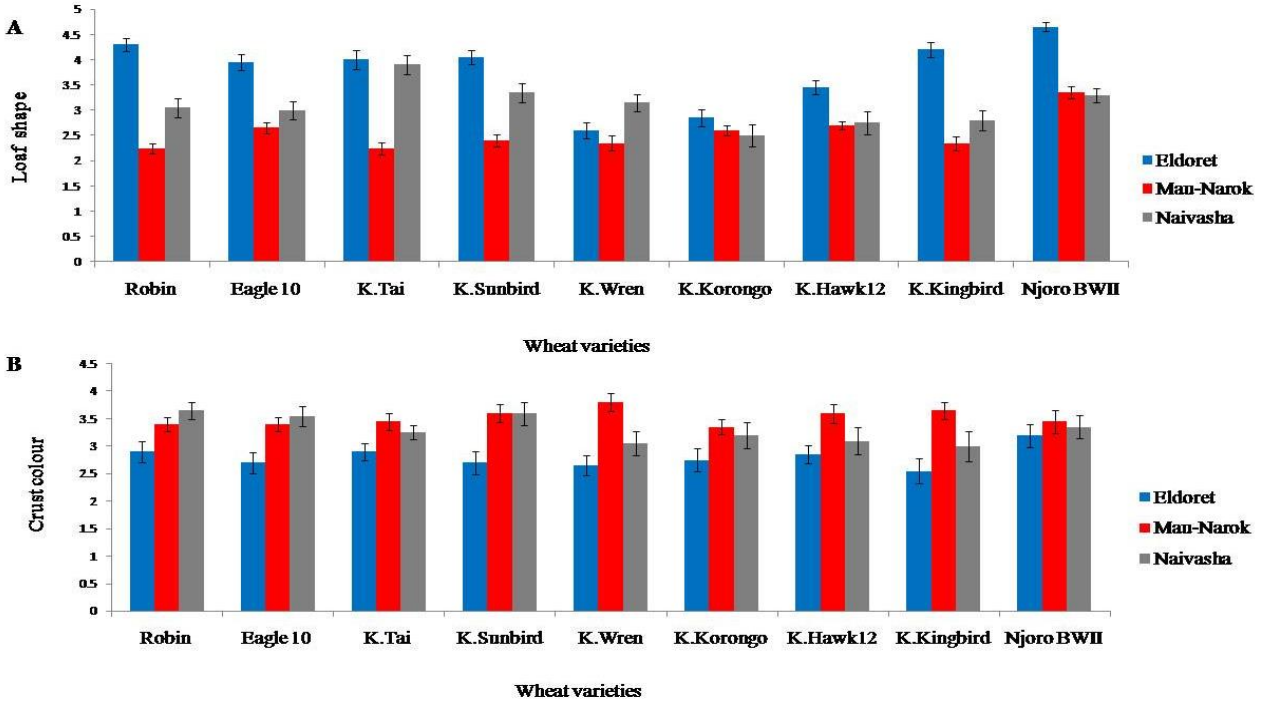

Figure 5.2: External whole meal loaf characteristic. **A-** Whole meal loaf shape **B-** Whole meal bread crust colour.

5.4.2 Internal loaf characteristics

Analysis of crumb colour for all the varieties grown in the three sites ranged between 2.75 to 4.00 (Fig. 5.3a) and were significantly ($p<0.05$) influenced by site (Appendix 19). Crumb colour for all the breads scored moderately (>3.00) except for K.Wren, K.Tai and K.Korongo grown in Eldoret, Mau-Narok and Naivasha, respectively (Fig 5.3a). Site comparison of crumb colour showed that loaves from varieties grown in Mau-Narok had the most preferred crumb colour and those in Naivasha had the least preferred crumb colour while varietal comparison showed that the control Njoro BWII had the most preferred crumb colour compared to the rest (Fig. 5.3a).

Results for analysis of crumb softness of bread showed no significant differences neither for the varieties or the sites (Fig. 5.3b). However, the control Njoro BWII recorded lower values especially in Mau-Narok and Naivasha while K.Wren recorded averagely higher values irrespective of site (Fig. 5.3b). Comparison of crumb softness across the 3 sites revealed that bread baked from varieties grown in Eldoret scored higher values compared to varieties grown in Mau-Narok and Naivasha (Fig. 5.3b).

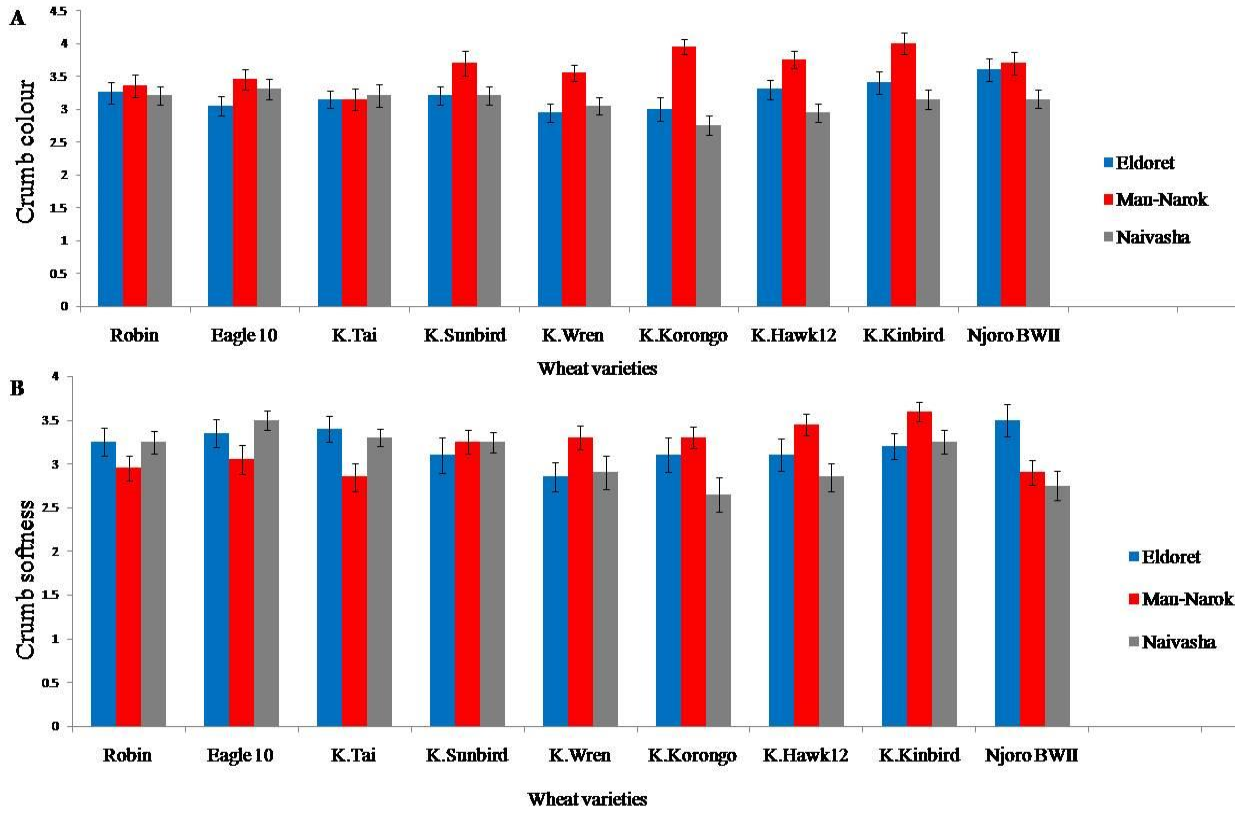

Figure 5.3: Internal loaf characteristics **A-**Whole meal bread crumb colour **B-** Whole meal bread crumb softness.

5.4.3 Flavor characteristics

Regardless of growing site, results for aroma and taste ranged between 2.55 to 3.80 and 2.15 to 3.05, respectively (Fig. 5.4) and results for aroma were significantly ($p<0.05$) determined by site (Appendix 20). Comparison of aroma results across the 3 sites indicated that bread baked from varieties grown in Mau-Narok and Naivasha scored high values compared to the same varieties when in Eldoret (Fig. 5.4a). The control Njoro BWII scored the highest value in Eldoret with a score of 3.20 while K.Kingbird scored the lowest in Eldoret and Naivasha respectively (Fig. 5.4a). Generally the control Njoro BWII recorded moderate results (>3.00) irrespective of site (Fig. 5.4a).

Results for taste analysis across the three sites were low though bread baked from varieties grown in Eldoret scored much higher while those from varieties grown in Mau-Narok scored the least (Fig. 5.4b). Bread baked from the control Njoro BWII when grown in Eldoret and K.Kingbird when grown in Mau-Narok recorded the highest values at 3.05 and 3.11, respectively (Fig. 5.4b).

Noticeably, Eagle 10 and K.Tai recorded very low scores for bread baked from varieties grown in Mau-Narok while in K.Wren scored the lowest both in Mau-Narok and Naivasha (Fig. 5.4b).

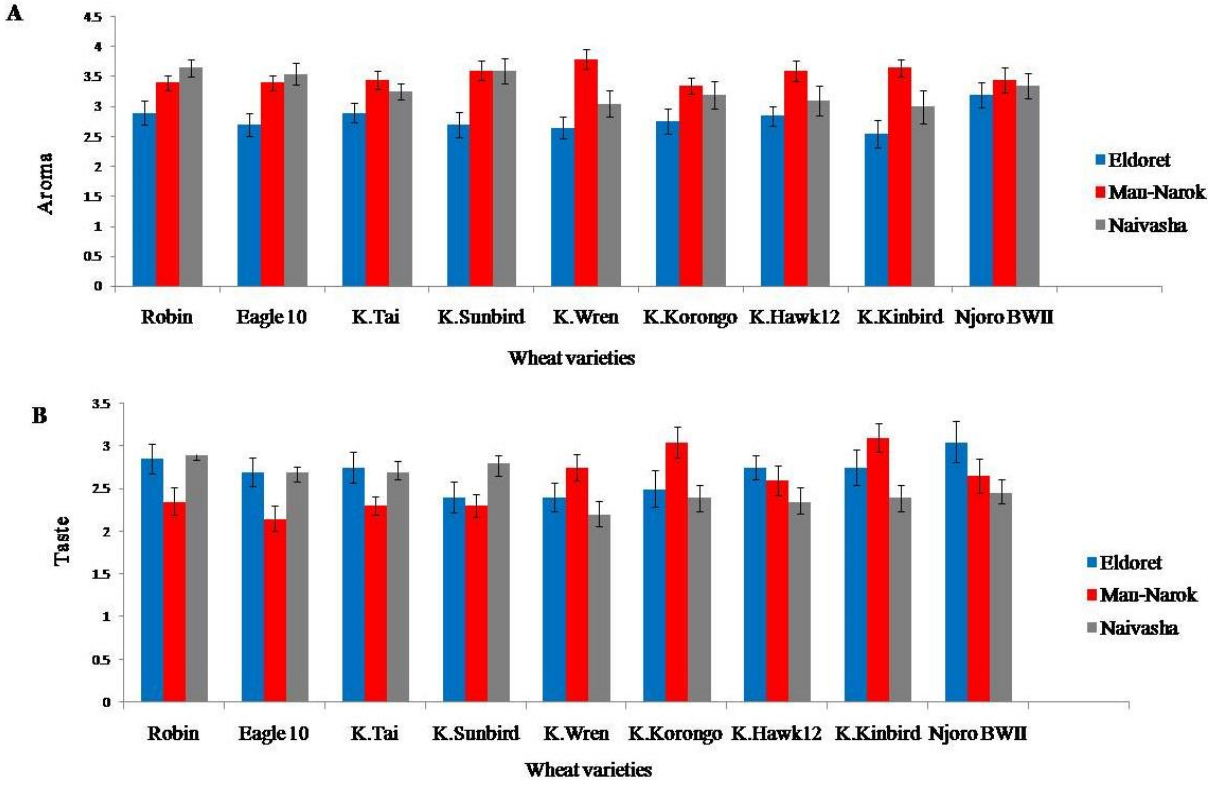

Figure 5.4: Whole meal bread flavor characteristics **A-** Aroma of whole meal bread **B-** Taste of whole meal bread.

5.4.4 Evaluation of general acceptability

Irrespective of growing site, analysis of scores obtained for general acceptability ranged from 2.55 to 3.5 (Fig. 5.5) and the results were significant ($p<0.05$) for site x variety (Appendix 21). Robin, Eagle 10 and K.Sunbird had little variations on general acceptability across the 3 study sites (Fig. 5.5). Acceptability of varieties grown in Eldoret was higher compared to the other two study sites. The control Njoro BWII recorded highest scores when it was grown in Eldoret and lowest when grown in Mau-Narok (Fig. 5.5).

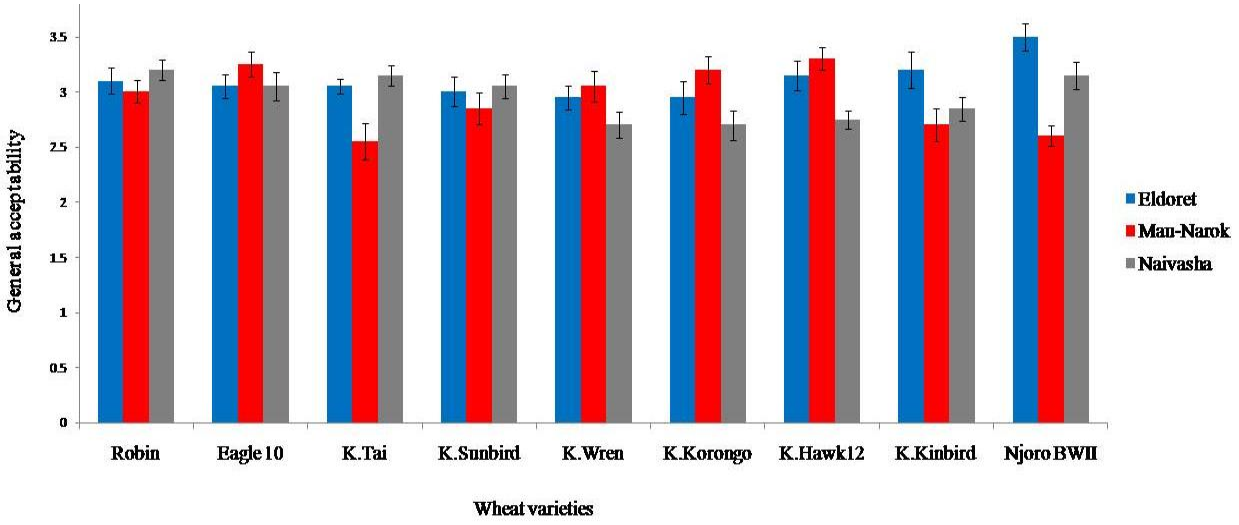

Figure 5.5: General acceptability of bread baked from whole meal flour from Kenyan wheat varieties grown in Eldoret, Mau-Narok and Naivasha.

5.4.5 Overall organoleptic analysis

Cluster analysis for organoleptic analysis using Minitab software from the means obtained during the study revealed that there were 3 distinct clusters namely A, B and C (Fig. 5.6). Robin, Eagle 10, K.Tai, K.Sunbird and K.Hawk12 formed cluster A while cluster B comprised of K.Wren and K.Korongo at a similarity level of about 60%. Cluster C was not clearly distinct and was comprised of K.Kingbird and Njoro BWII at a similarity level of 42%. The 1st cluster further alienated into two sub-clusters with the first comprising of Robin and K.Hawk12 at a similarity level of approximately 62% while the second sub-cluster at a similarity level of approximately 58% comprised of Eagle 10, K.Sunbird and K.Tai.

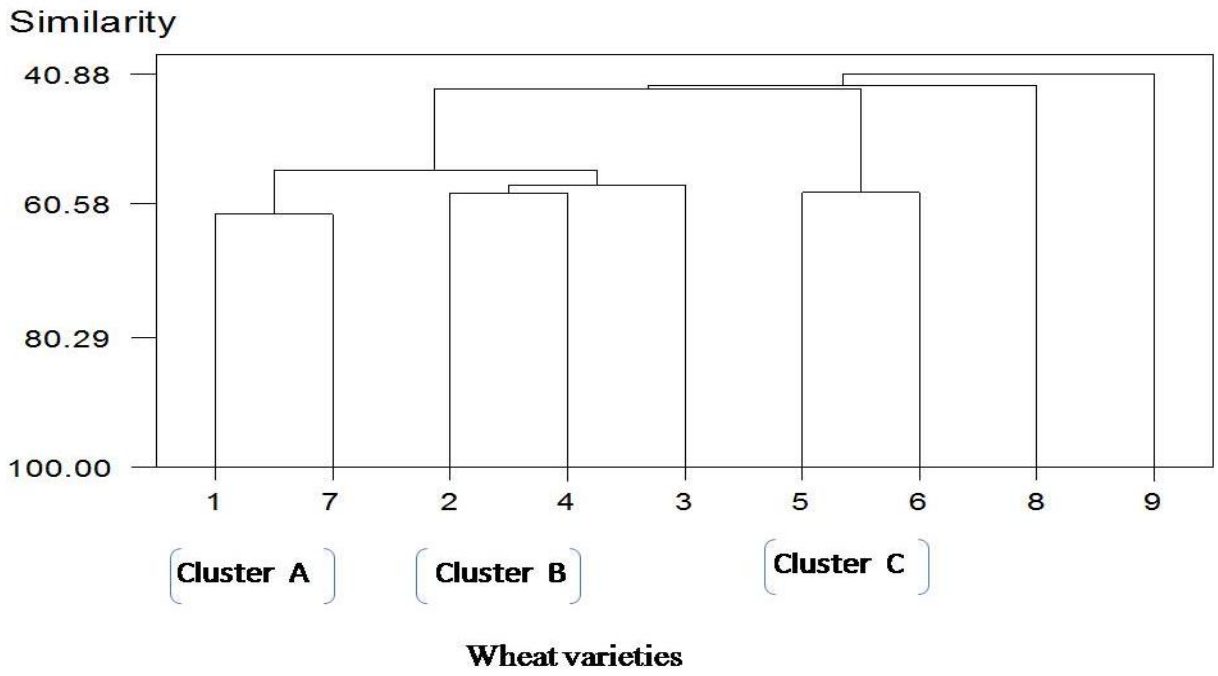

Figure 5.6: Dendogram showing differential clustering of Kenyan wheat varieties based on their organoleptic properties. Legend 1-9 = Variety 1 to variety 9. 1- Robin, 2- Eagle 10, 3- K.Tai, 4- K.Sunbird, 5- K.Wren, 6- K.Korongo, 7- K.Hawk12, 8- K.Kingbird and 9- Njoro BWII.

5.5 Discussions

5.5.1 External loaf characteristics

Results of loaf shape for varieties grown in Eldoret had the highest scores compared to varieties grown in Mau-Narok and Naivasha. Loaf shape is highly dependent on the integrity of gluten especially the ability to withstand pressure from CO_2 produced and the high temperatures during the baking process (Peighambardoust *et al.,* 2010). Presence of bran and germ particles interferes with the continuity of the dough creating many weak points that easily give in when exposed to extreme conditions resulting to flat breads as observed in loaves baked from varieties grown in Naivasha and Mau-Narok.

Crust colour of bread is usually as a result of browning reaction and caramelization reactions which when controlled give rise to an attractive brown colour (Purlis and Salvadori, 2009). Breads baked from varieties grown in Mau-Narok had the best scores compared to the rest.

Popular breads usually have attractive brown colour on their crust and are usually associated with the browning reaction and caramelization reactions products (Ahrne *et al*., 2007). The browning reaction is a chemical reaction between an amino acid and a reducing sugar, while caramelization is a complex group of reactions that take place when sugars are subjected to high temperatures in the absence of amino acids (Tsai *et al*., 2009). Crust browning occurs when the baking temperature is greater than 110^{o}C (Mondal and Datta, 2008). At high temperatures, water is quickly removed from the dough surface and the high levels of proteins in whole meal flour offers optimum conditions for Maillard reaction. The end products of Maillard reactions are melanoidins and they are responsible for browning of the crust (Michalska *et al*., 2008). Varieties from all the three sites had moderate scores for crust colour at levels above 3 an indicator that browning reaction occurred to all the varieties at an almost similar magnitude.

5.5.2 Internal loaf characteristics

Crumb colour scored well bordering between moderate and good. It is highly influenced by temperatures inside the dough. The temperatures in the dough are usually much lower than that of crust while water activity is usually high inside the dough and this causes the light coloration observed in the crumb colour (Borrelli *et al*., 2003). Crumb softness was moderate for all the varieties and across the three sites and is usually as a result of moisture retention inside the crust. All varieties were moderately soft indicating good moisture retention however, the crumb softness decreased with prolonged exposure to light during sampling time. Vey dry crumbs break easily giving rise to small bread particles and it is not easy to slice or divide the bread into portions as it easily breaks down. Crumb softness also gives bread the springiness or sponginess which is an indicator of high quality loaves.

5.5.3 Flavour characteristics

The melanoidins formed impact aroma to the baked breads and other products. Scores for all the varieties in all the three sites were above 3, an indicator that the product aroma was appealing irrespective of site of growth. For whole meal bread the scores were within the commonly acceptable range since no additives to improve aroma were included when dough was prepared.

Analysis on taste showed all varieties scored lower values irrespective of site of growth. This might be because of the compounds formed during the prolonged time in the oven. When

overexposed to high temperatures, the melanoidins formed impacts a bitter taste which might have resulted in the taste parameter having lower scores. The bitter undesired taste experienced could also have resulted from the formation of bitter compounds such as acrylamide formed in prolonged maillard reactions (Ahrne *et al*., 2007; Gokmen *et al*., 2007).

5.5.4 General acceptability

Bread acceptability levels for most varieties were highly influenced by the shape and taste of the varieties. For varieties that had bread with irregular shapes and scored low on taste, their score on general acceptability was greatly reduced. This is because for baked products, the shape and taste of the product greatly influence the desire and preference of the consumer.

5.6 Conclusions

Organoleptic properties contribute to the consumers preferences and influence their decision on bread consumption. The low score on bread shape and taste greatly contributed to the low score on general acceptability of the whole meal bread. However, based on the fact that no improvers for taste were added and no strengthening agents were used, it is fair to conclude that wheat varieties used in this study should be used to bake nutritious and healthier whole meal bread. The whole meal bread scored highly on aroma and crumb softness some of the parameters which also greatly contribute to consumer preferences.

5.7 References

Ahrne L., Andersson C.G., Floberg P., Rosen J. and Lingnert H. (2007). Effect of crust temperature and water content on acrylamide formation during baking of white bread: steam and falling temperature baking. *Journal of Food Science Technology*, **40:**1708-1715.

Borrelli R.C., Mennella C., Barba F., Russo M., Russo G.L., Krome K., Erbersdobler H.F., Faist V. and Fogliano V. (2003). Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Journal of Food Chemicals and Toxicology*, **41:**1367-1374.

Crina M., Laura S., Simona M., Stancuta S. and Sevastita M. (2012). Sensory evaluation of bakery products and its role in determining of the consumer preferences. *Journal of Agroalimentary Processes and Technologies*, **18(4):**304-306.

Gokmen V., Acar O.C., Koksel H. and Acar J. (2007). Effects of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies. *Journal of Food Chemistry*, **104:**1136-1142.

Kent J. (2002). Technology of wheat. Pergaman Press Oxford, **5:** 109-136.

Maneju H., Udobi C.E. and Ndife J. (2011). Effect of added brewers dry grain on the physico-chemical, microbial and sensory quality of wheat bread. *American Journal of Food Nutrition* **1(1):**39-43.

Michalska A., Amigo-Benavent M., Zielinski H. and Castillo M.D. (2008). Effect of bread making on formation of Maillard reaction products contributing to the overall antioxidant activity of rye bread. *Journal of Cereal Science*, **48:**123–132.

Mondal A. and Datta A.K. (2008). Bread baking. A review. *Journal of Food Engineering*, **86:**465-474.

Moskowitz H.R., Beckley J.H. and Ressureccion A.V.A. (2006). *Sensory and consumer research in food product design and development*. IFT Press.

Muler A. and Steinhart H. (2007). Recent developments in instrumental analysis for food quality. *Journal of Food Chemistry*, **101:**1136-1144.

Ndife J. and Abbo E. (2009). Functional foods: Prospects and challenges in Nigeria. *Journal of Science and Technology*, **1(5):**1-6.

Ness R.M., Ness M., Brennan M., Oughton E., Ritson C. and Ruto E. (2010). Modeling consumer behavioural intentions towards food with implications for marketing quality low input and organic food. *Journal of Food Quality and Preference*, **21:**100-111.

Peighambardoust S.H., Fallah E., Hamer R.J., Van Der Goot A.J. (2010). Aeration of bread dough influenced by different way of processing. Journal of Cereal Science, **51:**89–95.

Purlis E. and Salvadori V.O. (2009). Modeling: the browning of bread during baking. *Journal of Food Research International*, **42:**865-870.

Tsai P.J., Yu T.Y., Chen S.H., Liu C.C. and Sun Y.F. (2009). Interactive role of colour and antioxidant capacity in caramels. *Journal of Food Research International*, **42:**380-386.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

6.1.1 Rheological properties of whole meal flour from 9 Kenyan bread varieties and correlation with nutritional qualities.

Past studies have indicated that rheological properties of wheat flour are mainly influenced by the varietal composition especially that of protein and gluten (Rao *et al.,* 2000). These two components greatly determine the water absorption, DDT, mixing tolerance index and resistance to both elasticity and extensibility. Correlation analysis among the nutritional traits showed that starch and phytic acid were positively correlated at R=0.67 ($p<0.047$*). Similarly glucose and starch were also positively correlated at R=0.87 ($p<0.002$**), (Appendix 22). This correlation is largely due to the fact that more starch molecules provide free glucose molecules which are then redirected to the rate limiting step conversion of d-glucose to myo-inositol phosphate and ultimately the synthesis of phytic acid (Francesca and Eleonora, 2015). Noticeably in this study, correlation analysis between the nutrients and rheological parameters showed no significant correlation at apart from the positive correlation (0.69 at $P<0.05$) between zinc and weight (Appendix 23). This might have resulted from lower levers of zinc, iron, phytic acid and resistant starch. In a study conducted by Majzoobi *et al.,* (2014), addition of RS to the batter reduced the density. In their study, the changes were more pronounced with larger substitution percentages. The same effect of resistant starch was reported Baixauli *et al.* (2008b) after the incorporation of RS to muffin batter.

While consumption of bio-fortified bread or nutritionally enhanced wheat flour has been advocated for, there has been an underlying fear that the additives might affect the rheological properties of flour and to some extent. Most studies have focused mainly on the effect of individual additives such as chemical biofortification of nutrients and not in their natural form. With this in mind, this study was set up to evaluate the combined effect of the naturally occurring nutrients in whole meal flour. The results obtained suggest that the nutritional quality does not affect the rheological behaviour of dough and are in line with findings from a study by Sudha and Leelavathi, (2008).

6.1.2 Organoleptic properties of whole meal bread from Kenyan wheat varieties and its correlation with nutritional quality

Organoleptic qualities greatly influence consumer's preference to a given commodity (Ali, 2015). Results obtained for these properties on the whole meal products showed variations are influenced by site. Taste of the whole meal breads scored moderately and this was highly attributed to the ingredients used. Notably, correlation analysis between nutrition quality of wheat and the organoleptic properties showed significant effect between phytic acid and crust colour and also with crumb colour at (r=0.68 $p<0.05$ and r=0.7 both at $p<0.05$, respectively and between aroma and shape at r=0.*67* $p<0.05$ (Appendix 24). This might be due to high levels of bran which improves the crust colour (Majzoobi *et al.,* 2013). Phytic acid is highly correlated with bran size and particles, thus an increase in bran levels leads to high phytic acid and this may lead to improved crust colour. The shape of the bread is mainly influenced by the quality of gluten in wheat and the positive correlation with zinc might be as a result of complexing of zinc with most structural proteins. Aroma and shape are highly dependent on protein levels of the flour used. More protein in the flour provides free amino groups that react with water molecules leading to the formation of aromatic compounds (Bekedam *et al.,* 2006), while in relation to shape, the protein resists escape of CO_2 leading to the loaf structure hence the correlation. This information proves that nutritional improvement of wheat has little or no significant effect on the organoleptic properties and be encouraged without reservations.

In a study conducted on sensory evaluation of enriched muffins, those rich in RS were significantly darker in color, denser and had a well-done crust but was less liked overall (Mindy *et al.,* 2013). However for the Kenyan varieties reported herein this thesis RS did not significantly influence the sensory attributes when compared with control. An Asian study on effect of fortification on sensory attributes of bread concluded that zinc and iron had no significant effect on their acceptability (Aaron *et al.,* 2011). This is in line with results from this study which revealed that there was no significant correlation between the sensory attributes tested and the levels of zinc and iron.

6.2 General conclusion

Wheat is a major cereal crop consumed by most people irrespective of their social-economic status due to the different forms in which it can be processed for consumption. Increasing

the desired nutrition qualities would play a big role in managing micronutrient deficiencies, since wheat is a popular cereal crop. The analysis of zinc and iron in the nine wheat varieties showed that wheat contained considerable levels of the two elements thus it should continue finding a place in our diets with increasing frequencies. While whole meal flour contains more nutrients and desired fibers, most bakery products are usually made from finer white flour which during its processing looses most of the important nutrient and thus reduces bread nutrition value. This is because whole meal flour contains bran and germ particles which interferes with its elasticity and extensibility making its dough weak for making raised bakery products.

The levels of resistant starch in the nine varieties were highly influenced by the site though some varieties recorded lower levels. Varieties with high levels of resistant starch could be used in managing diabetes, improving insulin response, prevention of colonic cancer and regulation of blood glucose, if they are consumed as diets made from whole meal flour. The levels of resistant starch in the varieties were quite promising although further research to improve concentrations in the varieties may be necessary.

In the case of phytic acid low levels are desired in food crops mostly consumed in our diets and the results obtained showed that concentrations present in the nine varieties were low and as such could not adversely affect the absorption of microelements. Results for phytic acid concentrations did not differ greatly for whole meal flour from varieties grown in the three sites and was safe for consumption. The glucose levels obtained were in moderate concentrations indicating that whole meal products made from these varieties would not produce a sudden increase of blood glucose levels. This would make the body be in a better position to handle the blood glucose levels thus preventing increased chances of diabetes development. Starch levels were also moderate indicating that those varieties would provide adequate fibers which would increase the bulk of food in the digestive system and in the process improve peristaltic movements.

It is evident from the correlation analysis that there exist little or no significant between the nutritional quality, rheological and organoleptic properties hence improving the nutrition quality wouldn’t affect the rheological or organoleptic properties of whole meal flour. This is encouraging since breeders focusing on nutrition quality will not be hindered by fear of affecting the said properties.

6.3 Recommendations

1. Varieties with high levels of resistant starch, zinc, iron and low levels of phytic acid should be adapted in the areas in which the concentration for the desired traits was enhanced.
2. Consumption of whole meal products should be advocated for due to the superiority in the nutrition quality.
3. Breeders to devise ways to translocate the elements contained in the bran and germ parts of the wheat grain to the starch endosperm to cater for people who prefer processed flours.

REFERENCES

Aaron G.J., Ba L.N.., Hess S.Y., Guiro A.T., Wade S., Ndiaye N.F., Guinard J.X. and Brown K.H. (2011). Acceptability of complementary foods and breads prepared from zinc-fortified cereal flours among young children and adults in Senegal. *Journal of Food Science*, **76(1):**S56-S62.

Abdoulaye S., Issoufou A. and Wei-Rong Y. (2013). Application of resistant starch in bread: processing, proximate composition and sensory quality of functional bread products from wheat flour and African locust bean (*Parkia biglobosa*) flour. *Journal of Agricultural Sciences*, **4(5):**122-129.

Ali A. (2015). Production and evaluation of organoleptic characteristics of fruit juice and low sugar pulp of Behbahan variety dates of Kasi and Kabkab. *African Journal of Food Science*, **9(5):**322-325.

Alloway B.J. (2004). Zinc in soil and crop nutrition. *International Zinc Association. Brussels, Belgium.*

Alvarez F., Paniagua A., Abadia J.P.and Abadia A. (2003). Effects of Fe deficiency chlorosis on yield and fruit quality in peach (*Prunuspersica L. Batsch*). *Journal of Agriculture and Food Chemistry*, **51(19):**5738-5744.

Angioloni A. and Rosa M.D. (2005). Dough thermo-mechanical properties: influence of sodium chloride, mixing time and equipment. *Journal of Cereal Science,* **41:** 327-331.

Arnold T., Kirk G.J.D., Wissuwa M., Frei M., Zhao F.J., Mason T.F.D., et al (2010). Evidence of mechanisms of zinc uptake by rice using isotope fractionation. *Journal of Plant Cell and Environment,* **33:**370-381.

Arun Shunmugam (2011). Mapping of QTL associated with seed phytic acid concentration in pea recombinant inbred lines. Crop Development Centre University of Saskatchewan 1-17.

Barbara T., Hua J., Arnaud L., and Ann F. (2013). Absorption studies show that phytase from *Aspergillus niger* significantly increases iron and zinc bioavailability from phytate-rich foods. *The Food and Nutrition Bulletin,* 34(2): s90-s101.

Bashir K., Inoue H., Nagasaka S., Takashi M., Nakanishi H., Mori S. et al. (2006). Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. *Journal of Biological Chemistry*, **281:**32395-32402.

Bashir K., Ishimaru Y. and Nishizawa N.K. (2010). Iron uptake and loading into rice grains. *Journal of Rice,* **3:**122-130.

Bashir K. and Nishizawa N.K. (2006). Deoxymugineic acid synthase: a gene important for Fe-acquisition and homeostasis. *Journal of Plant Signaling and Behavior,* **1:**290-292.

Baixauli R.S., Salvador T., and Fiszman, S.M. (2008b). Muffins with resistant starch: baking performance in relation to the rheological properties of the batter. *Journal of Cereal Science,* **47:** 502–509.

Beard J. and Han O. (2009). Systemic iron status. *Journal of Biochimica et Biophysica Acta*, **1790:**584–588.

Bekedam E.K, Schols H.A., Boekel M.A.J.S., and Smith G. (2006). High molecular weight melanoidins from coffee brew. *Journal of Agricultural and Food Chemistry***, 54:**7658-7663.

Biesalski H.K. (2013). First international conference on hidden hunger, Hohenheim, Stuttgart, Germany. *Journal of Food Security*, **5:**457-473.

Bollain C. and Collar C. (2004). Dough viscoelastic response of hydrocolloid/enzyme/surfactant blends assessed by uni-axial and bi-axial extension measurements. *Journal of Food Hydrocolloids* **18**: 499-507.

Bo Lonnerdal (2002). Phytic acid, trace element (Zn, Cu, Mn) interactions. *International Journal of Food Science and Technology,* **7:** 749-758.

Bouis H.E. (2003).Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? *Proceedings of the Nutrition Society*, **62**: 403-411.

Bouis H.E. (2007). The potential of genetically modified food crops to improve human nutrition in developing countries. *Journal of Developmental Studies*, **43**: 79-96.

Brennan R.F. (2005). Zinc application and its availability to plants. Ph.D. Dissertation, School of Environmental Science, Division of Science and Engineering, Murdoch University.

Brown I. (1996). Complex carbohydrates and resistant starch-A review. *Journal of Nutrition*, **54:** S115-S119.

Bruno B., Erin M., Ines E. and Mary C. (2008). Worldwide prevalence of anaemia 1993-2005. W.H.O global database on anaemia. ISBN 9789241596657.

Cakmak I. (2000). Role of zinc in protecting plant cells from reactive oxygen species. *Journal of New Phytology*, 1**46:**185-205.

Cakmak I. (2008). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification. *Journal of Plant Soil*, **302**:1-17.

Cakmak I. (2010). Biofortification of cereals with zinc and iron through fertilization strategy. *19th World congress of soil science, soil solutions for a changing World 1-6 August 2010, Brisbane, Australia*.

Caspi R., Altman T., Dale J.M., Dreher K., Fulcher C.A., Gilham F., Kaipa P., Karthikeyan A.S., Kothari A., Krummenacker M., Latendresse M., Mueller L.A., Paley S., Popescu L., Pujar A., Shearer A.G., Zhang P. and Karp P.D. (2010). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Journal of Nucleic acids research*, **38:**473-479.

Conte S.S. and Walker E.L. (2011). Transporters contributing to iron trafficking in plants. *Journal of Molecular Plant Advance*, **4(3):** 464-476.

Cornell H (2003). The chemistry and biochemistry of wheat. In: Cauvain SP, ed. Bread making: improving quality. Sawston: Woodhead Publishing Limited; PP. 31-70

Crichton R. and Pierre J.L. (2001) Old iron, young copper: From Mars to Venus. *Journal of Biometals*, **14:** 99-112.

Curie C., Panaviene Z., Loulergue C., Dellaporta S.L., Briat J.F. and Walker E.L. (2001). Maize yellow stripe1 encodes a membrane protein directly involved in Fe (III) uptake. *Journal of Nature*, **409(6818):**346-349.

Debjit B., Chiranjib K.P. and Sampath K. (2010). A potential medicinal importance of zinc in human health and chronic disease. *International Journal of Pharmacy and Biomedical Science*, **1:**5-11.

Dobraszczyk B.J. (2004a). The physics of baking: rheological and polymer molecular structure function relationships in breadmaking. *Journal of Non- Newtonian Fluid.* **124(1-3):**61-69.

Erin L.C., Nathan H.C., Natasha G., Charis L.P. and Mary-Lou G. (2003).Over expression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncovers posttranscriptional control. *Journal of Plant Physiology*, **133:**1110-1112.

Federico B. (2006). From genomics to proteomics in *Azoarcus* sp. BH72, a N_2 fixing endophytic bacterium. Thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in (natural) science. Laboratory of general microbiology, the faculty of biology and chemistry University of Bremen, Germany. *https://www.deutsche-digitale-bibliothek.de/binary/.../full/1.pdf.*

Ferguson L.R., Tasman-Jones C., Englyst H., and Harris P.J. (2000). Comparative effects of three resistant starch preparations on transit time and short chain fatty acid production in rats. *Journal of Nutrition and Cancer*, **36:** 230-237.

Fernandez V., Victor D.R., Javier A.B. and Anunciacion A.B. (2006). Foliar iron fertilization of peach (*Prunuspersica (L.) Batsch*): Effects of iron compounds, surfactants and other adjuvants. *Journal of Plant Soil*, **289:**239-252.

Francesca S. and Eleonora C. (2015). Seed biofortification and phytic acid reduction: A conflict of interest for the plant. *Journal of Plants,* **4(4):**728-755.

Fuentes-Zaragoza E., Riquelme-Navarrete M.J., Sanchez-Zapata E., Perez-Alvarez J.A. (2010). Resistant starch as functional ingredient: A review. *Journal of Food Research International*. doi:10.1016/j.foodres.2010.02.004.

Ganna V. and Ross T.A.M. (2013). Multi copper oxidases and human Iron metabolism. *Journal of Nutrients,* **5:** 2289-2313.

Gill B.S., Appels R., Botha-Oberholster A.M. and Robin B.C. (2004). A workshop report on wheat genome sequencing: international genome research on wheat consortium. *Journal of Genetics*, **168(2):** 1087-1096.

Goldberg N.D. (2013). Iron deficiency anemia in patients with inflammatory bowel disease. *Journal of Clinical and Experimental Gastroenterology*, **6:** 61-70.

Gomez M., Ronda F., Blanco C.A., Caballero P. A. and Apesteguia A. (2003). Effect of dietary fibre on dough rheology and bread quality. *Journal of European Food Research and Technology,* **216:**51-56.

Hafeez B., Khanif Y.M. and Saleem M. (2013). Role of zinc in plant nutrition: A review. *American Journal of Experimental Agriculture*, **3(2):** 374-391.

Hamdollah E. (2011). The importance of iron (Fe) in plant products and mechanism of its uptake by plants. *Journal of Applied Environmental and Biological Sciences*, **1(10):** 448-452.

Hoover R. and Zhou Y. (2003). *In vitro* and *in-vivo* hydrolysis of legume starches by α-amylases and resistant starch formation in legumes. *Journal of Carbohydrate Polymers* **54(4):**401-417.

Hulisz D. (2004). Efficacy of zinc against common cold viruses: an overview. *Journal of America Pharmacy Association*, **44:**594-603.

Hung P.V., Maeda T. and Morita N. (2007). Dough and bread qualities of flours with whole waxy wheat flour substitution. *Food Research International*, **40:**273-279.

Igbabul B., Num G. and Amove J. (2014). Quality evaluation of composite bread produced from wheat, maize and orange fleshed sweet potato flours. *American Journal of Food Science and Technology*, **2(4):**109-115.

Ihsan M.Q., Said W., Anwar A.S., Alam Z. and Ayuab M. (2003). Effect of different fermentation time and baking on phytic acid content of whole wheat flour bread. *Asian Journal of Plant Sciences,* **2(8):**597-601.

Jeong J., Cohu C., Kerkeb L., Pilon M., Connolly E.L. and Guerinot M.L. (2008). Chloroplast Fe (III) chelate reductase activity is essential for seedling viability under iron limiting conditions. *Journal of Proceedings of the National Academy of Sciences*, **105(30):**10619-10624.

Jeremy M.B., John L.T. and Lubert S. (2002). *Biochemstry*. **5:** 806. W.H.Freeman & Co Ltd.

Jerome N. (2007). Zinc deficiency in human health. Elsevier B.V.

Jideani V. and Onwubali F. (2009). Optimisation of wheat-sprouted soybean flour bread using response surface methodology. *African Journal of Biotechnology*, **8:**6364-6373.

Jingyuan X., Jerold A.B. and Craig J. C. (2007). Viscoelastic properties of wheat gliadin and glutenin suspensions. *Journal of Food Chemistry*, **101:**1025-1030.

Joe M. and Mary L.G. (2009). Iron uptake and transport in plants: The good, the bad and the ionome. *Journal of Chemical Review*, **109(10):**4553-4567.

Juyun L., Alison W. and Barry G. G. (2009). Derivation and Evaluation of a Labeled Hedonic Scale. *Journal of Chemical Senses*, **34:**739-751.

Katina K., Salmenkallio-Marttila, M., Partanen R., Forssell P., Autio K. (2006). Effects of sourdough and enzymes on staling of high-fibre wheat bread. *Journal of Lebensmittel Wissenchaft und Technology*, **39:**479-491.

King J. (2011). Zinc: an essential but elusive nutrient. *American Journal of Clinical Nutrition*, **94**: 679-684.

Kumar P., Yadava R.K., Gollen B., Kumar S., Verma R.K. and Yadav S. (2011). Nutritional Contents and Medicinal Properties of Wheat. *Journal of Life Sciences and Medicine Research*, **22:**1-10.

Leszczynski W. (2004). Resistant starch- classification, structure, production. *Polish Journal of Food and Nutrition Sciences*, **13(54):**37-50.

Loewus A.F. and Pushpalatha P.N.M (2000). Myo Inositol metabolism in plants. *Journal of Plant Science*, **150:**1-19.

Lopez De Romana D., Brown K.H., and Guinard J.X. (2002). Sensory trial to assess the acceptability of zinc fortificants added to iron-fortified wheat products. *Journal of Food Science*, **67(1):**461-465.

Lopez H.W., Charles C. Jacques B., Hassan Y., Christian D., and Christian R. (1998). Intestinal Fermentation Lessens the Inhibitory Effects of Phytic Acid on Mineral Utilization in Rats. *The Journal of Nutrition*, **128(7):** 1192-1198.

Mahagayu M.C., Kamwaga J., Ndiema A.C., Kamundia J. and Gamba P. (2007). Wheat productivity, constraints associated in the eastern parts of Kenya Timau division. *Journal of Crop Science Conference Proceedings*, **8**:1211-1214.

Majzoobi M., Hedayati S., Habibi M., Ghiasi F., and Farahnaky A. (2014). Effects of Corn Resistant Starch on the Physicochemical Properties of Cake. *Journal of Agriculture Science and Technology*, **16:**569-576.

Majzoobi M., Farahnaky A., Nematolahi Z., Mohammadi H., and Taghipour A.M.J. (2013). Effect of different levels and particle sizes of wheat bran on the quality of flat bread. *Journal of Agriculture Science and Technology*, **15:**115-123.

Maret W and Sandstead H (2006). Zinc requirements and the risks and benefits of zinc supplementation. *Journal of Trace Elements in Medical Biology*, 20(1): 3-18.

Martins S., Evita S. and Ruta G. (2011). Assessment of the rheological properties of flour using the Mixolab. *Baltic Conference on Food Science and Technology*, **6:**33-38.

McDonald G.K., Graham R.D., Lloyd J., Lewis J., Lonergan P. and Khabaz-Saberi H. (2001). Breeding for improved zinc and manganese efficiency in wheat and barley. *Proceeding of the 10th Australian Agronomy Conference*. Department of Plant Science, Waite Institute, Glen Osmond, SA, Hobart.

Mindy M., Melanie S., Shanil J., Chandan P., Victorine I. and Parakat V. (2013). Sensory characteristics of high-amylose maize-resistant starch in three food products. *Journal of Food Science and Nutrition*, **1(2):**117-124.

Mirsaeedghazi H., Emam-Djomeh Z. and Mousavi S.M.A (2008). Rheometric measurement of dough rheological characteristics and factors affecting It. *International. Journal of Agriculture and Biology*, **10:** 112-119

Mohammed A., Khan A., Eric B., Michel B., Monika B. et al., (2010). World Health Organization, (W.H.O). *World Health Statistics 2010*.

Monroy L., Mulinge W. and Witwer M. (2013). Analysis of incentives and disincentives for wheat in Kenya. *Technical notes series*, MAFAP, FAO, Rome.

Moreira R., Chenlo F., Torres M.D., Prieto D.M. (2010). Influence of the particle size on the rheological behavior of chestnut flour dough. *Journal of Food Engineering*, **100:** 270-277.

Mosharraf L., Mahdi K and Mohammad S. (2006). Effect of hydrothermaled bran on physicochemical, rheological and microstructural characteristics of Sangak bread. *Journal of Cereal Science*, **49:**398-404.

Nathalie S. (2013). Regulatory effects of Cu, Zn, and Ca on Fe absorption: The intricate play between nutrient transporters. *Journal of Nutrients*, **5:** 957-970.

Ndife J. and Abbo E. (2009). Functional Foods: Prospects and Challenges in Nigeria. Journal of Science and Technology, **1:**1-6.

Neil, C.D (2012). Value added opportunities and alternative uses for wheat and barley. Agricultural Utilization Research Institute. *Dotty N.C and Associates LLC*, pp1-60.

Neelam G. and. Khatkar B.S. (2015). Quantitative and qualitative assessment of wheat gluten proteins and their contribution to instant noodle quality. *International Journal of Food Properties*, **18**:1648-1663.

Nielsen V.F.A., Inge T. and Anne S. M. (2013). Potential of phytase mediated iron release from cereal based foods: A quantitative view. *Journal of Nutrients*, **5:**3074-3098.

Ottoline L. (2010). The power of auxins in plants. *Journal of Plant physiology*, **154(2):**501-505.

Peck A.W. and McDonald G.K. (2010). Adequate zinc nutrition alleviates the adverse effects of heat stress in bread wheat. *Journal of Plant Soil*, **337(355):**374-389.

Philip J.W. and Martin R.B. (2009). Biofortification of crops with seven mineral elements often lacking in human diets iron, zinc, copper, calcium, magnesium, selenium and iodine. *Journal of New Phytologist*, **182:** 49-84.

Prasad A.S. (2009). Impact of the discovery of human zinc deficiency on health. *Journal of the American College of Nutrition*, **28(3):** 257-265.

Ponte P.I., Mendes I., Quaresma M., Aguiar M.N., Lemos J.P., Perreira L.M., Soares M.A., Alfaia C.M., Prates J.A., Fontes C.M. (2004). Cholesterol levels and sensory characteristics of

meat from broilers consuming moderate to high levels of Alfa. *International Journal of Poultry Science*, **83:**810-814.

Prasad, A.S. (2012). Discovery of human zinc deficiency: 50 years later. *Journal of Trace Elements in Medicine and Biology*, **26(3):**66-69.

Raboy V., Paola F.G., Kevin A.Y., Sierra D.S., Suewiya G.P., Andrew T.B., Pushpalatha P.N. M., William F.S., and David S.E. (2000). Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. *Journal of Plant Physiology*, **124**: 355-368.

Rajagopal A., Rao A.U., Amigo J., Tian M., Upadhyay S.K., Hall C., Uhm S.M., Fleming, M.D., Paw B.H., et al. (2008). Heme homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins. *Journal of Nature*, **453:** 1127-1131.

Rao V.K., Mulvaney S.J. and Dexter J.E. (2000). Rheological characterisation of long and short mixing flours based on stress-relaxation. *Journal of Cereal Science*, **31(2):**159-171.

Rosell M.C., Collar C., Haros M. (2007) Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab. *Journal of Food Hydrocolloids*, **21:** 452-462.

Saeed A., Anjum F.M., Saleem U.R. and Munir A.S. (2008). Effect of mineral fortification on rheological Properties of whole wheat flour. *Journal of Texture studies*, **40:**51-65.

Saha A., Perumall A.V., Lee Y., Meullenet J.F., Owens C.M. (2009). Tenderness, moistness, and flavor of pre- and postrigor marinated broiler breast fillets evaluated by consumer sensory panel. *International Journal of Poultry Science*, **88:**1250-1256.

Sajilata M.G., Rekha S.S. and Pushpa R.K. (2006). Resistant starch: A review. *Journal of Comprehensive Reviews in Food Science and Food Safety*, **5(1):**1-17.

Salim U.R., Nawaz H., Hussain S. and Ahmad M.M. (2007). Effect of sourdough bacteria on the quality and shelf life of bread. Pakistan Journal Nutrition, **6 (6):** 262-265.

Sands D.C., Morris C.E., Dratz E.A. and Pilgeram A. (2009). Elevating optimal human nutrition to a central goal of plant breeding and production of plant based foods. *Journal of Plant Science*, **177:** 377-389.

Sangnark A. and Noomhorn A. (2004) Chemical, physical and baking properties of dietary fibre prepared from rice straw. *Journal of Food Research International*, **37**:66-74.

Sarret G., Balesdent J., Bouziri L., Garnier J.M., Marcus M.A., Geoffroy N., *et al* (2004). Zn speciation in the organic horizon of a contaminated soil by micro X-ray fluorescence, micro and powder EXAFS spectroscopy, and isotopic dilution. *Journal of Environmental Science and Technology*,

38: (2): 792- 801.

Sayed R.M., Mohammad G. and Maryam R. (2013). Zinc importance for crop production. *International journal of Agronomy and Plant Production*, **4 (1):**64-68.

Schlemmer U., Frolich W., Prieto R.M. and Grases F. (2009). Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. *Journal of Molecular Nutrition and Food Research*, **53(S2):** S330-S375.

Sharp P. and Surjit K.S. (2007). Molecular mechanisms involved in intestinal iron absorption. *World Journal of Gastroenterology*, **13(35):** 4716-4724.

Shayeghi L.D., Oakhill G.O., Laftah J.S., Takeuchi A.H., Halliday K., Khan N., Warley A., McCann F.E. and Hider R.C. (2005). Identification of an intestinal heme transporter. *Journal of Cell*, **122:** 789-801.

Shewry P.R. (2009). The health grain programme opens new opportunities for improving wheat for nutrition and health *Nutrition Bulletin*, **34(2):** 225-231.

Shiau S.Y. and Yeh A. (2001). Effect of alkali and acid on dough rheological properties and characteristic of extruded noodles. *Journal of Cereal Science*, **33:** 27-37.

Shittu S.T., Oyeyemi W.A., Okewumi T.A. and Salman, T.M. (2013). Role of oxidative stress in therapeutic administration of artesunate on sperm quality and testosterone level in male albino rats. *African Journal of Biotechnology*, **12 (1):**70-73.

Shittu T.A., Dixon A., Awonorin S.O., Sanni L.O. and Maziyadixon B. (2008). Bread from composite cassava genotype and nitrogen fertilizer on bread quality. *Journal of Food Research International*, **41:** 568-578.

Stevenson-Paulik J., Odom A.R. and York J.D. (2002). Molecular and biochemical characterization of two plant inositol polyphosphate 6-3-5-kinases. *Journal of Biological Chemistry*, **277(45):**42711-42718.

Sudha M.L. and Leelavathi K. (2008). Influence of micronutrients on rheological characteristics and bread-making quality of flour. *International Journal of Food Science and Nutrition*, **59(2):**105-115.

Suzuki M., Michiko T., Takashi T., Satoshi W., Shinpei M., Junshi Y., Naoki K., Shoshi K., Hiromi N., Satoshi M. and Naoko K.N. (2006). Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc deficient barley. *The Plant Journal*, **48:**85-97.

Svec I. and Hruskova M. (2010). Evaluation of wheat bread features. *Journal of Food Engineering*, ***99*(4):**505-510.

Takahashi M., Nozoye T., Kitajima B., Fukuda N., Hokura N., Terada Y., et al.(2009). *In vivo* analysis of metal distribution and expression of metal transporters in rice seed during germination process by microarray and X- ray fluoresence imaging of Fe, Zn, Mn, and Cu. *Journal of Plant Soil*, **325:**39-51.

Tharanathan R.N. and Mahadevamma S. (2003). Grain legumes: A boom to human nutrition. *Journal of Trends in Food Science and Technology*, **14:** 507-518.

Nenova V. (2006). Effect of iron supply on growth and photosystem II efficiency of pea plants. *Journal General and Applied Plant physiology*, **32(1-2):**81-90.

Velu G.I., Ortiz M., Cakmak I., Hao Y. and Singh R.P. (2013). Biofortification strategies to increase grain zinc and iron concentrations in wheat. *Journal of Cereal Science*, **59(3):**365-372.

Wang X. and Bing Z. (2010). Dietary zinc absorption: A play of Zips and ZnTs in the gut. *Journal of Life*, **62**: 176-182.

Wang K., Zhou B., Kuo Y.M., Zemansky J. and Gitschier J. (2002). A novel member of a zinc transporter family is defective in *Acrodermatitis enteropathica*. *American Journal of Human Genetics*, **71**:66-73.

Welch R.M. and Graham R.D. (2004). Breeding for micronutrients in human nutrition perspective. *Journal of Experimental Botany*, **55:**353-364.

Weria W., Yaghoub R. and Kaveh H.A. (2013). Role of some mineral nutrients in biological nitrogen fixation. *Bulletin of Environment, Pharmacology and Life Sciences,* **2(4):**77-84.

Widodo B.M.R., Rose T., Frei M., Tanaka J.P.., Yoshihashi T, et al. (2010). Response to zinc deficiency of two rice lines with contrasting tolerance is determined by root growth maintenance and organic acid exudation rates, and not by zinc transporter activity. *Journal of New Phytologist*, 186:400-414.

Wood PJ, Beer MU, Butler G (2000). Evaluation of role of concentration and molecular weight of oat *β*-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition*, **84 (1):**19–23.

Yasushiro I., Khurram B. and Naoko K.N. (2011). Zn uptake and translocation in rice plants. *Journal of Rice,* **4:**21-27.

Yellamandha R.T. and Sankara-Reddi G.H. (2002). Principles of Agronomy. Kalyani Publishers, Ludhiana, **3:** 204-206.

Yogesh P., Sachin H.P., Sharu J., and Jitendra S.K. (2013). Biochemistry of metal absorption in human body: Reference to check impact of nano particles on human being. *International Journal of Scientific and Research Publications,* **3(4):1-9**

Yunde Z. (2010). Auxin biosynthesis and its role in plant development. *Journal of Plant physiology,* **61:**49-64.

Zhou J.R. and Erdman J.W. Jr. (1995). Phytic acid in health and disease. A critical review. *Journal of Food Science Nutrition*, **35(6):**495-508.

APPENDICES

Appendix 1: List of intended publications.

1. Evaluation of grain zinc, iron, phytic acid and resistant starch concentration in selected Kenyan bread wheat varieties. (submitted to the African crop science journal)
2. Evaluation of the effect of the nutritional quality on the rheological properties of stem rust resistant wheat varieties (*Triticum aestivum* L.) released in Kenya. (Under preparation)

Appendix 2: Calculations for resistant starch, soluble glucose and total starch assay

Calculations

Calculate resistant starch, non-resistant (solubilised) starch and total starch content (%, on a dry weight basis) in test samples as follows:

Resistant Starch (g/100g sample) (samples containing > 10% RS):

= ΔE x F x 100/0.1 x 1/1000 x 100/W x 162/180

= ΔE x F/W x 90.

Resistant Starch (g/100g sample) (samples containing < 10% RS):

= ΔE x F x 10.3/0.1 x 1/1000 x 100/W x 162/180

= ΔE x F/W x 9.27.

Non-Resistant (Solubilised) Starch (g/100g sample):

= ΔE x F x 100/0.1 x 1/1000 x 100/W x 162/180

= ΔE x F/W x 90.

Total Starch = Resistant Starch + Non-Resistant Starch.

Where:

ΔE = absorbance (reaction) read against the reagent blank.

F = conversion from absorbance to micrograms (the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction is determined, and F = 100 (µg of D-glucose) divided by the GOPOD absorbance for this 100 µg of D-glucose.

100/0.1 = volume correction (0.1 mL taken from 100 mL).

1/1000 = conversion from micrograms to milligrams.

W = dry weight of sample analysed

= “as is” weight x [(100-moisture content)/100].

100/W = factor to present RS as a percentage of sample weight.

162/180 = factor to convert from free D-glucose, as determined, to anhydro-D-glucose as occurs in starch.

10.3/0.1 = volume correction (0.1 mL taken from 10.3 mL) for samples containing 0-10% RS where the incubation solution is not diluted and the final volume is ~ 10.3 mL

Appendix 3: ANOVA table for Fe concentration.

Source	DF	Sum of Squares	Mean square	F Value	Pr > F
Site	2	57783.48	28891.74	19.91	<.0001
Variety	8	23319.15	2914.89	2.01	0.0635
Site*Variety	16	138200.25	8637.52	5.95	<.0001

Appendix 4: ANOVA table for Zn concentration.

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	721.46	360.73	6.63	<0.0027
Variety	8	15651.31	1956.41	35.93	<.0001
Site*Variety	16	12136.59	758.54	13.93	<.0001

Appendix 5: ANOVA table for RS, soluble glucose and total starch concentration.

RS

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	721.46	360.73	6.63	<0.0027
Variety	8	15651.31	1956.41	35.93	<.0001
Site*Variety	16	12136.59	758.54	13.93	<.0001

Soluble glucose

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	721.46	360.73	6.63	<0.0027
Variety	8	15651.31	1956.41	35.93	<.0001
Site*Variety	16	12136.59	758.54	13.93	<.0001

Total starch

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	721.46	360.73	6.63	<0.0027
Variety	8	15651.31	1956.41	35.93	<.0001
Site*Variety	16	12136.59	758.54	13.93	<.0001

Appendix 6: ANOVA table for phytic acid content.

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	721.46	360.73	6.63	<0.0027
Variety	8	15651.31	1956.41	35.93	<.0001
Site*Variety	16	12136.59	758.54	13.93	<.0001

Appendix 7: ANOVA table for whole meal flour protein levels.

Source	DF	Sum of squares	Mean Square	F value	Pr>F
Site	2	83.98	41.99	6521.34	<.0001
Variety	8	18.82	2.35	365.28	<.0001
Site*variety	16	25.35	1.58	246.05	<.0001

Appendix 8: ANOVA table for whole meal flour gluten concentration.

Source	DF	Sum of squares	Mean Square	F value	Pr>F
Site	2	704.53	352.27	7668.75	<.0001
Variety	8	347.81	43.48	946.45	<.0001
Site*variety	16	553.66	34.60	753.32	<.0001

Appendix 9: ANOVA table for whole meal flour moisture content**.**

Source	DF	Sum of squares	Mean Square	F value	Pr>F
Site	2	704.53	352.27	7668.75	<.0001
Variety	8	347.81	43.48	946.45	<.0001
Site*variety	16	553.66	34.60	753.32	<.0001

Appendix 10: ANOVA table for WA of whole meal flour**.**

Source	DF	Sum of squares	Mean Square	F value	Pr>F
Site	2	704.53	352.27	7668.75	<.0001
Variety	8	347.81	43.48	946.45	<.0001
Site*variety	16	553.66	34.60	753.32	<.0001

Appendix 11: ANOVA table for MTI of whole meal flour.

Source	DF	Sum of squares	Mean Square	F value	Pr>F
Site	2	704.53	352.27	7668.75	<.0001
Variety	8	347.81	43.48	946.45	<.0001
Site*variety	16	553.66	34.60	753.32	<.0001

Appendix 12: ANOVA table for DDT of Kenyan bread wheat varieties.

Source	DF	Sum of squares	Mean Square	F value	Pr>F
Site	2	704.53	352.27	7668.75	<.0001
Variety	8	347.81	43.48	946.45	<.0001
Site*variety	16	553.66	34.60	753.32	<.0001

Appendix 13: ANOVA table for stability of whole meal flour.

Source	DF	Sum of squares	Mean Square	F value	Pr>F
Site	2	704.53	352.27	7668.75	<.0001
Variety	8	347.81	43.48	946.45	<.0001
Site*variety	16	553.66	34.60	753.32	<.0001

Appendix 14: ANOVA tables for L, P and P/L values of whole meal flour.

L value

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	2165.65	1082.83	310.32	<.0001
Variety	8	7639.36	954.92	273.65	<.0001
Site*Variety	16	7825.23	489.08	140.15	<.0001

P value

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	583.27	291.63	100.99	<.0001
Variety	8	2024.69	253.09	87.64	<.0001
Site*Variety	16	3425.46	214.09	74.14	<.0001

P/L ratio

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	6.54	3.27	304.15	<.0001
Variety	8	8.18	1.02	95.06	<.0001
Site*Variety	16	17.38	1.09	101.03	<.0001

Appendix 15: ANOVA table for whole meal bread weight and volume.

Bread weight

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	455.82	227.91	75.82	<.0001
Variety	8	391.42	48.93	16.28	<.0001
Site*Variety	16	1721.03	107.56	35.78	<.0001

Bread volume

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	142767.28	71383.64	380.30	<.0001
Variety	8	25637.65	3204.71	17.07	<.0001
Site*Variety	16	30954.94	1934.68	10.31	<.0001

Appendix 16: Bread sensory evaluation score card

Sample code	External loaf characteristics		Internal loaf characteristics		Flavor	
	Loaf symmetry (5)	Crust colour (5)	Crumb colour (%)	Crumb softness (5)	Aroma (5)	Taste (5)

Instructions

Evaluators were provided with bread samples which they were to evaluate each sample consisted of a full loaf; for evaluation of external characteristics and a sliced section; for assessment of internal characteristics (crumb color and texture) and cubes for flavor analysis.

The numbers in brackets represented the maximum scores that could be awarded for each attribute; loaf shape (5), crust color (5), uniformity of bake (5), texture (5), crumb color (5), grain appearance (5), aroma (5), and taste (5).

Appendix 17: ANOVA table for loaf shape of whole meal bread.

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	138.11	69.41	108.67	<.0001
Variety	8	56.37	7.05	11.03	<.0001
Site*Variety	16	63.76	3.98	6.24	<.0001

Appendix 18: ANOVA table for whole meal bread crust colour.

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	24.00	12.00	16.62	<.0001
Variety	8	13.90	1.74	2.41	0.01
Site*Variety	16	17.83	1.11	1.54	0.08

Appendix 19: ANOVA table for whole meal bread crumb colour.

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	26.83	13.41	21.33	<.0001
Variety	8	7.36	0.92	1.46	0.17
Site*Variety	16	16.07	1.00	1.60	0.07

Appendix 20: ANOVA table for whole meal bread aroma.

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	49.45	24.72	26.74	<.0001
Variety	8	4.20	0.53	0.57	0.804
Site*Variety	16	14.75	0.92	1.00	0.46

Appendix 21: ANOVA table for whole meal bread general acceptability.

Source	DF	Sum of squares	Mean square	F value	Pr > F
Site	2	2.91	1.46	3.55	0.03
Variety	8	3.75	0.47	1.14	0.33
Site*Variety	16	20.49	1.28	3.12	<.0001

Appendix 22: Correlation analysis between nutritional content.

	PA	Zinc	Iron	RS	Glucose	Starch
PA	1.00	0.33	0.15	0.24	0.55	0.67
		0.39	0.70	0.53	0.13	0.047*
Zinc		1.00	-0.13	-0.23	0.60	0.49
			0.73	0.55	0.09	0.18
Iron			1.00	-0.36	0.20	0.02
				0.34	0.60	0.96
RS				1.00	-0.25	0.25
					0.51	0.51
Glucose					1.00	0.87
						0.002**
Starch						1.00

Levels of significance * P<0.05, **P<0.01, ***P<0.001 PA-phytic acid RS-resistant starch

Appendix 23: Correlation analysis between the nutritional content and rheological properties of whole meal flour of Kenyan wheat varieties.

	PA	Zn	Fe	RS	WA	MTI	STA	DDT	P	L	W	V
PA	1.00	0.3	0.15	0.24	-0.45	-0.59	0.13	0.55	0.42	0.43	-0.06	-0.33
		0.9	0.70	0.53	0.22	0.10	0.74	0.12	0.26	0.25	0.87	0.38
Zn		1.0	-0.13	0.23	0.56	-0.14	0.02	0.30	0.04	0.44	0.69	0.09
			0.73	0.55	0.11	0.72	0.96	0.44	0.92	0.24	0.04*	0.81
Fe			1.00	-0.36	-0.03	0.14	-0.03	-0.17	-0.02	0.51	-0.10	-0.36
				0.16	0.94	0.34	0.71	0.93	0.66	0.97	0.80	0.35
RS				1.00	-0.20	-0.61	0.07	0.43	0.58	0.04	-0.30	0.14
					0.61	0.08	0.86	0.24	0.10	0.92	0.43	0.73
WA					1.00	0.28	0.004	0.09	-0.06	0.37	0.53	0.22
						0.46	0.99	0.81	0.88	0.33	0.14	0.57
MTI						1.00	-0.52	-0.50	-0.81	-.41	0.38	0.40
							0.15	0.17	0.008**	0.27	0.31	0.29
STA							1.00	0.56	0.69	0.47	-0.67	-0.83
								0.12	0.04*	0.20	0.048*	0.006**
DDT								1.00	0.71	0.61	-0.29	-0.48
									0.03*	0.08	0.45	0.19
P									1.00	0.55	-0.49	-0.55
										0.12	0.18	0.13
L										1.00	-0.07	-0.59
											0.85	0.10
W											1.00	0.69
												0.04*
V												1.00

Levels of significance are * P<0.05, ** P<0.01, ***P<0.001. R values without * are not significant.

PA-phytic acid, , WA-Water absorption, MTI- Mixing tolerance index , STAB-Stability, DDT- Dough development time, P- Resistance to elasticity, L-Resistance to extensibility, W=Bread weight, V=Bread volume

Appendix 24: Correlation analysis between nutritional quality and organoleptic properties of whole meal bread from Kenyan wheat varieties

	PA	Zn	Fe	RS	Aroma	Shape	Taste	CC	ACC	CRC	Soft
PA	1.00	0.33	0.15	0.24	0.24	0.65	0.35	0.68	-0.02	0.75	0.07
		0.39	0.70	0.53	0.54	0.06	0.35	0.047*	0.97	0.02*	0.85
Zn		1.00	-0.13	-0.23	0.61	0.78	0.58	0.40	0.77	0.54	0.23
			0.73	0.55	0.08	0.01*	0.10	0.28	0.02*	0.14	0.55
Fe			1.00	-0.36	-0.32	-0.20	0.07	0.09	-0.01	0.40	0.65
				0.34	0.40	0.61	0.85	0.81	0.98	0.28	0.06
RS				1.00	-0.31	0.11	0.03	0.59	-0.38	-0.12	-0.13
					0.42	0.78	0.94	0.09	0.32	0.77	0.73
Aroma					1.00	0.67	-0.05	0.03	0.59	0.07	-0.16
						0.047*	0.90	0.94	0.09	0.86	0.69
Shape						1.00	0.35	0.54	0.41	0.49	0.24
							0.35	0.13	0.27	0.19	0.53
Taste							1.00	0.62	0.17	0.60	0.12
								0.08	0.66	0.09	0.75
CC								1.00	0.13	0.57	0.20
									0.74	0.11	0.61
ACC									1.00	0.18	0.08
										0.64	0.83
CRC										1.00	0.39
											0.30
Soft											1.00

Levels of significance are *P<0.05, **P<0.01, ***P<0.001. R values without * are not significantly correlated.

PA- Phytic acid Zn- Zinc, Fe- Iron, RS-Resistant starch, CrustC- Crust colour, Accep- Acceptability, CrumbC- Crumb colour, Soft- Crumb softness