DEBRE BRHAN UNIVERSITY

COLLEGE OF NATURALAND COMPUTATIONAL SCIENCE

DEPARTMENT OF CHEMISTRY

DETERMINATION OF SOME SELECTED HEAVY METALS AND NUTRITIONAL COMPOSITIONS OF WHEAT (TRICTUM AESTIVUM) IN MORETNA JIRU DISTRICT, ETHIOPIA

MSc THESIS

BY:

GIRMAW ABEJE ALENE

February: 2021

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A MASTER THESIS SUBMITTED TO THE DEPARTMENT OF CHE MISTRY IN PARCIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

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A thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance by Girmaw Abeje entitled "DETERMINATION OF SOME SELECTED HEAVY METALS AND NUTRITIONAL COMPOSITIONS OF WHEAT IN MORETNA JIRU DISTRICT, ETHIOPIA" and Recommended that it be submitted as fulfilling the thesis requirement.

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Dedication

This work is sincerely dedicated to:

My beloved Father and mother for their Support and True love, as well as to my siblings!

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Abstract: The objectives of this research was to assess the concentration of some selected heavy metals (Fe, Zn, Mn, Co) and the proximate nutritional composition (moisture, ash, fat, fiber, protein and carbohydrate) of wheat. The samples were purchased from local farmers at Segenet, Kusaye and Mangudo kebele in Moretna Jiru district. The wheat sample were cleaned of foreign materials by washing it with distilled water and then dried, powdered and sieved. The wheat samples were packaged in plastic containers prior to analyses. The samples were digested and analyzed for their level of Fe, Zn Co & Mn by using ICP-OES and their proximate nutritional compositions by using AOAC methods (AOAC,2000). The results showed that the specific amount of metals for SW wheat 65.653mg/L,9.669mg/L,1.506mg/L, and 0.111mg/L to Fe, Zn, Mn, & Co respectively; for KW wheat <0.013mg/L to Fe and Zn, 0.021mg/L and 0.024mg/L to Mn and Co respectively; and for MW wheat <0.013mg/L to Fe and Zn, 0.021mg/L and 0.024mg/L to Mn and Co respectively. In proximate compositions; the moisture content of SW>KW>MW; the ash content of SW>KW>MW; the fat content of MW>SW>KW; the crude fiber content of; the crude protein content of MW>SW>KW and the carbohydrate content of KW>SW>MW.

Keywords: Wheat, ICP-OES, Heavy Me Metals, Nutritional composition

Chapter One

1 Introduction

1.1 Back ground of the study

Today, wheat is a dominant cereal crop in the world (Davidson and Passmor, 1986). Total world wheat production is about 250 grams per person per day. It nourishes more people than any other type of cereal grains. Wheat production in Sweden has varied between 1.55 and 2.28 million metric tons during 1995-2003 and about 1/3 of the Swedish wheat production is used for human consumption (FAO data base, 2004). Ethiopia is among is the top three wheat producers in Africa, with wheat accounting for 20% of the nation's total cereal production. The nation has more than 600 small and large flour mills, with a total production capacity of 4.2 million tonnes per year (Susan Reidy, 2019). Examples of wheat food products are bread, pasta, macaroni, crackers, spaghetti etc. Bread is an important product of wheat flour and part of daily food consumption in many parts of the world. It is an important source of carbohydrates, proteins, minerals and antioxidants. Consumers demand for quality food is continuously increasing and thereby also the need of information about nutritional aspects. Evaluation of the quality of wheat includes functional properties for bread making and nutritional composition. About 70% of consumers use food choice to demonstrate their attitude (Hansen et al., 2001). In the Nordic countries 50% of the consumers are conscious about ethical issues, environmental problems and animal welfare. Reasons of buying organic food are related to health security and environmental safety (Meier-Ploeger, 2005). Nutritional composition of bread might influence health of humans. The main factors that influence the nutritive value of wheat include: genetics, environment (soil type, structure and microorganisms; climate, management practices) and post-harvest practices (harvest time, handling and processing method) (Hornick, 1992).

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 (Abd-El-Haleem et al., 1998; Adams et al., 2002; Shewry et al., 2009). Wheat flour is used to prepare bread, produce biscuits, confectionary products, noodles and vital wheat gluten or seitan (FAO, 2010). Many people like wheat-based products because of the taste, and particularly the texture.

The nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources. The importance of wheat is mainly due to the fact that its seed can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pastas, and thus it presents the main source of nutrients to the most of the world population (Lindsay 2002; Welch and Graham 2002).

Wheat grains are generally oval shaped, although different wheats have grains that range from almost spherical to long, narrow and flattened shapes. The grain is usually between 5 and 9mm in length, weighs between 35 and 50mg and has a crease down one side where it was originally connected to the wheat flower. The wheat grain contains 2-3%germ, 13-17% bran and 80-85% mealy endosperm (all constituents converted to a dry matter basis) (Belderok et al., 2000).

Wheat was originated in Southwest Asia, Tigris and Euphrates river valley, in the area known as the Fertile Crescent (Smith and Wayne, 1995). Sowing of grains from wild grasses, cultivation and repeated harvesting led to domestication of wheat. Selection of mutant forms with tough ears which remained intact during harvesting, larger grains, and a tendency for the spikelets to stay on the stalk until harvested was the set-off of modern agriculture (Dubcovsky et al., 1997). Bread wheat (Triticum aestivum) produces one-seeded fruits, which are called grains or kernels (Hoseney, 1994). The pleasant flavor, long shelf-life and unique gluten-forming characteristics of wheat products like pasta, noodles, bread, chapatti etc. make them very attractive among other cereals (Nelson, 1985).

Wheat is considered a good source of protein, minerals, B-group vitamins and dietary fiber [Simmonds DH, 1989, Shewry PR, 2007] although the environmental conditions can affect nutritional composition of wheat grains with its essential coating of bran, vitamins and minerals; it is an excellent health-building food. Wheat is used to prepare bread, produce biscuits, confectionary products, noodles and vital wheat gluten or seitan. Wheat is also used as animal feed, for ethanol production, brewing of wheat beer, wheat based raw material for cosmetics, wheat protein in meat substitutes and to make wheat straw composites. Wheat germ and wheat bran can be a good source of dietary fiber helping in the prevention and treatment of some digestive disorders [Simmonds DH, 1989]. The antioxidant activity and phytochemical content were studied in milled grain of eleven varieties which included a range of red and white wheat and durum wheat. Whole-wheat bread is good for health. There is no doubt that the adaptability and high yields of

wheat have contributed to its success, but these alone are not sufficient to account for its current dominance over much of the temperate world. The key characteristic, which has given it an advantage over other temperate crops, is the unique properties of dough formed from wheat flours, which allow it to be processed into a range of breads and other baked products (including cakes and biscuits), pasta and noodles, and other processed foods. These properties depend on the structures and interactions of the grain storage proteins, which together form the 'gluten' protein fraction. Lutein is the predominant carotenoids present in wheat (Abdel-Aal ESM,etal 1993) and the bran/germ fractions of wheat contained greater amounts of carotenoids and antioxidant activity than the endosperm fractions (Alan JB, etal 2000).

The awareness about the safety of food is increasing in several parts of the world. Many chemical compounds, such as acrylamide, pesticides, nitrosamines, and heavy metals, are considered as toxic contaminants when they occur at certain levels in the food (Rather, etal,2017). Many studies have shown that heavy metals have toxic effects even at very low concentrations. Heavy metals are natural components of the Earth's crust and cannot be degraded nor destroyed (McIntyre, T. 2003). They enter the human body through food, water and air. The consequence of heavy metal pollution can be hazardous to man through his food. Therefore, it is important to monitor heavy metals in aquatic environments such as water, sediment and biota (-Doiphode, V. V. 2002, Gogtay, etal,2002, Yang, etal,2002, Ernst, E ,2002, Chronopoulos etal,1997). Some elements including arsenic, cadmium and chromium are carcinogenic. Others, such as lead and mercury have a health implication and been associated with developmental abnormalities including autism in children when their levels in food proceed the acceptable (Lane, T.W,2005, Kim, K.R, etal,2010). The problem of heavy metals in food is rather complicated since their levels in food depend on several factors ranging from environmental conditions to the methods of production and processing. Plant might absorb heavy metals from soil, water or air. Food might be easily contaminated during processing. High metals' concentration like lead (Pb), cadmium (Cd), copper (Cu) and nickel (Ni) in food have been correlated with the metal pollution in soil, air and water. Heavy metals are dangerous in their form of captions and highly toxic when bonded to their short chains of carbon atoms. Therefore, controlling the heavy metal concentration in food like cereals, fish and vegetables should be made to ensure their safety (Piccolo, A. 2003, Allafaji, S.H. 2012). Heavy metals are ubiquitous; therefore, they tend to bio-accumulate and, hence, cause an increase in their concentration in a biological system. The interested heavy metals include lead, Arsenic, cadmium,

copper and nickel. The levels of the interested metals in the selected food will be interpreted to determine the toxicity of such heavy metals according to the international regulations and recommendations like codex alimentarius commission (Food and Agriculture ,1985) and European commission regulations (Kabelitz, L., & Sievers, H. 2004).

In current perspectives of environmental pollution, heavy metal contamination of the environment is most burning issue for pollution control agencies. Rapid industrialization and urbanization leads to a constant and rapid input of these heavy metals in the surrounding environment of human concern. Role of heavy metals in plant and human biology is now quite understood. Trace metals such as copper and zinc are classified as essential to life due to their involvement in certain physiological process. Elevated level of these metals has however been found to be toxic [Pretoria, 2nd ed. (1) 1996]. The criteria for essentiality of elements for plant growth and development were first established by Arnon and Scott & Meyer and Anderson & Bennett [D.I. Arno,1939; D. Van Nostrand, 1939, Minnesota, 1993].

Plants are considered as intermediate reservoirs through which heavy metals/ elements/ nutrients are transferred from soil to other organisms via a food chain. Several heavy metals are toxic to human beings [K. Tsuchiya,1976; L. Friberg, etal,1983]. Although some of the heavy metals such as Zn, Mn, Ni, and Cu act as micronutrient at lower concentrations, they become toxic at higher concentrations [O. Rapheal, etal,2011]. The metals are not toxic as the condensed free elements but are dangerous in the form of cations and when bonded to short chains of carbon atoms [N.A. Ward,1995]. Potential toxicity of trace metals results from the fact that they are transitional elements able to form stable coordinated compounds with a range of both organic and inorganic ligands [J.E. Fergusson,1990] and [P.A. Spear,1981]. Many metals act as biological poisons even at parts of per billion (ppb) levels. The toxic elements accumulated in organic matter in soils are taken up by growing plants [S.S. Dara,1993]. In studying the heavy metal interrelationships in natural systems i.e. between soil, vegetation and animals, in general, there is a greater correlation for the certain elements (Cu and Zn) between soil and vegetation than between vegetation and animals or between soil and animals. Excellent reviews on the food chain or soil plant animal aspects of heavy metals have been conducted by Underwood [E.J. Underwood,1977] and Nicholas and Egan [D.J.D. Nicholas,1975]. The toxicity of these metals may result in blocking the essential biological functional groups of the biomolecules, displacing the essential metal ion present in

biomolecules or and modifying the active confirmation of the biomolecules like polypeptides etc. The polypeptides store genetic information and their disruption can have serious results such as cancer or congenital deformation [B.L. Vallee,1972] and [T. Yamane,1961].

1.2 Statement of the problem

The wheat is the main staple food for most of the population; so it is the main source of trace and essential elements in addition to the major nutrients (B.S.Hetzel etal,1986; K.M.Hambidge ,1986). The food due to environment pollution (L.Freberg etal, 1985) may also contain certain toxic elements for which body has a tolerance level and above this level these may adversely affect the biological system (H.W Numberg,1985;E.Friden,1984). In this area there is no industrial site but wheat food may toxic due to the type of fertilizers used and other farming conditions. Therefore, it is necessary to assess the adequacy and safety of common diet to take daily such as wheat by monitoring the concentration of essential and toxic elements (heavy metals); percent of moisture, fiber, ash, fat, protein and carbohydrate; and to establish their base line level.

This study was aimed to assess the safety of human food diet that is consumed by people. It is therefore important to determine the concentration of the selected heavy metals and the percent of moisture, fiber, ash, fat, protein and carbohydrate in wheat in order to establish a base line level of these elements and nutrients. In order to carry out these objectives, the research tried to identify and find answers for the following questions;

- ✓ Which heavy metals are present in Moretna-Jiru wheat?
- ✓ What are the moisture, ash, protein, carbohydrate, fat and fiber content of wheat present in Moretna-Jiru?
- ✓ What are the concentration of heavy metals present in wheat?

1.3 OBJECTIVE OF THE STUDY

1.3.1 General objective

The general objective of this study was to assess some selected heavy metals and nutritional composition of wheat in Moretna-Jiru district.

1.3.2 Specific objective

The specific objective of this research was:

- To determine some selected heavy metals such as Fe, Zn, Mn, and Co in wheat found in Moretna-Jiru.
- To determine the quantity of some selected heavy metals (Fe, Zn, Mn, Co) of wheat in Moretna Jiru.
- To determine the percentage composition of ash, dietary fiber, carbohydrate, protein, moisture content and lipid present in wheat of Moretna Jiru.
- To compare the concentration of Fe, Zn, Mn and Co of wheat with others reported result
- To compare the proximate composition of ash, dietary fiber, carbohydrate, protein, moisture and fat of wheat with others reported result.

1.4 Scope and Limitation of the study

The level of heavy metals and proximate composition in cereal crop samples (wheat) collected from Moretna-jiru, (Segenet, Kusaye and Mangudo) districts were carried out using atomic absorption spectrophotometer ICP-OES. The study was particularly focused on wheat as these sampling sites are main traffic density and more crop areas to Debre Berhan city and surrounding north Shewa Amhara. The results were analyzed and compared for selective determined heavy metal concentration among wheat sample.

However, to obtain detailed information and draw strong conclusions on the level of heavy metals and nutritional composition in these cereal crops may need collection of samples from all parts of the country. In addition, may also need other additional different techniques used to check accuracy of the result. But this study was delimited to Moretna-jiru north Shewa zone Amhara regional state of Ethiopia. This is due to financial and time problems.

1.5 SIGNIFICANT OF THE STUDY:

The aim of this study was to determine the levels of Mn, Fe, Co and Zn in wheat and to obtain information about proximate nutritional composition accumulation of wheat in Moretna-Jiru. The significance of studying the type and quantity of heavy metals and nutritional compositions exist in wheat was to investigate the positive and negative impact of these heavy metals for different consumers like human beings and animals, to protect the toxic effect of some heavy metals, to protect nutritional deficiency diseases by feeding wheat.

Chapter Two

2 Review literature

2.1 Wheat (Triticum aestivum)

Wheat domestication started 10,000 years ago with cultivation of einkorn, a diploid wheat with seven chromosome pairs. Later tetraploid wheats known as emmer and durum evolved (Heun et al., 1997). Common wheat, which is used to make most of the wide variety of products today, is hexaploid. Three species of wheat are grown today, common or bread wheat (Triticum aestivum), Durum wheat (Triticum durum) and spelt wheat (Triticum spelta). In USA and Canada, the common or bread wheat (Triticum aestivum), form five classes: hard red winter, hard red spring, soft red winter, hard white and soft white. Different wheat classes show variation in nutritional characteristics and uses (Table 1). Durum wheat (Triticum durum) includes today, the durum and red durum wheat classes, and spelt wheat (Triticum spelta). Currently about 4000 wheat cultivars are grown all over the world (Posner, 2000). In the marketing year of 2019/2020, the global production volume of wheat amounted to over 765 million metric tons. This was an increase of over 30 million tons compared to the previous marketing year (M. Shahbandeh, Jan 20, 2021).

Table 1: Wheat classes, their general characteristics and uses (Atwell, 2001).

Class	General Characteristics	General uses
Hard red winter (HRW)	High protein, strong gluten, high water absorption	Bread and related products
Soft red winter (SRW)	Low protein, weak gluten, low water absorption	Cakes, cookies, pastries, pie crusts, crackers, biscuits
Hard red spring (HRS)	Very high protein, strong gluten, high water absorption	Bread, bagels, pretzels and related products
Hard white	High protein, strong gluten, high water absorption, bran lacks pigment	Bread and related products
Soft white	High protein, strong gluten, high water absorption	Noodles, crackers, wafers and other products in which specks are undesirable
Durum	High protein, strong gluten, high water absorption	Pasta

2.2 Nutritional Contents of wheat

The nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources. The importance of wheat is mainly due to the fact that its seed can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pastas, and thus it presents the main source of nutrients to the most of the world population.

A huge increase in demand for cereals is predicted if the food needs for the estimated world population growth are to be met. But there is another potentially great benefit to these communities and that is the possibility to ensure such staple crops are nutritionally-balanced and help remove the millions of cases of nutritionally-related deficiency disease that afflict them. It should be

emphasized that in the past there has not been a single instance where plants have been bred to improve their nutritional content. If this has occurred, it is purely by accident not design (Lindsay 2002; Welch and Graham 2002).

Over three billion people are currently micronutrient (i.e. micronutrient elements and vitamins) malnourished. 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 (Welch 2005). One sustainable agricultural approach to reducing micronutrient malnutrition among people at highest risk (i.e. resourcepoor women, infants and children) globally is to enrich major staple food crops with micronutrients through plant-breeding strategies. Available research has demonstrated that micronutrient-enrichment traits are available within the genome of wheat (as well as other food crops) that could allow for substantial increases in the levels of minerals, vitamins and other nutrients and health-promoting factors without negatively impacting crop yield. Importantly, micronutrient bioavailability issues must be addressed when using a plantbreeding approach to eliminate micronutrient malnutrition. Enhancing substances (e.g. ascorbic acid, S-containing amino acids, etc) that promote micronutrient bioavailability or decreasing antinutrient substances (e.g. phytate, polyphenolics, etc) that inhibit micronutrient bioavailability, are both options that could be pursued in breeding programs (Welch 2002; Welch and Graham 2004; Welch 2005).

Grain anatomy

The fruits of most plants contain one or more seeds, which, at ripeness, can be easily separated from rest of the fruit tissue. For Germineae this is different: fruit wall (pericarp) and seed coat are united. As a result, the seed and fruit cannot be separated. This type of fruit, which is characteristic for all grasses, including cereals, is given the botanical term of caryopsis.

Wheat grains are generally oval shaped, although different wheats have grains that range from almost spherical to long, narrow and flattened shapes. The grain is usually between 5 and 9mm in length, weighs between 35 and 50mg and has a crease down one side where it was originally connected to the wheat flower. The wheat grain contains 2-3% germ, 13-17% bran and 80-85% mealy endosperm (all constituents converted to a dry matter basis) (Belderok et al., 2000).

The bran (outer layers of wheat grain) is made up of several layers, which protect the main part of the grain. Bran is rich in B vitamins and minerals; it is separated from the starchy endosperm during the first stage of milling. In order to protect the grain and endosperm material, the bran comprises water-insoluble fibre. More than half the bran consists of fibre components (53%). Chemical composition of wheat bran fibre is complex, but it contains, essentially, cellulose and pentosans, polymers based on xylose and arabinose, which are tightly bound to proteins. These substances are typical polymers present in the cell walls of wheat and layers of cells such as aleurone layer. Proteins and carbohydrates each represent 16% of total dry matter of bran. The mineral content is rather high (7,2%). The two external layers of the grain (pericarp and seed coat) are made up of dead empty cells. The cells of the inner bran layer- aleurone layer are filled with living protoplasts. This explains the rather high levels of protein and carbohydrate in the bran. There are large differences between the levels of certain amino acids in the aleurone layer and those in flour. Glutamine and proline levels are only about one half, while arginine is treble and alanine, asparagine, glycine, histidine and lysine are double those in wheat flour (Cornell 2003).

The endosperm is surrounded by the fused pericarp and seed coat. The outer endosperm, the aleurone layer, has a special structure: it consists of single layer of cubic shaped cells. The aleurone layer is rich in proteins and enzymes, which play a vital role in the germination process. The inner endosperm, i.e. the endosperm without the aleurone layer, is referred to as mealy or starchy endosperm. The endosperm mainly contains food reserves, which are needed for growth of the seedling, it is rich in energy-yielding starch. Apart from carbohydrates, the mealy endosperm contains fats (1,5%) and proteins (13%): albumins, glubulins and the major proteins of the gluten complex- glutenins and gliadins - proteins that will form the gluten at dough making. The contents of minerals (ash) and of dietary fibers are low; 0,5% and 1,5%, respectively (Belderok et al., 2000).

The germ lies at one end of the grain. It is rich in proteins (25%) and lipids (8-13%). The mineral level is also rather high (4,5%). Wheat germ is available as a separate entity because it is an important source of vitamin E. Wheat germ has only one half the glutamine and proline of flour, but the levels of alanine, arginine, asparagine, glycine, lysine and threonine are double (Cornell 2003).

Globally, there is no doubt that the number of people who rely on wheat for a substantial part of their diet amounts to several billions. Therefore, the nutritional importance of wheat proteins

should not be underestimated, particularly in less developed countries where bread, noodles and other products (e.g. bulgar, couscous) may provide a substantial proportion of the diet. Wheat provides nearly 55% of carbohydrate and 20% of the food calories. It contains carbohydrate 78.10%, protein 14.70%, fat 2.10%, minerals 2.10% and considerable proportions of vitamins (thiamine and Vitamin-B) and minerals (zinc, iron). Wheat is also a good source of traces minerals like selenium and magnesium, nutrients essential to good health [Adams ML,2002; Fraley RT, 2003; Shewry PR,2006; Topping D, 2006]. Wheat grain precisely known as caryopsis consists of the pericarp or fruit and the true seed. In the endosperm of the seed, about 72% of the protein is stored, which forms 8-15% of total protein per grain weight. Wheat grains are also rich in pantothenic acid, riboflavin and some minerals, sugars etc. The barn, which consists of pericarp testa and aleurone, is also a dietary source for fiber, potassium, phosphorus, magnesium, calcium, and niacin in small quantities.

The kernel of wheat is a storehouse of nutrients essential to the human diet. Endosperm is about 83% of the kernel weight; it is the source of white flour. The endosperm contains the greatest share of the protein in the whole kernel, carbohydrates, iron as well as many B-complex vitamins, such as riboflavin, niacin, and thiamine. Bran is about 14.5% of the kernel weight [Drankham K,etal 2003; Shewry PR, Jones etal 2005; Uauy C, 2006]. Bran is included in whole-wheat flour and is available separately. Of the nutrients in whole wheat, the bran contains a small amount of protein, larger quantities of the B-complex vitamins listed above, trace minerals, and indigestible cellulose material called dietary flour. Wheat germ is the embryo of the wheat kernel. The germ or embryo of the wheat is relatively rich in protein, fat and several of the B-vitamins [Adams ML,etal 2002].

2.2.1 Proteins

Dietary protein performs mainly the three functions of nutrients such as growth, maintenance and repair of body tissues. It regulates the key processes within the body, it is required for the maintenance of appropriate pH, it helps in detoxifying action and only excess protein can be used as a source of energy; one gram of protein provides 4 kilocalories (Saradha, 2010). Proteins are broken down by the body into their constituent amino acids which are then used for rebuilding protein structures in the body. Their most notable use is in muscle repair (Cawley and Haworth, 2015).

Proteins are an important source of energy and an essential component of our diet. Proteins from different foods in our diet contain a number of amino acids. There are 22 amino acids, divided into essential and non-essential amino acids. Essential amino acids must be included in our diet since they cannot be synthesized within the body. Nutritional value of the food depends on proteins composition and upon their specific amino acid composition (Friedman, 1996). Wheat is an important source of proteins since large amounts of wheat is often included in the diet, and wheat contains 8-20% proteins. Wheat proteins are classified into several groups on the basis of their solubility properties, genetic background and amino acid composition etc (Loponen et al., 2004). The most well-known classification system classifies the wheat proteins into albumins, globulins, gliadins and glutenins on the basis of solubility (Osborne, 1924) (Table 2).

Table 2. The systematics of wheat proteins is classified into groups on the basis of their solubility by Osborne (1924).

Proteins		Soluble in	Location in
Non-gluten protein	Albumins	Water	Embryo (metabolic proteins) and endosperm cells (cytoplasmic proteins)
	Globulins	Dilute salt solutions (0.5 M NaCl)	Embryo and aleurone layer (storage proteins) and endosperm cells (cytoplasmic proteins)
Gluten proteins	Gliadins	70-80% ethanol	Endosperm (storage proteins)
	Glutenins	Dilute acids or alkali solutions (0.05 M acetic acid)	

Albumins are the smallest wheat proteins, followed in size by globulins. The separation of albumins and globulins turned out to be not as clear as initially suggested by Osborne. Gliadins and glutenins are complicated high-molecular weight proteins. Most of physiologically active proteins (enzymes) in wheat grains are found in the albumin and globulin groups. In cereals, the

albumins and globulins are concentrated in the seed coats, the aleurone cells and the germ, with a somewhat lower concentration in the mealy endosperm. The albumin and globulin fraction cover about 25% of the total grain proteins (Belderok et al., 2000).

Gliadins and glutenins are storage proteins and cover about 75% of the total protein content. The wheat plant stores proteins in this form for future use by the seedling. Gliadins and glutenins are mainly located in the in the mealy endosperm and are not found in the seed coat layers nor in the germ. Storage proteins in wheat are unique because they are technologically active. They have no enzyme activity, but they have a function in the formation of dough as they retain gas, producing spongy baked products (Belderok et al., 2000).

2.2.2 Carbohydrates

Carbohydrates are the most abundant and diverse class of organic compounds occurring in nature. Chemically they are composed of carbon, hydrogen, and oxygen in the ratio Cn: H2n: On (Herrero et al., 2010). They present in the diet are an important source of energy in the human diet with intakes ranging from 40 to 80 % of total energy requirements. They are necessary for the metabolism of other nutrients (Herrero et al., 2010). Other important effects of carbohydrates on human physiology are satiety and gastric emptying, control of blood glucose, insulin metabolism and serum cholesterol, and influencing colonic microflora and gastrointestinal processes such laxation and fermentation (Muir et al. 2009).

From the food which we eat wheat provides carbohydrate which is an important source of energy and its standard differs from a country to a country. The Nigerian regulatory standards for carbohydrate are 48%(SON, 2004), according to Ayub et al., 2003, Pakistan standard of carbohydrate are from 45 - 58%, For USA percent of carbohydrate are 50%(World book of encyclopedia, 1992), according to Wisal et al., 2013 carbohydrates contents of bread are 86.38%.

Starch polysaccharides

Cereal grains store energy in the form of starch. The amount of starch contained in a wheat grain may vary between 60% and 75% of the total dry weight of the grain. Starch occur in seed in the form of granules. Wheat has two types of starch granules: large (25-40 um) lenticular and small

(5-10um) spherical ones. The lenticular granules are formed during the first 15 days after pollination. The small granules, representing about 88% of the total of granules, appear 10-30 days after pollination (Belderok et al 2000).

Starch is basically a polymer of glucose. Chemically, at least two types of polymers are distinguishable: amylose and amylopectin. The molecular weight of amylose is around 250.000 (1500 glucose molecules) but varies widely. Amylose is a mostly linear α-(1,4) linked glucose polymer with a degree of polymerization (DP) of 1,000–5,000 glucose units. The structure of this polymer was assumed to be mainly linear, but this appears to be true for only part of the amylose, the remainder is slightly branched.

Amylopectin is branched to a much greater extent than amylose. So much that, on the average, the unit chain in amylopectin is only 20-25 glucose molecules long. Amylopectin has a molecular weight of about 108. The ratio of amylose to amylopectin is relatively constant, at about 23. Amylopectin is a much larger glucose polymer (DP 105–106) in which α-(1,4) linked glucose polymers are connected by 5–6% α-(1,6)-linkages. Normal wheat starch typically contains 20–30% amylose and 70–80% amylopectin (Konik-Rose et al., 2007).

Starch is the most important polysaccharide and found in abundance in many plants. Starch is also a major component of wheat grain and present in its endosperm. Wheat grain contains about 63-66% of starch, figures being higher for soft wheats than for hard wheats (Toepfer et al., 1972). The major components of starch are amylose and amylopectin. The contents of amylose and amylopectin are significantly different among varieties of cereals. The ratio of amylose and amylopectin differs among starches. The level of amylose and amylopectin in wheat flour is 25–28 and 72–75%, respectively (Shibanuma et al., 1994). However, some mutant wheat genotypes as well as maize, barley, rice etc. contain either increasing amylose content or increasing amylopectin content (Kiribuchi-Otobe et al., 1997).

Properties of wheat starch

Starches have different distribution of the granule size among crops. The largest granule size of starch is about 50μm and the smallest 2μm. Wheat starch granules show concentric shells under scanning electron microscopy when treated with enzymes (French, 1984). The lipid content of wheat starch is roughly proportional to the surface areas of the fractions, thereby strongly

suggesting that the lipid is concentrated near the surface of the granules (Whattham and Cornell, 1991).

Uses of starch

Starch is important in homes and industrial uses. In the food industry its pasting properties are utilized for gravies, soups, custards and desserts of various types. Wheat, corn and potato starches and their derivatives are commonly used. These derivatives have special properties, such as starch ethers and esters (e. g. phosphates) giving better clarity and stability to the product (Englyst and Hudson, 1997). Baked products like biscuits and cakes etc have starch in them. The addition of starch to flours with less protein, improves the lightness of texture required in baked goods such as sponges and pastry. Syrups from starch are used in the confectionery and brewing industries. Its adhesive properties provide low cost bonding to make cardboard boxes. Starch and its modified forms are also used for the sizing of paper and fabrics (Cauvain, 2003).

Non-starch polysaccharides:

Cellulose

Cellulose contents in wheat are about 3% (Toepfer et al., 1972). It is a β -1,4' glycan, also called an equatorial group when the oxygen attached to carbon 1 is more in the plane of the ring. The structure brings considerable difference to the physical and chemical properties of the polymer. Cellulose is more fibrous than starch and α –amylase has no activity on it. Cell walls of the lignified bran layers have more amounts of cellulose (Matz, 1991). Cellulose contents of white flour are less than 1%, but other nonstarch polysaccharides are also present to the extent of about 3%. Wheat bran contains more percentage of non-starch materials, normally about 9% cellulose and 29% of other non-starch polysaccharides (Fincher and Stone, 1986). Thus, whole meal flour is a better source of these polysaccharides than is white flour.

Pentosan

Pentosan is considered as the main component of the non-starch polysaccharides in cereal. Pentosan in wheat dominantly are comprised of pentosan sugar that is L- arabinose and D-xylose. Pentosan are normally called hemicelluloses and play an important role in food absorption by

decreasing absorption of lipid and cholesterol, therefore pentosan is very useful in human diet (Mohammadkhani, 2005; Lineback and Rasper, 1988).

Wheat grain pentosanes contribute to water absorption of flour and viscosity of doughs. In bread making industry loaf volume, improved crumb and crust characteristics are due to pentosans, especially when treated with pentosanase enzymes (Higgins, 2002). Dough rheological characteristics and macaroni production processing are correlated with the amounts of pentosans in flour (Menger, 1976). Pentosan increase the dough extensibility in durum wheat and also have effect on time of dough development and dough viscosity (Jelaca and Hlynka, 1971; 1972).

2.2.3 Fiber content of wheat

Numerous studies (McKee and Latner 2000; Philippe et al., 2006; Weickert and Pfeiffer 2008; Rave et al., 2007) have demonstrated the beneficial effects of fiber consumption in protection against heart disease and cancer, normalization of blood lipids, regulation of glucose absorption and insulin secretion and prevention of constipation and diverticular disease. Dietary fiber is defined as lignin plus the polysaccharide components of plants which are indigestible by enzymes in the human gastrointestinal tract (Bermink, 1994). These components are typically divided into two categories. Soluble dietary fiber is those components that are soluble in water and includes pectic substances and hydrocolloids. Insoluble dietary fiber is those components that are insoluble in water and includes cellulose, hemicellulose and lignin. Whole grains are good sources of insoluble fiber. Arabinoxylans (AX) and (1→3), (1→4)-β-glucans are major components of wheat endosperm cell walls. Arabinoxylan (insoluble type of fibre) is considered to be an optimal substrate for fermentative generation of short-chain fatty acids (SCFAs)—in particular, of butyrate in the colon. Butyrate at high concentrations in the colon is hypothesized to improve bowel health and lower cancer risk by several possible mechanisms (Philippe et al., 2006).

The increasing awareness of the potential benefits of high-fibre diets has promoted a growing interest for the consumption of whole-grain breads and bran breads. Supplementation has been used to enhance fiber content of foods. Some fiber-fortified baked goods have been available for years. While supplementation has focused on cookies, crackers and other cerealbased products, enhancement of fiber content in snack foods, beverages, spices, imitation cheeses, sauces, frozen foods, canned meats, meat analogues and other foods has also been investigated (Hesser 1994).

Traditionally, fiber supplementation has focused on the use of milling by-products of cereal grains. All of the milling by-products of wheat, corn, sorghum and other grains, as well as the by-products from the wet milling of corn and wheat, have been investigated as possible fiber supplements (Matz 1991).

Dietary fiber is a composition of Non-Starch Polysaccharides, Resistant Starch and Lignin. It promotes one or more of the following beneficial physiological effects; laxation, reduction in blood cholesterol; or modulation of blood glucose (British Nutrition Foundation.2018).

Furthermore, it has the following additional uses to humans.

1. Fiber adds bulk to the diet and prevents constipation and increases transit time in the gut. (British Nutrition Foundation.2018)
2.Dietary fiber is known to be associated with reduced incidence of coronary heart disease. The mechanism of its action is attributed to its binding to bile salt and thus preventing its re absorption and in reducing cholesterol level in circulation (Morenga, Lisa Te; et al, 2019).

3. Fiber is also known to reduce blood glucose levels and is often recommended for the management of certain types of diabetes (Food and Nutrition Board 2008).

2.2.4 Fats and Oils

Oils and fats have always been part of human food, being essential for health (David and Klonoff, 2007). They are recognized as essential nutrients in both human and animal diets. Nutritionally, they are concentrated sources of energy (9 Cal/gram); provide essential fatty acids which are the building blocks for the hormones needed to regulate bodily systems; and are a carrier for the oil soluble vitamins A, D, E, and K. They also enhance the foods we eat by providing texture and mouth feel, imparting flavor, and contributing to the feeling of satiety after eating (Aziah et al., 2012). Fats, like protein and carbohydrates, are a nutrient energy to the body. Fats are organic compounds that come in liquid or solid form. They are composed of saturated and unsaturated fatty acids. Saturated fats cause high levels of cholesterol, known as LDL, and can be found in animal products and vegetable oils. Unsaturated fats help to lower bad cholesterol, but still must be consumed in moderation because of their caloric content. Unsaturated fats are found in most liquid vegetable oils. (Kiki M, 2013) this shows what percentage of the sample is fat.

2.2.5 Ash content of wheat

The ash content is an inorganic residue remaining after the removal of water and organic matter by heating, which provides a measure of the total amount of minerals in wheat. It is the residue remaining all the moisture has been removed and organic matter has been burned away at 550°C. The ash content is lowest in the center of the kernel and increases towards the outer parts because the bran layer contains several times more minerals than does the pure endosperm (Fjell et al., 1996).

Ash is a measure of mineral content and is used to grade flour into different varieties. For example, whole wheat flour has a higher ash level than white flour. Ash in analytical chemistry, the compound that remains after a scientific sample is burned. The ash content shows just how much of the leaf sample is ash when burned at extreme temperature. The ash content is then calculated by dividing the weight of the ash sample by the weight of the sample before ashing. The weight is represented in grams (Moreland and Heil, 2011).

2.2.6 MOISTURE CONTENT

The moisture content is the loss in weight of a sample when heated under specified conditions. Wheat flour moisture is influenced by weather and environmental or storage conditions such as humidity and storage temperature. Such conditions affect the keeping quality of a flour. Higher moisture may lead to spoilage and lump formation during storage. Lower moisture content, on the other hand, cause loss to the baker in terms of low dry matter. (Brady Carter,2016)

2.2.7 Minerals

Wheat grain and its products are known to be important sources of minerals for man and livestock (Iskander and Murad, 1986). Mineral are vital components of plant metabolism and often stored in seeds (Peterson et al., 1983). For plant growth and development, minerals play an important role either as essential nutrients or through their effect on enzyme systems (El Gindy et al., 1957). In wheat grain 1.6 percent consists of minerals but the contents decrease to 0.4 percent after milling to white flour (Fujino et al., 1996). There is variability in mineral element contents among different parts of wheat grains. Except for amounts of C and O in grains, grain cortex contains mainly K, P

and Se while amounts of Cl, Si, S, Mg and Ca are low. The aleurone layer of wheat grains contain high amount of P, K and Mg while amounts of Si, Se, S, Ca, Cl and Fe are low. The concentration of minerals in the endosperm layer was low except for C and O.

Mineral composition of wheat grown under different environments revealed that there were differences in ash, K, Mn and Mg contents, while only minor differences in Fe, Zn, P and Cu contents were found. Organic and conventional farming were shown to have a great effect on mineral contents of wheat grain (Bourn and Prescott, 2002). The extractability of P and Mg was significantly higher in wheat varieties grown under inorganic conditions as compared with those grown under organic conditions. The reason for higher amount of P and Mg in inorganic conditions is high application of fertilizers. Correlation between P and Mg contents was found significant (Koivistoinen et al., 1974). Significant differences have also been found for the contents of Ca, Cu, Mn, P and Zn between cultivars grown organically and inorganically. Significantly higher contents of Cu and Mn was found in inorganically as compared to organically grown wheat (Punia and Khetarpaul, 2007; Ryan et al., 2004).

Heavy metals are chemical elements with a specific gravity that is at least five times that of water. The specific gravity of cadmium is 8.65, lead 11.34, arsenic 5.73, mercury 13.633, zinc 7.2, copper 8.89 (Lide, 1992). Heavy metals like copper and manganese occur naturally in plants. They could serve as plant nutrients depending on their concentrations. However, lead and many other heavy metals are directly distributed as a result of human activities which could be toxic even at low concentrations.

Plant parts take up these heavy metals absorbing them from airborne deposits in the part exposed to the air from polluted environment as well as contaminated soils through root systems (Elbagemi et al., 2012). These heavy metals can be accumulated in the shoots, fruits and roots of plants at low, medium and high levels. Heavy metals contamination of cereals may also occur due to their irrigation with contaminated water (AL jassir et al., 2005).

Additional sources of heavy metals for plants are rainfall in atmospheric polluted areas, traffic density, use of oil fossil fuels for heating, atmospheric dusts, plants protection agents and fertilizers which could be absorbed through leaves blades (Sobukola, 2008).

CHAPTER TREE

3 Materials and Methods

3.1 Apparatus and Instrument

The materials and apparatus used to study this research were:

Polyethylene plastic bags were used for collection of wheat samples. Drying oven (DHG-9070A, shanghi, china) and Muffle furnace were used for drying and ashing samples respectively. Mortar and pestle, Electric Blast Drier and 0.25mm stainless steel sieve were used to grind and homogenize, dried and sieved the powder wheat samples respectively. Electronic analytical balance (AAS-200DS Deriver instrument company, Germany) was used for weighting the samples.

Volumetric flasks (25ml, 50ml,100ml, 500ml, and 1000ml were used for preparing working standard solutions and dilute the final digested blanks, spiked samples, un spiked samples, and stock solution. Micro-pipette was used for measuring volumes of standard solutions. Funnel used for transfer solutions. Stirrer was used for stirring the solutions. ICP-OES is the instrument that used to analysis selected heavy metals in wheat samples. Other apparatuses used in the study includes; conical flask, watch glass, volumetric flasks, filter paper (what man #42), Desiccators, crucible, Soxhelet apparatus, distillation apparatus, reflux setup, digestion unit, distillation unit, titration unit, Erlenmeyer flask,and beakers.

3.2 Chemicals and Reagents

The chemicals and reagents used in the research are: 65% w/v HNO_3, hydrogen per oxide, deionized water, cotton, hexane, universal indicator, 1.25% H_2SO_4, 1.25% NaOH, conc.H_2SO_4, $CuSO_4$, K_2SO_4, 4% boric acid, 50%NaOH, $(NH_4)_2SO_4$, 0.1N HCl, methyl red indicator and 9.25% HCl.

3.3 Description of the study areas

The study areas are three different kebeles (Segenet, Kusaye and Mangudo) in Moretna-Jiru district Amhara administrative region located around Debre Berhan(North-Shewa) zone. Moretna-

Jiru distric lies between 10^{o} 09’ 60.00’’ N latitude and 39^{0} 00’ 0.00’’ E longitude. It is the major wheat producing district in Debre Berhan zone (Amhara Region censes 2007). Enewari is the administrative center of this district.

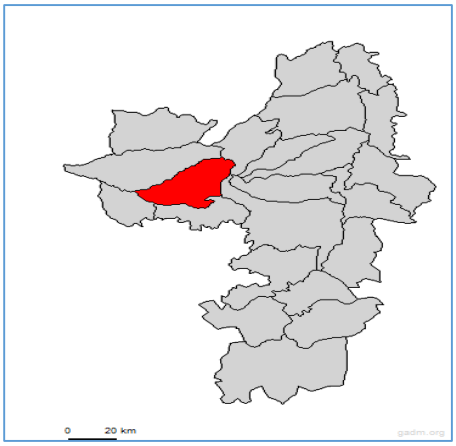

Figure 1: Location map of the studying area (the shaded part)

3.4 Sample Collection and Handling

Wheat samples which are grown in Moretna-Jiru were collected randomly at three different areas (Segenet, Kusaye and Mangudo). Three sub sampling sites were selected in each kebele based on the availability of the crop and the collected samples (about 20g) samples from each site were pooled together to form a composite sample of 60g for each kebele. Then, wheat grains were stoking in plastic containers and transported to Debre Berhan university analytical chemistry laboratory for analysis.

3.5 Sample Preparation and treatment

The freshly collected samples were washed with deionized water to eliminate visible dirt and removed the water quickly with a blotting paper. Then the samples were ground into small pieces using domestic grinder, homogenized and accurate amounts were weighed as required for different analysis.

3.6 Determination of Heavy Metals

The term heavy metal refers to any metallic element that has a relatively high density. Examples of heavy metals include Fe, Cu, Zn, Hg, Cd, Pb, Cr and As (Afshin and Farid ,2007). They were determined by analysis of the digested sample using ICP-OES.

3.6.1 Sample Digestion

The sample collected from Moretna –Jiru was washed well with distilled water and dried at $105c^0$ at 2hr in oven. The samples were grounded in a domestic grinder separately. 2g of grounded powder samples were weighted and transferred to a clean crucible, which is labeled according to the sample number and dry-ashing process was carried out in a muffle furnace by stepwise increase of temperature up to$550c^o$ and then left to ash at this temperature for 6hr. The samples were removed from the furnace and allowed to cool. The ash was wetted with distilled water and 2.5ml of concentrated HNO_3 was added. The crucible was covered with watch glass and placed on hot plate. The digestion performed at a temperature of 90 to $95c^o$ for 1hr. The ash was dissolved in 5ml of 9.25%HCl and digested again on hot plate until the white fumes ceased to exist and sample reached to 2ml. After cooling 20ml of distilled water was added and filtered using whatman filter #42. The filtered sample was then diluted up to the mark of 50ml standard volumetric flask and stored in polyethylene container until analysis. All samples were prepared identically in triplicates. Blanks were prepared to check for back ground contamination by the reagents used.

3.6.2 Preparation of standard solutions and Analysis of samples

All working standards were prepared by diluting stock standard solutions (1000ppm) of the metals to be analyzed. Determination of the metal concentration in experimental solution would be based on the calibration curve. Plotting the calibration curve stock solutions of 1000mg/L was prepared. The calibration standard solutions were used to calibrate instrument response with respect to analyte concentration followed standard procedure (USEPA, 2001). Calibration curves were prepared for each of the metals by running a range of concentration of freshly prepared standard solution in their respective linear ranges. In this study, for the linear dynamic range, the calibration

sample of Fe (iron), Zn (Zinc), Mn (Manganese), and Co (Cobalt) were prepared from their sock standard solutions containing 1000mg/L for each metal in 3N HNO_3. The ICP-OES working conditions are given in table 3.

Table 3: instrument operating conditions for the determination of selected heavy metals in wheat samples by ICP-OES.

Type of Instrument	Arcos _SOP
Plasma Power	1400 W
Pump Speed	30rpm
Coolant Flow	13 L/min
Auxiliary Flow	0.8 L/min
Nebulizer Flow	0.73 L/min
Optic Temperature	15.05 C^0 (14.0-16.0)
OSC. Exhaust	285.70 Imp/S (Min 170.0)
OSC Temperature	51.53 C^0 (40-70)
Osc Impedance	5365 Ohms
HVPS Current	607 m A
HVPS Voltage	3260v
Flow light tube	0.90 L/min (0.8-1.8)
Nebulizer Pressure	1.96 bar (2.0-4.0)
Main Argon Pressure	6.75 bar (6.0-8.0)

3.6.3 Instrumental Calibration

All working standards were prepared by diluting stock standard solutions (1000ppm) of the metals to be analyzed. Determination of the metal concentration in experimental solution would be based on the calibration curve. Plotting the calibration curve stock solutions of 1000mg/L was prepared. The calibration standard solutions were used to calibrate instrument response with respect to analyte concentration followed standard procedure (USEPA, 2001).

3.6.4 Precision and Accuracy

3.6.4.1 Precision

Precision means the values gathered from repeated measurements are close to each other in cluster, when the procedure will apply repeatedly to multiple aliquots of a single homogeneous volume of sample matrix. Precision of analytical procedure is usually expressed as a relative average deviation (RAD) of the three replicate results and spiked samples are subjected to the same digestion procedure like actual mass (Mengesha., 2008). The percentage relative average deviations (%RAD) of the samples were calculated as;

$$\%RAD=(AD/X)\ x100 \quad \text{----------1}$$

Where RAD= relative average deviation; X = mean; AD = average deviation

Relative average deviation was parameter of choice for expressing precision level should not exceed 15% of the relative average deviation (RAD).

3.6.4.2 Accuracy

Accuracy is an analytical method describes the values gathered from repeated measurements are close to the target (true value concentration of the analyte). Accuracy was determined by replicate analysis of samples containing known amounts of the analyte. Accuracy was expressed as matrix spike recovery and percent recovery results were calculated by the following equation (Javed et al., 2010). The spiked samples are then subjected to the same digestion procedure like actual sample.

$$\%R = \frac{(CM\ spiked - CM\ un\ spiked) \times 100}{Amount\ of\ Add} \quad \text{----------2}$$

Where CM = metal concentration

3.6.5 Method of Detection Limit (LOD) and Limit of Quantification (LOQ)

3.6.5.1 Method of Detection Limit (LOD)

Method of detection limit is a minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, and is determine from analysis of sample in a given matrix containing the analyte (Shrivasta and Gupta, 2011). The concentration at which we can decide whether an element is present or not. The limit of detection (LOD) is a measure of how sensitive the analytical method is and is the lowest concentration or weight of analyte that can be measured at a specific confidence level. For the determination of limit of detection of the analytical method (LOD), triplicate (n = 3) eight blanks were prepared in parallel and analyzed for their metal contents.

The standard deviation (SD) of the eight blanks was calculated and multiplied by three to determine the method detection limit (Boke et al., 2015). Hence, method of detection limit for each metal would be estimated by digesting eight replicates (n = 8) of method banks with the optimum procedure for wheat samples.

$$LOD = 3SDblank \quad \text{----------3}$$

3.6.5.2 Limit of Quantification (LOQ)

Limit of quantification is the lowest concentration of analyte that can be determined with acceptable level of uncertainty. The limit of quantification (LOQ) is the smallest quantity of analyte that can be measured with acceptable accuracy and precision and obtained from analysis of eight replicate blank which is digest, in the sample digestion procedure as actual samples. In this study limit of quantification will obtain from triplicate analysis of eight reagents blanks which are digests in the same digestion procedure as actual sample. Limit of Quantification is ten times of the standard deviation can be calculated;

$$LOQ = 10SDblank \quad \text{----------4}$$

3.6.6 Statistical Analysis

All the samples analysis in the study were carried out in triplicate and the results were reported as mean and standard deviation.

3.7 Determination of the nutritional composition of wheat

Nutritional composition parameters (carbohydrate, fat, protein, ash) of the wheat were determined using the Association of Official Analytical Chemists (AOAC, 2002). The nitrogen content of the sample was determined by micro Kjeldhal method. The nitrogen value obtained was multiplied by 5.7 to convert it to crude protein. The weight difference methods were used to determine moisture and ash content levels while crude fat of the wheat was determined using the AOAC procedure with petroleum ether or hexane as a solvent. The carbohydrate content was determined by calculation using the different method.

3.7.1 Determination of moisture content

The grinded wheat samples were weighed(2g) using analytical balance. The weights of the samples were recorded and given a code to identify it easily after drying in oven. The wheat samples were placed in the drying oven at $105C^0$ for 3hr and then cooled outside oven and reweighed.

Then, the moisture content was estimated by the formula (AOAC,2002):

$$\text{Moisture content (\%)} = \frac{(W1 - W2)\ x\ 100}{\text{Weight of fresh sample}};\ \text{----------}5$$

Where; W1 =weight of sample and dish before drying

W2 =weight of sample and dish after drying

3.7.2 Determination of ash content

The crucible which is used for the analysis was cleaned and dried at $130C^0$. It was removed from the oven and cooled in desiccators. The mass of the crucible was measured by analytical balance (M_1). About 2 grams of the sample was weighed in to crucible (M_2). The sample was then placed in furnace at $550C^0$ overnight. Then the crucible was removed from the furnace and placed in the desiccators. Finally, the mass was weighed (M_3).

%Ash can be calculated using the following formula (AOAC,2002):

$$Ash\ (\%) = \frac{M3-M1}{M2-M1} X100\% \text{ ----------6}$$

Where; M1 = weight of the dish

M2 = weight of fresh sample and dish

M3 = weight of ash and dish

3.7.3 Determination of crude fat content

Soxhelet extraction

The sample was grounded in to powder. About 5 grams of each sample was weighed with analytical balance. The sample was put in extraction thimble and closed with fat-free cotton. The thimble was inserted in to Soxhelet extractor. 300mL of the solvent (hexane) was poured in to solvent vessel. The extraction was carried out at a temperature of 110-130 c^0 for 6 hours. The solvent and the fat mixture was separated by simple distillation heating the solvent vessel until all the solvent was evaporated and condensed in the receiver. The recovered solvent was reused for subsequent extractions.

The fat in the vessel was transferred in to 50mL beaker and was heated until the solvent is evaporated. After evaporation of the solvent the beaker that contains the fat was weighed in analytical balance and the weight is recorded.

The fat content was computed according to the following formula (AOAC,2002).

$$\%Fat = \frac{(m2-m1)\,x100}{E} \text{ ----------7}$$

Where: m_1 is the weight of the dry empty beaker, m_2 is the weight of the beaker, containing the fat after evaporation of the solvent and E is the sample weight in grams.

3.7.4 Determination of Fiber content

Two grams of the sample was weighed using analytical balance and put in to a 250ml round bottom flask. Then 200ml of 1.25% H_2S0_4 was added and the mixture was boiled under reflux for 30minutes. The solution was filtered with whatman filter paper; the residue was rinsed thoroughly with hot water until it is no more acidic when tested using universal indicator.

The residue was transferred into a 250ml beaker and 200ml of 1.25% NaOH was added and boiled for 30 minutes in a digestion apparatus after which it was filtered and rinsed with distilled water until the filtrate was neutral when tested with universal indicator. The residue was transferred into a crucible and placed in electric oven at $100C^0$ for eight hours to dry. It was then removed and placed in desiccators to cool before weighing(W1). The crucible was transferred to a muffle furnace (Galenkamp, size 3) and incinerated for 30 minute at $550c^0$.

Afterwards, the sample was cooled again in desiccators and re-weighed (W2).

The crude fiber content was determined using equation(AOAC,2002):

$$\%\text{Crude fiber} = \frac{[(w1-w2)X100]}{w3} \text{----------8}$$

Where: W1 = weight of (Crucible + sample) after drying;

W2 = weight of (Crucible + sample) after ashing;

W3 = weight of fresh sample.

3.7.5 Determination of crude protein content

Kjeldahl method

This method is used for the quantitative determination of crude protein in all types of foods. Crude protein is total nitrogen multiplied by protein factor. The system consists of three units, namely Digestion unit, Distillation unit and Titration unit (The ASEAN Manual of Food Analysis, 2011 and AOAC,2002).

Digestion: is the decomposition of a sample into liquid form by treatment with enzymes or strong acids or alkalis.

2.5 g of each sample was Weighed and placed in digestion tube. 4.5 g of catalyst (a mixture of $CuSO_4$ and K_2SO_4) and 15mL of concentrated H_2SO_4 was added.

The digestion tube was placed in the digester. The mixture was digested initially at low temperature to prevent frothing and boiled briskly until the solution was cleared and freed of carbon or until oxidation is completed. Digestion was continued until a clear digest is obtained. It was heated for another hour after the liquid has become clear to complete breakdown of all organic matter.

Distillation: is the process of separating the components or substances from a liquid mixture by using selective boiling and condensation (Harwood & Moody, 1989).

A 250 mL Erlenmeyer flask was placed containing 50 mL of 4% boric acid with indicator as receiver on the distillation unit. 100 mL of water and 70 mL of 50% sodium hydroxide were added to the digest and started distillation. It was distilled until all ammonia had been released or approximately 150 mL distillate was obtained. By lowering the receiver flask so that the delivery tube was above the liquid surface and was continued the distillation for 2 minutes, the delivery tube was rinsed with water.

Titration: is a technique where a solution of known concentration is used to determine the concentration of an unknown. Typically, the titrant (the known solution) is added from a buret to a known quantity of the analyte (the unknown solution) until the reaction is complete. Knowing the volume of the titrant added allows the determination of the concentration of the unknown. Often an indicator is used to usually signal the end of the reaction, the end point (Whitney, W.D.; Smith, B.E., 2008)

The distillate was titrated with the standardized 0.1 N hydrochloric acid until the first appearance of the pink color. The volume of the acid was recorded (ASEAN, 2011 & AOAC, 2002).

$$\text{N (\%)} = \frac{(\text{vs}-\text{vb}) \text{ x N HCl x1.4}}{\text{Weight of sample}} \text{ ----------9}$$

Crude Protein (%) = % total nitrogen x appropriate nitrogen conversion factor(pf). pf=5.7

NB: N=normality of the acid used for titration; vs=volume of 0.1N HCl sample; vb=volume of 0.1N HCl blank

3.7.6 Determination of crude carbohydrate

Total carbohydrate content of the samples can be determined by subtraction of the tested parameters from 100% using the following formula(FAO/WHO,2002).

Total carbohydrates [%] =100- [% Protein+ fiber +% oil+ % Ash+%Moisture] ----------10

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Concentration of the selected Heavy metals in wheat

To analyse samples by the ICP-OES techniques, the calibration curve of each element must be determined first with good linear regression. Figures 2-5 show the obtained calibration curves by ICP-OES measurements of the interested metals. These elements include iron Fe, zinc Zn, manganese Mn and cobalt Co, respectively.

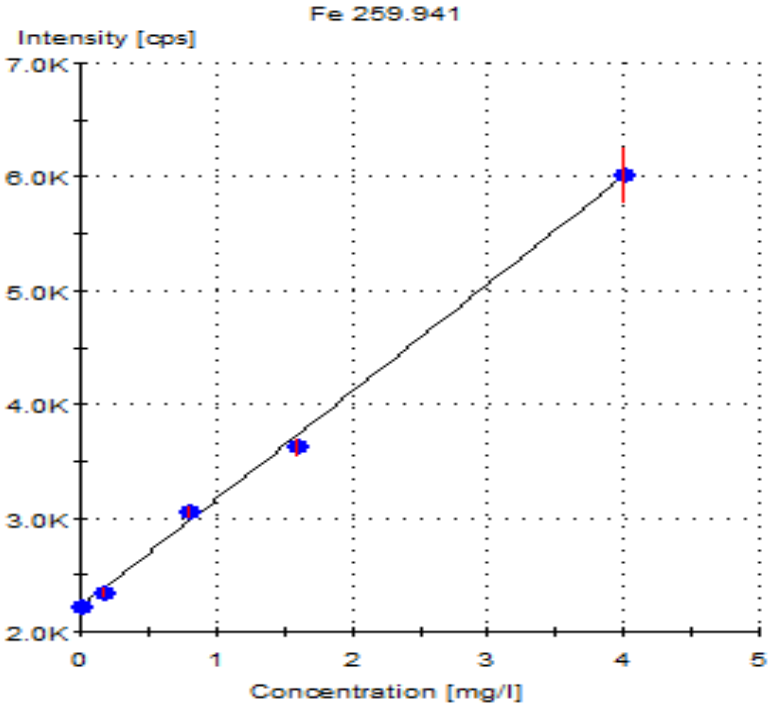

Figure 2: Intensity vs Concentration Calibration curve for iron metal

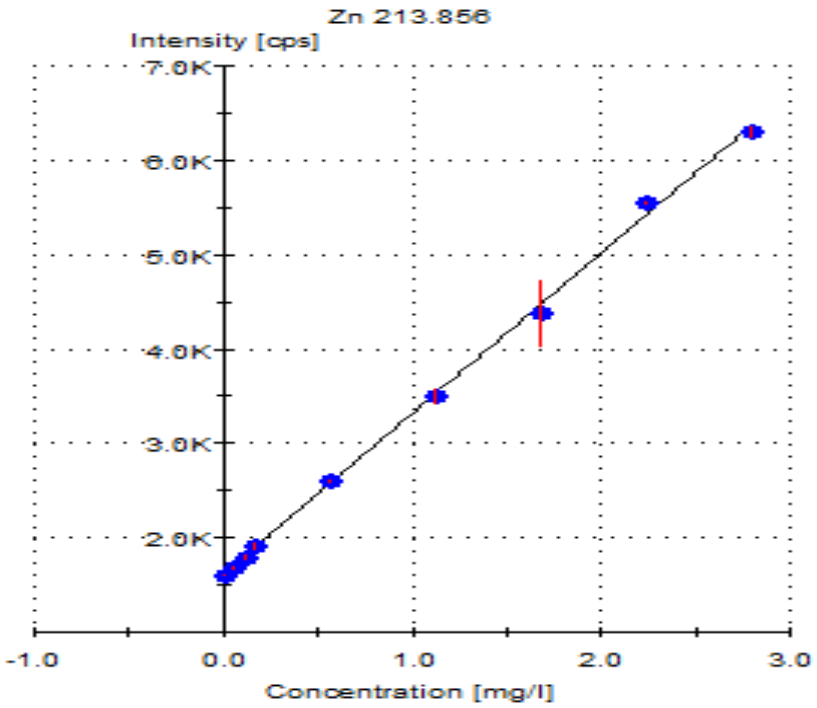

Figure 3: Intensity vs Concentration Calibration curve for zinc metal

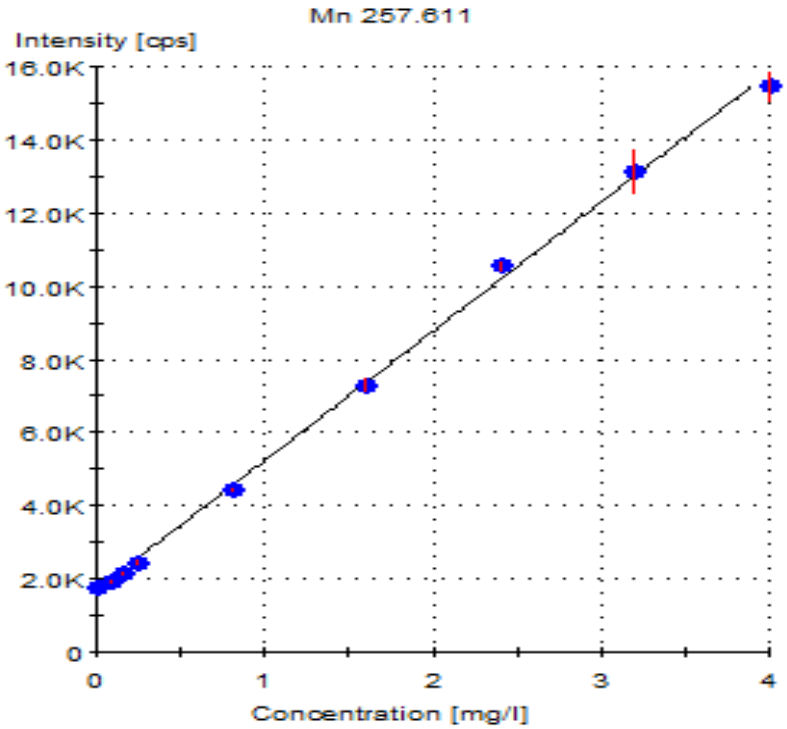

Figure 4: Intensity vs Concentration Calibration curve for manganese metal

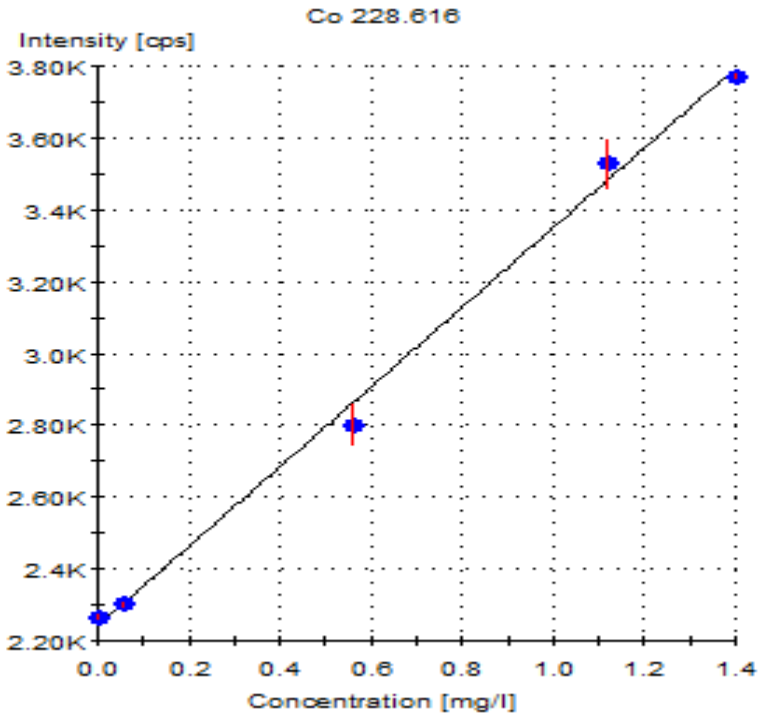

Figure 5: Intensity vs Concentration Calibration curve for cobalt metal

The correlation coefficient values that listed in Table 4 show the degree of linear association between the concentration of standards and absorption of the radiation. The Calibration Correlation factor on a regular base falls between 0.996 to1.00 for all elements as follow.

So that the accuracy and precision of our method was the R^2 of the Calibration, the spike test and the recovery of our control sample which is the concentration limit.

Table 4: The correlation coefficients of the obtained calibration curves from the ICP-OES measurements.

Element	Instrumental detection limit mg/kg	Wave length (nm)	R^2	Range
Fe	0.0018	259.941	0.9999	0.0018 - 4.8
Zn	0.0009	213.856	0.9993	0.0009 - 3.36
Mn	0.0002	257.611	0.9998	0.0002 - 4.8
Co	0.0025	228.616	0.9982	0.0025 - 3.36

4.1.1 Limit of Detection (LOD) and Limit of Quantification (LOQ)

In this study, LOD and LOQ for each metal were determined from analysis of triplicates of laboratory control samples (method of blank) which were digested in the same digestion procedure as actual samples. Therefore, eight blank samples (n = 8) were digested for wheat samples and the blank were analyzed for the contents of Fe, Zn, Mn, and Co by ICP-OES. The standard deviation for each element was calculated from blank measurement. Below Limit of Detection (LOD), the analyte is not detected and above limit of quantification it is measured. In present study, method of detection was determined from the standard deviation obtained from the eight blank samples.

Hence, LOD is 3SDblank and LOQ is 10SDblank. From (Table 5) the LOD values for all metals that were analyzed by ICP-OES wheat samples were ranged from 1.917-3.15 and LOQ values were also ranged from 6.39-10.5 and. Whereas IDL ranged from 0.0002-0.0025.

Table 5: Method detection limit, limit of quantification and instrument detection limit of wheat samples determined by using blank solutions

Elements	SD	LOD (ppm)	LOQ (ppm)	IDL (mg/kg)
Fe	1.05	3.15	10.5	0.0018
Zn	0.996	2.988	9.96	0.0009
Mn	1.05	3.15	10.5	0.0002
Co	0.639	1.917	6.39	0.0025

4.1.2 Accuracy and Precision of Analytical Results

The %RAD did not differ by more than 15% of the mean which indicate that the analytical method used was precise and reliable (Extreme Preparatory Chemistry,2008). In brief, accuracy measured by percentage of the recovery and precision is also measured by percentage of relative average deviation. In this study, triplicates of each sample was used to evaluate analytical method for accuracy and precision and the obtained percentage recovery varied from 87.25% to 123.20% in the three wheat samples which were almost in acceptable ranges (Javed et al., 2010). The %RAD

in present study did not differ by more than 10% of the mean which indicate that the analytical method used was also precise and reliable.

Table 6: Recovery test results for heavy metal determination in wheat sample (mean & SD, n= 3)

Description	Element	Un spike conc. mg/l		Spike conc. mg/l		Amount of added	Accuracy %R	Precision %RAD
		mean	SD	mean	SD			
Sample taken from Segenet	Fe	65.653	5.8120	145.540	9.6217	80	99.86	4.95
	Zn	9.669	0.2102	11.834	0.0766	2	108.25	0.467
	Mn	1.506	0.0593	3.625	0.0286	2	105.92	0.543
	Co	0.111	0.0057	2.467	0.0544	2	117.83	1.686
Sample taken from Kusaye	Fe	<0.013	---	1.7450	0.0894	2	87.25	3.78
	Zn	<0.013	---	2.4640	0.0032	2	123.20	0.284
	Mn	0.021	0.0072	2.4073	0.1209	2	120.37	3.863
	Co	<0.024	---	2.4508	0.0233	2	122.54	O.723
Sample taken from Mangudo	Fe	<0.013	---	1.980	0.0566	2	98.98	2.2
	Zn	<0.013	---	2.441	0.0110	2	122.07	0.34
	Mn	0.021	---	2.475	0.1335	2	122.70	3.636
	Co	<0.024	---	2.166	0.1098	2	108.32	3.892

Table 7: Shows the concentration of selected heavy metals (Fe, Zn, Co and Mn) in wheat using ICP-OES in mg/L

Description	Concentration	Elements			
		Mg/l	Mg/l	Mg/l	Mg/l
Sample taken from Segenet		Fe	Zn	Mn	Co
	Conc.1	71.920	9.896	1.438	0.105
	Conc.2	64.600	9.481	1.544	0.116
	Conc.3	60.440	9.630	1.537	0.111
	mean	65.653	9.669	1.506	0.111
Sample taken from Kusaye	SD	5.8120	0.2102	0.0593	0.0057
	Conc.1	<0.013	<0.013	0.018	<0.024
	Conc.2	<0.013	<0.013	0.015	<0.024
	Conc.3	<0.013	<0.013	0.029	<0.024
	mean	<0.013	<0.013	0.021	<0.024
Sample taken from Mangudo	SD	---	---	0.0072	----
	Conc.1	<0.013	<0.013	0.024	<0.024
	Conc.2	<0.013	<0.013	0.018	<0.024
	Conc.3	<0.013	<0.013	0.021	<0.024
	mean	<0.013	<0.013	0.021	<0.024
	SD	------	-----	------	-------

Iron (Fe)

Iron is s the second most abundant on the earth's crust. It is also an essential element in man and plays a vital role in the formation of hemoglobin, oxygen and electron transport inhuman body iron (Al-Khashman, 2012). Iron was found to have the highest concentration in Segent wheat sample analyzed (Table 7). The result obtained from Segenet wheat sample was 65.653mg/L and <0.013mg/L from Kusaye and Magudo wheat samples.

Zinc (Zn)

Zinc is an air borne pollutants so its majority accumulates above-earth crops. It is also an essential to all organisms and has an important role in metabolism, growth, development and general wellbeing. It is an essential cofactor for a large number of enzymes in the body. Zinc deficiency leads to coronary heart diseases and various metabolic disorders (Saraf and Samant, 2013). As can

be seen from (Table 7), the average concentration levels of zinc in at Segenet was 9.669mg/L and <0.013mg/L at Kusaye and Mangudo. But Segenet wheat sample contains high concentration of Zn than Kusaye and Mangudo. The average concentration Zn in Kusaye and Mangudo wheat samples was similar (<0.013mg/L) and its concentration was similar to iron in Kusaye and Mangudo wheat samples.

Manganese (Mn)

Manganese is essential element required for various biochemical processes. The kidney and liver are the main storage places for the manganese in the body. Mn is essential for the normal bone structure, reproduction and normal functioning of the central nervous system. Its deficiency causes reproductive failure in both male and female (Saraf and Samant, 2013). As can be seen from (Table 7), the average concentration levels of Manganese in at Segenet was 1.506mg/L and <0.021mg/L at Kusaye and Mangudo. But Segenet wheat sample contains high concentration of Mn than Kusaye and Mangudo. The average concentration Mn in Kusaye and Mangudo wheat samples was similar (<0.021mg/L).

Cobalt (Co)

Cobalt is an integral component of the vitamin B-12 molecules. It is required in the manufacture of red blood cells and preventing anemia. An excessive intake of cobalt may cause the over production of red blood cells (Winther, 2012). The average concentration of Cobalt in wheat sample at Segenet was 0.111mg/L and <0.024mg/L at Kusaye and Mangudo. Segenet wheat sample contains high concentration of Co than Kusaye and Mangudo wheat sample. The content of Co obtained in Kusaye and Mangudo wheat samples was similar.

4.1.3 Comparison of Heavy Metals in Present Study with other Reported Results

Table 8: Comparison of heavy metals in present study with reported literatures (mg/L)

Samples	Metals				Reference
	Fe	Zn	Mn	Co	
Wheat	10.64	8.54	7.67	1.72	Wadaje,2015
	3.45	1.88	3.55	1.32	E.D. Doe et al 2013
Barley	31.85	3.85	1.67	0.15	Wadaje,2015

Maize	0.4	0.66	0.34	0.13	Wadaje,2015
Wheat of Segenet	65.653	9.669	1.506	0.111	The present study
Wheat	425	100	500	50	FAW/WHO;2001
Wheat of Kusaye	<0.013	<0.013	0.021	<0.024	The present study
Wheat of Mangudo	<0.013	<0.013	0.021	<0.024	The present study

4.2 Nutritional Composition of Wheat (%)

4.2.1 *Moisture Content of Wheat* (mean ± SD, n=3)

Table 9; Shows the moisture content of wheat

sample	Moisture content (%)			
	T1	T2	T3	Mean± SD
SW	5.34	5.36	5.51	5.40 ± 0.148
KW	3.52	3.53	3.51	3.52 ± 0.0141
MS	3.51	3.53	3.51	3.516 ±0.0188

NB: Where T1= trial one; T2= trial two; T3=trial three

The moisture content of wheat collected from Segenet (SW) was the highest and was significantly different from KW and MW wheat sample. The moisture content of KW wheat (3.52%) and MW wheat (3.516 %) were lower than 5.540 % obtained for SW wheat sample. The moisture content of wheat flour was within the acceptable limit of not more than 10% for long term storage of flour [Singh, A. etal, 2005]. Moisture content of foods is influenced by type, variety and storage condition [Eshun, G 2012]. The low moisture content of wheat flour would enhance its storage stability by avoiding mould growth and other biochemical reactions [Singh, A. etal,2005]. The moisture content of wheat in this study was range from 3.516%-5.40% and which was quite lower than 7.75% reported by (Sui, Z. etal, 2006).

4.2.2 Ash Content of Wheat (mean ± SD, n=3)

Table 10: Shows the ash content of wheat (%)

Sample	Ash content (%)			
	T1	T2	T3	mean ± SD
SW	1	1.5	1.3	1.27 ± 0.251
KW	1	1.3	1.4	1.23 ± 0.208
MW	0.5	1.2	1.3	1.00 ± 0.436

The ash content of the wheat ranged between 1.00 and 1.27% (Table 4). The ash content for SW wheat in this study was higher than the KW wheat (1.23%) and MW wheat (1.00%). The ash content (1.40%) of wheat reported by (Leach HW, etal ,1959) was close to 1.27% ash content of wheat reported for this studies. Ash content is an indication of mineral content of a food. This therefore suggests that the wheat collected from Segenet (SW) could be important sources of minerals than KW and MW wheat.

4.2.3 Crude Fat Content of Wheat (mean ± SD, n=3)

Table 11: Shows the Crude Fat Content of Wheat (%)

Sample	Crude fat content %			
	T1	T2	T3	mean ± SD
SW	2.4	2.5	2.3	2.40 ± 0.141
KW	2.0	1.7	1.7	1.80 ± 0.283
MW	2.8	2.4	2.6	2.60 ± 0.283

The crude fat content of wheat ranged between 1.80 and 2.60% (Table 11).MW wheat had higher (2.60%) fat content than SW wheat (2.40%) and KW wheat (1.80 %). The fat content of wheat from this study was found to be higher than 1.5% reported for wheat flour [Sefa-Dedeh, S. 1997]. The differences in fat content may be due to location and varietal differences [Moss, R.,1987]. Diets with high fat content contribute significantly to the energy requirement for humans. High fat content of MW wheat in this study would make it a better source of fat than the SW wheat and KW wheat. High fat wheat flours are also good for flavor enhancers and useful in improving palatability of foods in which it is incorporated [Aiyesanmi, A. F. and Oguntokun, M. O. 1996].

4.2.4 Crude Fiber Content (mean ± SD, n=3)

Table 12: Shows the crude fiber content of wheat (%).

Samples	Crude fiber content (%)			
	T1	T2	T3	mean ± SD
SS	6.50	8.10	7.20	7.27 ± 1.18
SK	7.00	6.90	7.50	7.13 ± 0.516
SM	9.00	8.40	8.60	8.67 ± 0.474

The crude fiber content of wheat of Moretna-jiru in this study varied from 7.13% to 8.67% (table 12). The crude fiber content of SW wheat, KW wheat and MW wheat in this study were 7.27%,7.13% and 8.67% respectively. The crude fiber content of all of the three wheat samples in the present study were higher than 0.85% reported by Leach et al (1959). Crude fiber helps in the prevention of heart disease, colon cancer, diabetes, etc. MW wheat would be a better source of fiber content; since it had significantly higher crude fiber content as compared to SW wheat and KW wheat. Therefore, it will be useful if MW wheat is added to meal diet and used in food formulation to help relieve constipation.

4.2.5 Crude Protein Content of Wheat

Table 13: shows the crude protein content of wheat (mean ± SD, n=3)

Samples	Crude protein content (%)=%N x 5.7						
	T1		T2		T3		mean ± SD
	%N	%P	%N	%P	%N	%P	
SW	2.237	12.75	2.258	12.87	2.337	13.32	12.98 ± 0.311
KW	2.356	13.43	2.352	13.41	2.302	13.12	13.32 ± 0.283
MW	2.493	14.21	2.542	14.49	2.559	14.59	14.43 ± 0.481

The crude protein content of the wheat samples ranged between 12.98% and 14.43% (Table 13). The protein content of wheat reported in this study was found to be lower than the 14.70% [Adams, M.L., et al 2002] and higher than 12.86% [Moorthy, S. N., et al 1996] for wheat flours. The crude protein content differences can be attributed to the geographical location. Since soils with high nitrogen levels can influence protein levels [Brown, K. H.,1991]. The protein content of the wheat in this study suggests that they may be useful in food formulation systems especially with the MW wheat (14.43%) sample due to the greater crude protein content as compared to SW wheat (12.98%) and KW wheat (13.32) samples. The crude protein content and quality of SW wheat and KW wheat flour can be improved by blending SW and KW wheat flours with MW wheat flours and used as composite flours.

4.2.6 Carbohydrate Content of Wheat (mean ± SD, n=3)

Table 14: Shows the carbohydrate content of wheat.

Samples	Carbohydrate content (%)			
	T1	T2	T3	mean ± SD
SW	72.01	69.67	70.37	70.68 ± 1.15
KW	73.05	73.16	72.77	72.99 ± 0.164
MW	69.98	69.98	69.40	69.79 ± 0.620

The total carbohydrate content of wheat taken from Moreta-jiru found in this research ranged between 69.79% and 72.99% (table 14). The carbohydrate content of SW wheat, KW wheat, and MW wheat obtained in the research were 70.68%,72.99%, and 69.79% respectively. The carbohydrate content of all wheat samples determined in the present study were lower than 74.22% reported by Ahmed, Lydia, and Campbell (2012). It can be observed that the KW wheat sample used for this study found a high carbohydrate content. Carbohydrates are a good sources of energy and that a high concentration of this is desirable in breakfast meals and weaning food formulas. In this regard, therefore, the high carbohydrate content of KW wheat sample would make it a good source of energy and use in breakfast formulation.

CHAPTER FIVE

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study has characterized the level of some selected heavy metals (Fe, Zn, Co and Mn) and the proximate nutritional compositions (moisture, ash, fat, fiber, protein and carbohydrate) of wheat samples. The level of these metals and nutritional compositions of wheat cultivated on that area of Amhara regional state, north Shewa zone, Moretna Jiru district in a particular area of Segenet, Kusaye and Mangudo was determined; heavy metals by using ICP-OES and proximate nutritional compositions by AOAC method (AOAC 2002). The results obtained in the study were expressed in terms of mean and standard deviation. The level of Fe, Zn, Mn and Co in SW wheat 65.653mg/L, 9.669mg/L,1.506mg/L, and 0.111mg/L; in KW wheat <0.013mg/L, <0.013mg/L,0.021mg/L, and <0.024mg/L respectively; and in MW the level of these metals was similar to that of Kusaye wheat sample. The SW wheat is a good sources of Fe, Zn, Co and Mn because it contains higher amount of Fe, Zn, Co and Mn than KW and MW wheat. In moisture content KW and MW wheat samples were similar. They have lower moisture content than SW wheat. The difference of the moisture content may be due to storage condition. In ash content SW wheat is higher than KW and MW wheat due to the difference mineral content of the wheat samples. KW and MW wheat have almost similar ash content. The percentage of: MW > SW > KW wheat for fat content and fiber content; MW > KW > SW wheat for protein content; and KW > SW > MW wheat for carbohydrate content. In general, it is concluding that wheat sample is a good source of Fe, Zn, Co, Mn, moisture, ash, fat, fiber, protein and carbohydrate content. The concentration difference may be due to storage condition, geographical location of soil and farming conditions.

5.2 Recommendation

Hence this study was the first to the areas on the determination of some selected heavy metals; iron, zinc, manganese, cobalt and proximate nutritional compositions; moisture, ash, fat, fiber, protein and carbohydrate content in wheat samples feature studies should focus on the determination of other nutritionally important minerals like Magnesium, Copper, potassium…etc. In addition, iron, zinc, cobalt and manganese determination was performed by using ICP-OES

instrument such that other researchers must look for this result with other more advanced instruments if there.

Even though the study result showed the reason why SW wheat is more acceptable than KW and MW wheat is due to its high iron, zinc, manganese and cobalt content this may not be the only reason such that more and more studies are required. So it was recommended to other researchers to conduct research on these study areas for determining other cases in order to explore the reason more scientifically.

It was also recommended that feature studies must targeted to compare the nutritional composition like carbohydrate, protein, fat and fiber content of wheat with other food crops.

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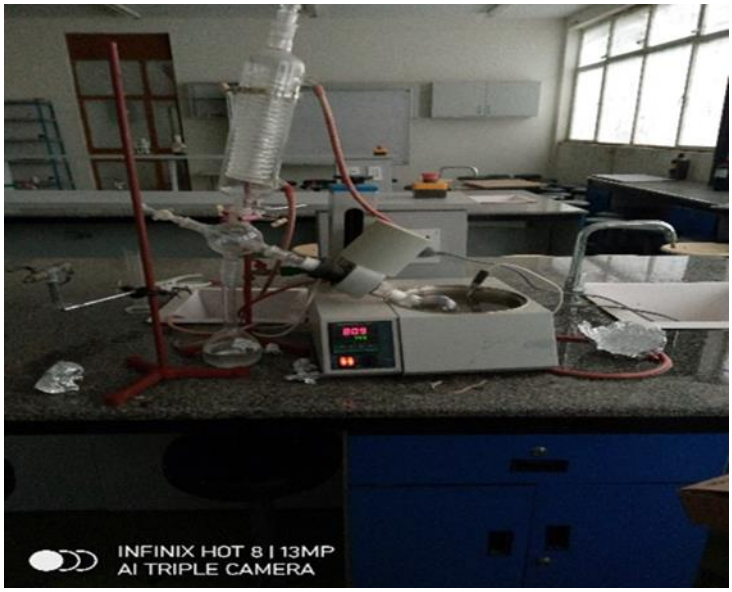
APPENDEXIES

Appendence 1: Digestion of samples to determine metal on hot plate

Appendence 2: Sample in muffle furnace (ignite samples)

Appendence 3: Rotatory evaporator (evaporate solvents present in oil)



Appendence 4: Determination of moisture content in wheat

Sample	Trial	Mass of sample & dish before drying W1 (g)	Mass of sample & dish after drying W2 (g)	Mass of fresh sample W3 (g)	%Moisture
SW	1	59.63	59.52	2	5.34
	2	59.65	59.54	2	5.36
	3	59.67	59.56	2	5.51
KW	1	39.83	39.76	2	3.52
	2	39.86	39.79	2	3.53
	3	39.81	39.74	2	3.51
MW	1	70.54	70.4798	2	3.51
	2	70.54	70.4694	2	3.53
	3	70.54	70.4798	2	3.51

Appendence 5: Determination of ash content in wheat

Sample	Trial	Mass of dish M1 (g)	Mass of fresh sample & dish M2 (g)	Mass of ash & dish M3 (g)	%Ash
SW	1	22.37	24.37	2	1
	2	22.38	24.38	2	1.5
	3	22.39	24.39	2	1.3
KW	1	22.68	24.68	2	1
	2	22.70	24.70	2	1.3
	3	22.69	24.69	2	1.4
MW	1	20.43	22.43	2	0.5
	2	20.45	22.45	2	1.2
	3	20.44	22.44	2	1.3

Appendence 6: Fat content of wheat

Sample	Trial	Mass of beaker M1 (g)	Mass of beaker & Fat M2 (g)	Mass of fresh sample E (g)	%Fat
SW	1	53.20	53.320	5	2.4
	2	53.20	53.325	5	2.5
	3	53.20	53.315	5	2.3
KW	1	27.57	27.670	5	2.0
	2	27.57	27.655	5	1.7
	3	27.57	27.655	5	1.7
MW	1	27.57	27.710	5	2.8
	2	27.57	27.690	5	2.4
	3	27.57	27.700	5	2.6

Appendence 7: Reflux apparatus used to extract fibers

Appendence 8: Fiber contents of wheat

Sample	Trials	Weight of crucible & sample after drying (W1)	Weight of crucible & sample after ashing (W2)	Weight of fresh sample (W3)	%Crude fiber
SW	1	103.110	102.980	2	6.50
	2	103.550	103.388	2	8.10
	3	103.340	103.196	2	7.20
KW	1	103.230	103.090	2	7.00
	2	103.210	103.072	2	6.90
	3	103.540	103.390	2	7.50
MW	1	103.690	103.57	2	9.00
	2	103.420	103.252	2	8.40
	3	103.580	103.408	2	8.60

Appendence 9: Carbohydrate contents of wheat

Sample	Trial	%Moisture: a	%Ash: b	%Fat: c	%Fiber d	%Protein e	%Carbohydrate =100_(a+b+c+d+e
SW	1	5.34	1	2,4	6.50	12.75	72.01
	2	5.36	1.5	2.5	8.10	12.87	69.67
	3	5.51	1.3	2.3	7.20	13.32	70.37
KW	1	3.52	1	2.0	7.00	13.43	73.05
	2	3.53	1,3	1.7	6.90	13.41	73.16
	3	3.51	1.4	1.7	7.50	13.12	72.77
MW	1	3.51	0.5	2.8	9.00	14.21	69.98
	2	3.53	1.2	2.4	8.40	14.49	69.98
	3	3.51	1.3	2.6	8.60	14.59	69.4

Appendence 10: Plant analysis certificate

Lab Code	Description	Concentration	Fe mg/l	Mn mg/l	Zn mg/l	Co mg/l
HWA2458/20	Sample Taken from Signet (2g/50m)	Conc.1	71.920	1.438	9.896	0.105
		Conc.2	64.600	1.544	9.481	0.116
		Conc.3	60.440	1.537	9.630	0.111
		Mean	65.653	1.506	9.669	0.111
		Stdv	5.8120	0.0593	0.2102	0.0057
Added Concentration			80.00	2.00	2.00	2.00
HWA2458/20	After Spike	Conc.1	156.580	3.652	11.751	2.439
		Conc.2	141.100	3.627	11.849	2.530
		Conc.3	138.940	3.595	11.902	2.433
		Mean	145.540	3.625	11.834	2.467
		Stdv	9.6217	0.0286	0.0766	0.0544
Recovery %			99.86	105.92	108.25	117.83
HWA2459/20	Sample Mangudo 2g by 50ml	Conc.1	< 0.013	0.024	< 0.013	< 0.024
		Conc.2	< 0.013	0.018	< 0.013	< 0.024
		Conc.3	< 0.013	0.021	< 0.013	< 0.024
		Mean	< 0.013	0.021	< 0.013	< 0.024
		Stdv	-	-	-	-
Added Concentration			2.00	2.00	2.00	2.00
HWA2459/20	After Spike	Conc.1	1.946	2.472	2.454	2.293
		Conc.2	2.045	2.610	2.435	2.099
		Conc.3	1.948	2.343	2.435	2.107
		Mean	1.980	2.475	2.441	2.166
		Stdv	0.0566	0.1335	0.0110	0.1098
Recovery %			98.98	122.70	122.07	108.32
HWA2460/20	Sample Kusaye 2g by 50ml	Conc.1	< 0.013	0.018	< 0.013	< 0.024
		Conc.2	< 0.013	0.015	< 0.013	< 0.024
		Conc.3	< 0.013	0.029	< 0.013	< 0.024
		Mean	< 0.013	0.021	< 0.013	< 0.024
		Stdv	-	0.0072	-	-
Added Concentration			2.00	2.00	2.00	2.00
HWA2460/20	After Spike	Conc.1	1.721	2.469	2.466	2.466
		Conc.2	1.670	2.268	2.465	2.424
		Conc.3	1.844	2.485	2.460	2.462
		Mean	1.7450	2.4073	2.4640	2.4508
		Stdv	0.0894	0.1209	0.0032	0.0233
Recovery %			87.25	120.37	123.20	122.54

Appendence11: Preparation of standard solution

Standared Preparation with thire concentration and intensities

we prepare the standared sries as follows and all concentration are in mg/l taken from the original stock.

STD	Con/n (Zn) mg/l	Intensity (Zn)	Con/n (Co) mg/l	Intensity (Co)	Con/n (Mn)	Intensity (Mn)	Con/n (Fe)	Intensity (Fe)
STD 0	**0**	2823.35	0	3789.83	0	2985.1	0	4102.63
STD 1	**0.056**	3211.79	**0.028**	4366.39	**0.08**	5225.8	**0.08**	4900.39
STD 2	**0.112**	3619.41	**0.056**	4715.89	**0.16**	7954.64	**0.16**	5903.83
STD 3	**0.168**	**4043.38**	**0.084**	**5114.76**	**0.24**	**10851.3**	**0.24**	**6813.31**
STD 4	**0.56**	6647.12	**0.28**	7683.86	**0.8**	29106.6	**0.8**	12888.5
STD 5	**1.12**	10315.4	**0.56**	11070.1	**0.4**	56798.8	**0.4**	21721.3
STD 6	**1.68**	14370.2	**0.84**	14859.5	**1.6**	85914.1	**1.6**	31279
STD 7	**2.24**	**18997.2**	**1.12**	**18844.2**	**2.4**	**115795**	**2.4**	**40529**
STD 8	**2.8**	23153.7	**1.4**	22437.2	**4**	145107	**4**	49983.9