



**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
CENTER FOR FOOD SCIENCE AND NUTRITION**

**Effect of Refined milling on the Nutritional Value and
Antioxidant Capacity of Wheat types Common
in Ethiopia: A Recovery Attempt with Bran Supplement**

**A thesis submitted to the School of Graduate Studies of Addis Ababa
University in partial fulfillment of the requirement for the Degree of
Master of Science in Food Science and Nutrition**

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and
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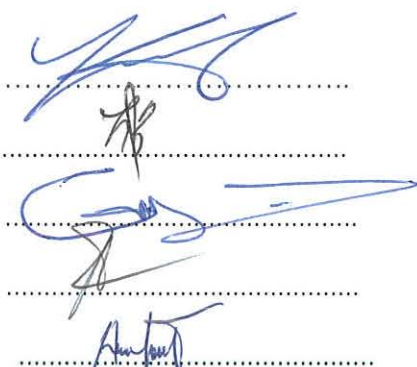
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List of abbreviations and Acronyms

AACC	American Association of Cereal Chemists
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BHA	Butylated Hydroxyl Anisole
BHT	Butylated Hydroxy Toluene
CSA	Central Statistics Authority
CVD	Cardio vascular disease
DNA	Deoxyribonucleic acid,
DPPH	Di phenyl picryl Hydrazyl
FAO	Food and Agricultural Organization
FC	Folin Ciocatteu
FDRE	Federal Democratic Republic of Ethiopia
GAE	Gallic Acid Equivalent
IAOM	International Association of Operative Millers
IBS	Irritable Bowel Syndrome
MCF	Moisture Correction Factor
MOA	Ministry of Agriculture
PPM	Parts per million
RPM	Round per minute
ROS	Reactive oxygen species
ROS	Reaction Oxygen Specious
RSA	Radicals Scavenging Activity
TAC	Total antioxidant capacity
TPC	Total phenolic content
UK	United Kingdom
WB	Wheat Brown

Abstract

The effect of wheat flour extraction on nutritional quality of two types of wheat grown in Ethiopia (hard and soft) was evaluated. Whole wheat flour (100% extraction) and white wheat flour (68% extraction) were subjected to proximate, mineral and antioxidant analysis. Results indicated that at low extraction rate (68 %) the value of protein, fat , fiber , ash , Iron, zinc and phosphorous and antioxidant content of the samples were significantly affected (decreased) ($P < 0.05$) by milling. The total phenolic content (TPC) of white wheat flours, which ranged from 3.34 to 3.49 mg GAE/g were significantly lower ($p < 0.005$) than those of whole wheat flours (range 7.66 to 8.20 GAE/g). At the concentration of 50 mg/mL used, the scavenging effect of wheat extracts, on the DPPH radical scavenging decreased in the order of soft whole , hard whole, soft white and hard white wheat flour , which were 90.39 , 89.89, 75.80, 57.57%, respectively. Bread was prepared from different levels of substitution of white wheat flour by 0 to 25 % of wheat bran. The proximate composition, mineral content and sensory properties of the bread were investigated. The results indicated that protein, fat, ash, fiber, iron and zinc contents of the bran supplemented breads increased significantly ($P < 0.05$) with the progressive increase in the bran level of the bread. The highest value for protein, fat, fiber ,ash, iron and zinc was 12.04,2.61,2.48,3.27 g/100g and 4.84 and 2.33 mg/100g respectively were found in 25 % bran supplemented bread. The sensory evaluation of bread showed that all level of supplementation had mean score above 4 on over all acceptances. The results indicated that refined milling at 68% extraction significantly reduce the nutritional and antioxidant activity of wheat flour and Bread of good nutritional and sensory qualities could be produced from 10 % and 20 % bran supplementation.

Key words: Whole wheat flour, white flour, bran, extraction rate, free radical scavenging activity.

CHAPTER ONE

1. INTRODUCTION

Wheat has accompanied humans since remote times (as far back as 3000 to 4000 BC) in their evolution and development, evolving itself (in part by nature and in part by manipulation) from its primitive form (emmer wheat) into the presently cultivated species (Curtis *et al.*, 2002). Wheat crop is widely adapted to a variety of environments and is cultivated in tropical, subtropical and temperate areas (Hussain *et al.*, 2013). It is widely consumed by humans, in the countries of primary production (which number over 100 in the FAO production statistics for 2004) and in other countries where wheat cannot be grown (Shewry, 2009). It also occupies 27 percent of the total cereal production worldwide (Curtis *et al.*, 2002). It is thus, an important agricultural commodity which is consumed in large amount all over the world among all grains.

Ethiopia is the largest wheat producer in sub-Saharan Africa (MOA, 2011). Nationally, wheat ranks fourth in total area coverage (1,389,215.00 ha). It is also third in productivity (after maize and sorghum) among cereals (CSA, 2005). It is one of the most important crops grown and consumed in Ethiopia both in terms of total production (2.85 million MT in 2010/11) (CSA, 2011) and the proportion of total calories consumed in the country (19.6% of calories consumed) (Rashid *et al.*, 2010).

Wheat possesses several health benefits, especially when utilized as a wholegrain product. According to Kumar *et al.* (2011) wheat provides protection against diseases such as constipation, ischaemic heart disease, diverticulum, appendicitis, diabetes, and

obesity .These benefits are attributed in part to the presence of different compounds such as dietary fibers, phytochemicals, proteins, vitamins and minerals (Ragae *et al.*, 2012).

Whole wheat grain consists of bran, germ and endosperm. When refined, only carbohydrate rich endosperm is retained. This results in a big loss of many nutritionally valuable biochemical compounds such as dietary fiber, vitamins, minerals and antioxidant compounds which play an important role in reducing cardiovascular disease (CVD) (Mellen *et al.* 2008).

When white flour is produced, many important nutrients and fibre are removed, because these components are mainly located in bran and germ (Iuliana *et al.*, 2012). Wheat bran is rich in protein (~14%), carbohydrates (~27%), minerals (~5%) and fat (~6%) Anwarul, *et al.*, 2002). In addition, bran is the main by-product produced by milling. Wheat bran is a most important fiber source which is inexpensive and available. It is a good source of not only dietary fiber but also for other major nutrients. The loss of vitamins and minerals in the refined wheat flour has led to widespread prevalence of constipation and other digestive disturbances and nutritional disorders (Kumar *et al.*, 2011).

The process of milling entails separating endosperm from the bran and germ and reducing it to flour. Milling processes have an effect on the nutritional value of the various products produced from the kernel and therefore it is important to understand the extent of refining effect on the nutritional content of the wheat flour. It is also vital to

research possibilities and methods of the introduction of bran to recover the loss of minerals during its extraction.

1.1 Statement of the Problem

Milling is the critical process affecting the concentrations of nutrients in wheat-derived food products. The outer parts of the kernel, especially the aleurone layer and the germ are richer in minerals. Conventional milling reduces nutritional content in flour and concentrates them in the milling residues (Cubadda *et al.*, 2009).

White flour with the extraction rate 68% meaning up to 32 % of the original grain is not in the flour . Whole grain flour includes all parts of the seed and is 100% extraction. Milling of wheat into highly refined flours not only preclude considerable amounts of nutrients from human consumption, but the remaining flours have a much poorer nutritive value than flour made from whole wheat.

Over the past twenty years, wheat production and consumption have both increased in Ethiopia (Bergh, *et al.*, 2012). On top of this wheat flour covers a substantial proportion of the population. According to the survey conducted in Ethiopia, 28% of consumers purchase flour and flour products. It is estimated that wheat flour reaches about 22 million people (FDRE, 2011) However, no published information is available regarding the effect of refining on nutritional and antioxidant capacity of wheat that are commonly grown in Ethiopia though it is widely distributed and consumed. The nutritional value and antioxidant properties of wheat grain are significantly influenced by Soil type and

richness, growing temperatures, moisture levels, other climatic differences, and genotype (Adom *et al.*, 2003).

It is therefore, very important to understand the nutritional value of wheat grown in Ethiopia and evaluate if we are getting most out of our grain after refined milling. Besides, it is necessary to find a way to improve the nutrient quality of the refined wheat flour products without compromising the palatability of the product. Hence, in this study an attempt was made to recover the nutrients through supplementation of bran.

1.2. Significance of the Study

Research in the effect of refining of wheat on the nutritional value and supplementation of bran will introduce and expand the use of wheat bran as value-added food ingredient and thereby enhance the nutritional value of bread. Results from this study will provide useful information on the utilization of wheat bran in manufacturing bread.

1.3 Objectives of the Study

1.3.1 General Objective

To examine the effect of refined milling on the nutritional and antioxidant capacity of wheat (hard and soft), that are common in Ethiopia and to recover the loss of nutrients during milling through bran supplementation.

1.3.2 Specific Objectives

- Evaluate the extent of loss of fiber, protein, iron, zinc and phosphorous and antioxidant capacity on two types of wheat (hard and soft) as a result of refined milling.
- Study the effect of supplementing wheat flour with wheat bran on the nutritional value.
- Investigate the effect of supplementing wheat flour with wheat bran on the overall acceptability of the bread.

1.4 Limitation of the study

Determination of vitamins were not included in the study due to shortage of time and financial resource. Furthermore, the conclusion made on the sensory result was not based on panelists who prefer to eat brown bread .

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Wheat

Wheat grains are generally oval shaped, although different wheat have grains that range from almost spherical to long, narrow and flattened shapes wheat grain is a seed, and each grain of wheat is made up of three distinct parts. There is the coarse outer bran layer then, inside the bran layer, the grain is made up of two main parts (Fig.1). The smaller part is called the germ (which would grow into a new plant) and the larger part the endosperm (Šramková *et al.*, 2009).

The wheat grain contains 2-3% germ, 13-17% bran and 80-85% endosperm on dry basis. A fruit coat, or pericarp, surrounds the seed and adheres closely to the seed coat. This type of fruit is commonly called a kernel or grain. The pericarp surrounds the entire seed and acts as a protective covering. It is composed of several layers (IAOM, 2010a). The outer seed coat and bran layers and the crease are high in mineral material (Blakeney *et al.*, 2009), which is about (7.2%) and also the protein and carbohydrate level is high.(Šramková *et al.*, 2009).

The endosperm is the functional material for making end products. Endosperm cells are packed with starch granules and remnants of protein bodies and cell membranes (A.B. Blakeney *et al.*, 2009). The endosperm is not a homogenous piece of starch. It is divided into countless cells separated from each other by thin walls of cellulose. Each one of the

cells contains starch granules which are embedded in a protein matrix. This protein matrix forms the gluten. The gluten is the component, which gives strength to the flour. It is formed from the proteins gliadin and glutenin when water is present and gives elasticity to the dough (IAOM, 2011).

Fig. 1. The anatomical structure of a wheat kernel (Slavin *et al.*, 2001).

Wheat germ, the main fatty component of wheat grain, is characterized by consisting of polyunsaturated fatty acids (Umar *et al.*, 2008). Wheat grain comprises about 2-3g/100g of germ that produces about 11g/100g oil, rich in unsaturated fatty acids especially linoleic acid (Naz & Sadiq, 2011).

2.1.1 Types of Wheat

Roughly 90 to 95 percent of the wheat produced in the world, about 600 million tones is common wheat (*Triticum aestivum*) which is better known as hard wheat or soft wheat, depending on grain hardness (Curtis, 2002) Wheat is considered of to be the greatest importance among cereals because of its processing characteristics (Marie & Ivan, 2009). Wheat hardness variation is (hard or soft grain texture) an important trait in the determination of end-use quality of wheat flours for food industry. Common classifications differentiate 'soft' and 'hard' (*T. aestivum*) wheat from 'very hard' or durum wheat (*T. durum*) (Topin *et al.et al.*, 2008).

2.1.1.1 Hard Wheat

Kernel hardness in bread wheat (*Triticum aestivum* L.), a staple food source for humans, is a major grain quality trait as it determines the physical properties and hence the end-uses of flour (Lesage *et al.*, 2012). Hardness is defined as "difficult to penetrate or reduce to smaller fragments (Daniela, 2007). The hardness of the grain appears to be determined by the degree of adhesion between the starch granules and the protein matrix (Csóti *et al.* 2005) but possibly also between the matrix proteins and the cell walls. Due to strong adhesion, starch granules fragmentize during the milling of hard wheat. This fragmentation is called starch damage. It is the most important factor in determining the

water absorption of flour. It also determines the amount of carbohydrates available to yeasts for fermentative activity. Therefore, it positively affects gas production, loaf volume and, as a result, baking quality. Thus, hardness is one of the most important characteristics that affect the functionality of wheat. It affects a range of characters including the milling (tempering, milling yield, flour particle size, shape and density of flour particles), baking and end-use properties (Daniela, 2007).

Milling of a hard variety requires more energy and thus severity of grinding results in higher percentage of damaged starch in hard wheat flours. By contrast, lesser energy is needed to grind soft wheat grains to desired particle size flour and hence the soft wheat flour generally has lesser percentage of damaged starch (Al-Dmoor 2013).

Hard white wheat has a hard endosperm, white bran, and a medium to high protein content (10.0 to 14.0 percent) (Blakeney *et al.*, 2009). Hard wheat is known for its high gluten content, which is important in bread making, products (Ragaei *et al.*, 2012). Despite its relatively recent origin, bread wheat shows sufficient genetic diversity to allow the development of over 25 000 types (Feldman *et al.*, 1995) which are adapted to a wide range of temperate environments (Shewry 2009).

2.1.1.2 Soft wheat

Soft wheat kernel structure is typically larger and plumper than hard wheat. In general, the soft wheat kernel tends to be softer and less glassy in appearance than a hard wheat kernel (IOAM, 2011). Soft wheat on milling yields weaker flour with relatively lower

protein content. Weak flour proteins do not form a continuous gluten matrix when flour is mixed with water due to smaller quantity and basic quality characteristics of gluten proteins in weak flour (Hanee, 2013).

An important functional difference between hard and soft wheat is in their water absorption. Soft wheat have the softer endosperm texture (require less energy to mill) and yield smaller particles with less starch damage upon milling than do hard wheat (Daniela, 2007). Soft wheat is used for pastries, (Ragae *et al.*, 2012). Soft white wheat has low protein (8.5 to 10.5 percent) and low moisture. It is used in flat breads, cakes, biscuits, pastries, crackers, and snack foods (Blakeney *et al.*, 2009).

2.1.1.3 Durum wheat

Durum grains are amber-colored and larger than those of other types (IOAM, 2011). Durum wheat is the hardest of all wheat classes with a high protein content (12.0 to 15.0 percent), yellow endosperm, and white bran (Blakeney *et al.*, 2009). While common wheat is milled to produce flour, the objective of milling durum wheat is to produce semolina and to minimize the production of durum flour. Semolina is the coarse, granular particles of endosperm used for pasta processing (Dariusz & Janusz, 2005).

The key features of durum wheat include its hardness, intense yellow colour and nutty taste. After conversion to pasta, durum wheat produces products with good cooking quality and stability to overcooking with unmatched eating quality (Mike, 2008).

2.2 Wheat Milling Process

The objective of milling is to separate the starchy endosperm from the kernel, and to ground it into flour (Iuliana *et al.*, 2012). Wheat is commonly used in refined form excluding its outer husk 'The bran' (Mohsin *et al.*, 2012). When milling wheat to produce flour for bakeries, the customer may require finely milled white flour that contains mostly the starchy material with little or none of the bran, germ or husk (Fellows *et al.*, 1995). The wheat milling process involves cleaning, tampering, milling and finishing the product as discussed here under.

2.2.1 Cleaning

In modern milling considerable attention is given to preliminary screening and cleaning of the wheat or blend of wheat to exclude foreign seed and other impurities (Fellows *et al.*, 1995) Wheat undergoes a cleaning process in order to get rid of foreign materials and impurities. These various steps of cleaning involve a number of cleaning machinery like aspirators, de-stoners, and seed removers, are used at various places and in different combinations varying from mill to mill, to get rid of foreign materials (Anon.1993).

2.2.2 Tampering

After cleaning, the wheat is transferred to tempering bins for extensive conditioning when water is added in order to facilitate the efficient separation of the endosperm from the bran (Anon.1993). The wheat is dampened and washed if it is too dry for subsequent efficient grinding, or if it is too damp it is gently dried to avoid damaging the physical

state of the protein present, mainly in the form of the elastic substance gluten (Fellows *et al.*, 1995).

2.2.3 Milling

The first step in grinding for the gradual reduction process is performed between steel cylinders, with grooved surfaces, working at differential speeds. The wheat is directed between the first “break,” or set of rolls, and is partially torn open. Then separated by suitable sieving and the branny impurities can be removed by air purifiers and other devices. The cleaned semolina is reduced to fine flour by grinding between smooth steel rolls, called reduction rolls. The flour produced in the reduction rolls is then sieved out (IAOM, 2010a).

The various sieving and purification processes free more and more endosperm in the form of flour. Flour is obtained from a whole series of processing operations. The flour is sieved out after each reduction roll. The sieving plant generally employs machines called plan-sifters, and the air purifiers (IAOM, 2010b).

2.2.4 Finished flour

Wheat flours are characterized by the flour extraction, which may be defined as the proportion of flour by weight, derived by milling from a known quantity of wheat (Slavin *et al.*, 2001). The extraction rate is used to define different types of wheat flours. The flour with less than 75% extraction rate is typical white. If the extraction rate exceeds 80%, the flour will contain non endosperm particles, and if the extraction rate approaches 100%, whole meal flour can be made (Clydesdale, 1994). In the milling process, the grains may be fractionated into different types of flour. With decreasing extraction rates

in milling, more and more of the outer grain layers are removed resulting in the loss of dietary fiber and associated bioactive compounds such as vitamins B group, tryptophan and lignin (Nilsson *et al.*, 1997).

The extraction rate of wheat flour has marked effect upon its nutritional content. As a result of milling process, nutrient level of high extraction rate flour increases directly with increase in flour ash values (Matthews and Workman, 1977).

2.3 Bread

Bread may be described as a fermented product produced mainly from wheat flour, water, yeast and salt by a series of process involving mixing, kneading, proofing, shaping and baking. Bread products and their production techniques differ widely around the world (Dewettinck *et al.*, 2008).

Bread is a staple processed food that dates back over 12000 years (Anusooya *et al.*, 2010). The consumption of bread and other baked goods such as biscuits, doughnuts and cakes produced from wheat flour is very popular (Joel *et al.*, 2011).

Wheat flour is one of the major conventional ingredients in bread making due to its gluten fraction, which is responsible for the elasticity of the dough by causing it to extend and trap the carbon dioxide generated by yeast during fermentation (Mepba *et al.*, 2007). The gluten permits bread to rise since it strengthens the dough and allows it to trap and hold the small pockets of gas formed by the fermentation action of the yeast during the baking process (IOAM, 2011). Only the flour of bread wheat is able to form dough that

exhibits the rheological properties required for the production of leavened bread. This property results from the ability of wheat storage proteins, gliadins and glutenins, to form special protein complex known as gluten.

The use of white flour derived from the processing of whole wheat grain, which is aimed at improving the aesthetic value of white bread has also led to the drastic reduction in the nutritional density and fiber content when compared to bread made from whole grain cereals (Maneju *et al.*, 2011). As bread is an important part of the diet, the loss of vitamins during transformation of wheat grain to bread is an important nutritional issue (Dewettinck *et al.*, 2008).

2.4 The Importance of Wheat Bran and its Application

Bran is created as a by-product in milling industries and one million tons of wheat can produce up to 0.25 million tons of wheat bran. According to Caballero *et al* (2003) wheat bran contains protein (13-18%), fat (3-6%), ash (6-7%) and total dietary fiber (35-58 %) of dry weight. Furthermore, wheat bran also contains several important compounds such as phenolic acids, carotenoids, lignans, phytosterols, flavonoids, tochoopherol and phytic acid which are distributed unequally in different WB tissues. Precise composition of macro and micronutrients may vary from cultivar to cultivar and the extraction technique of these compounds from bran. Wheat ash content is usually measured for quantification of bran (Naeem *et al.*, 2009).

Wheat bran has relatively diverse application in food, feed, medicine and fermentation industries due to its richness in carbohydrates (mostly fibers), protein and fats which

makes it an important dietary element (Mohsin *et al.*, 2012) and antioxidants (Stevenson *et al.*, 2012). Wheat whole grain and wheat bran extracts possess antioxidant properties against oxidation of biologically important molecules such as DNA, proteins and membrane lipids (Magdaléna *et al.*, 2013).

Wheat bran is used as a supplemental source of dietary fiber for preventing colon diseases (including cancer), preventing gastric cancer, treating Irritable Bowel Syndrome (IBS) (Daniela *et al.*, 2012; Kumar *et al.*, 2011). Compounds, such as vitamins, lignans, phenolic acids and alkylresorcinols, present in bran are desirable ingredients to be added in daily diet to improve the nutritional value of food. So WB can be used as natural and cheaper source of value added products in preparation of functional food ingredients or for fortification of certain products. 20% WB supplementation to flour and baked product offer an inexpensive and interesting alternative to the synthetic vitamins and nutrient supplements for poor people by adopting some pretreatment procedures (Gomez *et al.*, 2003). Pretreatment (grinding to obtain smaller particle size, heat treatment, pre-fermentation with yeast and lactic acid bacteria and extrusion) of bran improves the nutritional as well as physical quality of bread and baked products (De Kock *et al.*, 1999; Salmenkallio *et al.*, 2001). Wheat bran can be used in fermentation industry, as a substrate for production of a range of products such as biomass, biofuel and production of other metabolites (Mohsin *et al.*, 2012).

2.5. Nutritive Value 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. Wheat is

rich in the basic nutrients such as energy, protein, vitamins and minerals and also contains balanced amounts of bioactive compounds such as dietary fiber and antioxidants (El-Sayed *et al.*, 2008) and thus it presents the main source of nutrients to the most of the world population.

2.5.1 Macronutrients

Wheat has higher protein content to maize, rice, and other major cereals (Adejumo, 2013). Protein is considered the most important nutrient for humans and animals, as manifested by the origin of its name, from the Greek *proteios* for primary (Zuzan, *et al.*, 2009). The protein content of wheat grains may vary between 10% - 18% of the total dry matter (Zuzana, *et al.*, 2009). Carbohydrates have special significance in cereals which usually comprise about 50-80% carbohydrate on a dry weight basis (Shelton and Lee, 2000). Wheat flour is an excellent source of complex carbohydrate. (Adejumo, 2013).

Cereals, such as wheat, are rich in insoluble dietary fibre, which increases fecal weight, bulk and softness increases the frequency of defecation and reduces the intestinal transit times (Muralikrishna & Rao, 2007). According to Haripriya & Premakumari (2010) wheat is lower in soluble fiber than most grains. The refining of grains removes proportionally more of the insoluble fiber than soluble fiber, although refined grains are low in total dietary fiber.

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. The

germ has the highest amount of lipids (11%), but significant amounts are also associated with the bran and the starch and proteins of the endosperm. (Šramková, *et al.*, 2009)

2.5.2. Micronutrients

Minerals, classified as micronutrients are needed by our body in small amounts. Minerals are important components required by humans in their daily food. All body processes depend upon the action of minerals to activate enzymes performing metabolic functions. Deficiency of specific minerals may lead to various chronic diseases (Abrar *et al.*, 2010). Deficiency in minerals, however, can have a major impact on health such as anemia and osteoporosis that commonly occur in both developed and developing countries.

Iron deficiency is the most widespread nutrient deficiency in the world, estimated to affect over 2 billion people (Stoltzfus & Dreyfuss, 1998). Although many of these people live in less developed countries, it is also a significant problem in the developed world. Zinc deficiency is also wide spread, particularly in Sub-Saharan Africa and South Asia, and has been estimated to account for 800 000 child deaths a year (Micronutrient Initiative, 2006). Wheat and other cereals are significant sources of both of these minerals, contributing 44% of the daily intake of iron (15% in bread) and 25% of the daily intake of zinc (11% in bread) in the UK (Henderson *et al.*, 2007).

Cereals and derived products are among the major dietary sources of essential elements for humans (Cubadda *et al.*, 2009) Due to the high consumption of wheat in a variety of

food products all over the world, wheat is considered an important source of minerals (Abrar *et al.*, 2010). The contribution of cereal products to the estimated dietary intake of several minerals and nutritionally beneficial trace elements is about 20–30% of their total intake in Western countries (Carcea *et al.*, 2007).

Wheat is one of the cereals which is classified as rich sources of phosphorus (200-1200 mg/ 100 g) (Dewettinck *et al.*, 2008). It is also a moderate source of calcium (100-200 mg/100 g), magnesium (100-200 mg/100 g), iron (1-5 mg/100 g), zinc (1-5 mg/100 g) and copper (0.1-1 mg/100 g) (Dewettinck *et al.*, 2008).

2.6 Free radicals and antioxidants

2.6.1 Free radicals

Free radicals are atoms or molecules that have a single unpaired electron in their outer orbit. The unpaired electron in the free radical makes it very unstable and capable of reacting with other molecules such as proteins, DNA, and fatty acids, leading to change in structure and function (Tuppo & Forman, 2001).

Oxygen is an element indispensable for life. Oxidative properties of oxygen play a vital role in diverse biological phenomena. Oxygen has double edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events (Mahantesh *et al.*, 2012). Free radicals are produced as part of normal cellular function. They may also be produced by endogenous and environmental sources. Endogenous sources include mitochondrial leak, respiratory burst, enzyme reactions, and auto-oxidant

reactions. Environmental sources include cigarette smoke, pollutants (such as ozone and nitrogendioxide), ultraviolet light, and ionizing radiation (Young *et al.*, 2001). The most important free radicals produced are the oxygen derivatives such as the superoxide radical and the hydroxyl free radical (Tuppo & Forman, 2001).

Oxidative stress occurs when there is an imbalance between the free radicals and their scavengers; either there is an increase in the production of free radicals, a decrease in free radical scavengers (such as antioxidant enzymes and antioxidants), or both (Ehab & Lloyd 2001). The elevated cellular levels of ROS or free radicals cause damage to nucleic acids, proteins, and membrane lipids and have been associated with many aging-related health problems including carcinogenesis and heart diseases (Halliwell *et al.*, 1992).

Overproduction of free radicals can be responsible for tissue injury. Cell membranes are made of unsaturated lipids and these unsaturated lipid molecules of cell membranes are particularly susceptible to free radicals. Oxidative damage can direct to a breakdown or even hardening of lipids, which composition of all cell walls. Breakdown or hardening is due to lipid peroxidation leads to death of cell or it hinders the cell to properly get its nutrients. (Mahantesh *et al.*, 2012). The most exposed to the free radicals are cellular membranes that are rich in fatty acids polyunsaturated oxidable easily and can form lipoperoxids. ((Olariu *et al.* 2009). The brain is particularly very sensitive to oxidation stress possibly because of its high lipid content, high aerobic metabolic activity and low catalase activity (Mon *et al.*, 2011).

2.6.2 Antioxidants

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction (Mahantesh *et al.*, 2012). Most of the literature on plant phenolics focuses mainly on those present in fruit and vegetables, wines and teas. However, many phenolic compounds in fruit and vegetables (i.e. phenolic acids and flavonoids) are also reported in cereals (Emanuela *et al.*, 2012).

There is positive relationship between the consumption of whole-grain products and reducing the incidence of chronic diseases. These beneficial effects have been attributed to the unique phytochemicals of the grains. Endogenous and exogenous antioxidants act as “free radical scavengers” by preventing and repairing damages caused by ROS and RNS, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases (Mahantesh *et al.*, 2012).

Antioxidants may exert their activity by several mechanisms, like by suppressing the production of active species by reducing hydro peroxides and H_2O_2 , by sequestering metal ions, termination of chain reaction by scavenging active free radicals and also caused repairing and/or clearing damage of cell. Biosynthesis of other antioxidants or defense enzymes also induced by some antioxidants. Therefore, antioxidant synthesized in body or supplied from outside like phyto constituents plays important role to protect the body from free radical induced injury (Mahantesh *et al.*, 2012).

2.6.2.1 Classification of Antioxidants

Generally, antioxidants can be classified as natural and synthetic antioxidants (Shui & Leong, 2004).

Natural and synthetic Antioxidants

Natural antioxidants constitute a broad range of compounds including phenolic or nitrogen species and carotenoids. The body produces different antioxidants (endogenous antioxidants) to neutralize free radicals and protect the body from different disease leads by the tissue injury. Exogenous antioxidants are externally supply to the body through food also plays important role to protect the body. The body has developed several endogenous antioxidant defense systems classified into two groups such as enzymatic and non enzymatic (Mahantesh *et al.*, 2012).

Free radicals cause autoxidation and development of rancidity. During the storage of food, transition metals, light or other factors may initiate the generation of free radicals leading to lipid peroxidation or degradation of proteins, vitamins and pigments, cross-linked lipids and other macromolecules into non-nutritive polymers. As a result, lipid peroxidation leads to the formation of off-flavors, off-color and undesirable chemical compounds. Antioxidants prevent foods from becoming rancid, browning, or developing black spots by suppressing the reaction that occurs when foods are exposed to oxygen in the presence of light, heat, and transition metals.

Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free-radical-induced tissue injury (Mon *et al.*, 2011).

2.6.2.2 Antioxidant capacity of wheat

In 1972, Fulcher first found that wheat grain contained antioxidants. Since then, a number of studies have detected antioxidant properties in wheat and wheat-based food products. Wheat contains a diverse array of bioactive compounds that may contribute to its antioxidant capacity (Magdaléna *et al.*, 2013). These bioactive components include carotenoids, tocopherols, tocotrienols, phenolic acids, phytic acid, phytosterols and flavonoids (Abdel-Aal & Rabalski, 2008).

The majority of beneficial antioxidant phytochemicals (including phenolic acid and alkylresorcinols) in whole wheat grain are present in the germ/bran fractions (Stevenson *et al.*, 2012). Health-beneficial properties of whole-wheat grains can be largely ascribed to the presence of phytochemicals in the diet that reduce oxidative stress, thereby reducing the risk of chronic diseases. Among the different antioxidants present in wheat, phenolic compounds seem to have the greatest potential of being beneficial to health (Archie *et al.*, 2006). Studies have shown that wheat whole grain and wheat bran

extracts possess antioxidant properties against oxidation of biologically important molecules such as DNA, proteins and membrane lipids (Abdel-Aal & Rabalski, 2008).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Samples

Hard wheat (Kubsa) and soft wheat (ET-13-A2) samples were obtained from Kebron food complex, Oromia region and Wedera farmers cooperative ,Debrebrhan, respectively in Ethiopia . Bran sample was obtained from universal food complex (Addis Ababa, Ethiopia).



Fig 2. Hard wheat

bran

soft wheat

3.1.1 Milling of wheat

One kg of each sample from both soft and hard wheat were cleaned and tempered separately to 14% moisture level and kept for 6 and 24 h respectively at ambient temperature in a closed plastic jar. The amount of water required for tempering was calculated according to AACC (2000) as follows:

$$\text{Water to be added (ml)} = \frac{100 - \text{original moisture\%}}{100 - \text{desired moisture\%}} \times \text{wt. of sample (g)}$$

After tempering, wheat samples were milled at the extraction rate of 68% based on the capacity of the milling machine and 100% by using Buhler Automatic mill (Deutschland). The milling of flour was conducted at Kokeb flour and Pasta factory and The extraction rate was calculated according to Slavin et al., (2000) as follows:

$$\text{Extraction (\%)} = \left(\frac{w_1}{w_2} \right) \times 100$$

Where W1 = weight of refined white flour ; W2 = Weight of the wheat before milling

3.1.2 Formulation of bread

Flour blends were prepared by mixing wheat flour with wheat bran in the proportions of 100: 0 ,90:10, 80:20 and 75:25 (wheat flour to bran) using homogenizer and 100% white wheat flour was used as control. The formulation was made based on the preliminary test.

The four flour samples were packaged in black low density polyethylene bags and stored in plastic containers at room temperature from where samples were taken bread production.

3.1.3 Processing of bread

Bread was prepared by partially replacing the wheat flour with 0 %, 10%20%, 25% of bran sample. The dough was prepared based on the method described by Hertzberg (2007) with some modification by weighing 400 g of wheat flour, 8g sugar, 4 g salt, 8g of oil, 1.6g yeast. A mixer (Linkrich-B15) was used to mix the ingredients in order to homogenize the mixture for 30 min water was added to the dough until the desired

consistency was achieved. The dough was weighed and divided into 3 equal portions. These were placed in baking pans and left for 1 h. Then they were transferred into an oven pre-heated to about 180 - 250°C and allowed to bake for 20 min. The baked products were left to cool (Aboaba & Obakpolor, 2010).

3.2 Proximate analysis of wheat flour and bread

All samples were analyzed for moisture, crude protein, crude fat, crude fiber and total ash according to their respective standard methods as described below.

3.2.1. Determination of moisture content

The dish used for the moisture determination were dried at 105 °c for 1hour in drying oven . It then transferred to the desiccators cooled for 30 minutes, and weighed. The prepared samples were mixed thoroughly and about 5 g of the flour samples were transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 3 hrs at 105⁰C, and then the dishes and their contents were cooled in desiccators to room temperature and reweighed.

Calculation

$$MC = \left(\frac{w_2 - w_3}{w_2 - w_1} \right) \times 100$$

W_1 = mass of the dish. W_2 = mass of the dish and the sample before drying, and W_3 = mass of the dish and the sample after drying (AOAC,2000).

3.2.2. Determination of total ash content

Total ash was determined according to AOAC(2000).

The porcelain dishes used for the analysis were placed into a muffle furnace for 30 min at 550⁰C. The dishes were removed and cooled in a desiccators for about 30 minutes to room temperature; each dish was weighed. About 2.5g of the flour and bread samples were added into each dish. The dishes were placed on a hotplate under a fume hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550⁰ C for 5 hours and removed from the muffle and cooled in the desiccator and weighed. The amount of total ash was calculated by using the following formula.

Weight of total ash was calculated by difference and expressed as percentage of sample

$$\text{Calculation: \% Ash} = \left(\frac{m_3 - m_1}{m_2 - m_1} \right) \times 100$$

Where, m_1 = mass of crucible

m_2 = sample mass with crucible

m_3 = final mass of sample with crucible

3.2.3. Determination of crude protein (AOAC, 2000)

Protein ($N \times 6.25$) was determined by the Kjeldahl method. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid containing copper

sulfate and potassium sulfate as a catalyst. The ammonia released after alkalization with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

Digestion: About 0.5g of the flour and bread samples were taken in a Tecator tube and 6ml of concentrated sulfuric acid was added, mixed, thoroughly and 3.5ml of 30% hydrogen peroxide was added step by step. As soon as the violet reaction had ceased, the tubes were shaken for a few minutes and placed back into the rack. A 3 g of the catalyst mixture (0.5g of copper sulfate with 100g of potassium sulfate) was added into each tube, and allowed to stand for about 10 min before digestion. When the temperature of the digester reached 370⁰C, the tubes were lowered into the digester. The digestion was continued until a clear solution was obtained about 4hr. The tubes in the rack were then transferred into the fume hood for cooling; 15ml of de ionized water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml deionized water and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed down with a small amount of water, stoppered and the steam switched on. A 100ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condenser is above the surface of the distillate.

The distillation was continued until a total volume of 150ml is collected. The tip was rinsed with a few milliliter of water before the receiver was removed.

Titration: The distilled solution was then titrated with 0.1N hydrochloric acid to a reddish color.

Nitrogen (mg) in the sample = $V \times N \times 14$

Nitrogen (g/100g) sample mg of nitrogen $\times$ 100/mg sample

Total nitrogen (%) = $(V - V_b) \times N \times 14/W$

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

Where: V = volume of hydrochloric acid consumed to neutralize the sample; V_b = the volume of acid consumed to neutralize the blank; N = normality of the acid; 14 = Eq.wt of nitrogen; 6.25 = conversion factor from total nitrogen to crude protein.

3.2.4. Determination of crude fat content (AOAC, 2000)

Crude fat was determined by exhaustively extracting a known weight of sample in diethyl ether (boiling point, 55⁰C) in a soxhlet extractor. The ether was evaporated from the extraction flask. The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage.

The extraction flasks were cleaned, dried in drying oven at 70⁰C for 1 hour, cooled in desiccators (with granular silica gel) for 30 minutes, and then weighed. The bottom of the extraction thimble was covered with about 2cm layer of fat free cotton. About 2 gram of

flour / bread samples were added into the extraction thimbles, and then covered with about 2cm layer of fat free cotton. The thimbles with the sample content were placed into soxhlet extraction chamber. The cooling water was switched on, and a 50 ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for about 4 hrs. the extraction flasks with their content were removed from the extraction chamber and placed in the drying oven at 70⁰C for about 1 hr, cooled to room temperature in the desiccators for about 30 minutes and re-weighed.

$$\% \text{ Fat content} = \left(\frac{W_2 - W_1}{W} \right) \times 100$$

Where: W = weight of fat; W₂ = weight of extraction flask after extraction (wt. of flask and fat); W₁ = weight of extraction flask before extraction (wt. of flask); W₀ = weight of fresh sample.

3.2.5. Determination of crude fiber content (AOAC, 2000)

1 g of sample (W₁) and 1 g of celite (sand) was weighed into a crucible and placed the crucible in fibertec hot extraction and added 150ml of hot 0.64N of sulfuric acid. Add 2 - 4 drops of n-octanol to prevent foaming & boiled for 10 min. In step 2 of hot extraction add 150ml of 0.556N NaOH & put 2 - 4 droplets of n-octanol and boil it for 10 min. In both process washing with hot deionized water was applied. Evaporate solvent, dry the crucibles at 130⁰c for 2 hours and cooled in dessicator and weigh (W₂). Ash the sample in the crucible at 550⁰c for 3 hours and cooled down and weigh (W₃).

$$\% \text{ crude fiber} = \left(\frac{W_2 - W_3}{W_1} \right) * 100$$

3.3 Determination of Mineral contents

3.3.1 Determination of Fe, and Zn

Iron and zinc were determined according to the standard method of AOAC(2000). Ash was obtained from dry ashing of the samples. The ash was wetted completely with 5ml of 6N HCl, and dried on a low temperature on hot plate. A 7ml of 3NHCL was added to the dried ash and heated on the hot plate until the solution just boiled. The ash solution was cooled to room temperature in a hood and was filtered using filter paper (Whatman45,). A5ml of 3N HCl was added into each crucible dishes and was heated until a solution boiled ,then cooled and filtered into the flask. The crucible dishes are again washed three times with deionized water, the washing was filtered into a flask .Then the solution was cooled and diluted to 50 ml with de-ionized water. A blank was prepared by taking the same procedure as the sample.

3.3.2 Determination of Phosphorus

Phosphorus was determined using the molybdovanadate method (AOAC, 1990). Briefly, Five ml of aliquot was pippeted from the sample digest into a 100 ml volumetric flask. Ten ml of the molybdate and vanadate solutions was added to the samples and the standards and made up to volume with distilled water. After 10-30 minutes color developed and measured on the absorbance of the blank, sample and standards by spectrophotometer at a wavelength of 460 nm.

$$p(ppm) = \left(\frac{c \times v_1 \times v_2 \times mcf}{S \times A} \right)$$

Where:

C = P concentration in sample digest read from the curve, ppm.

V₁ = Volume of the digest (100 ml).

V₂ = Volume of the dilution.

S = Weight of the plant material calcinated in g .

A = Aliquot (5 ml).

mcf = moisture correction factor

3.4 Antioxidant capacity determination

3.4.1. Sample extraction

Samples were extracted based on the procedures as outlined by Woldegiorgis, *et al.*, (2014). The powdered wheat samples were homogenized and weighed in to ten gram. was then extracted by stirring with 100 ml of methanol at 25⁰ C at 150 rpm for 24 h using an incubator shaker (ZHWY-103B) and then filtered through Whatman No. 1 filter paper. The residue was then extracted with two additional 100 ml portions of methanol as described above. The combined methanolic extracts were evaporated at 40⁰C to dryness using a rotary evaporator and re-dissolved in methanol at a concentration of 50 mg/ml and stored at 4⁰C for further use.

3.4.2 Determination of free radical Scavenging Activity

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH (Gursoy *et al.*, 2010). Antioxidant activity of the methanol extracts was determined by DPPH radical scavenging method as described by Woldegiorgis, *et al.*, (2014). A 0.004% solution of DPPH radical solution in methanol was prepared and then 2 ml of DPPH solution was mixed with 1 ml of various concentrations (0.1-50 mg/ml) of the extracts in methanol. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid was used as a standard and mixture without extract was used as the control. Inhibition of free radical DPPH in percent (I %) was then calculated:

$$\text{Radical Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The extract concentration providing 50% of radicals scavenging activity (IC_{50}) was calculated from the graph of RSA percentage against extract concentration.

The percentage yield extracts were calculated based on dry weight as:

$$\text{Yield (\%)} = (W_1 / W_2) \times 100$$

Where W_1 = weight of extract after solvent evaporation; W_2 = Weight of the grinded wheat.

3.4.3 Total phenolics determination

Phenolic compounds concentration in the wheat was estimated with Folin-Ciocalteu reagent according to the Singleton & Rossi method(1965) as described by Woldegiorgis, *et al.*, (2014) with some modification. One milliliter of sample (5000 µg) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725nm. Gallic acid was used to construct the standard curve (5-80µg/ml). The results were mean values $\pm$ standard error of mean and expressed as mg of gallic acid equivalents/g of extract (GAEs). Total content of phenolic in wheat in gallic acid equivalent (GAE) was calculated by the following formula:

$$C = \frac{c \times V}{m}$$

Where C is the total content of phenolic compounds, mg/g fresh material, in GAE; c is the concentration of gallic acid established from the calibration curve($Y = 0.0085x + 0.06492$; $R^2 = 0.9966$), V the volume of extract ,L ;m is the weight of extract, g.

3.5. Sensory Evaluation of bread

Sensory evaluation was conducted for the freshly baked breads by 30 semi-trained panelists consisting of students male and female aged from 23-43 years old from Food Science and Nutrition Center of Addis Ababa University.

The samples were presented randomly in identical containers, coded with three digit numbers. The sensory was conducted using a seven point hedonic scale. Where 1=dislike

very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately and 7= like very much. The sensory attributes which were evaluated were taste, odour ,colour, texture and overall acceptability .Those samples were considered as acceptable which their average score for the overall acceptability were greater than 4 which mean neither like nor dislike. (Lazardiou *et al.*, 2007).

3.6 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests were used for mean separation at $p < 0.05$. Linear regression analysis was used to calculate IC₅₀ value. Pearson correlation between DPPH scavenging (%) and total phenolic content was considered at $p < 0.05$.

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1 Proximate composition of wheat, wheat bran and bread

The mean value for moisture, crude protein, crude fat, total ash and crude fiber of wheat bran, wheat flour (hard and soft), white wheat flour (hard and soft) and bran supplemented bread are presented in Tables 4.1 and 4.2 and 4.3.

4.1.1 Proximate composition of wheat flour

4.1.1.1 Moisture content of wheat flour

The mean values for moisture contents of different whole wheat and white wheat flours are presented in Table 4.1. It ranged from 10.48 to 12.30 %. The highest moisture level, 12.30%, was found in hard white wheat flour. The moisture content varies significantly between whole and white flour and between hard and soft white flour ($P<0.05$). The increment on moisture content of both soft and hard white wheat as compared to the whole wheat flour could be due to the addition of water during tampering process to facilitate milling of wheat which resulted in retaining more water in refined wheat flour than whole wheat flour.

4.1.1.2 Ash content of wheat flour

The statistical analysis (Table 4.1) showed significant difference ($P<0.05$) on total ash contents of all flour samples. The result indicated that highest ash content ranged from 1.62 to 1.41%. The highest ash content (1.62 %) was found in hard whole wheat flour

where as the soft white flour showed the lowest (0.38 %) ash content. The results were comparable to the values obtained from different extraction rate of wheat flour which ranged 1.51 % to 0.54 % ash content at 93% and 70 % extraction rate respectively (Azizi *et al.*, 2006).

Table 4.1 Proximate composition of wheat

Parameters	Wheat samples			
	HWF	HWWF(refined)	SWF	SWWF(refined)
Moisture (%)	10.75±0.38 ^a	12.30±0.09 ^c	10.48±0.10 ^a	11.60±0.23 ^b
Ash (%)	1.62±0.03 ^d	0.65±0.01 ^b	1.41±0.07 ^c	0.38±0.07 ^a
Fat (%)	1.82±0.04 ^b	1.43±0.18 ^a	1.78±0.10 ^b	1.32±0.11 ^a
Protein (%)	14.40±0.30 ^d	11.91±0.087 ^c	9.11±0.12 ^b	7.13±0.06 ^a
Fiber (%)	2.6±0.08 ^b	0.42±0.06 ^a	2.5±0.08 ^b	0.36±0.07 ^a

HWF- Hard whole wheat flour, HWWF- Hard white wheat flour(refined), SWF -Soft whole wheat flour, SWWF- Soft white wheat flour(refined). Data are average of triplicate ±SE .Mean value with different superscript in the same rows are significantly different (P<0.05).

4.1.1.3 Fat content of wheat flour

The result for crude fat content is shown in Table 4.1 and the values showed significant difference (P<0.05) between whole wheat flours and white wheat flours. The fat content decreased in white wheat flour. The highest fat content, 1.83%, was found in whole wheat flour (100% extraction rate); whereas, the lowest, 1.32%, was

found in white wheat flour (low extraction rate). The high percentage of fat in whole wheat flour is because wheat germ is ground along with endosperm during milling (Farooq *et al.*, 2001).

4.1.1.4 Protein content of wheat flour

The results of this study indicated that the protein contents for all flours varied significantly ($P < 0.05$). The protein contents decreased with in both hard and soft white wheat flour; at the same time there was significant difference between hard and soft whole wheat flour. The highest protein content found on hard whole wheat flour was 14.40 %; whereas the protein content of soft whole wheat flour was 9.11 %. The result of this study was in line with the value of hard and soft whole wheat reported by Blakeney *et al.* (2009).

4.1.1.5 Fiber content of wheat flour

The mean value for fiber content of hard whole wheat, hard white wheat, soft whole wheat and soft white wheat flour are 2.6, 0.42, 2.5 and 0.36 g/100g, respectively (Table 4.1). The result of this study showed that there is significant difference ($P < 0.05$) between whole wheat flour and white wheat flour. Both hard and soft whole wheat flour exhibited high crude fiber content (2.6 and 2.5 %).

The white wheat flour showed less fiber content because wheat bran was removed during milling process which decreased the amount of fiber in flour. The result of this

study is in agreement with Azizi *et al*, (2006), who reported crude fiber in the range of 0.30 to 2.24 % white wheat and whole flour respectively.

4.1.2. Proximate composition of wheat bran

The proximate composition of wheat bran samples are given in Table 4.2. Wheat bran was found to contain highest amounts of crude protein, fat, fiber and ash with mean values of 15.26 %, 3.12%, 9.97%, and 4.45% respectively (Table 4.2).

The objective of milling is to separate the bran and germ from the starchy endosperm so that the endosperm can be ground into flour. The aleurone layer, which is rich in protein, minerals and vitamins, usually breaks away with the outer layer of the bran in the milling process (Pederson *et al.*, 1989), thus, contributing significantly to the nutritional quality of the bran fraction (Posner, 2000).

Table 4.2 Proximate composition of wheat bran

Parameter	Composition (g/100g)
Protein	15.26±0.35
Fat	3.12±0.7
Fiber	9.97±0.27
Ash	4.5±0.16

4.1.3 Proximate compositions of bread

Proximate composition of different bran supplemented bread and control were analyzed for proximate composition. The statistical analysis of proximate composition of the samples is given in Table 4.3.

Table 4.3 Proximate composition of Bran supplemented bread and control (Dry weight basis)

Samples	Moisture content %	Protein content %	Fat content %	Ash content %	Fiber content %
Control (WFB)	30.92±0.38 ^a	9.42±.22 ^a	1.56±.0.06 ^a	1.38±0.10 ^a	0.38 ±0.02 ^a
WF:BR (90:10)	33.14±0.26 ^b	10.70±0.55 ^b	2.14±0.032 ^b	2.04±0.32 ^b	2.13±0.05 ^b
WF:BR (80:20)	33.24±0.69 ^b	11.66±0.89 ^c	2.36±0.04 ^c	2.29±0.04 ^c	3.11±0.06 ^c
WF:BR (75:25)	32.83±0.58 ^b	12.04±0.84 ^c	2.61±0.06 ^d	2.48±0.02 ^d	3.27±0.008 ^d

WFB-(white wheat flour bread)- control, WF:BR 90:10, 10 % bran supplemented bread ,WF:BR 80:20- 20% bran supplemented bread, WF:BR 75:25 - 25% bran supplemented bread Data are average of triplicate ±SE. Mean value with different superscript in the same column are significantly different (P<0.05).

4.1.3.1Moisture content of bread

The mean values for moisture contents of the bread samples are presented in Table 4.3 which ranged from 30.92 to 32.83%. The highest moisture level, 32.83%, was found in

25% bran supplemented bread. The moisture content of the control bread decreased from the three samples bread significantly ($P<0.05$).

4.1.3.2 Protein content of bread

The statistical analysis for crude protein is presented in Table 4.3. The mean value for protein content of all the study samples of bread ranged from 9.42 for control to 12.04 for WBB (75:25). The protein contents for three of the breads (0%, 10%, 20% bran supplemented bread) varied significantly ($P<0.05$). However, there was no significant difference between 20% and 25 % bran supplemented bread. The result of protein contents are in agreement with the findings of Butt *et al.*, (2004) who reported an increase contents of protein with an increase in bran proportion.

4.1.3.3. Fat content of bread

The result of this study indicate that crude fat showed significant difference ($P<0.05$) among all breads. The fat content increased with an increase in bran level. The highest fat content, 2.61%, is found in 25% bran supplemented bread, whereas, the lowest, 1.56%, was found in the control bread. The increase in fat content is because of the germ which is grounded along with bran and endosperm during milling, results in bread with higher fat content than the control bread (Farooq *et al.*, 2001).

4.1.3.4. Fiber content of bread

The mean values of crude fiber contents of different bread samples are given in table 4.3. The statistical analysis showed significant ($P<0.05$) effect on the quantity of crude fiber.

The crude fiber contents ranged from 0.38 % to 3.27 %. The 25% bran supplemented bread exhibited the highest crude fiber (3.27%), whereas, the control bread contain the lowest crude fiber (0.38%). The crude fiber increased with an increase in bran supplementation rate. The control showed less fiber contents because the bread was made from refined bread with no additon of bran.

4.1.3.5 Ash content of bread

Ash is the mineral residue remaining after a sample has been completely oxidized in a manner such that all organic volatile material is driven off, while preventing any mineral from being lost (Posner, 2000). Ash varied significantly among all the bread (Table 4.3). The statistical analysis showed significant ($P<0.05$) effect on total ash contents. The results indicate that ash content ranged from 1.38 to 2.48 %. The highest ash content (2.48%) was found in 25% bran supplemented bread, whereas the control showed the lowest (1.38%) ash content. The addition of 10 to 25% wheat bran to the bread increased the ash content.

4.2 Mineral content of wheat and Bread

4.2.1 Mineral analysis of wheat

4.2.1.1 Iron contents

The mineral content of the whole and white flour samples are shown in Table 4.4. According to the results of this study, the iron content level in hard and soft whole wheat flour is significantly different ($p<0.05$) from hard and soft white wheat flour. The iron

content of whole wheat flour ranged from 2.95 to 4.15 mg/100g where as the iron content of the white wheat flour ranged from 2.51 to 3.35 mg/100g.

Dewettinck *et al.*, (2008) reported that the iron content of the whole wheat was (1-5 mg/100g) which is in agreement with the result of this study. However, the level of iron in the white flour decreases significantly. The milling process removes many important nutrients when white flour is produced. The bran and the germ are relatively rich in minerals and the milled products contain less of these than the original grain. As a result of milling the palatability is increased but the nutritional value of the products is decreased (Hoseney, 1992).

Table.4.4 Mineral composition of whole and refined wheat flour

Whole and refined wheat Sample	Mineral content mg/100g		
	Iron	Zinc	Phosphorous
HWF	4.15±0.12 ^c	3.59±0.063 ^d	337.99±0.56 ^d
HWWF	2.51 ±0.16 ^a	1.39± 0.036 ^b	144.69±0.61 ^b
SWF	3.35±0.17 ^b	2.47±0.04 ^c	313.98±1 ^c
SWWF	2.95±0.26 ^a	0.58±0.01 ^a	77.03±0.51 ^a

HWF- hard whole wheat flour, HWWF- Hard white wheat flour, SWF -soft whole wheat flour. SWWF- Soft white wheat flour. Mean value with different superscript in the same column are significantly different (P<0.05).

4.2.2.2 Zinc content

Zinc content varied significantly among all whole wheat and white wheat flour (Table 4.4). The zinc content of the whole wheat ranged from 3.59 to 2.47 mg/100g. However, the white flour contained in the range of 0.58 to 1.39 mg/100g. The highest Zn content

3.59 mg/100g was found in hard whole wheat flour. This study showed that low rate of extraction of wheat reduce the zinc content of the wheat significantly ($P<0.05$). The hard whole wheat flour zinc content was 3.59 mg/100 g where as milling reduced the zinc content to 1.39 mg/100g. Where as incase of soft wheat the decrease was from whole wheat to white wheat flour 2.47 to 0.58 mg/100g of zinc respectively. According to Lopez et al.,(2003) 80% of the total amounts of minerals are concentrated in the aleurone layer of pericarp (bran) which was removed during milling process while only 20% minerals are present in endosperm .

4.2.2.3 Phosphorous content

The mean total content of phosphorous on hard whole and soft whole wheat flour was 337.99 and 313.98 mg/100g respectively however, there was significant decrease of phosphorous content ($P<0.05$) on the refined milled product of hard and soft white wheat flour which was 144.69 and 77.03 mg/100g respectively. Wheat is one of the cereals which is classified as rich sources of phosphorous. The result of the whole wheat flour was in line with the finding of Dewettinick *et al.*, (2008) which reported phosphorous from 200-1200 mg/100g.

4.2.2 Mineral analysis of bread

4.2.2.1 Iron contents

The replacement effect of different levels of wheat bran on the mineral content of bread is shown in Table 4.5. All the mean value for iron content varied among the bread samples. Results showed that mineral progressively increased when levels of bran were

increased. Control contained 1.98 mg/100 g iron and at the level of 25% bran replacement, the value increased to 4.84mg/100g of iron. The result of this study was in agreement with the study of Butt, *et al.*, (2004).

Table 4.5 Mineral analysis of bread (Dry weight basis)

Samples	Iron mg/100g	Zinc mg/100g
Control (WFB)	1.98±0.056 ^a	0.93±0.064 ^a
WF:BR(90:10)	2.34±0.159 ^b	1.697±0.108 ^b
WF:BR (80:20)	2.95±0.0753 ^c	2.28±0.131 ^c
WF:BR (75:25)	4.83±0.074 ^d	2.33±0.066 ^c

WFB-(white wheat flour bread)- control, WF:BR 90:10, 10 % bran supplemented bread ,WF:BR 80:20- 20% bran supplemented bread, WF:BR 75:25 - 25% bran supplemented bread. Data are average of triplicate ±SE Mean value with different superscript in the same column are significantly different (P<0.05)

4.2.2.2 Zinc contents

The replacement effect of different levels of wheat bran on the zinc content of bread is shown in Table 4.5. Results showed that zinc progressively increased when levels of bran were increased. The result ranged from 0.93 to 2.33 mg /100 g of zinc. The value of zinc at the bran supplementation of between 20 and 25 % was not significantly different (P>0.05).

Micronutrient malnutrition greatly increases mortality and morbidity rates, diminishes cognitive abilities of children, lowers labor productivity and reduces the quality of life for

all those affected (Welch & Graham 1999). Deficiency of micronutrients, such as iron and zinc, is critical and major problem

It could be concluded that the addition of bran improves the nutritional quality of bread and could be a means of providing adult their daily requirements of iron and zinc. Although supplementation of bran improves the mineral content of the bread due to increase of phytic acid content, the availability of these minerals may be reduced. Therefore, such issues would need further evaluation.

4.3 Antioxidant capacity of wheat

4.3.1. Extraction yield and total phenolic content

4.3.1.1 Yield of extracts

The percentage yields of extracts were 7.58 % w/w (Hard refined wheat), 8.2% w/w (Hard whole flour wheat) and 7.7% w/w (Soft refined wheat flour) and 7.3% w/w (soft whole flour wheat).

Table 4.6. Percent yield and total phenolic content

Sample	Mean $\pm$ SEM(mg GAE/g) $\pm$ SEM	Yield % (g/100g)
HWF(whole)	7.66 $\pm$ 0.70 ^b	8.2
HWWF(refined)	3.49 $\pm$ 0.86 ^a	7.58
SWWF(refined)	3.34 $\pm$ 0.14 ^a	7.7
SWF(whole)	8.20 $\pm$ 0.35 ^b	7.3

HWF- hard whole wheat flour, HWWF- Hard white wheat flour(refined), SWF -Soft whole wheat flour. SWWF-Soft white wheat flour(refined) Data are average of triplicate $\pm$ SE. Values in the same column with different superscript are statistically significant ($p < 0.05$).

4.3.1.2 Total Phenolic Content

It has been recognized that the total phenolic content of plant extracts is associated with their antioxidant activities due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Total phenolic content (TPC) was expressed as milligrams of Galic acid equivalent (GAE) per gram (mg/g) of dry flour samples. As shown in table 4.6, the total phenolic content (TPC) in whole wheat was highest. The TPC of white wheat flours (refined), which ranged from 3.34 to 3.49 mg GAE/g which were significantly lower ($p < 0.005$) than those of whole wheat flours (range 7.66 to 8.20 mg GAE/g). However, the mean content did not vary much between whole hard and soft wheat type. Also there was no significant variation between soft and hard white wheat flour. The difference in the total phenolic content between whole and white wheat flour could be due to the process of milling. Research found antioxidants in wheat concentrated mostly in the aleurone layer of bran with some in the pericarp, nucellar envelope and germ (Fulcher and Duke, 2002; ŽILIC *et al.*, 2012a).

4.3.2 DPPH scavenging activity of wheat

The ability of wheat extracts to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity test. The antioxidants can react with DPPH, a violet coloured stable free radical, converting it into a yellow coloured α, α -diphenil- β -picrylhydrazine. The discoloration of the reaction mixture can be quantified by measuring the absorbance at 517 nm, which indicates the radical-scavenging ability of

the antioxidant. The antioxidant capacity whole and refined wheat was measured as the DPPH• scavenging activity.

The DPPH radical scavenging effects of wheat methanol extracts was shown in Figure 3. As the concentration of sample increased, the percent inhibition of DPPH radical also increased (Huang *et al.*, 2005). At the concentration of 50 mg/mL, the scavenging effect of ascorbic acid, and wheat extracts, on the DPPH radical scavenging decreased in the order of L- ascorbic acid > soft whole > hard whole > soft white > hard white wheat flour, which were 92.53, 90.39, 89.89, 75.80, 57.57 % respectively. Therefore, the percentage of DPPH radical scavenging capacity of soft whole and hard whole wheat extracts are comparable with commercial antioxidants, L-ascorbic acid at concentration of 50 mg/mL. This suggested that whole wheat contain compounds that can donate electron/hydrogen easily and stabilizes free radicals.

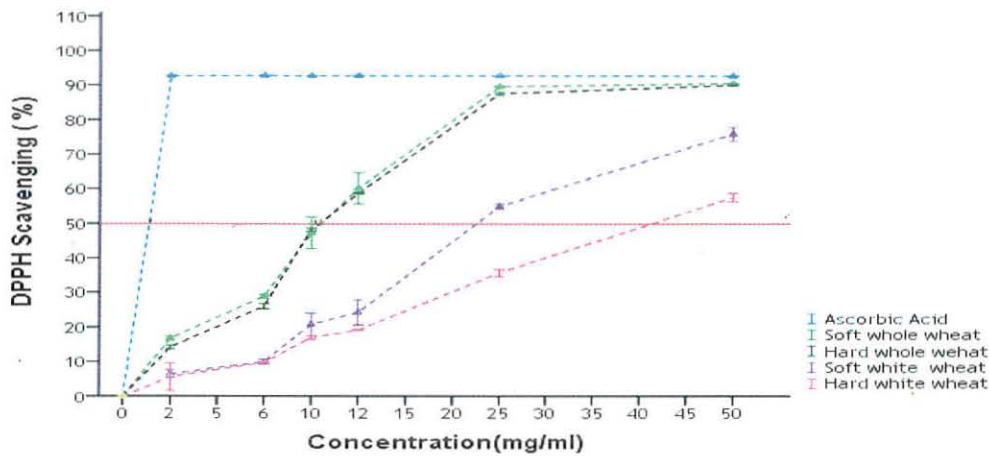


Figure 3. DPPH radical scavenging activity (%) of wheat extracts and standard.

Values are average of triplicate measurements (mean $\pm$ SEM).

The IC50 values of all the extracts were calculated from plotted graph of percentage scavenging activity against concentration of the extracts (Fig .3) The lower the IC50 value, the higher is the scavenging potential. The IC50 values ranged from 10.56 mg/mL for whole wheat extracts to 41.25 mg/mL for hard white wheat extracts. Strongest scavenging activity (lower IC50 values) was recorded for whole hard and soft wheat extracts which appeared more than four times stronger than that of hard white flour and two times stronger than that of soft white wheat extracts. IC50 value of ascorbic acid well known antioxidant, was relatively more pronounced than that of the extracts (figure 3). The results of this study demonstrate that the antioxidant content of wheat has been affected by the refined extraction/milling process. According to Fikreyesus *et al.*, (2013) the DPPH (IC 50) for whole wheat flour is 15.56 which is different from the result obtained in this research. It is known that the antioxidant properties of wheat grain are significantly influenced by the genotype and environmental conditions (Adom *et al.*, 2003).

To the best of my knowledge, there are no or few studies conducted on antioxidant content of Ethiopian wheat and particularly on comparison of antioxidant content on the whole and white wheat flour.

A relationship between phenolic content and antioxidant activity was extensively investigated, and both positive and negative correlations were reported. Bakchiche *et al.* (2013), Petra *et al.* (2012) and many other research groups stated that there was a positive

correlation. However, a few evidences of no significant correlation were confronted (Mohammed *et al.*, 2008). In this study, the dependence of DPPH scavenging activity (%) in relation to the total phenolic content was also evaluated. The total phenolic content correlated significantly (Fig 4) with DPPH scavenging activity ($R^2 = 0.637$, $p < 0.05$). Thus the phenolics from the wheat extracts showed a good hydrogen-donating capacity, as well as high reactivity to free radicals, leading to the stabilization and termination of the radical chain reactions.

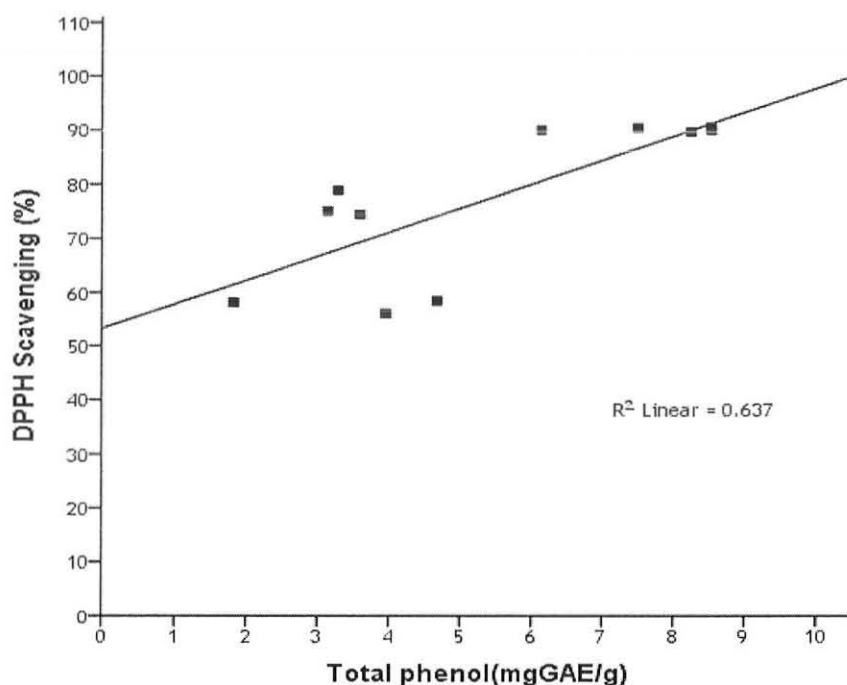


Fig. 4 Correlations between total phenolic content of extracts and DPPH scavenging activity of whole and white wheat flour.

4.4. Sensory analysis of the Bread

The sensory attributes of bread made from bran (Fig.5) using different ratio were evaluated using 7-point hedonic scale at Addis Ababa University; Center for Food

Science and Nutrition by semi-trained panelists of first year M.Sc program students of Food Science and Nutrition stream and the mean scores of evaluated sensory attributes were presented in Table 4.7



Fig.5 Wheat bran supplemented bread.

4.4.1 Taste

Taste is an important parameter when evaluating sensory attribute of food. The product without acceptable good test is likely to be unacceptable by consumers. The observed mean score of taste in experiential bran supplemented bread ranged from 4.43 to 5.93 (Table 4.7). Control (100% white wheat flour bread) had the highest mean score in taste (5.93) followed by 10% bran supplemented bread (5.93). The 100% white wheat flour (control) bread had significant difference ($p < 0.05$) with 10% bran (5.26), 20% bran bread (4.80) and 25% bran bread (4.43). The control bread had significant difference ($p < 0.05$) from three of the bran supplemented bread (10%, 20%, 25%). The 10% bran

supplemented bread scores > 5 indicating that it is moderately likable by panelists and 20% and 25% bran supplemented bread were rated as neither like nor dislike by the panelists (scored as 4.80 and 4.43 respectively). This is due to the addition of the bran to the bread.

4.4.2 Odour

The mean score of odour of bread ranged from 4.76 to 5.53 (Table 4.7). Most of the samples were similar in odor while only 25% supplemented bread significantly ($P < 0.05$) decreased. Three of the samples were liked moderately whilst the 25 % bran supplemented bread was rated as neither like nor dislike.

Table 4.7 Sensory characteristics of wheat bran supplemented Bread.

Sample	Test	Odour	Colour	Texture	Overall acceptability
Control (WFB)	5.93±0.14 ^c	5.53±0.21 ^b	5.93±0.18 ^c	5.70±0.19 ^b	5.93±0.11 ^c
WF:BR (90:10)	5.26±0.16 ^b	5.43±0.18 ^b	5.46±0.14 ^c	5.30±0.13 ^b	5.43±0.15 ^b
WF:BR (80:20)	4.80±0.24 ^b	5.33±0.23 ^b	4.76±0.22 ^b	4.60 ±0.20 ^a	4.96±0.16 ^b
WF:BR (75:25)	4.43±0.30 ^a	4.76±0.22 ^a	4.13±0.24 ^a	4.36±0.23 ^a	4.50±0.22 ^a

WFB-(white wheat flour bread)- control, WF:BR 90:10, 10 % bran supplemented bread ,WF:BR 80:20- 20% bran supplemented bread, WF:BR 75:25 - 25% bran supplemented bread. Data are average of triplicate ±SE Mean value with different superscript in the same column are significantly different ($P < 0.05$).

4.4.3 Colour

As is shown in Table 4.7 colour of bread had low score as a result of increasing the level of wheat bran. The colour of control and 10% supplemented bread were similar in appearance while 20% and 25% bran supplemented bread were decreased (4.76 and 4.13) significantly ($p < 0.05$).

4.4.4 Texture

The results indicated that no significant difference ($p > 0.05$) were observed by panelists between the control and 10 % supplemented bread. The texture of the control and 10% bran supplemented bread were relatively most preferred (liked moderately) by the panelists. While the bread prepared from increasing level of bran supplement from 25 % were scored as 4.5.

4.4.5 Overall acceptability

Generally, among the bread products, the control were highly acceptable by the panelists, with a score of 5.93. Next to this the 10 % and 20% bran supplemented bread scored, 5.43 and 4.96 respectively. These two are liked moderately. And the result obtained for 25 % bran supplemented bread was significantly different from the previous two. The latter was neither like nor disliked by the panelists and it scored 4.5. In relation to this Lazardiou *et al.*, (2007) reported that those samples were considered as acceptable which their average score for the overall acceptability were greater than 4 which mean neither like nor dislike.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Wheat and wheat products are important staple foods that are commonly consumed in Ethiopia. Consumption of whole grains as part of the diet is recommended for health reasons because they are good source of minerals, fibers, protein and antioxidants.

There are no studies on the effect of refining on the nutritional content and antioxidant capacity of wheat grown in Ethiopia. Therefore, this study was conducted to evaluate the effect of refining on the nutritional content and the antioxidant capacity of two types of wheat. The study also attempted to utilize bran which is the milling by product as a value added food ingredients to make nutritious bread.

The study showed that wheat extraction/refining at the lower rate has significantly reduces the proximate composition as well as the antioxidant content of wheat in both hard and soft wheat samples.

Addition of wheat bran to white wheat flour improves the nutritional value of the bread. Based on obtained results, the incorporation of wheat bran in the ratio of 10 to 20 % showed better sensory acceptability though the proximate composition and mineral content increased at 25% bran supplimented bread. This indicates that bread of good

nutritional and sensory qualities could be produced from 10 % and 20 % bran supplementation. The result of this study also indicated that wheat bran as a good source of minerals and fibers and can be used to supplement bread.

5.2 Recommendations

The following recommendations are made based on the study conducted

- Whole wheat flour with high extraction rate (100 %) needed to be given high emphasis by consumers because of its nutritional and antioxidant capacity of the product.
- Commercialization of wheat bran as a value added food ingredient to benefit from its high nutrition content.

Recommendation for future work

- Antioxidant properties of wheat are significantly influenced by the genotype and environmental conditions therefore the antioxidant capacity of wheat varieties in Ethiopia should be studied
- Study need to be conducted on the pretreatment of wheat bran for to improve the physical quality of the product that can be produced form wheat bran.

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Annexes

SENSORY EVALUATION SCORE SHEET

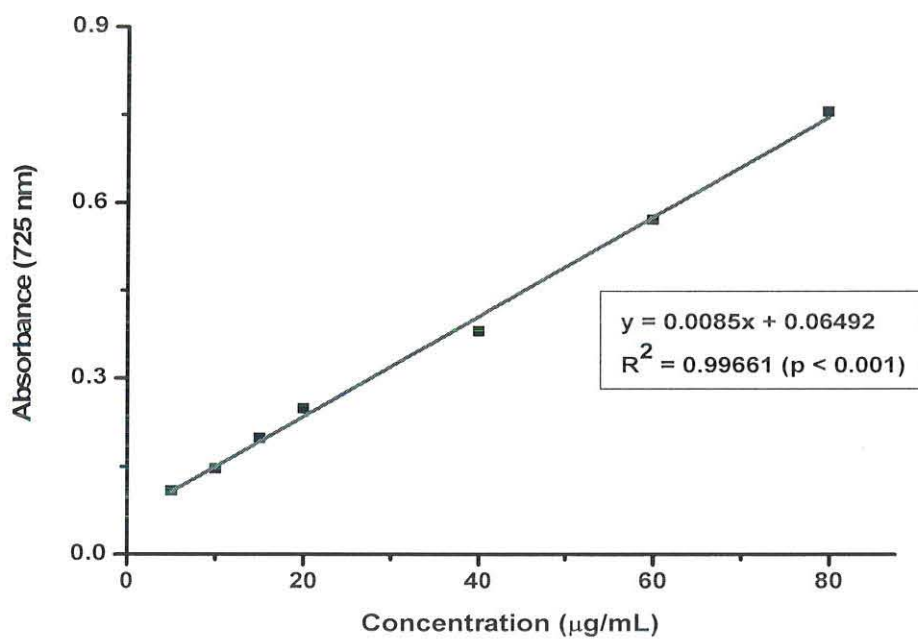
Sample Code _____

Date _____

Sex _____ **age** _____

After drinking some water, please taste the sample and tick the box in the table below to indicate your attitude about the product.

Attributes	Dislike extremely	Dislike very much	Dislike moderately	Neither like or dislike	Like moderately	like very much	Like extremely
Test							
Color							
Odor							
Texture							
Over all acceptability							



Galic acid calibration curve.

Declaration

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

Name: Genet Gebremedhin Heshe_____

The thesis has been submitted with our approval as advisors:

Dr Gulelat Desse Haki (Ph.D)_____

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