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Effect of Processing and Tef Varieties (*Eragrostis tef* (Zucc.)) on the Antioxidant Properties of Ethiopian Traditional Bread, *Injera*.

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 program

By

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LIST OF ABBREVIATION AND ACRONYMS

AAPH - 2, 2'-Azobis(2-Amidinopropane) Dihydrochloride

ABTS – 2, 2′-Azino-di-(3-ethylBenzthiazoline Sulphonate)

BHA - Butylated Hydroxy Anisole

BHT - Butyl Hydroxy Toluene

DPPH – Di Phenyl Picryl Hydrazyl

EDTA - Ethylene Di-amine Tetra Acetate

EHNRI - Ethiopian Health and Nutrition Research Institute

ET – Electron Transfer

FIC - Ferrous Ion Chelating effect

FRAP - Ferric ion Reducing Antioxidant Power

FRSA – Free Radical Scavenging Activity

g – gram

GAE - Gallic Acid Equivalent

HAT – Hydrogen Atom Tranfer

l - litre

ml – millilitre

ORAC -- Oxygen Radical Absorbance Capacity

POH - Phenolic Compounds

RSA- Radical Scavenging Assay

RON – Reactive Nitrogen Species

ROS – Reactive Oxygen Species

RPA – Reducing Power Assay

TAC – Total Antioxidant Capacity

TEAC - Trolox Equivalence Antioxidant Capacity

TFC – Total Flavonoid Content

TPC - Total Phenolic Content

TRAP - Total Radical trapping Antioxidant Parameter

TRP – Total Reducing Power

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A.1.2. Standard curve for total flavonoid determination

A.2. Two ways ANOVA table for total phenols and flavonoids of three tef varieties and their processed product, *injeras*.

ABSTRACT

Imbalanced production and consumption of reactive oxygen species, leading to oxidative stress such as cancer, arteriosclerosis neurodegenerative diseases and ageing processes. The protective effects of plants have long been attributed to their antioxidant compounds, such as phenolic compounds and flavonoids. The purpose of this study was comparing antioxidative potential of methanolic extracts of three tef varieties (white, brown and red tef) and their *injeras* (partly fermented and fully fermented) and their enriched one with fenugreek. The antioxidant capacities of tef varieties and their flat bread, *injeras* were evaluated by using different methods, namely DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, total reducing power, total phenol contents and total flavonoid levels. Total phenol content (TPC) was determined by using folin-ciocalteau reagent. Total flavonoid content (TFC) was determined by using colorimetric assay method, the absorbance of all parameters measured using UV-viz-spectrophotometry. The level of IC $_{50}$ values of free radical and total reducing power of various tef varieties varied from 0.6 mg/ml to 0.88 mg/ ml and 2.25 mg/ml to 2.5 mg/ml respectively. The results showed the highest scavenging activities (0.6 mg/ml) and reducing power (2.25 mg/ml) observed in red tef while the lowest scavenging activities and reductive potential potent in white tef (0.86 mg/ml) and (2.5g/ml). Current results of total phenolic content assay indicate that total phenol content was higher in red tef (11.47 mg GAE/g) as compared to brown (9.715 mg GAE/g) and white tef (8.28 mg/ GAE/g). In addition, total flavonoids for white, brown and red tef were 1.03 mg/CE/g, 1.78 mg CE/g and 2.13 mg CE/g respectively. The processing of tef flour in to partly or fully fermented *injeras* and their enrichment with fenugreek affect the tef antioxidant levels this has implications for their bioactive properties and potential health benefits. The IC$_{50}$ - values for free radical scavenging activities and reducing power of partly fermented, fully fermented, enriched partly fermented and enriched fully fermented white tef *injeras* were 2.8 mg/ml ; 2.75 mg/ml, 3.25 mg/ml ; 3.3 mg/ml, 2.63 mg/ml; 2.7 mg/ml; 3 mg/ml ; 3 mg/ml respectively. Phenolic composition in the tef *injeras* examined. The results showed that enriched partly fermented red tef *injeras* had remarkable phenol levels (up to 9.12 GAE/g). The total phenol contents for partly fermented, fully fermented, and enriched fully fermented red tef *injeras* were found to be 8.75, 7.36, and 8.24 mg GAE/g respectively. In brown tef *injeras*, the highest flavonoid content was noticed in enriched partly fermented *injera* (1.9 mg CE/ g).

The lowest content was in brown fully fermented *injeras* (1.18 mg CE/ g). The flavonoid content of brown partly fermented and brown enriched fermented *injera* were 1.33 mg CE/ g and 1.264 mg CE/g respectively. The analysis of variance and LSD test done on the total phenols and flavonoids contents, the result revealed that the phenol and flavonoid contents of three tef varieties were significantly affected ($P < 0.05$) by processing and variety, however their interaction was found to be insignificance ($P>0.05$). In all four total antioxidants parameters, antioxidant activities of *injeras* decreased in the order of red tef *injeras* > brown tef *injeras* > white tef *injeras* in the same processing conditions. The study showed that partly fermented tef *injeras* had high antioxidant capacity than fully fermented tef *injeras* among the same tef varieties. However, partly fermented tef *injeras* extracts showed lower antioxidant activities than raw tef flour. The enrichment of tef flour with fenugreek flour at the ratio of 95: 5 respectively had high antioxidant levels respect to unenriched *injeras*.

Key words: *Injera,* tef, fenugreek, antioxidant activity, DPPH, free radical scavenging activity, total reducing power, total phenol, total flavonoid, enrichment,

1. Introduction

1.1. Back ground of the study

Africa is the centre of origin and still today the major producing area for several cereal crops, notably sorghum, pearl millet, finger millet, tef, fonio and African rice. These traditional African cereals are sometimes called "Orphan Crops", or even '' Lost Crops'' (National research council, 1996). This is despite the fact that they are staple foods for millions of people in the semi-arid regions of the world, and especially particularly those who live by subsistence farming.

Tef, *Eragrostis tef* (Zucc.) Trotter is a self-pollinated, annual, warm season grass that is used throughout the world as grain for human consumption and as forage for livestock. The amount of tef produced in the world is increasing rapidly due to the plant's popularity as an especially nutritious grain. Tef grain does not contain gluten and is an increasingly important dietary component for individuals who suffer from gluten intolerance. Tef is an ancient grain that was believed to have been domesticated in Ethiopia between 4000 and 1000 BC. When grown as a grain it is normally ground into flour, which is used to make *injera*, flat bread eaten with every meal. It is also used as porridge, similar to cream of wheat or fermented and used to make an alcoholic beverage (Ketema, 1997).

There are few different varieties of tef that vary in color from light to dark. The color can be ivory, light tan to deep brown or dark reddish brown purple, depending on the varieties. According to EHNRI (1997) the various types of injera produced from the different varieties of tef do not have significant variation in their calorie, moisture, protein, carbohydrate, or phosphorus nutrients. Significant variations are, however, observed in the other nutrients. There is no well established among their antioxidant difference.

Some researchers suggested that fenugreek (Trigonella foenumgraecum) could be good supplement used with tef. In some regions of Ethiopia, e.g. Welo, women usually prepare *Injera* by adding some fenugreek to tef flour to improve its baking quality. Because of this, *Injera* is believed to become softer and possess a shiny appearance. Thus, it should be encouraged to keep on this traditional practice and users be made aware that such practice not only has the benefit of improving the baking quality of the *Injera* but also of supplementing its protein content, especially lysine (Ketema, 1997).

In recent years, nutritionists and the general public have come to regard cereals as more than sources of energy and essential nutrients. Certain minor components of foods are now recognized for their health-promoting properties, in particular for their roles in preventing or alleviating the effects of some of the chronic diseases such as cardiovascular disease and certain cancers. The biological activities of many phytochemicals are attributed to their antioxidant properties (Kelawala and Ananthanarayan, 2004). Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. The free radicals produced by oxidation reactions start chain reactions that damage cells. Antioxidant terminates these chain reactions by removing free radical intermediates and inhibits other oxidation reactions by being oxidized themselves (Sies, 1997). Antioxidants play a major role in the prevention and treatment of a variety of diseases. The proposed mechanism by which antioxidants protects cells from oxidative stress is by scavenging free radicals, chelating catalytic metals and halting lipid per oxidation chain reactions. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases (Nikolova *et al*., 2007).

Vegetables and fruits are the most important sources of these antioxidants. However, grains have largely been ignored as important contributors of dietary antioxidants, despite the fact that they are a staple dietary component for most of the world's population. Antioxidants found in whole grain foods are polyphenol including phenolic acids and flavonoids, which are responsible for the high antioxidant activity (Nahapetian,and Bassiri, 1976).

Although, many studies have been done for their cereals antioxidant activities in many other parts of the world, very little information is available about the antioxidant properties of tef. A major reason is that it is under-researched. Thus, the challenge for researcher working on Ethiopian tef is to think and act smarter; hence, study was undertaken to determine the antioxidant content of three tef varieties (white tef, red tef, and brown tef) and their each processed products (*Injera*) with and without enrichment with fenugreek.

1.2.Statement of the problem

Natural antioxidants have drawn increasing attention in the prevention of various oxidative stress associated diseases. Cereals are known to posses' antioxidant activity. Many of the biological functions, such as anti-mutagenicity, anti-carcinogenicity, and anti-aging originate from antioxidant properties (Cook and Samman, 1996). Natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects.

Most of the literature on plant antioxidants focuses mainly on those fruits, vegetables, wines, and teas (Pennington, 2002). However, many antioxidants found in fruits and vegetables are also detected in cereal grains. The different species of grains have a great deal of diversity in their germplasm resources, which can be exploited. However, many of these compounds are not quantifiable and unidentifiable. Therefore, further research is needed to quantify, isolate and characterize the components that contribute to health, which is challenging because many of these compounds are bound to the matrix of the cereals, making their extraction difficult (Spiller, 2002).

Obtaining antioxidants from cereals have several attractive advantages: Compared to fruits and vegetables; cereal grains are (1) dry, (2) easy to store for long periods of time, and (3) possibly easier to process in to shelf-stable concentrates. Cereals can provide viable alternatives to diversify sources of healthy components in foods (Blandino *et al.*, 2003).

Identifying and quantifying tef antioxidants help us to select tef with increased levels of health promoting compounds. There has not been well established research for antioxidants of Ethiopian tef varieties so research is needed to determine their stability during processing, quantity, characterization and health contribution in humans. Within tef varieties, great variations in colours occur among genetic materials and it may provide large quantities of potentially health promoting substances.

As antioxidants are susceptible to oxidation and degradation, exposure to light, oxygen and heat, conditions normally present during food processing, influence the properties of antioxidants, so processed foods are mainly enriched with antioxidant rich food ingredients to enhance the asthetic nutritional quality and antioxidant contents. Pulses, spices and green leafy vegetables were added as antioxidant rich food ingredients in acceptable proportion, by using standard methods of preparation (Swaminathan *et al.*, 1981). Therefore information on the stability of antioxidants during food processing is important for evaluating the potential health benefits of foods containing phytochemicals. Hence, this study was designed to investigate in the antioxidant potential of tef varieties and their processed products, *injeras*.

1.3. Objectives of the study

1.3.1. General objective

The general objective of the study is to evaluate the effect of processing and tef varieties on the antioxidant properties of Ethiopian traditional bread, *Injera*.

1.3.2. Specific objectives

1. To determine the free radical scavenging activities of tef varieties and their *injeras*; white, brown and red tef.
2. To determine the total reducing power among three tef varieties and their *injeras*.
3. To quantify total phenols from three tef varieties and their processed products, *injeras* (partly and fully fermented).
4. To quantify the total amounts of flavonoids in three tef varieties and their *injeras*.
5. To evaluate the total antioxidant activities (free radical scavenging, reducing power, total phenols and flavonoids) of enriched three tef varieties *injeras* (with fenugreek).

2. Literature review

2.1. Plant antioxidants

Secondary plant metabolites, including antioxidants, are produced by plants to regulate Physiology and patterns of growth (Daniel *et al.*, 1999). Some help plants deal with environmental extremes, deter pest attacks, or respond to damage caused when insects or plant pathogens reach damaging levels. Some play a role in repairing injured leaf or fruit tissue through the formation of pigments. Antioxidant-driven plant defences and wound-healing processes account for the distinctive and elevating antioxidant levels in food sometimes remarkably rich colour and flavour of certain fruits and vegetables grown in some regions. After harvest and during storage cereals, fruits and vegetables with higher antioxidant levels are typically able to more effectively slow the onset and progression of post-harvest infections. This property can help extend the shelf life of product and lessen mycotoxin risks (Daniel *et al.*, 1999). Besides, antioxidants are substances produced by plants in order to provide protection to their cells from particularly harmful UV rays and other plant pathogens. These materials can be useful to human beings, since they play similar protective functions in the human body, and thus their consumption may protect the body from harm.

2.2. Free radicals and antioxidants

2.2.1. Free radicals

Free radicals are oxygen-based or nitrogen-based molecules with unpaired electrons that are generated by a number of metabolic processes within the body. For example, when the body turns foods into energy, free radicals are formed by normal oxidation reactions. Vigorous exercise increases free radical production, as does inflammation, exposure to certain chemicals, cigarette smoke, alcohol, air pollutants and high-fat diets (Alarcon-Aguilara *et al.*, 1998). Besides environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods cause the formation of free radicals. Free radicals cause depletion of the inmune system, the change in gene expression and induce abnormal proteins. The oxidation process is one of the most important routs for producing free radicals in food, drugs, and even living systems (Pourmad *et al.*, 2006; Dillard *et al.*, 2000; Turkoglu *et al.*, 2007).

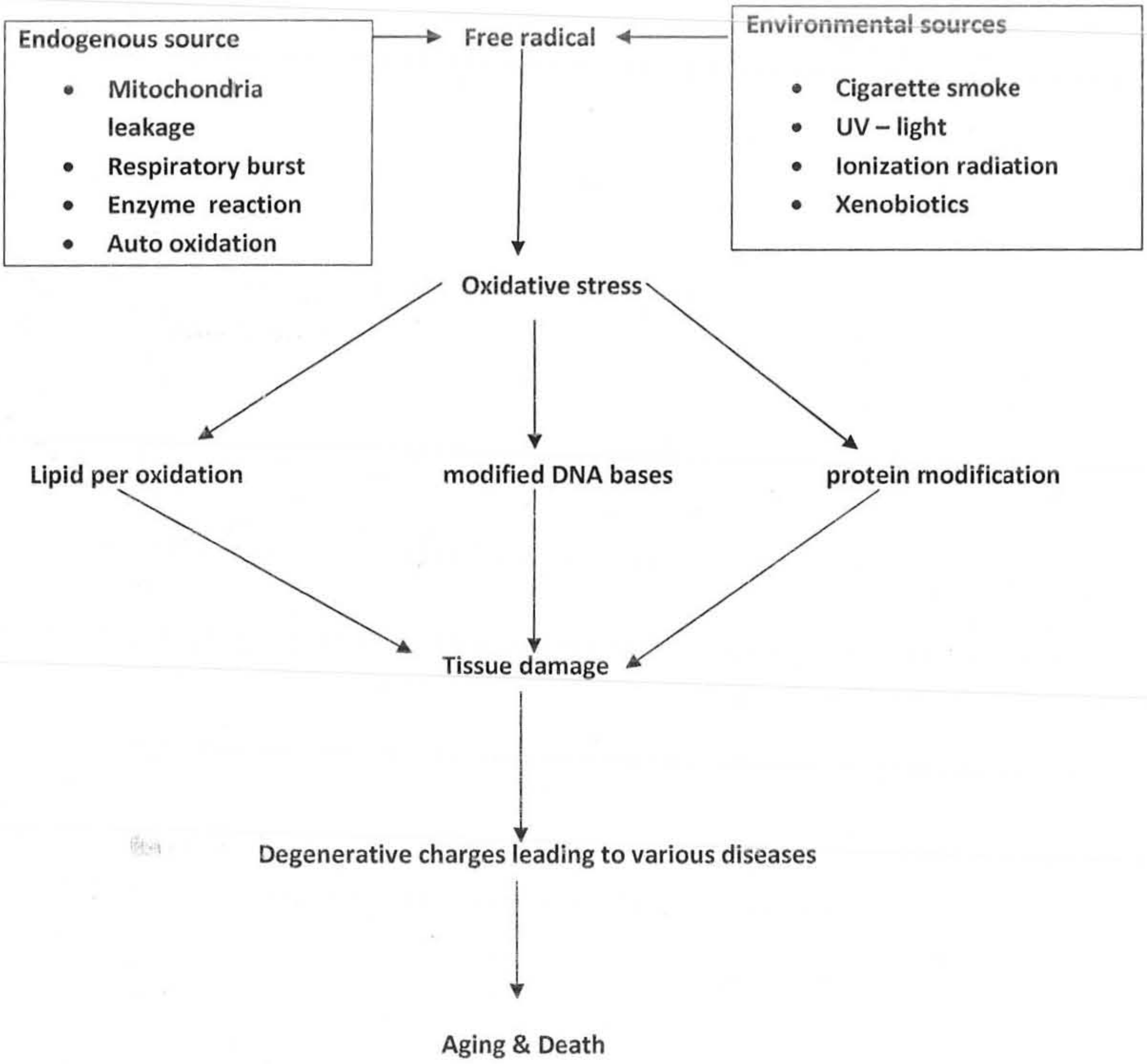


Figure 1. Major sources of free radicals in the body and the consequences of free radical damage.

According to the Mitochondrial Free Radical Theory of Aging (1999), free radicals are a class of molecule with a very simple definition. The nature of atomic structure and of the covalent chemical bond (the features that give an atom its valency) are fixed by the rule that electrons occupy orbital's of atoms, such that an orbital can contain zero, one or two electrons, and that electrons carry less energy when they are one of a pair in an orbital than when they are unpaired. A molecule is only a free radical if it possesses one or more unpaired electrons.

Once free radicals formed, these highly reactive radicals can start a chain reaction like dominoes. As one free radical interacts with another molecule, in effect stealing part of it, a new free radical is created. These reactions often occur in or near cell membranes and can erode the cell's internal integrity. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis and cancer (Pourmad *et al.*,2006: Wong *et al.*, 2006; Su *et al.*,2007; Tepe *et al.*, 2007). These free radicals cause DNA damage and lipid peroxidation, leading to cancerous cells and some targeting mitochondria inside cells, affecting their energy-producing capability so cells may function poorly or die if this occurs (Fennema, 1996).

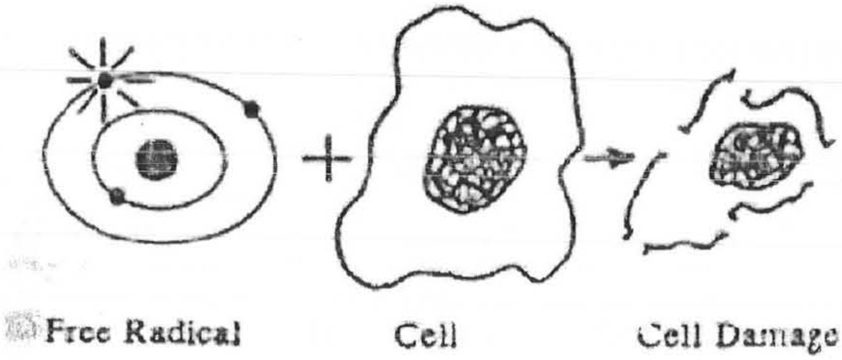


Figure 2. Cell damage by free radicals (Source: Simone, 1992)

Within the human body, millions of processes are occurring at all times. These processes require oxygen. Unfortunately, that same life giving oxygen can create harmful side effects, or oxidant substances, which cause cell damage and lead to chronic disease. Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species (Davies, 1995).

Reactive oxygen species encompass both true free radicals and molecules with paired electrons like hydrogen peroxide. Other important reactive oxygen species include singlet oxygen and superoxide anions, hydroxyl, and peroxyl radicals. Free radicals and reactive oxygen species cause cell damage, trigger inflammation, and promote abnormal cell growths, including many kinds of cancer. Antioxidants help prevent tissue damage by combining with free radicals and neutralizing them (Apel and Hirt, 2004).

Organisms have to counteract these negative effects of free radicals by diverse effective enzymatic and nonenzymatic mechanisms (Apel and Hirt, 2004). Several enzymes like superoxide dismutase, catalase peroxidase are able to scavenge ROS (Blokhina *et al.*, 2003). Trace-elements, such as selenium zinc, copper and manganese play an important catalytic role for the enzymatic activity. Carotenoids and fatty acids are two examples for non-enzymatic classes of substances which are able to protect the organism from oxidative damage (Sies and Stahl, 1995). Tocopherol, flavonoids, phenolics, tannin and alkaloids are other examples for substances belonging to this group of non-enzymatic substances.

2.2.2. Antioxidants

In recent years, food scientists and nutritionists and the general public have come to regard foods as more than sources of energy and essential nutrients. Certain minor components of foods are now recognized for their health-promoting properties, in particular for their roles in preventing or alleviating the effects of some of the chronic diseases such as cardiovascular disease and certain cancers. The biological activities of many phytochemicals are attributed to their antioxidant properties. Plant-based foods contain antioxidants, which complement the antioxidants produced by the body. When ingested, some portion of plant antioxidants is absorbed in the gut lining and enters the bloodstream (Arts and Hollman, 2005).

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, antioxidants play great role in oxidation termination. The body cannot manufacture phytochemicals which scavenge free radicals from the body so they must be supplied in the diet like from fruits, vegetables and cereals (Sies *et al.*, 1996).

Several classes of compounds that occur in plant foods may have antioxidant properties in the body. These include vitamin E, vitamin C, lignans, carotenoids, flavonoids and phenolic compounds and other pigments. Evidence for important roles of vitamin E and C is strong, whereas the roles of other classes of antioxidants are still being elucidated. Vegetables and fruits are the most important sources of these antioxidants. However, grains have largely been ignored as important contributors of dietary antioxidants, despite the fact that they are a staple dietary component for most of the world's population. Antioxidants found in whole grain foods are minerals (Ca, Mg, K, P, Na and Fe) and phytochemicals (phytates and phenolic compounds, flavonoids), which are responsible for the high antioxidant activity of whole grain foods (Nahapetian and Bassiri, 1976).

A sufficient ingestion of natural antioxidants in food has great consequence for the defence of macromolecules against oxidative damage. According to Gorinstein (2007) the cells most frequently damaged by oxidative stress are unsaturated fatty acids in lipids, cholesterol, different functional polypeptides and proteins, and nucleic acids. Mechanisms of antioxidants consist of free radical quenching, transition metal chelating, reducing peroxide, and simulation of in vivo antioxidative enzyme activities. In living systems, the antioxidants may elevate the levels of endogenous defences. The action of antioxidants in foods and biological systems is reliant on the systems' composition, interfacial phenomena, and partitioning properties of the antioxidants between lipid and aqueous phases (Anderson, 2009).

2.2.2.1.Natural and synthetic antioxidants

Generally, antioxidants can be classified as natural and synthetic antioxidants. Based on their functions, antioxidants are further classified as primary or chain breaking antioxidants and synergists or secondary antioxidants. Antioxidants containing a phenol group play a prominent role in biological and food system (Khosla, 1995).

Synthetic antioxiants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), and natural antioxidants such as phenolics, flavonoids, tannin, tocopherol and ascorbic acid, are widely used in food industries due to their protecting ability against oxidation-reduction reactions (Roberto *et al.,* 2000). It is known that BHT and BHA retard lipid oxidation, however, due to increasing consumer awareness of health aspects; their used is slowly replaced by alternative antioxidants, which are without toxic effect. Recently, there is growing interest in the use of natural antioxidant in food products. Natural antioxidants are perceived as safe, less toxic and beneficial for human health; however it is very expensive and not widely commercialized. Sources of natural antioxidants are spices and herbs, and such materials have been used throughout history for flavouring and preservative agent (Kikuzaki and Nakatani, 1993).

Results of toxicological and nutritional studies which link some synthetic antioxidants to cancer and other diseases have forced regulatory agencies to impose severe restrictions on their use in human foods. Consumer preference also had led to increased interest in natural antioxidants by food manufacturers. The growing interest in the substitution of synthetic food antioxidants by natural one has fostered research on whole grain for identifying new antioxidants. Studies related to natural antioxidant compounds of cereals varieties and their products and the effect of enrichment with natural antioxidants to elevate their own antioxidants levels should be promoted (Mirald and Mostaghimi, 2001).

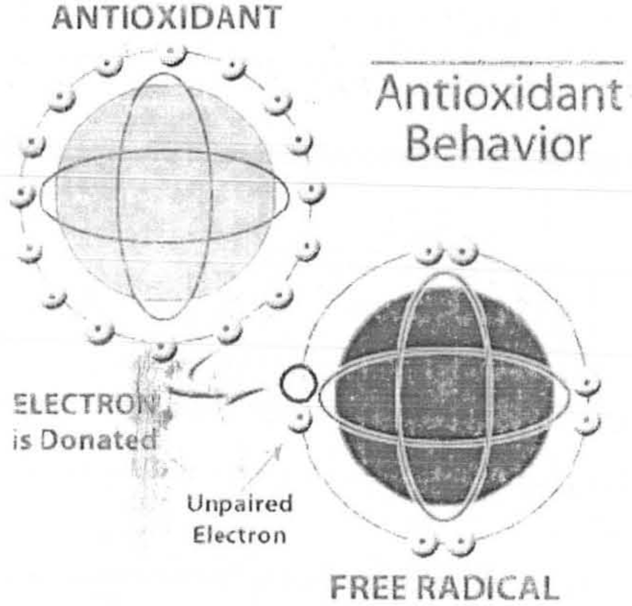


Figure 3. Free Radical Molecule
Source:- www.biomatrixone.com

2.3.Antioxidants capacity assay

Antioxidants are present in plants at concentrations up to several grams per kilogram (Daniel *et al.*, 1999). In general, levels are higher in the rinds and skins of produce compared to the inside of fruits or seeds. A number of chemical assays have been developed to measure different antioxidants. Others are designed to measure total antioxidant capacity, or TAC. In vitro assays are designed to test antioxidant levels in foods.

The measurement of antioxidants is complicated because the biochemistry of antioxidants is so complex. Plants have multiple mechanisms to produce and metabolize antioxidants, just as mammals do. The diversity of polyphenolic secondary plant metabolites reflects the many differences in the carbon skeletal structure of phenolic molecules, as well as differences in their oxidative state (Antolovic *et al.*, 2002). In addition, antioxidants in foods and in people are continuously changing form and even function as a result of glycosylation (reactions with sugar molecules), hydroxylation of aromatic phenolic rings, through polymerization, and as a result of the biosynthesis of various stereoisomers.

Most natural antioxidants are multifunctional in complex heterogeneous foods; their activity cannot be assessed by any one method (Amarowicz *et al.*, 2004). No single assay will accurately reflect all of the radical foundations or all antioxidants in a mixed or complex system, and it must be appreciated at the outset that there are no simple universal methods by which antioxidant capacity can be measured accurately and quantitatively also too many analytical methods result in inconsistent results, inappropriate application and interpretation of assays, and improper specifications of antioxidant capacities.

There is increasing interest in the use and measurement of antioxidant capacity in the food, pharmaceutical, and cosmetic industries. Much of this interest is derived from the increasing evidence of the importance of reactive oxygen/nitrogen species (ROS/RON) in aging and pathogenesis (Brand-Williams *et al.*, 1995). For foods the ideal antioxidant evaluation method should be conducted under the chemical, physical, and environmental conditions expected in food systems in order to accurately evaluate antioxidant potential. However, in food products, these conditions vary widely so individual evaluation methods are needed (Decker *et al.*, 2005). Many simplistic one dimensional method that use a broad range of conditions, oxidants and methods to measure end points of oxidation have been developed to measure the free radical scavenging or "antiradical" ability of antioxidants (Frankel, 2005; Brand-Williams *et al.*, 1995).

Antioxidant capacity assays can generally be classified into two types: hydrogen atom transfer (HAT) reactions or electron transfer (ET) assays. HAT assays, such as oxygen radical absorbance capacity (ORAC) and total radical trapping antioxidant parameter (TRAP), apply a competitive reaction scheme where antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds.

ET assays involve two components in the reaction mixture: the antioxidant and the oxidant (which is the probe). The probe will abstract an electron from the antioxidant causing a color change of the probe. The colour change is used to monitor the reaction and works as an indicator of the reaction endpoint. Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) are examples of ET assays (Huang *et al.*, 2005; Frankel, 2007; Sanchez-Moreno, 2002).

Oxygen radical absorbance capacity (ORAC) assay measures the ability of antioxidants to scavenge peroxyl radicals (Kuti and Konuru, 2004). Cao *et al.*, (1993) developed the method which measures antioxidant scavenging activity against peroxyl radical production induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) at 37°C (Ou *et al.*, 2001). Various probes can be utilized as the fluorescent probe including phycoerythrin and fluorescein. The loss of fluorescence of the probe is an indication of the extent of damage from its reaction with the peroxyl radical. The protective effect of an antioxidant is measured by calculating the area under the time recorded fluorescence decay curve and the antioxidant capacity is expressed as μmoles of Trolox equivalents (Ou *et al.*, 2001; Frankel, 2007; Huang *et al.*, 2005).

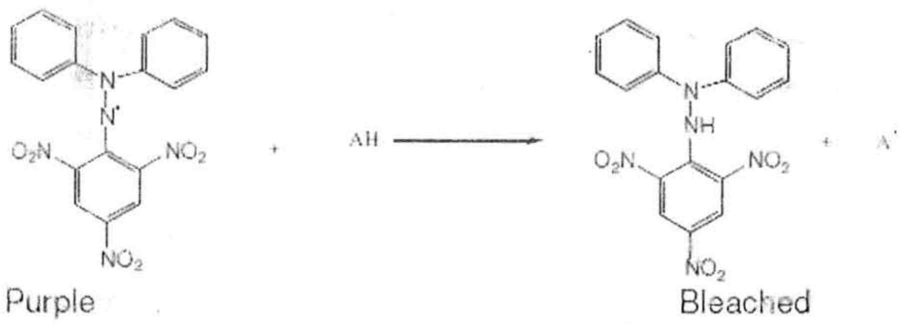


Figure .4 DPPH structure
Source: Chu *et al.* (200).

2.4. Antioxidants in Cereals

Health aspects of grains have long been known. In the 4th century B.C Hippocrates, the father of medicine, recognized the health benefits of whole grain bread. Physicians and scientists in the early 1800s to mid 1900s recommended whole grains to prevent constipation and chronic diseases. The 'fibre hypothesis', published in the early 1970s, suggested that whole foods, such as whole grains, fruits and vegetables provide fibre, along with the other constituents that have health benefits (Spiller, 2002).

Historically, Africa's indigenous cereal grains have been a major food for humans and other animals and as constituents of nutritional and technological importance, cereal nutritional constituents have been studied extensively. Cereal grains varieties contain high levels of antioxidant compounds (Harborne, 1980). Most of the literatures on plants phytochemicals focus mainly on those in fruits, vegetables, wines and teas however; many antioxidant compounds in fruits and vegetables (i.e., phenolic acids and flavonoids) are also reported in cereals.

All plant-derived foods contain phytochemicals such as polyphenols which affect their organoleptic and nutritional properties. Awareness of their importance in human nutrition has been aroused because of their potential beneficial effects on human health (Anderso *et al.*, 2009). Phytochemicals represent a heterogeneous group of substances including glucosinolates, the wide group of polyphenols and carotenoids. Benzoic and cinnamic acids-derivatives are the two main groups of phenolic acids present in cereal. Polyphenols in cereals have been receiving considerable attention largely because of their adverse influence on colour, flavour, antioxidants and nutritional quality.

A wide range of factors can influence the mix of antioxidants that a plant manufactures, as well as the levels the plant produces at any given point. These factors include soil type and chemistry, available nitrogen and levels of other plant nutrients, moisture levels, temperature, and pest pressure (Brandt *et al.*, 2002; Daniel *et al.*, 1999; Romero et al., 2004; Wang *et al.*, 2000; Wang *et al.*, 2002). In general, factors that impose stress on plants tend to trigger a plant's innate defence mechanisms and these mechanisms are driven by and/or entail the synthesis of antioxidants.

2.4.1. Total antioxidants in cereals

Sripriya *et al.* (1996) studied the free radical quenching action of finger millet on 1, 1-diphenyl-2-picrylhydrazyl and hydroxyl radicals by electron spin resonance spectrometry. DPPH radical quenching with 50 ml of the extracts showed that the brown finger millet quenched 94 per cent whereas the white finger millet quenched only 4 per cent. The phenolic content of brown finger millet was 96 per cent higher than the white variety. Processing of the brown finger millet by germination and/or fermentation decreased the quenching activity. In comparison, foxtail millet, pearl millet and sorghum, quenched 91, 59 and 52 per cent respectively, while wheat, rice (dehusked) and rice husk quenched 18, 1.8 and 20 per cent respectively. Brown finger millet also quenched 77 per cent of hydroxyl radicals.

Zielinski and Kozlowska (2000) examined the antioxidant properties of water and 80 per cent methanolic extracts of cereal grains and their different morphological fractions. Wheat (*Triticum aestivum* L.) cv. Almari and cv. Henika, barley (*Hordeum vulgare* L.) cv. Gregor and cv. Mobek, rye (*Secale cereale* L.), cv. Dankowskie zlote, oat (*Avena sativa* L.) cv. Slawko and buck wheat (*Fagopyrum esculentum* Moench) cv. Kora were used. Among the water extracts, only the buckwheat exhibited antioxidant activity at the concentration analysed. The antioxidant activity for 80 percent methanolic extracts for whole grains was in the following order buckwheat > barley > oat > wheat ≥ rye.

Qin Liu and Yang Qiu (2010) determined the extracts from six wheat varieties (three purple, one yellow, two red, and one white) were evaluated and compared for their antioxidant capacities against oxygen radical and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Phenolic composition in the extracts was examined by high-performance liquid chromatography and mass spectrometry. The results showed that Charcoal purple wheat had remarkable antioxidant activity (up to 6899 μmol/100 g) followed by Red Fife wheat and yellow Luteus wheat. White AC Vista wheat, due to its lowest phenolic content, exhibited the weakest antioxidant property.

Velioglu (1998) determined the antioxidant activities and total phenolics of 28 plant products, including sunflower seeds, flaxseeds, wheat germ, buck wheat and several fruits, vegetable and medicinal plants. The total phenolic content, varied from 169 to 10.548 mg/100 g of dry product. Antioxidant activity of methanolic extract ranged from 0.05, 53.7, 0.009 and 5.17 to

0.26, 99.1, 0.46 and 969.3, respectively. The correlation coefficient between total phenolics and antioxidative activities was statistically significant.

Wong *et al.*, (2006) evaluated the total antioxidant activity of selected natural food materials like ragi, amaranth, wheat, sesame seed and flax seed. Ragi showed greater antioxidant activity with least percentage lipid peroxidation when compared to other plant material.

Table 1. Total antioxidants activity (ABTS) of some selected cereals

Antioxidants activity (ABTS) levels of cereal grains	**u mol Trolox equiv.**
Black sorghum	75
Red sorghum	60
Millets brown tef	40
Red corn	25
Blue corn	25
Black barley	37
White corn	20
Yellow corn	18

Source: Guajardo-Flores *et al.,* 2006

Brunswick laboratories (2006) determined antioxidant capacities of different cereals including two tef varieties by adding the sample to the peroxyl radical generator, 2,2'-azobis(2-amidinopropane) dihyd rochloride (AAPH) and inhibition of the free radical action is measured using the fluorescent compound and the values were showed that ivory tef had more antioxidants activities than brown tef.

Table 2. Oxygen radical absorbance of some selected cereals

Whole grain type	**ORAC, umole TE/100g (hydrophilic)**
Sorghum whole flour	1800
Quinoa seed, white	3200
Quinoa seed, black	4800
Quinoa seed, red	3900
Teff whole flour, ivory	3600
Teff whole flour, brown	3400
Amaranth seed, white	900

Source: Brunswick laboratories, Norton, MA (2006)

In vitro antioxidant activity levels of a wide array of cereal grains, different antioxidant capacity assay were used. In general, tannin-containing grains (like Sorghum and rice) and pigmented cereal grains had the highest levels of phenols and antioxidant activity in each grain category. Tannin sorghums and black rice had the highest levels of phenols and antioxidant activity whereas none pigmented cereals (i.e. rice, wheat, and waxy barley) had the lowest levels. These results suggest that condensed tannins and pigment-contributing compounds such as the anthocyanins increase phenols and antioxidants activity. In vitro methods used to measure antioxidant activity (ABTS, DPPH, total reducing power and ORAC) do not give information about the bioavailability or metabolism of these compounds in biological systems. How-ever, these methods are useful to screen and compare antioxidant activity levels among a wide variety of samples.

2.4.2. Phenolics compounds

Phenolic compounds (phenolics) in cereal grains encompass a diverse group of secondary plant metabolites and primarily located in the grain outer layers. The general definition of a phenolic compound is any compound containing a benzene ring or derivatives of benzoic or cinnamic acids. They contain hydroxyl or methoxyl groups substituted at various positions on the aromatic ring (Hahn *et al.*, 1984). Phenolic compounds can be conveniently divided into three broad groups, phenolic acids, flavanoids, and polymeric flavanols including condensed tannins (Harborne, 1980).

They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defence against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, *etc.*) and beverages (tea, coffee, beer, wine, *etc.*), and partially responsible for the overall organoleptic properties of plant foods. For example, phenolics contribute to the bitterness and astringency in foods because of the interaction between phenolics, mainly procyanidin, and the glycoprotein in saliva (Harborne, 1980).

Agronomically, the presence of phenolics is associated with diminished pre-harvest losses due to bird predation and post-harvest losses due to storage pests. However, tannins bind proteins, carbohydrates and minerals, thereby affecting the nutritional and functional value of the bound constituents. Phenolics may also impart undesirable colours in grain products during food processing. Recent investigations using sorghum grains have focused on examining the types and levels of phenolic compounds as constituents that adversely affect nutritional and sensory quality of food (Cotelle, 2001).

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelating potential (Rice-Evans *et al.*, 1995). The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Kähkönen *et al.*, 1999). The importance of natural phenolic compounds from plants materials is also raising interest among scientists, food manufacturers, and consumers due to functional food with specific health effects (Löliger, 1991). The total phenolics content and antioxidant activity of cereals are significantly correlated.

Regarding functionality as health ingredients, the favourable redox potentials and the relative stability of their phenoxyl radical make phenolics good candidates as antioxidants (Simic and Jovanovic, 1994). Phenolic acids found in cereal grains are antioxidants in vitro (Thompson, 1993) while many naturally occurring simple phenolics scavenge radicals as effectively as vitamins A and E when tested in vitro, more complex phenolics such as free- or protein-complexed proanthocyanidins seem to be most effective (Hagerman *et al.*,1998). It is of some interest then that the proanthocyanidins found in barley exhibit antioxidant activity (Tamagawa *et al.*, 1999). Recently it has been suggested that proanthocyanidin dimers and trimers could be absorbed in vivo (Deprez, 2001). However, there is still a paucity of studies on the bioavailability and metabolisms of phenolics.

Despite their wide distribution, the health effects of dietary phenolic compounds have come to the attention of nutritionists only in recent years. Researchers and food manufacturers have become more interested in phenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases (Manach *et al.*, 2004). The preventive effects of these second plant metabolites in terms of cardiovascular, neurodegenerative diseases and cancer are deduced from epidemiologic data as well as *in vitro* and *in vivo (*Rasmussen et al., 2005; Arts et al., 2005; Hertog et al., 1994; Cole et al., 2005) and result in respective nutritional recommendations. Furthermore, phenolics were found to modulate the activity of a wide range of enzyme and cell receptors. In this way, in addition to having antioxidant properties, polyphenols have several other specific biological actions in preventing and or treating diseases.

2.4.2.1. Phenolics as Free Radical Scavengers

Phenolic compounds (POH) act as free radical acceptors and chain breakers. They interfere with the oxidation of lipids and other molecules by rapid donation of a hydrogen atom to radicals (R):

$$R + POH \rightarrow RH + PO \qquad (1)$$

The phenoxy radical intermediates ($PO^{\cdot}$) are relatively stable due to resonance and therefore a new chain reaction is not easily initiated. Moreover, the phenoxy radical intermediates also act as terminators of propagation route by reacting with other free radicals:

$$PO^{\cdot} + R^{\cdot} \rightarrow POR \qquad (2)$$

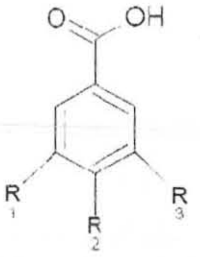
Phenolic compounds possess ideal structure chemistry for free radical scavenging activities because they have: (1) phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical; (2) extended conjugated aromatic system to delocalize an unpaired electron. Several relationships between structure and reduction potential have been established (Cotelle, 2001).

2.4.2.2. Phenolics characterization

Phenolic acids can be divided into two classes: derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall (D'Archivio *et al.*, 2007). Waniska *et al.*, (1989) identified ferulic, coumaric, cinnamic and gentestic acids as the major phenolic acids in pseudo cereals.

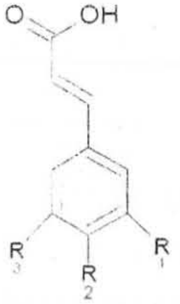
The two phenolics classes' are:

Benzoic Acid Derivatives	Cinnamic Acid Derivatives
Protocatechuic acid	Coumaric acid
Gallic acid	Caffeic acid
	Ferulic acid



Benzoic acid derivatives: protocatechuic: R1=H, R2=R3=OH; Gallic: R1=R2=R3=OH; *p*-hydroxybenzoic: R1=H, R2=OH, R3=H; vanillic: R1=H, R2=OH, R3= OCH_3.

Figure 5. Benzoic acid derivatives structure



Cinnamic acid derivatives: *p*-coumaric: R1=H, R2=OH, R3=H; ferulic: R1= OCH_3, R2=OH, R3=H; sinapic: R1=OCH_3, R2=OH, R3=OCH_3; caffeic: R1=R2=OH, R3=H.

Figure 6. Cinnamic acid derivatives structure

Source: Yu *et al.* (2001)

Sorghum phenolic acids include hydroxybenzoic (mainly protocatechuic and p-hydroxybenzoic acid) and hydroxycinnamic acids (mainly ferulic and p-coumaric acid) both free and bound as esters. Most of them are found in usual lager beers, issued either from barley malt or from hop (Yu *et al.*, 2001).

Ferulic acid is the most common phenolic acid in cereal grains, which account for most dietary intake of the phenolic acid and as much as 90 percent of total polyphenol content of wheat (Manach *et al.*, 2004). Wheat bran is a particular rich source of ferulic acid (Scalbert and Williamson, 2000), where it is found mostly in the outer parts of the grain. Hence, ferulic acid levels are far lower in baked foods derived from highly processed flour. The health benefits of wheat germ and bran are likely in part due to their relatively high concentrations of ferulic acid.

Barley contains 0.2 to 0.4% phenolics by weight of grain (Bendelow and LaBerge, 1979). Phenolic acids in barley grain include benzoic acid (p-hydroxybenzoic acid, vanillic, and protocatechuic acids) and cinnamic acid derivatives (caffeic, coumaric, ferulic, and chlorogenic acids) (Yu, *et al.*, 2001). Simple flavanoids (monomers, dimers and trimers) based on catechin and gallocatechin units account for 58-68% of the total phenolics (McMurrough *et al.*, 1983).

Dyke and Rooney (2006) found a new group of phenolic acids in aqueous alcoholic extracts of both oat groats and hulls. These acids occurred as conjugates covalently linked to the amine function of several different ortho-aminobenzoic acids. One of the conjugates was a place yellow crystalline solid with a molecular weight of 325 ($C_{18}H_{15}NO_5$). Two additional derivatives of avenalumic acid were also detected the 3'-hydroxy and 3'-methoxy analogues. These acid, which are the ethylenic homolgues of the well known p-coumaric, ferulic and caffeic acids may be widely distributed in cereal grains.

Fabjan *et al.*, (2003) isolated two avenanthramides belonging to a group of about 40 cinnamoylanthranilic acid derivatives in oat grains N-(4'-hydroxy-methoxy-(E)-cinnamoyl)-5- hydroxyanthranilic acid (A1) and N-(4'-hydroxy-3'-methoxy-(CE)-cinnamoyl)-5-hydroxy-4- methoxyanthranilic acid (A2). A1 had ~20 per cent of the activity exerted by β-tocopherol and A2 had ~60 per cent. A1 was preferentially located in the outer part of the grain.

Peterson and Qureshi (1993) analyzed for tocols in 12 oat and 30 barley genotypes each from three locations. Significant genotype differences existed for most tocols of both species. Total tocol concentrations for genotypes ranged from 19 to 30 mg kg-1 for oats and 42 to 80 mg kg^{-1} for barley. Location differences were significant for oats but not for barley. α- tocotrienol and α-tocopherol were the predominant tocol isomers in both species.

Watanabe (1999) isolated three antioxidative phenolic compounds, one serotonin derivative and two flavonoids, from an ethanol extract of Japanese barnyard millet. Their structures were established to be N-(p-coumaroyl) serotonin, luteolin and tricin. N-(pcoumaroyl)-serotonin exhibited a strong antioxidant activity almost equivalent to that of butylated hydroxyl anisole at the same concentration. Although the antioxidant activity of luteolin was lower than that of N-(p-coumaroyl) serotonin, it was nearly equal to that of quercetin, whereas as the activity of tricin was lower than that of luteolin.

2.4.2.3.Phenol levels in cereals

In the last decade a number of publications have been published in which antioxidant capacity of plant material, so as antioxidant characteristics of phenol compounds are tested, through different methods (Velioglu *et al.*, 1998; Miller *et al.*, 2000; Halvorsen *et al,*, 2002; Javanmardi *et al.*, 2003). Because of this it is difficult to compare final results, even though they are the same plant species. Conducted research shows that values of total phenolic compounds in buckwheat, rye, oats, barley, corn, wheat, and rice extracts vary from 2,95-20,35 mg GA/L of extracts on 20°C. Higher temperatures (40°C) affects the better extraction of total phenols and given values are in average 4, 29-30, 65 mg GA/L. The highest concentration is measured in buckwheat extract, followed by rye, oats, barley, corn, wheat, and rice, respectively.

The research of total phenols in earlier published papers differs in preparation method of samples (solvent selection, extraction time and temperature). The final values vary. However, if we compare values of total phenolic compounds in buckwheat, rye, oats, barley, corn, wheat, and rice, it can be concluded that content of total phenols in rice is the smallest. Brown rice is richer in phenols than white rice. It has been determined that buckwheat has very good antioxidant characteristics (Zielinski and Kozlowska, 2000; Adom and Liu, 2002). As Guajardo *et al.* (2006) have shown foxtail millet contained more phenolic acids over brown millet tef.

Table 3. Phenolic acid content in some cereal grains

Sample	**Amount (ug/g)**
Whole grains	
Barley	450 – 1346
Finger millet	612
Foxtail millet	3907
Maize	601
Oat	472
Pearl millet	1478
Rice	197 – 376
Rye	1362 – 1366
Sorghum	385 – 746
Wheat	1342

Source: (Dyke and Rooney, 2006)

The same varieties of different genes of cereals produce different antioxidants levels. As table1 Suggest that the more the cereals have colour seeds, the more the phenol content but not always. The following table lists some total phenol level of cereals among the same species.

Table 4. Total Phenol levels of cereal among the same varieties.

Cereals	**Phenol (mg gallic acid equiv./g)**	**Cereals**	**Phenol (mg gallic acid equiv./g)**
Black sorghum	5	Black rice	11
Red sorghum	4.5	Red rice	6
White Sorghum	1.9	White rice	0.8
Purple wheat	2.2	Yellow corn	2
Red wheat	2	Red corn	2.5
White wheat	1.2	White corn	2.2

Cereal	**Phenolic compounds**
Brown tef	?
Red tef	?
White tef	?

Source: - Guajardo-Flores *et al.* (2006).

2.4.3. Flavonoids

Flavonoids are a diverse group of chemicals found in all plants. About 4000 phytochemicals belong to the flavonoids group. They are most commonly known for their antioxidant activity. Flavonoids are also commonly referred to as bioflavonoids in the media – the terms are equivalent and interchangeable, for flavonoids that are biological in origin (Paśkoa, 2009).

Flavonoids are natural substances with variable polyphenolic structures, compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain. The flavonoids skeletons are characterized by its (C_6-C_3-C_6) to be differentiated according to the saturation level and opening of central pyran ring into 6 different subgroups (Yao *et al*., 2004). Both the oxidation state of the heterocyclic ring and the position of ring B are important in the classification.

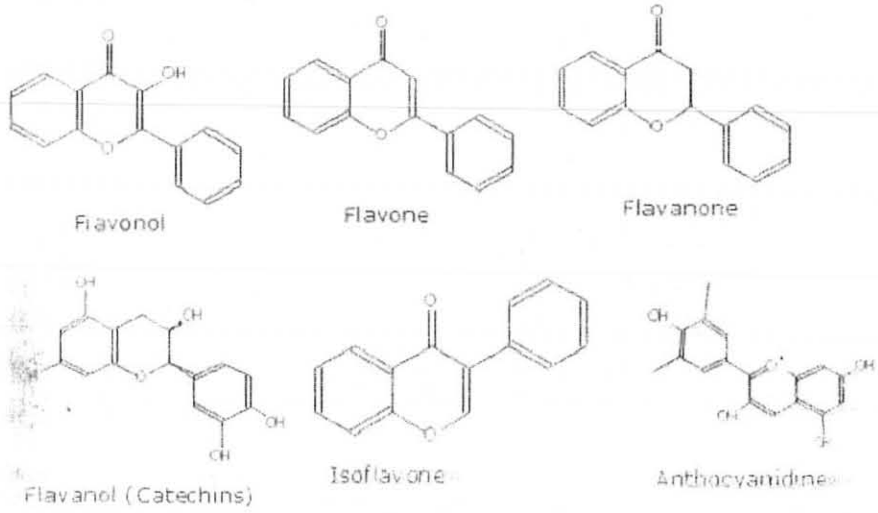
Flavonoids normally accumulate in plants as glycosylated derivatives, which are stable to hydrolysis and are biologically active both in planta and as dietary components. Several factors such as plant type, age of the plant or plant parts, stage of development, and environmental conditions govern the flavonoids contents in plants. Flavonoids, the most important of secondary metabolism products, occur in the plants. They have medicine and biological effects (Goren *et al*., 2001).

Flavonoids, the major factors that determine the radical-scavenging capability are:
(i) *The ortho-dihydroxy structure on the β-ring*, which has the best electron-donating properties and confers higher stability to the radical form and participates in electron delocalization.
(ii) *The 2, 3-double bond with a 4-oxo function in the C- ring*, which is responsible for electron delocalization from the B ring.
(iii) *The 3- and 5-hydroxyl groups with the 4-oxo function in A and C rings*, which are essential for maximum radical scavenging potential.
(iv) *The 3-hydroxyl group is important for antioxidant activity*. The 3-glycosylation reduces their activity when compared with corresponding aglycones (Shahidi and Wanasundara, 1992; Bors and Michel, 2002).

2.4.3.1.Characterization of flavonoids

Flavonoids are themselves divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C-ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation. The Isoflavonoids and the Neoflavonoids can be regarded as abnormal flavonoids (Chu et al., 2000).

The anthocyanins and flavones are the major class of flavonoids present in cereals and in general, this class of compounds contributes the blues, purples, and reds in plants. (Zielin ski *et al.*, 2000 ; Paśkoa *et al.*, 2009; Gorinstein *et al.*, 2007).


Flavonol

Flavone

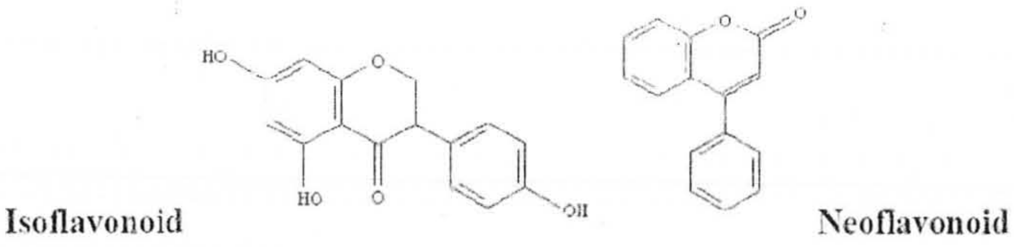
Flavanone

Flavanol (Catechins)

Isoflavone

Anthocyanidines

Figure 7. Structures of flavonoids
Source: (Zielin ski *et al.*, 2000)


Isoflavonoid

Neoflavonoid

Figure 8. Structures of abnormal flavonoids
Source: (Paśkoa *et al.*, 2009)

2.4.3.2. Anthocyanin

Anthocyanins are flavonoids. They are water-soluble vacuolar flavonoid pigments that appear red to blue, according to P^H. They are synthesized by organisms of the plant kingdom, and have been observed to occur in all tissues of higher plants, providing colour in leaves, stems, roots, flowers, and fruits. Anthocyanin pigments are responsible for the red, purple, and blue colours of many fruits, vegetables, cereal grains, and flowers. They have long been the subject of investigation by botanists and plant physiologists because of their roles as pollination attractants and phyto-protective agents (Deprez *et al.*, 2001).

An anthocyanin pigment has intensified because of their possible health benefits as dietary antioxidants. Anthocyanins are one class of flavonoid compounds, which are widely distributed plant polyphenols. Flavonols, flavan-3-ols, flavones, flavanones, and flavanonols are additional classes of flavonoids that differ in their oxidation state from the anthocyanins.

Anthocyanins are versatile and plentiful flavonoid pigments found in red/purplish fruits and vegetables, including purple cabbage, beets, blueberries, cherries, raspberries and purple grapes. Within the plant they serve as antioxidants and pigments contributing to the coloration of flower (Deprez *et al.*, 2001).

2.4.3.3. Flavonoids levels in cereals:

Goren *et al.*, (2001) have reported big differences in proanthocyanidin concentrations between red and white sorghum grains. Red sorghums showed an average of 9400 mg/kg, against 1300 mg/kg for white samples. In most samples, proanthocyanidins decreased after germination. The proanthocyanidin levels were revealed to be positively correlated to the total phenolic contents (Amarowicz *et al.*, 2004).

Flavan-4-ols such as apiforol (leucoapigeninidin) and luteoforol (leucoluteolinidin) are other interesting sorghum polyphenols, as precursors of sorghum 3-deoxyanthocyani (di)ns. Never reported in beer. they have been found at concentrations up to 4200 mg/kg in sorghum (Amarowicz *et al.*, 2004).

Although the typical aggregate antioxidants content of tef is little known, the natural variations existing among the types of antioxidants and amount existing among different varieties are poorly understood. It is important to be able to fractionate tef antioxidants efficiently and to identify fractions with certain beneficial health effects. The limitation on methods of quantifying cereal phenolics are the different responses given by different phenolics and the difficulty of procuring an appropriate standard.

2.4.4. Tannin

Tannins, which are also called proanthocyanidins or procyanidins, consists of polymerized flavanol units and they contribute to astringency in foods. These compounds are found in cereals sorghum with a pigmented testa layer like, red finger millets, and barley. Tannins bind to proteins, carbohydrates, and minerals, which decrease digestibility of these nutrients and reduce feed efficiency during feeding. Plants containing high tannin levels are not preferred by birds and insects. However, humans have acquired a taste for moderately astringent food stuff (i.e., dark chocolate and cranberries). Condensed tannins have high antioxidant activity in vitro compared to monomeric phenolic compounds. In addition these compounds may have anti-carcinogenic, cardiovascular, gastro protective, anti-ulcerogenic, and cholesterol-lowering properties, and they also promote urinary tract health (Kähkönen *et al.*, 1999).

Tannins are another major group of polyphenols in our diets and usually subdivided into two groups: (1) hydrolysable tannins and (2) condensed tannins. Hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid, also called gallotannins, or with hexahydroxydiphenic acid, also called ellagitannins. The great variety in the structure of these compounds is due to the many possibilities in forming oxidative linkage. Intermolecular oxidation reactions give rise to many oligomeric compounds having a molecular weight between 2,000 and 5,000 Daltons (Khanbabaee and Ree, 2008). Condensed tannins are oligomers or polymers of flavan-3-ol linked through an interflavan carbon bond. They are also referred to as proanthocyanidins because they are decomposed to anthocyanidins through acid-catalyzed oxidation reaction up on heating in acidic alcohol solutions. The structure diversity is a result of the variation in hydroxylation pattern, stereochemistry at the three chiral centers, and the location and type of interflavan linkage, as well as the degree and pattern of methoxylation, glycosylation and galloylation (Koleckar *et al.*, 2008).

Kelawala and Ananthanarayan (2004) studied isolated sorghum fractions with highest levels of tannins and total phenols and determined their antioxidant capacities relative to blue berries fractionations. Maximum values of phenols were observed in the white, black and brown brans respectively. Only the brown sorghum had catechins. The findings suggested that sorghum (especially high tannin types) is rich sources of catechins and total phenols. The bran gives high concentration of these compounds with antioxidant power similar to berries. These fractions could be source of antioxidants and other nutrients in foods.

Sorghums containing condensed tannins have consistently shown the highest antioxidant activity in vitro, and they approach or exceed the antioxidant levels of fruits and vegetables. Sorghums containing condensed tannins dominate production of grains in hot, humid regions of Africa because they have significantly improved resistance to grain moulds and birds, which allow for their successful production. Tannin sorghums contain a pigmented testa, which contributes astringency during grain maturation and causes birds to utilize other food sources. When other grains are unavailable birds consume tannin sorghum. These tannin sorghums are grown extensively in east and southern Africa (Khanbabaee and Ree, 2001).

2.5. Description of Tef

Ethiopia's major staple crops include varieties of cereals, pulses, oilseeds, and coffee. Grains are the most important field crops and the chief element in the diet of most Ethiopians. The principal grain is tef. Tef is an important food source in Ethiopian diet –used to make injera, flatbread. The word "tef" is thought to have been derived from the Ethiopian Amharic word *teffa*, which means lost—due to small size of the grain and how easily it can be lost if dropped. It is the smallest grain in the world, measuring only about 1/32 of an inch in diameter. It takes 150 grains of tef to weigh as much as one grain of wheat. Because the grains of tef are so small, the bulk of the grain consists of the bran and germ. This makes tef nutrient-dense (http:// www.beanslentils.com).

Tef *(Eragrostis teff* (Zucc.) Trotter) belongs to the family Poaceae, subfamily Eragrostoideae, tribe Eragrosteae and genus *Eragrostis*. The genus contains about more than 300 species (Costanza, 1974). Within the genus *Eragrostis* 43% of the species seem to have originated in Africa. 18% in South America, 12% in Asia, 10% in Australia, 9% in Central America, 6% in North America and 2% in Europe (Costanza, 1974). Of the 54 *Eragrostis species* in

Ethiopia, 14 (or 26%) are endemic (Cufodontis, 1974). The fact that the genetic diversity for tef exists nowhere in the world except in Ethiopia, indicates that tef originated and was domesticated in Ethiopia. Vavilov (1951) has identified Ethiopia as the centre of origin and diversity of tef.

2.5.1. Nutritional value of teff.

It is one of the cereals that constitute a major source of proteins, calories, minerals for millions of people in Ethiopian. It contains 11% protein, 80% complex carbohydrate and 3% fat. It is an excellent source of essential amino acids, especially lysine amino that is most often deficient in grain foods. Tef contains more lysine than barley, millet, and wheat and slightly less than rice or oats. Tef is also an excellent source of fiber and iron, and has high amount of calcium, potassium and other essential minerals. It is totally gluten free ideal for those with a gluten intolerance i.e. Coeliac Disease (http://www.wam.umd.edu/tes/tef/injera.html).

2.5.2. Ethiopian Common tef varieties.

There are several varieties of tef, each with characteristics best suited to specific conditions. But the common are:-white, red and brown tef.

2.5.2.1. White tef is the preferred type but only grows in certain regions of Ethiopia. White tef grows only in the Highlands of Ethiopia, requires the most rigorous growing conditions, and is the most expensive form of tef. It was reserved for the wealthiest and most prestigious families. The prestige associated with consuming white tef, as well as its more stringent growing conditions, contributes to the increased cost. The shelf life of *injera* is extended with the use of white tef (Mesfin, 2004).

2.5.2.2. Red tef; - the least expensive form and the least preferred type, has the highest iron content. In persons living in areas of the country where consumption of red tef is most prevalent, haemoglobin levels were found to be higher with a decreased risk of anaemia related to parasitic infection. As studies of the increased health benefits associated with high iron contents in red tef become elucidated, there is more acceptance of this grain in society. Today in Ethiopia, red tef is becoming more popular related to its increased iron content. The available data were not able to differentiate the iron content between red and white tef. The average iron content of tef is 62.71mg/4oz grain. Some of Studies indicate that the level of iron in the tef is related to the threshing of the grain on the soil. (http://ethnomed.org/cultures/ethiop/teff.html).

2.5.2.3. Brown tef and Ivory tef: - The third main type of tef, brown (mixed) tef is a mixture of both red and white tef without any standard proportions from each and locally it is called "mixed" tef (Mesfin, 2004). Wholesalers in the major markets divide tef into four, namely; *magna* (very white), *nech* (white), mixed (mixed between white and red) or brown and key. Four of the grades indicate the color of the tef variety. The market value gives magna the first while key the last grade position (Refera, 2009). Poor farmers usually sell the white tef at reasonably higher prices, while buying either the mixed or red tef for their own consumption. White tef is more expensive than the red tef. People who can afford the price usually consume white tef or *Magna* tef, while the medium and poor families consume the mixed and/or red tef. Farmers who grow white tef often do not consume it. Instead, they sell the white tef at reasonably higher prices and buy either the red or the mixed tef for home consumption According to the pilot study made by Kinde Kassegne (2009), the classification of tef is not uniform, what is referred to as *magna tef* (top quality) in some area of Ethiopia is sometimes sold as *mixed tef* (medium quality) in Addis Ababa commodity market. Quality attributes required in tef appear to be poorly understood with little documentation of quality requirements for products. This introduces some arbitrariness in the comparison of prices at different locations (Zeleke, 2009).

Different tef varieties have different colors this is might be due to heterogeneous phytochemicals found in germ of the seed. Hence, these give some clue how tef varieties are differencing in their antioxidant contents (http://www.efn.org/~sundance/TeffMillet.html).

2.6.Fenugreek

Fenugreek (Trigonella foenum graecum) is an annual herb that belongs to the family Leguminosae widely grown in Pakistan, India, Egypt, and Middle Eastern countries (Alarcon-Aguilara *et al.*, 1998). Due to its strong flavor and aroma fenugreek in one of such plants whose leaves and seeds are widely consumed in subcontinent including Ethiopia as a spice in food preparations, and as an ingredient in traditional medicine. It is rich source of calcium, iron, β-carotene and other vitamins (Sharma *et al.*, 1996). Both leaves and seeds should be included in normal diet of family, especially diet of growing kids, pregnant ladies, puberty reaching girls and elder members of family because they have haematinic (i.e. blood formation) value (Ody, 1993). Fenugreek seed is widely used as a galactagogue (milk producing agent) by nursing mothers to increase inadequate breast milk supply (Fleiss, 1998).

The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels (Billaud, 2001; Sauvaire *et al.*, 1991; Ribes *et al.*, 1986). Therefore, fenugreek seeds are used as a traditional remedy for the treatment of diabetes and hypercholesterolemia (Basch *et al.*, 2003; Miraldi *et al.*, 2001). It reported to have restorative and nutritive properties and to stimulate digestive processes, useful in healing of different ulcers in digestive tract (Khosla *et al.*, 1995). Fenugreek has also been reported to exhibit antioxidants properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive (Cowan, 1999; Shetty, 1997).

2.7.Preparation of *injera* from tef

2.5.1. *Injera* fermentation

Injera is fermented, sour leavened pancake-like bread made from tef *(Ergagrostis tef)*, wheat, barley, sorghum or maize or a combination of some of these cereals. *Injera* can be produced from any of the various cereals depending on availability and abundance of the cereals, which are cultivated in the agro-ecological zones suitable for their growth. Generally speaking, people on the high lands prepare *injera* from barley and wheat where as those on the low lands prepares it from maize, sorghum or millet.

The various *injera* types different varieties of cereals produced from the different varieties of cereals do not have significance variation in their calorie, moisture, protein, and carbohydrate or phosphorus contents. Significant variations are, however, observed in the other nutrients. The fermentation process results in significant reduction of most of the nutrients found in the cereal flour. However, in general, *injera* can be good source of energy, fiber, iron and vitamins (EHNR, 1997).

Injera baking consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures. Temperatures in the high lands of Ethiopian are generally between 17 and 25 ^{0} C. The only required ingredients are the tef flour and water.

An appropriate amount of flour is mixed with twice its weight of water. This is kneeded thoroughly to produce a thick paste. Inoculation is accomplished by consistently using partially cleaned fermentation container and by adding some *ersho*, the clear, yellow liquid that accumulates on the surface of the batter towards the final stage of a previous fermentation. The fermentation process of tef injera is described by Berhanu Abegaz Gashe (1985). The initial 18 hours are characterized by vigorous evolution of gas and maximum dough-rising. This is followed by the appearance of an acidic yellowish liquid on the surface of the dough at about 30 – 33 hours of fermentation. Gas evolution decreases after the P^H has fallen below 5.8 (31 hours). The liquid layer is discarded at the end of the first stage of fermentation. As soon as the liquid layer is poured off, about 10% of the fermenting dough is mixed with three parts of water and boiled for 2 to 5 minutes. This is called '*absit*', a dough enhancer, and it is mixed with the rest in fermentation vat. This process signals the initiation of the second stage of fermentation. By mixing the boiled dough with the rest in the vat, the dough-rising and gas formation processes are enhanced so they occur in short time. Maximum dough-rising, which normally takes 30 minutes to 2 hours, signals the termination of fermentation. At this stage the fermenting dough is thin enough to pour on to the hot flat pan, locally known as '*mitad*' for stam-baking in to *injera* (Steinkraus, 1983). The *injera* pan is made of clay and has diameter of 45 – 60 cm. baking is preceded by cleaning the heated pan with pieces of cloth after greasing the pan with kale and cotton seeds. Pouring of dough starts from the outer part of the pan to the centre moving clock wise direction. Bubbles start forming within seconds and the pan then covered with a lid. *Injera* is thus baked on the bottom side and the upper side is baked by stream. The total baking time for one *injera* is 2 ½ - 3 ½ minutes. The temperature in the middle of the *injera* during the baking process would reach around 90^0C. The *injera* is removed from the baking pan with the help of a straw plate and allowed to cool down. The weight of one tef *injera* is 350 – 450 g. *Injera* can be kept for 3 to 4 days. Longer keeping results in drying and mouldiness.

'*Absit*' ensures that *injera* will have the proper texture and consistency. *Injera* baked without '*absit*' or with less '*absit*' than the required will have fewer amounts of eyes on the upper surface. A higher number of larger eyes are a very desirable attribute of an attractive *injera*. It also tends to be brittle after few hours of baking. Too much '*absit*' makes baking difficult. *Injera* baked at 24 hours or less is called '*aflegna injera*' and has sweet taste. It is recommended for people suffering from gastritis and, thus, do not tolerate acidic foods.

2.8. Effect of processing on the antioxidant properties of cereals

Wide variety foods are processed from cereals and legumes using different processing methods. Food processing does impact on quality aspects such as sensory and nutritional. Various chemical reactions, physical and biological processes are set in motion during food processing. The phytochemical quality of foods as affected by processing becomes important in this regard. Phenolic compounds are a significant group of phytochemicals in cereals. They possess bioactive properties such as antioxidant activity and offer potential health benefits. Processing of cereals may enhance or reduce levels of phenolic compounds in foods and this has implications for their bioactive properties and potential health benefits they can offer (Beta *et al.*, 1999).

Processing like fermentation, heating, enrichment, maceration and various separation steps can result in oxidation, thermal degradation, leaching, and other events that lead to increase by dissociating of plant from plant matrix components or lower levels of antioxidants in processed food compared with fresh (Shi and le Maguer, 2000).

The way foods are processed, mixed together with other foods, cooked, preserved and prepared for final consumption can dramatically affect antioxidant levels. In the near term, increasing the retention of antioxidants in foods as they are processed and prepared is likely to offer the greatest potential to increase average antioxidant intakes, especially if consumer interest and purchasing patterns makes the retention of antioxidants a priority for the food processing and manufacturing industries. There are some significant differences in the food processing and manufacturing technologies that are allowed and typically used in companies manufacturing processed organic foods in contrast to conventional foods. Some of these differences are known to have a significant and consistent impact on antioxidant levels. For example, the synthetic chemical hexane is one of several chemicals used to promote extraction of oils from crops in conventional, high-temperature and high-pressure oil processing plants, but this synthetic chemical may not be used in the extraction of organic oils. Hexane is known to promote removal of antioxidants (Daniel *et al.*, 1999).

Antioxidant levels in many processed foods are a fraction of the levels in fresh foods. In some cases, food processing actually concentrates antioxidants (i.e., lycopene in most processed tomato products). Novel food processing methods and technologies could markedly lessen the loss of antioxidants in a variety of processed foods (Daniel *et al.*, 1999).

Attention is repeatedly drawn to the use of locally produced cereals for food processing. However, the lack of ideal grain quality parameters compounded with the scarcity of suitable processing technologies at household level should serve as an impetus for grain scientists to identify methods of utilizing the available cereals (Beta *et al.*, 1999). During processing, phenolics other than those endogenous in cereal grains may be formed as by-products of enzymatic or thermal degradation or as products of polymerization of simple phenolics. Some components in whole grains may be most important in the health benefit and should be retained in food processing.

As antioxidants susceptible to oxidation and degradation, exposure to light, oxygen and heat, conditions normally present during food processing, may accelerate or enhance the total antioxidants capacity. "Therefore information on the stability of antioxidants during food processing is important for evaluating the potential health benefits of foods (Hye-Min Han and Bong-Kyung Koh, 2008).

2.8.1 Fermentation

One way of processing the grains into food is through fermentation (Taiwo, 2009 and Blandino *et al.*, 2003). Campbell-Platt (1987) has defined fermented foods as those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. Over the years, fermentation became part of the cultural and traditional norm among the indigenous communities in most developing countries, especially in Africa. The rural folk have come to prefer fermented over the unfermented foods because of their pleasant taste, texture and colour. This popularity has made fermented foods one of the main dietary components of the developing world (Aderiye and Laleye, 2003; Mosha and Vicent, 2004; Nout and Sarkar, 1999).

Many researchers have demonstrated that microbial fermented food products obtained higher antioxidant capacities than unfermented food (Santiago *et al.*, 1992; Esaki *et al.*, 1994, 1999; Berghofer *et al.*, 1998; Lin *et al.*, 2006). The Difference in the antioxidants may be mainly because of the process of microbial fermentation and dissolution of antioxidants from the grains.

Hye-Min Han and Bong-Kyung Koh (2008) tested the effect of different wheat pizza dough fermentation times, ranging from zero to 48 hours, on antioxidant properties. Longer fermentation times also boosted antioxidant levels, in some cases by as much as 100 percent. The increase likely resulted from chemical reactions induced by yeasts, which had more time to release the antioxidant components that were bound in the dough.

The influence of fermentation on antioxidant activities and total phenolics of 4 cereals, namely buckwheat, wheat germ, barley and rye, was determined and compared with those of their unfermented counterparts. The total phenolic content (TPC) increased upon fermentation. Antioxidant activities (AOA) were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, ferric ion-reducing antioxidant power (FRAP) and thiobarbituric acid (TBA) methods. The presence of those microorganisms was enhanced levels of antioxidant activity. Thus fermentation offers a tool to further increase the bioactive potential of cereal products (Kähkönen *et al.*, 1999).

The fermentation of rice defatted soya flour, prepared in 40:60, 50:50 and 60:40 proportions and then mixed with butter milk was carried out at 25, 30 and 35°C for 12, 18 and 24 hrs. The unfermented cereal legume blends contained high amounts of phytic acid (375.00- 465.20 mg/100 g) and polyphenols (0.44-0.60 g/100 g). Indigenous fermentation at 35°C for 24 hrs reduced the phytic acid level of almost half in all the blends. Higher temperature and longer period of fermentation, reduced phytic acid content, on the other hand, polyphenols increased significantly or remained constant in the fermented blends (Kähkönen *et al.*, 1999). Lin *et al.*, (2006) have demonstrated that microbial fermented soybean products obtained higher antioxidant capacities than unfermented soybean.

2.8.2. Baking

It is generally well known that there can be significant losses of natural compounds during thermal processing due to the fact that most of the bioactive compounds are relatively heat labile. Therefore, heat processed food is considered to have a lower health promoting capacity than the corresponding fresh one. However, recent studies have shown that thermally processed food have higher biological activities due to their various chemical changes during heat treatment (Kim *et al.*, 2006).

Esaki *et al.*, (1999) studied the influence of heat treatment of phytase activity (microbial and intrinsic) on the phytate degradation. The feeds used in the trials were barley or wheat, heat treated or untreated. Approximately 50 per cent of the phytate in barley was degraded within 6 hrs of soaking. The rate of phytate degradation was decreased when soaking temperature was reduced from 20°C to 10°C. A relationship between phytate degradation and temperature were inversely proportional to each other

Kim *et al.*, (2006) studied the effect of heat on the antioxidant activities of tom-kha paste extract before and after heating. After heat treatment, tom-kha paste extract, pronounced more antioxidant activity in terms of DPPH assay. This may due to the fact that most of the compounds in this experiment were relatively heat stable. Or it could be that the heating process may enhance the antioxidant activity due to the formation of Maillard reaction products (Nicoli, 1999).

As Hye-Min and Bong-Kyung (2008) note that the Pizza wheat flour longer baking times or higher temperatures generally corresponded to higher levels of antioxidants in comparison to less intense baking conditions. Antioxidant levels of pizza wheat flour increased by as much as 60 percent during longer baking times and by as much as 82 percent during higher baking temperatures, depending on the type of wheat flour and the antioxidant test used. This because most of the antioxidants in wheat are found in the bran and endosperm components, which have been largely removed in high temperature. The exact mechanisms involved are not yet fully understood.

As Friedman and Dao (1990) observed in grains like sorghum, cooking and baking results in the partial loss of phenolics. Also phenolics can become modified such that their solubility and functional group properties are altered.

Rice-Evans *et al.* (1996) carried up on two cultivars of pearl millet grains (HC-4 and HHB-67) were milled and chapatis (unleavened bread) were made from the flour. The crude protein, ash, true protein nitrogen, non-protein, fat, phytic acid and polyphenol content of the samples were determined, as were the *in vitro* protein and starch digestibility. Milling of pearl millet grains changed its gross composition. Baking did not significantly change the nutrient content of raw pearl millet flour. Milling and heat treatment during chapati making, significantly lowered polyphenols and phytic acid and significantly improved the protein and starch digestibility.

Taiwo (2009) determined phytic acid in cereal (brans, flours and millet wheat products) and breads. The cereal flours showed values of 3-4 mg/g for soft wheat, 9 mg/g for hard wheat and 22 mg/g for whole wheat. Corn (maize), millet and sorghum flours had mean contents of 10 mg/g and oat, rice, rye and barley between 4 and 7 mg/g. Wheat brans had wide ranges (25-58 mg/g). The phytic acid for oat brans was half that of wheat bran (20 mg/g) and higher (58 mg/g) than that for rice bran. The milling products (semolinas) from hard wheat exhibited 10 mg/g and soft wheat a mean of 23 mg/g. The breads made with single or mixed cereal flours exhibited ranges between 1.5 and 7.5 mg/g. The loss of phytic acid relative to unprocessed flours was between 20 per cent for oat bread and 50 per cent for white bread.

2.8.3. Enrichment

Many researchers note that increasing consumer awareness of the health benefits of antioxidant has prompted the development of bakery products containing whole grains flour and the fortification and enrichment of bakery products with high natural antioxidant potentials.

Consumption of grains as part of diet is now recommended for health reasons because of rich source of antioxidants. However, nutritionally it has negative impact on health as lot of phytochemicals are removed during processing, specially antioxidants viz., polyphenols, phytic acid, vitamin E and trace minerals. Pulses, spices and green leafy vegetables were added as antioxidant rich food ingredients in acceptable proportion, by using standard methods of preparation (Swaminathan *et al.*, 1981)

Enriching of cereals with antioxidant rich food ingredients definitely replaces antioxidants which are lost during processing. Such methods are in practice in our heritage. In the present study *injera* was enriched with antioxidant rich seed, fenugreek to enhance the antioxidant activities.

2.9. Enrichment of staple foods with fenugreek

Fenugreek were attracted the attention of producers to meet manufacturing demands for "functional food" additives and Natural Health Products (NHP) in Canada (Fitzpatrick, 2004). Mansour and El-Adawy (1994) recommended that fenugreek seed could be added to foods like ground meat and baked cereals foods, not only as nutritional supplements but also as a potential health function.

Park *et al.* (1997) examined the stability of the antioxidants in dough and stored bread at room temperature. White bread was fortified individually with fat coated L-ascorbic acid (ASA), cold water-dispersible (CWD) β-carotene and CWD all rac-α-tocopheryl acetate (TOAC) at levels of 64, 5 and 100 mg, respectively of active ingredient per 100 g of flour (14% mb). The freshly baked pup loaves retained 76, 67 and 96 per cent of added antioxidant, respectively. In leaves stored at 25°C for one the seven days, ASA, disappeared rapidly and PE β -carotene disappeared, slowly, CWD β-carotene and ToAC were stable. When bread was fortified with both fat-coated ASA and CWD β -carotene and stored for five days, no protective effect on the retention of the antioxidants was found. Park *et al.*, (1997) studied the stability of antioxidants when fortified with wheat and psyllium husk fibre. A 7:3 (w/w) mixture of wheat fiber (WF) and psyllium husk fiber (PHF) was substituted for 10 wt per cent of flour on a 14 per cent mb and the protein in the blend was restored to 10.3 per cent by incorporating vital wheat gluten. After adding 0.5 per cent sodium stearoyl 2-lactylate, the blend (100 g) was fortified with a combination of fat, coated ascorbic acid (ASA), protein-encased (PE), β-carotene and cold water dispersible (CWD) all rac-2-tocopheryl acetate (TOAC) at levels of 72, 5.6 and 115 mg, respectively of active materials. Adding the fiber ingredients to the pup loaf formula increased water absorption 25 per cent and mixing time 50 per cent and imparted stickiness to the dough. The crumb of the fiber and antioxidant bread remained much softer than control bread during one to seven days of storage at room temperature. ASA (97%) was lost significantly faster than β -carotene (25%) and TOAC (<10%).

Fellow (1997) studied the effects of white, brown and black sorghum bran addition on bread quality. Among the three sorghum brans, brown sorghum bran dough had increased water absorption and mixing time. Sensory evaluation showed that the breads were of excellent quality and flavour compared to commercial white bread. Addition of 10 per cent white sorghum bran increased dietary fiber by 13 per cent per 25 g slice and added 4.5 mg phenols, brown bran increased fiber by 92 per cent and added 68 mg phenols and 267 mg catechins equivalent, black bran added fiber as well as phenols and catechins to breads, yielding products with desirable texture and flavour.

3. Materials and Methods

3.1. Sample

The Samples (white, red, and brown Quncho tef (DZ.Cr-387)) were brought from Bishoftu Agricultural Research Institute, Ethiopia with purpose. This sampling sited was chosen because of occurrence of diverse varieties of tef and to get pure breeding.

3.2. Sample preparation

All the samples were taken at one lot, cleaned, and stored in closed bins and used for entire study. The samples divided in to two flour portions. Half of the portion will had three tef flour varieties (White, red and brown) and prepared for further analysis of antioxidant capacity determination. The tef flour portions turned in to dough. The first part of dough fermented for 3 days while the second dough fermented for 18 hours called *aflegna injera.* The dough was baked in the electrical heated oven and become *Injeras. Injeras* dried at 40 ^{0}C over night, ground to a fine powder (to pass through a 40 mesh sieve) and stored in polyethylene bags for further extractions.

Sample preparation

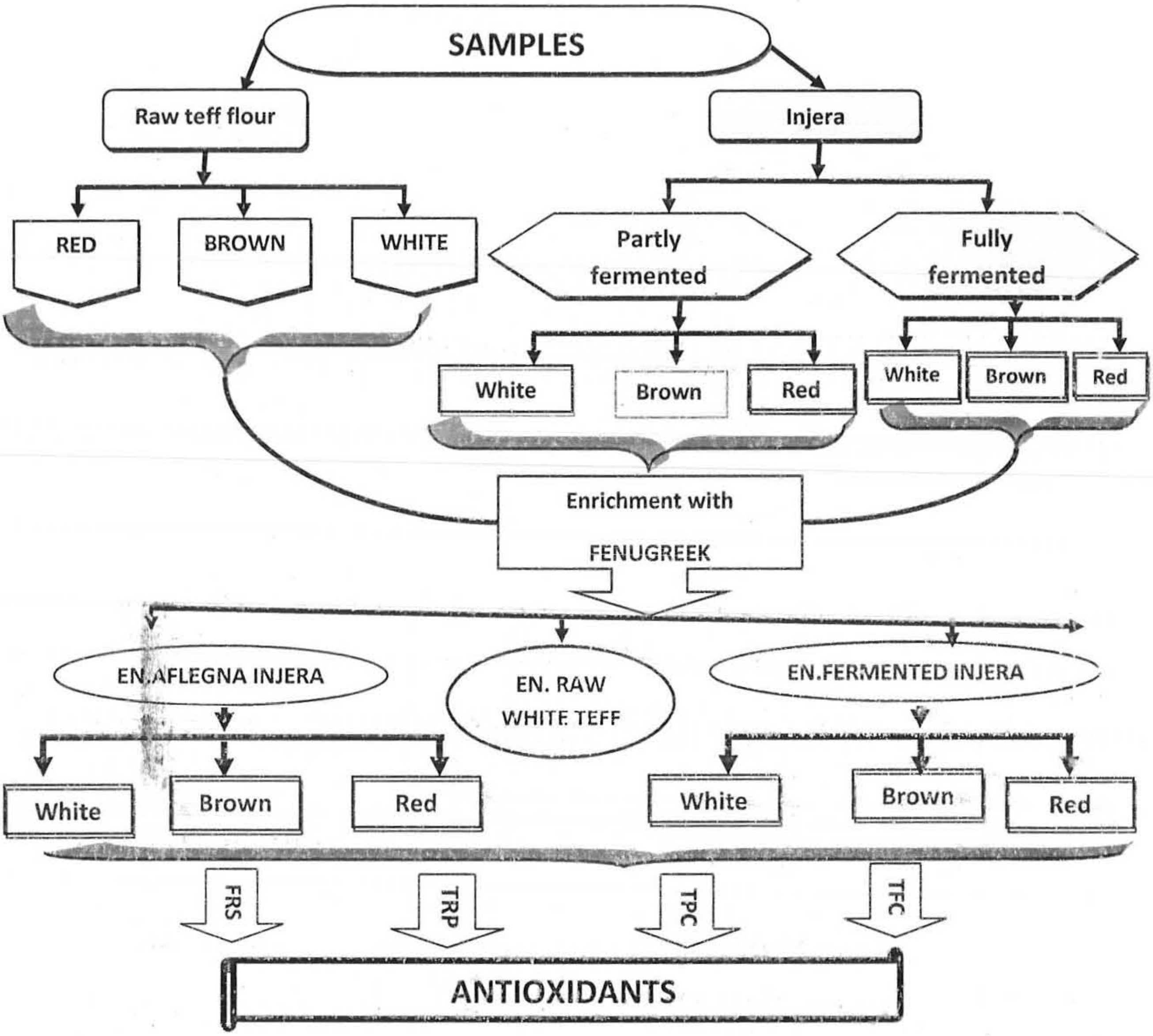


Figure 9. Flow chart of sample preparation

Injera preparation

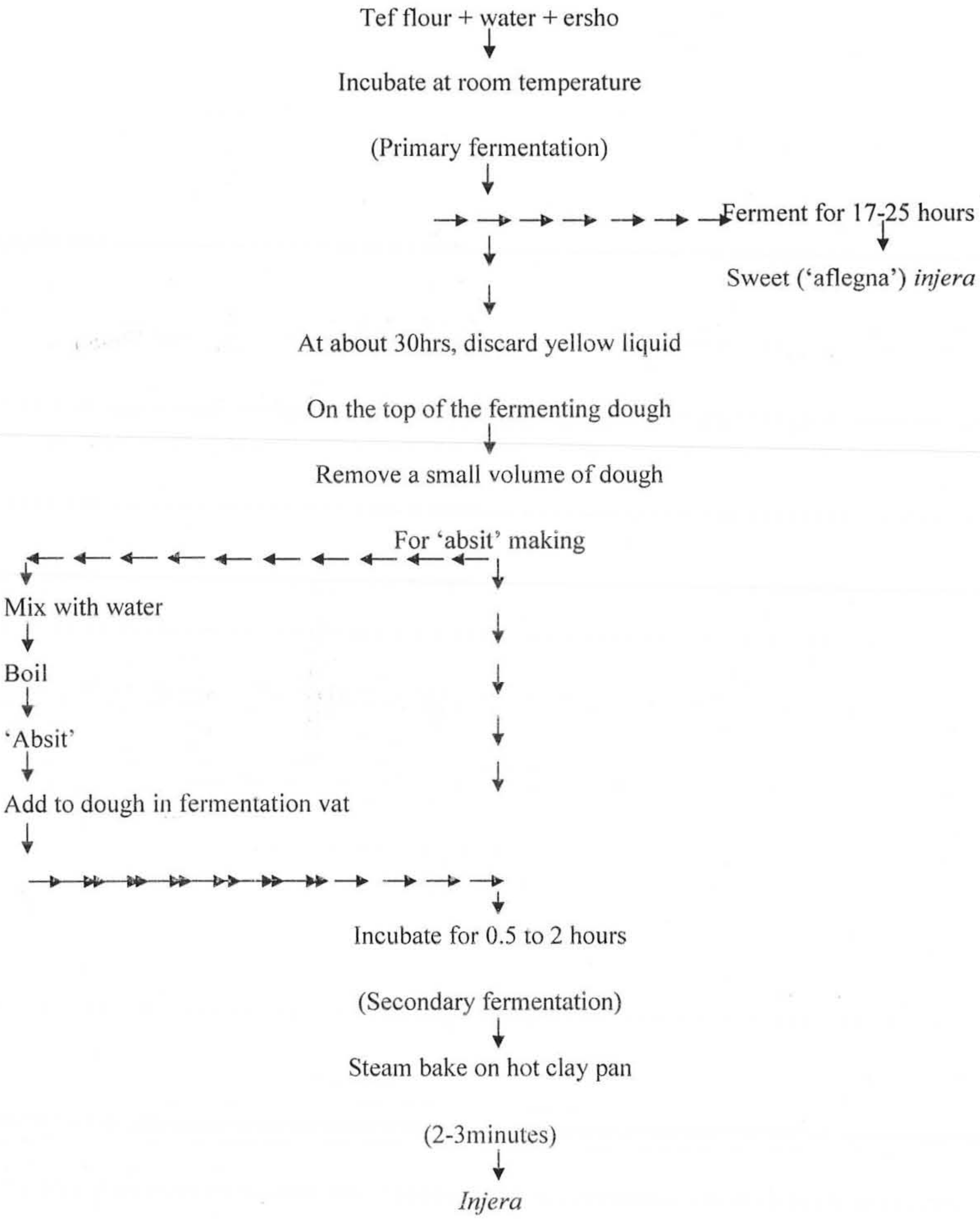


Figure 10. Flow chart for injera preparation

Source: (Steinkraus, 1983)

3.3. Chemicals

Standards BHT (2-tetra-butyl-4-hydroxyphenol), Gallic acid and reagents, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent (FCR), potassium hexacyanoferrate, and methanol were purchased from Sigma-Aldrich. Ferric chloride ($FeCl_3.6H_2O$), ferrous chloride ($FeCl_2$. $4H_2O$), trichloroacetic acid (TCA), aluminum chloride ($AlCl_3.6H_2O$), sodium nitrite ($NaNO_2$), phosphate buffer (K_2HPO_4/ KH_2PO_4) from (Fluka), and sodium carbonate (Na_2CO_3) (HiMedia).

3.4. Antioxidant Capacity Determination

3.4.1. Sample Extraction

Samples were extracted based on the procedures as outlined by Barros and Babtista *et al.* (2007) and Ferreira *et al.* (2007). The tef grain varieties processed into flour and *injera*, homogenized, weighed in to ten gram of dried powder. The powder was extracted by stirring with 100 ml of methanol at 25^0C at 150 rmp for 24 hrs using temperature shaker incubator (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 40 ^{0}C to dryness using rota evaporator (Stuart R3300) and re-dissolved in methanol at the concentration of 50 mg/ ml and stored at 4^0C 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). The effect of methanolic extracts on DPPH radical was estimated according to Kirby and Schmidt (1997). 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- 4 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 (Perkin Elmer Lamda 950 UV/V is/NIR) by monitoring the decrease in absorbance at 517 nm. This absorption maximum was first verified by scanning freshly prepared DPPH from 200-800 nm using the scan mode of the spectrophotometer. Butyl hydroxytoluene (BHT) 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:

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

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 (Barros & Baptista et al., 2007; Barros & Ferreira *et al.*, 2007).

3.4.3. Determination of Total Reducing Power

Total reducing power was carried out according to the method established by Oyaizu (1986). One millilitre of the extract at different concentrations (1- 6 mg/ml), phosphate buffer (0.2 M, pH 6.6, 2.5 ml) and potassium hexacyanoferrate solution (1% v/ m, 2.5 ml) were mixed in a test tube and incubated for 20 min at 50^0C. Then 2.5 ml trichloroacetic acid (10%) was added, and the mixture was centrifuged at 2000 x g for 10 min. The upper layer (2.5 ml) was transferred into another tube and mixed with 2.5 ml deionized water and 0.5 ml ferric chloride(0.1%) and left to react for 10 min. Finally, the absorbance of the reaction mixture was measured at 700 nm. Stronger absorbance at this wavelength indicates higher reducing power of the antioxidant. The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of RPA absorbance at λ = 700 nm against extract concentration. BHT was used as standard (Barros & Baptista et al., 2007; Barros & Ferreira et al., 2007).

3.4.4. Total Phenolics Determination

Phenolic compounds concentration in the tef and *injeras* methanolic extracts were estimated based on procedures described by Ferreira *et al.* (2007). One millilitre of sample (2000 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 725 nm. Gallic acid was used to construct the standard curve (7.5–50 µg/ml). The results were mean values ± standard error of mean and expressed as mg of gallic acid equivalents/g of extract (GAEs). Total content of phenolic in teff and its products; *injeras* extracts 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 the concentration of gallic acid established from the calibration curve ($y = 17.00x + 0.114$; $R^2 = 0.997$); V the volume of extract, L; m is the weight of extract, g.

3.4.5. Assay for total flavonoids

Total flavonoid was determined by a colorimetric method as described in Xu and Chang (2007). Briefly, 0.25 ml of sample (50 mg) was mixed with 1.25 ml of deionized water and 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the pink colour was measured at 510 nm against the blank. For calibration curve (+) - Catechin was used with a concentration range of 10–250 µg/ml. Results were expressed as mg (+)-catechin equivalent (CE)/g of extract. The standard curve equation $y = 2.657x + 0.049$, $R^2 = 0.997$ was constructed from catechin to establish the actual concentration of the extracts.

3.4.6. Data Entry and Analysis

The experimental results were in mean ± standard error (SE) of three parallel measurements. Data were evaluated by using two way variance analysis (ANOVA) and means were separated by Duncan's multiple range test ($p<0.05$) by using SPSS version 15.0. For the construction of graphs and interpolating IC_{50} of the respective antioxidant activities Microsoft Excel was used.

4. Results and discussions

4.1. Free Radical Scavenging Activity

The antioxidant activity of tef or tef *injera* extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH· method. Scavenging of different types of reactive oxygen species, mostly free radicals, is thought to be one of the main mechanisms of antioxidant action exhibited by phytochemicals. The synthetic nitrogen-centered DPPH radical is not biologically relevant but is often used as an "indicator compound" in testing hydrogen-donation capacity (Re *et al.*, 1999). The total antioxidant capacity assay was conducted to systematically evaluate the ability of tef and tef *injeras* extracts to scavenge free radicals in *Vitro*. Extracts of the samples at various concentrations (0.1, 0.5, 1, 2, 3, and 4) mg/ml were added to 2 ml of DPPH· solution. When DPPH· reacts with an antioxidant compound donating hydrogen found in the extract, it is reduced, resulting in a decrease in absorbance at 517 nm. The absorbance was recorded at 30 min using a UV–Vis spectrophotometer. The percentage of inhibition were calculated and plotted against the samples concentration to obtain the amount of antioxidant necessary to reduce the initial concentration of DPPH.

In this study, optimization of the concentration range required to scavenge DPPH was evaluated with pre-tests by tracking the purple to yellow color change of DPPH with increased concentration for each samples. This had been crucial part of the study since setting a much lower or much higher concentration range might not indicate the difference in scavenging power between samples clearly and more so, the IC_{50} (the concentration required to scavenging 50 % of the radical) might be skipped. It is after this optimization process that 0.1 - 4 mg/ml was chosen and prepared by diluting the stock solution (50 mg/ml) with methanol. This concentration optimization process was applied in similar way for reducing, total phenolics and total flavonoids assays.

The IC_{50} value expresses the amount of tef varieties and their *injeras* extracts necessary to decrease the absorbance of DPPH by 50% (Antolovich *et al.*, 2002). The value can be determined graphically by plotting percent of inhibition against the used extract concentration on excel. A lower IC_{50} value means a higher antioxidant capacity of the sample.

4.1.1. Free radical scavenging activity of tef varieties

The IC_{50} of white, brown, and red tef is 0.875, 0.75 and 0.6 mg/ml respectively as compared to standard: BHT (IC_{50} =0.056). Red tef was the most potent of all that could scavenge most free radical as shown had the lowest IC_{50} value while white tef with the highest IC_{50} , is the least potent (fig.14).

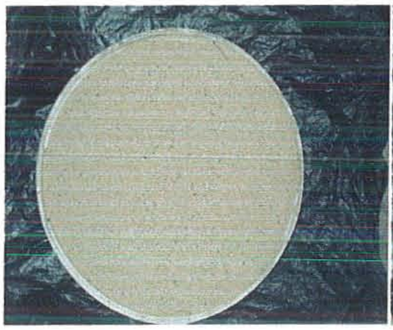


Figure 11. White tef

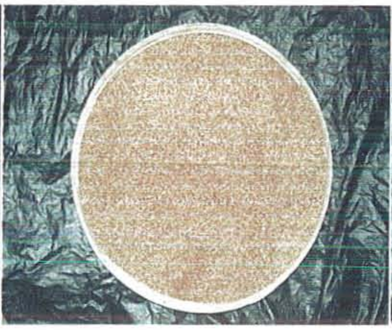


Figure 12. Brown tef

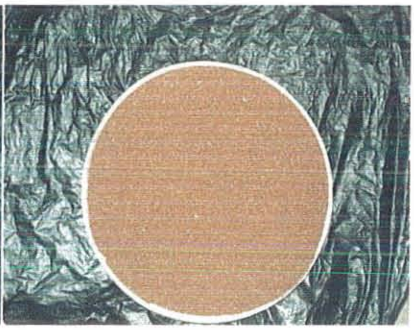


Figure 13. Red tef

The results obtained were in agreement with those Brunswick laboratories; Norton, MA (2006) studies showed that ivory tef (3600 umol TE/100) had strong antioxidant activity as compared to brown tef (3400 umol TE/100) by oxygen radical absorbance capacity (ORAC). However, it couldn't evaluate brown tef values with my findings since the antioxidant activity were tested through different methods (Velioglu *et al.*, 1998; Miller *et al.*, 2000; Halvorsen *et al.*, 2002; Javanmardi *et al.*, 2003). Guajardo-Flores *et al.* (2006) determined the total antioxidant of red corn, blue corn and white corn with ABTS the total antioxidants are 25, 24 and 20 u mol Trolox equivalents respectively. They suggested that pigmented cereals had the highest free radical scavenging activities. Sripriya *et al.* (1996) evaluated the free radical action of brown finger millets and white finger millets, DPPH radical quenching activity of brown finger greater than white finger millets.

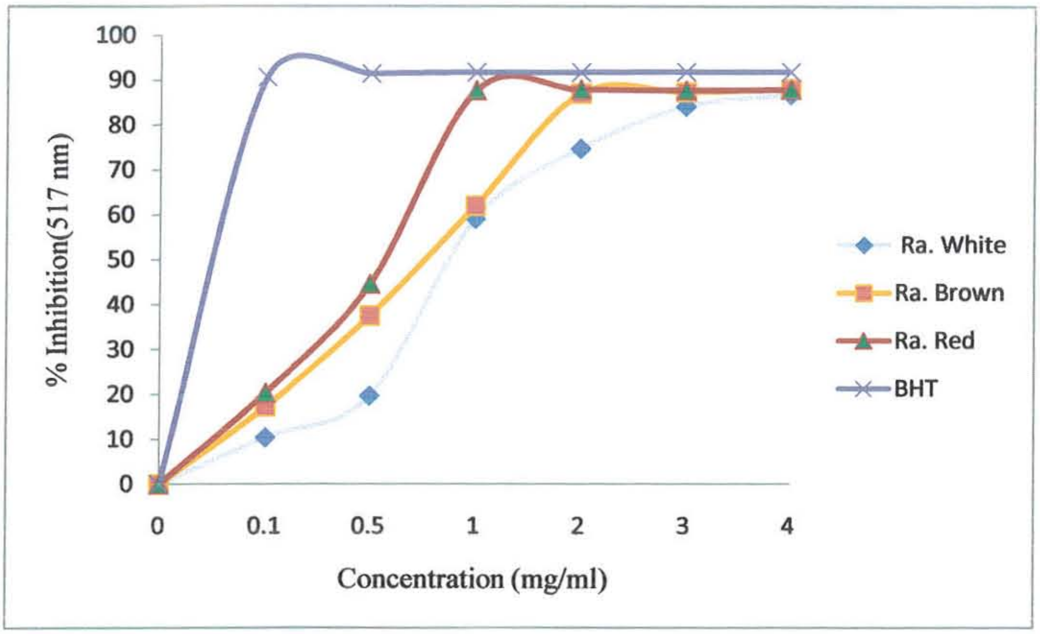


Figure 14. The DPPH scavenging activity of raw tef extracts.

4.1.2. Free radical scavenging activities of partly fermented *(aflegna)*, fully fermented tef *injeras* and their enriched one.

Enriched tef *injeras* with fenugreek were evaluated for sensory characteristics like appearance, colour, texture, taste, flavour and overall acceptability in comparison with control tef *injera* by woinishet (2010) (unpublished). As reported by Woinishet (2010) sensory quality of the supplemented tef *injera* has been observed to be significantly affected by blending proportion. From acceptability rating, she was concluded that 5% fenugreek flour could be incorporated up to 95% tef flour in the formulation of *injera* without affecting their sensory quality.

Methanol extracts from white tef *injeras* were evaluated for free radical scavenging activity by DPPH method (fig. 15). Among the extracted *injeras* the IC_{50} of the concentration of sample calculated and enriched partly fermented white tef *injeras* showed higher scavenger property (2.63 mg/ml). Fully fermented white tef *injera* sample had the highest IC_{50} value (3.25 mg/ml). The methanolic extracts of 50% inhibition value of white partly fermented, and enriched fully fermented white tef *injera* was 2.80 mg/ ml and 3.00 mg/ml respectively. The enrichment of white raw tef with fenugreek reduces 0.875 mg/ml to 0.81 mg/ml (IC_{50}).

From figure 16 processing of brown tef in to partly or fully fermented *injeras* affect scavenging activities. The IC_{50}- values for partly fermented brown tef *injera*, fully fermented brown tef, enriched partly fermented brown tef *injera* and enriched brown fully fermented tef *injeras* were 2.30, 2.75 2.25 and 2.50 mg/ml respectively.

Scavenging activities of enriched red tef *injeras* was stated in Figure 17, from the figure, it is observed that the IC_{50} ranges from 1.15 – 1.6 mg/ml. The lowest scavenging was found in red fully fermented *injeras* (1.6 mg/ml) while the highest was observed in enriched partly fermented *injera* (1.15 mg/ml). The IC_{50}-value for partly fermented red tef *injeras* and enriched fully fermented red tef *injeras* were 1.25 mg/ml.

As it observed from the results, the antioxidant contents of tef varieties had been affected by processing in to partly and fully fermented *injeras* and enrichment with fenugreek. There were slight decreases in the *injeras* (both partly and fully fermented) antioxidant levels with respect to the all raw tef varieties flour. The decrease in the free radical scavenging activities between raw flour varieties and their processed product, *injeras* may be due to a consequence of the thermal effect (90 ^{0}C) during baking and it might be some of the antioxidants in tef were relatively not heat stable. This observation is however similar to that obtained by (Oboh, 2005; Oboh and Akindahamus, 2004; Amic *et al.*, 2003). When cereals exposed to various conventional processing including thermal could bring a loss in their antioxidant contents.

The free radical retention capacity of partly fermented *injera* was higher than fully fermented *injeras* in all tef varieties in the same processing conditions. However, *aflegna injera* extracts showed lower activity than raw tef flour at the same concentration (protocol) in the same varieties. The order of free radical scavenging capacity was: raw teff extract > *Aflegna injera* > fully fermented *injera* among the same varieties of tef.

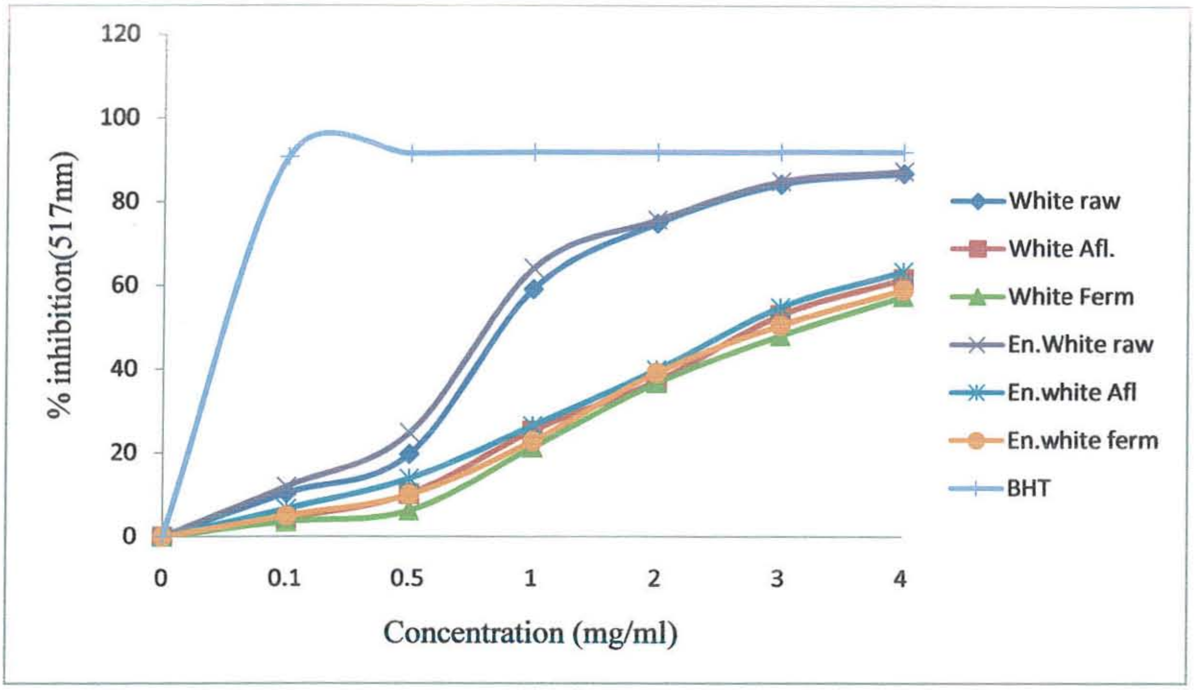


Figure 15. The DPPH Scavenging activity of white tef and its *injeras*

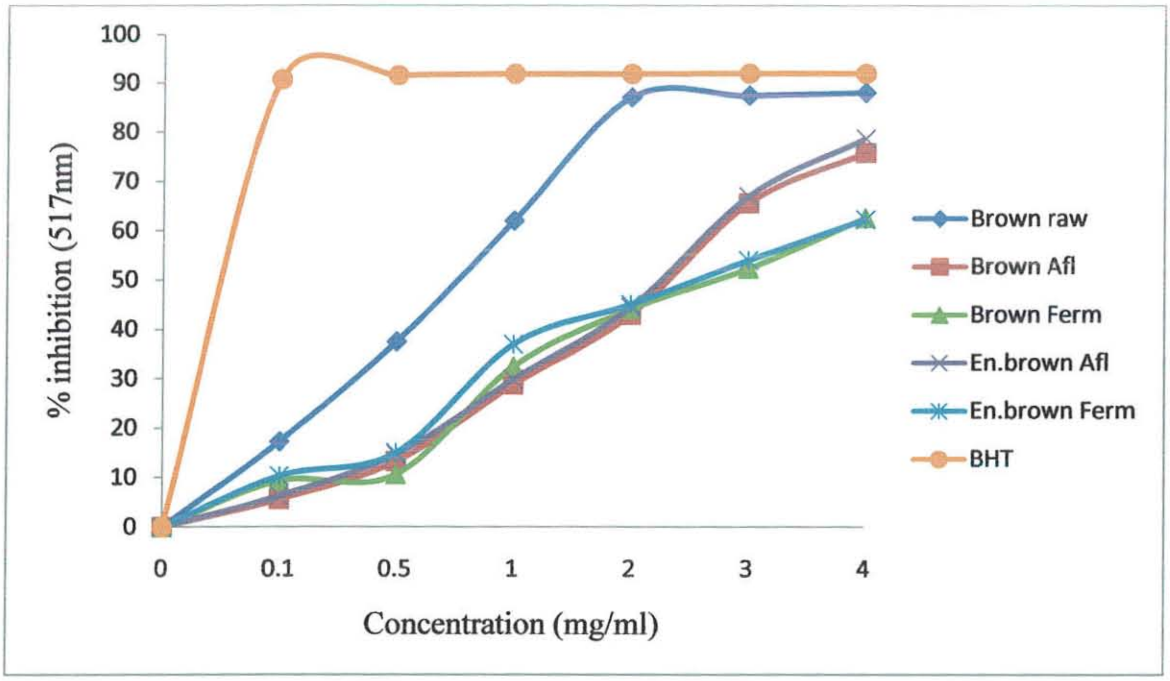


Figure 16. The DPPH scavenging activity brown tef and its *injeras*

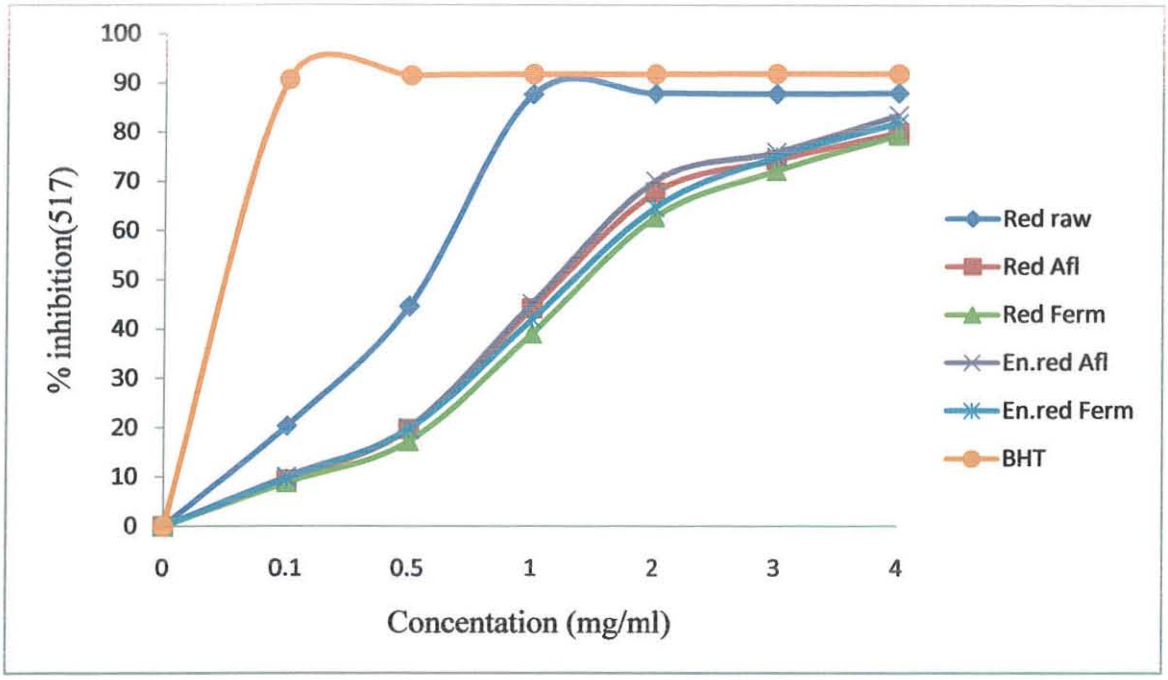


Figure17. The DPPH scavenging activity red tef and its *injeras*

4.2. Total reducing power.

Reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts (Gülçin *et al.*, 2003). The reducing power of methanolic extracts of tef and tef *injeras* were summarized in below Figures. From the figures, reducing power increased with an increased in extracts concentrations. The ability to reduce Fe (III) may be attributed from hydrogen donation from phenolic compounds (Shimada *et al.*, 1992) which is also related to the presence of reductant agent (Duh, 1998). In addition, the number and position of hydroxyl group of antioxidants compounds also rule their antioxidant activity (Rice-Evans *et al.*, 1995).

Similar to DPPH scavenging, the concentration range which shows difference between samples for reducing power was optimized. It was found that concentrations from 1-6 mg/ml were adequate enough for comparison and interpolating the IC_{50} (effective concentration at which the absorbance is 0.5). Effective concentration ($IC_{50\%}$) of the parameter representing the extracts concentration able to reduce 50% of the used reductant agent. It was determined by drawing a graph with the sample concentration at which the absorbance is 0.5. High IC_{50} value means lower antioxidant capacity of the sample and vice versal.

4.2.1. Total reducing power of tef varieties

From figure 18 it is observed that the highest reducing ability was seen in red tef (2.25 mg/ml), followed by brown tef (2.3 mg/ml) and the lowest reductive observed in white tef (2.5 mg/ml). High reductive potential in red tef may be due to the presence of high tannins and pigment-contributing compounds such as the anthocyanins or the existence of phytochemicals that are responsible to the variable distribution of structural parts such as bran, endosperm, and germ (srilakshmi, 2005).

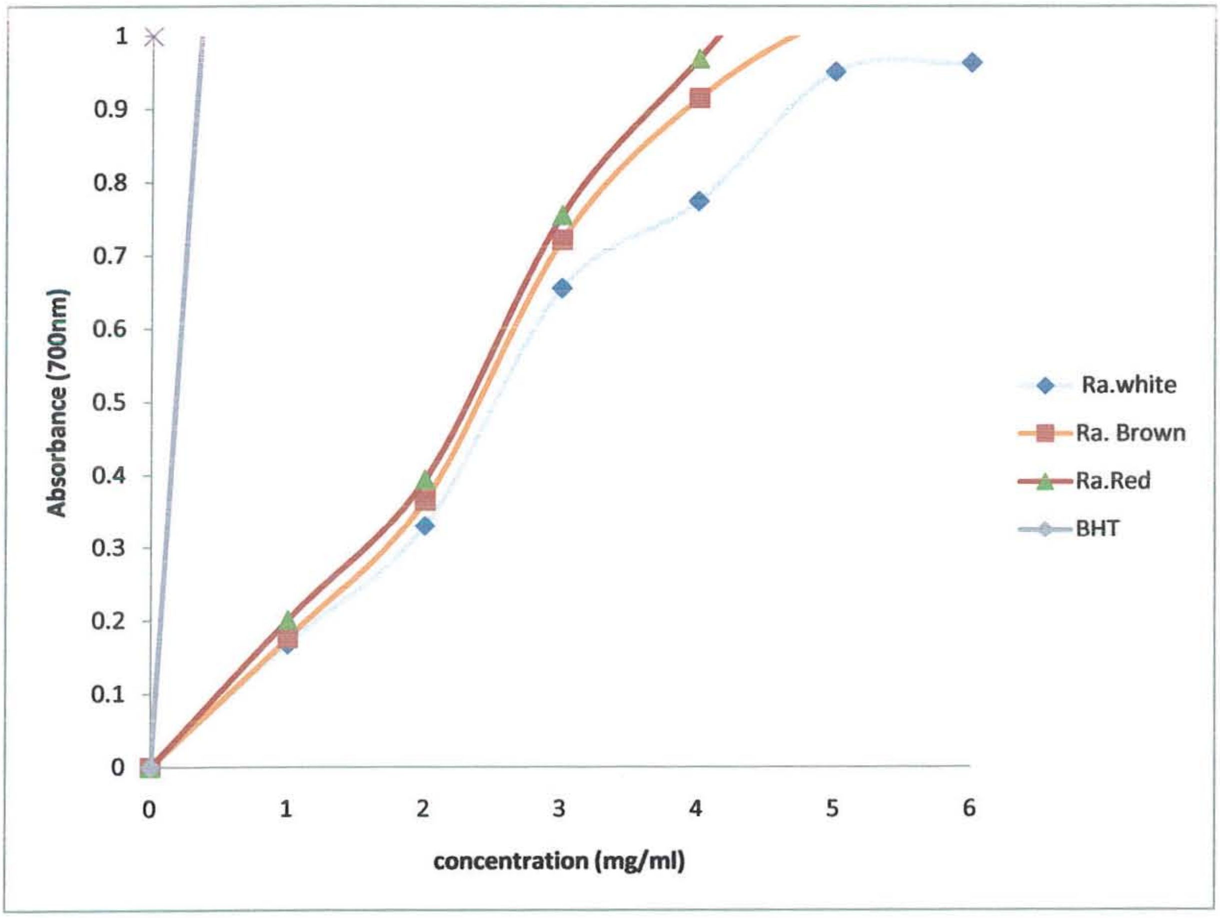


Figure 18. Total reducing power of tef varieties

4.2.2. Reducing power of partly fermented, fully fermented tef *injeras* and their enriched one.

As indicated in Figure 19 the IC_{50} - values for total reducing power of white tef *injeras* extracts were presented. Enriched partly fermented white tef *injeras* had the minimum IC_{50} (2.7 mg/ml) while fully fermented white tef *injeras* had the lowest reducing capability; it had 3.30 mg/ml. The IC_{50} value for partly fermented white tef *injera* was 2.75 mg/ml.

There were changes in the total reducing power; the extractions of brown tef *injeras* with fenugreek had higher reductive potential respect to the unenriched *injeras* (figure 20). The IC_{50} values for partly fermented brown tef *injera* (2.75 mg/ml), fully fermented brown tef *injera* (2.75 mg/ml), enriched partly fermented brown tef *injera* (2.50 mg/ml) and enriched fully fermented brown tef *injera* was 2.60 mg/ml.

As it can be seen from the figure 21 the IC_{50}- values for methanolic extracts of partly fermented red tef *injera*, fully fermented red tef *injera*, enriched partly fermented red *injera* and enriched fully fermented red tef *injera* were 2.5 mg/ml, 2.75 mg/ml, 2.20 mg/ml and 2.4 mg/ml respectively.

There were overlap in the values of reductive potential for white partly fermented *injera*, partly brown tef *injera* and fully fermented brown tef *injera* and red fermented *injera* (2.75 mg/ml) this might be due to the reducing power assay is considered to be a sensitive method for the quantitative determination of dilute concentrations of total antioxidants which participate in the redox reaction (Amarowicz, 2004).

There were decreases of reducing power of *injeras* in contrast to their flour. As Friedman and Dao (1990) observed in grains like sorghum, cooking and baking results in the partial loss of reductive potential. These observations however, contrary to the observation of Hye-Min and Bong-Kyung (2008), as they observed that grains (wheat flour) longer baking times or higher temperatures generally corresponded to higher levels of antioxidants in comparison to less intense baking conditions. This because most of the antioxidants in grains found in the bran and endosperm components, which had been largely obtained in high temperature.

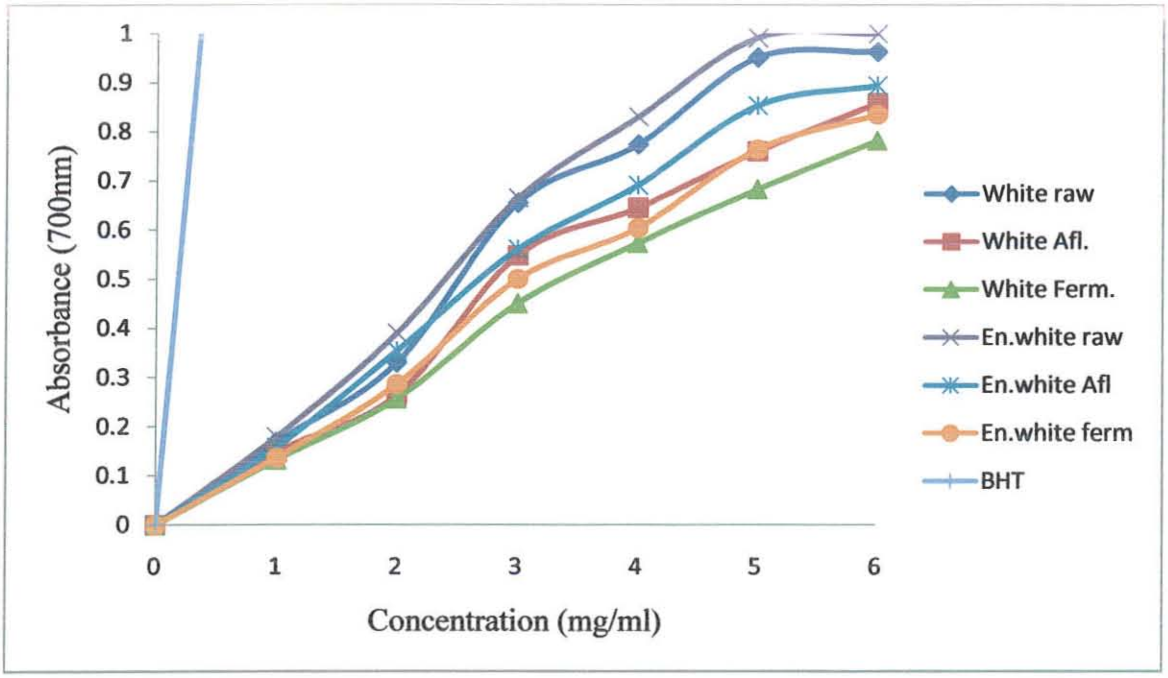


Figure 19. Reducing power of white tef and its *injeras*

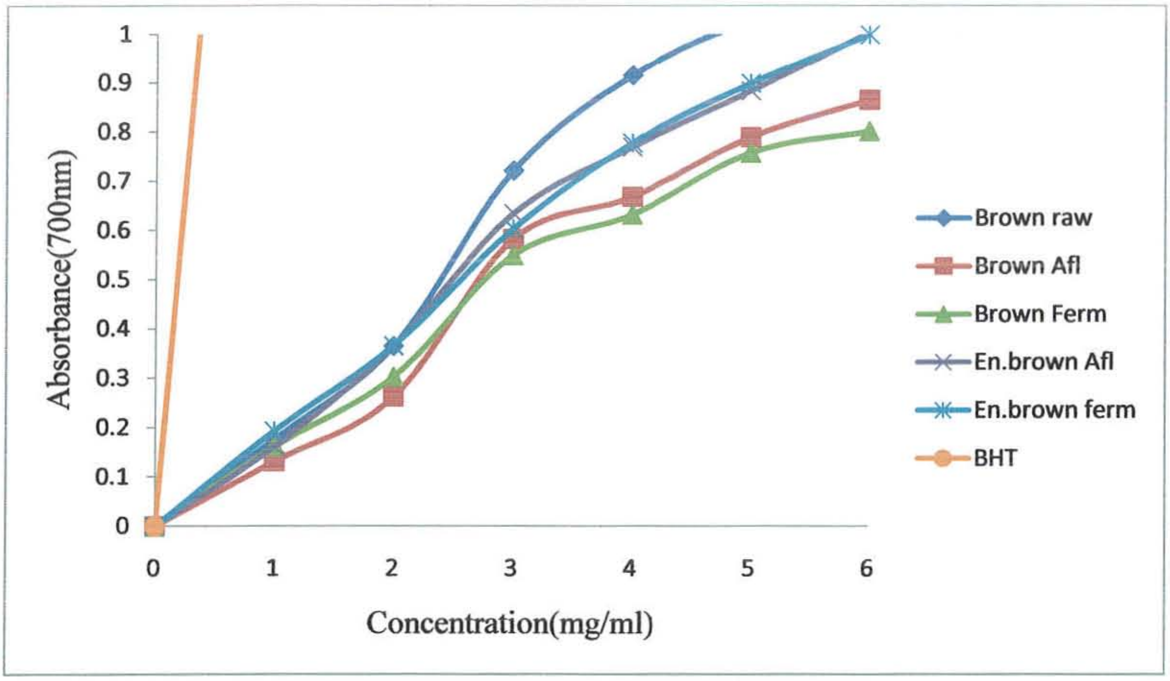


Figure 20. Reductive potential of brown tef and its *injeras*.

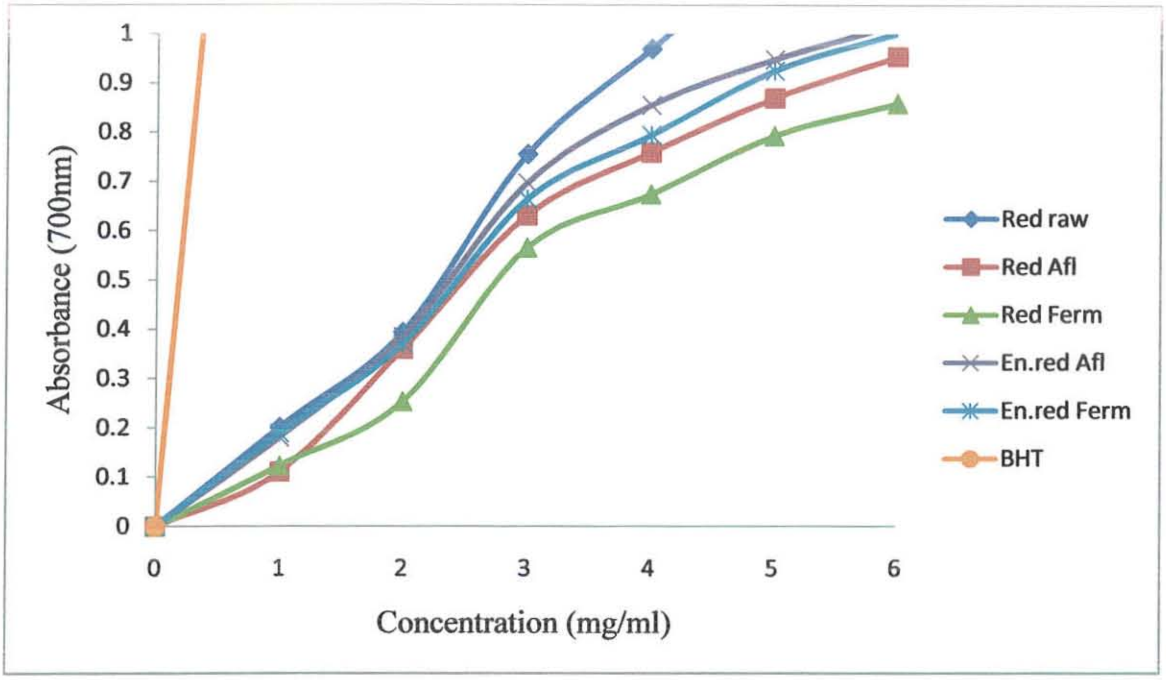


Figure 21. Reductive potential of red tef and its *injeras.*

Extracts of *aflegna injera* (partly fermented) had higher scavenging activities and reductive potential than fully fermented *injeras.* The reasonable evidence that less antioxidant in fully fermented *injeras* than partly fermented *injeras*' among the same varieties were; in case of fully fermented *injera* at about 30 hours, the yellow liquid on the top of the fermented dough would be discarded this result in the loss of water-soluble antioxidants. The finding of Rovio *et al.* (1999) suggested that water may be used as an extraction solvent for antioxidants and has gained an increasing attention due to its unique antioxidants solvation properties. Ibáñez *et al.* (2003) extracted the most active antioxidant compounds from *rosemary* including flavonoids by water extraction.

A number of studies have documented significant reduction in antioxidant capacity of cereals in fermented foods. In study of miller fermented foods, the fermentation reduce antioxidants levels (paredes-lopez and Harry, 1988) this is because antinutritional factors which sometimes act as antioxidants show a decline during fermentation. Contrary, in majority of cereals, fermentation often increases the content of soluble vitamins: well known antioxidant (paredes-lopez and Harry, 1988).

The finding of Iuliana (2010) indicates that antioxidant activity of the rye sour dough highly depends on the fermentation time. Long fermentation process is tools to increase the bioactive compounds and antioxidants activities. However, the finding of Iuliana (2010) contrary with my finding since the fully fermented tef *injeras* which fermented for 72 hours had low antioxidant activity than partly fermented tef *injera* which had been fermented only for 18 hours. This might be due to the out weight of the loss water soluble antioxidants with the liquid on the top of the fermented tef sour dough before fully fermented *injera* was baked over the enhancement of antioxidants during fermentation or may fermentation in tef couldn't result increase in the antioxidant levels.

Tef *injeras* such as unenriched and enriched partly fermented and fully fermented were evaluated for free radical scavenging activities and total reducing power, variations observed; the extracts from the enriched *injeras* appear to posses' greater antioxidant activity when compared to unenriched *injera*s. The reasons for the higher free-radical scavenging and total reducing ability of the enriched *injera* were that due to the presence of fenugreek. Fenugreek has higher antioxidants than cereals. This is in agreement with the result reported by park *et al.* (1997) studied the stability of antioxidants when wheat fortified with fenugreek; the fortified product increased its antioxidant levels. A number of studies have documented that fenugreek has high antioxidant activity (Zheng and Wang, 2001; Velioglu *et al.*, 1998). Mansour and El-Adawy (1994) recommended that fenugreek seed could be added to foods like ground meat and baked cereals, not only as nutritional supplements but also to enhance a potential functional food (antioxidants).

Table 5. Free radical scavenging activities and reducing power of three tef varieties and their *injeras*.

Extracts	**IC_{50} (mg /ml) for RSA**	**IC_{50} (mg /ml) for TRP**
White tef	0.875	2.50
Brown tef	0.75	2.30
Red raw tef	0.60	2.25
Partly fermented white tef *injera*	2.80	2.75
Fully fermented white tef *injera*	3.25	3.30
En. Raw white tef	0.81	2.3
En. partly fermented white tef *injera*	2.63	2.70
En. fully fermented white tef *injera*	3.00	3.00
Partly fermented brown tef *injera*	2.30	2.75
Fully fermented brown tef *injera*	2.75	2.75
En. partly fermented brown tef *injera*	2.25	2.50
En. fully fermented brown tef *injera*	2.50	2.60
Partly fermented red tef *injera*	1.25	2.50
Fully fermented red tef *injera*	1.60	2.75
En. partly fermented red tef *injera*	1.15	2.20
En. fully fermented red tef *injera*	1.25	2.40

4.3. Total phenols

Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, antiatherosclerotic, and anti-carcinogenic activities. These activities may be related to their antioxidant activity (Chung, Wong, Huang, & Lin, 1998). Moreover, it had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds. Therefore, it is important to consider the effect of the total phenolic content on the antioxidant activity of teff extracts.

The folin-ciocaltue reagent is used to obtain a crude estimate of the amount of phenolic compounds present in the extracts. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the reagent. However, the assay has been shown not specific to just phenolic compounds but to any other substance that could be oxidised by the folin reagent (Escarpa and Gonzalez, 2001; singleton *et al.*, 19999). In addition, phenolic compounds depend on the number of phenolic groups they have, responded differently to the Folin-ciocaltue reagent (Singleton *et al.*, 1999).

4.3.1. Phenol contents of tef varieties

Total phenolic compound contents of each tef varieties and its *injeras* were expressed as g gallic acid equivalent (GAE); the result showed that total phenolic compound content in red tef was highest reported as 11.47 mg GAE/g. Brown tef extract was ranked second (9.87 mg GAE/g) possessing higher antioxidant properties than white tef (8.248 mg GAE/g). The phenolic content of raw tef varieties were significantly different ($P<0.05$). This is in agreement with the result reported by Toyokuni *et al.* (2002); Pigmented grains have been shown to have higher phenolic compounds than the regular non-pigmented grains.

4.3.2. Phenol contents of partly fermented, fully fermented tef *injeras* and their enriched one.

The total phenolic content results of white tef *injeras* were reported in Table 6, the average phenol values of partly *(aflegna)* and fully fermented white tef *injeras* were 5.81 and 4.03 mg GAE/g respectively. Whereas the total phenolic content for enriched partly fermented white tef *injera* and enriched fully fermented white tef *injeras* were found to be 6.77 mg GAE/g and 5.59 mg GAE/g respectively.

Phenol content of partly and fully fermented brown tef *injeras* or enriched compared to unenriched brown tef *injeras* was stated in the Table 6. From the table, it is observed that there is enhancement in phenol content among enriched *injeras*. The total phenol of brown tef *injera* ranged between 5.81 mg GAE to.8.53 mg GAE/g. The highest increase in phenol content was observed in enriched partly fermented brown tef *injera* (8.53 mg GAE/g) and the lowest increase was in fully fermented white tef *injeras* (5.81 mg GAE/g). Total phenol values of partly fermented and enriched fermented brown tef *injera* were found to be 7.19 and 7.94 mg GAE/g respectively.

As Table 6 indicates processing of red tef in to partly or fully fermented *injeras* affect the amount of total phenol contents. The total phenol contents for partly fermented, fully fermented, enriched partly fermented and enriched fully fermented red tef *injeras* were found to be 8.75, 7.36, 9.12 and 8.24 mg GAE/g respectively.

The raw tef showed the highest total phenol contents in respect to both partly and fully fermented *injeras* in all three varieties. This may be due to treatment of foods with high thermal processing. According to Julkunen (1985), increasing the temperature above 60 ^{0}C, lowered the phenolic compounds considerably in the majority of cereals. The study also found that the total phenolic compounds of partly fermented *injera* was greater than fully fermented *injera* this is might be due to solubility of phenolics in water which discarded before *injera* was baked. However, there were no large phenolic differences between partly and fully fermented in the same varieties since the predominant phenolic compounds (Ferulic) in cereals were identified as lipid acid soluble derivatives. This in agreement with Collins (1986), he suggested that some phenolic acids and flavonoids are water soluble compounds, but lipid soluble derivatives are common to grains such as ferulic acid.

The highest increase in phenol content was observed in enriched *injeras as compared to unenriched one*. This enhancement of antioxidant content in enriched *injeras* due to attributed to the addition antioxidant rich food ingredients, fenugreek. Similar results were also observed by Polasa (1998) and Anilakumar *et al.* (2007) in *rotti,* Nigerian cereal staple food.

4.4. Total flavonoids

Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities (Cook & Samman, 1996). In food systems, flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides. Therefore, they present antioxidative efficiency in oils, fats and emulsions.

4.4.1. Flavonoid contents of tef varieties.

Results presented in the Table 6 show significant variation ($P<0.005$) in flavonoid contents of tef varieties. It was ranged between 1.03 mg CE/ g and 2.13 mg CE/ g. The results for white tef, brown tef and red tef were: 1.03, 1.78 and 2.12 mg CE/ g respectively. The results illustrated that red tef had highest flavonoids as compared to white and brown tef. This may due to seed colour. Seed colours affect flavonoid contents. Cereals seed grown on plants with red secondary color had higher levels of flavonoids than those less coloured seed. This was possible if and only if the anthocyanin responsible for red pigmented seed testa. The results were similar to the values obtained from different colour of sorghum (Beta *et al.*, 1999).

4.4.2. Flavonoids of partly fermented, fully fermented tef *injeras* and their enriched one.

Among white tef *injeras* (table 6) the flavonoids content varied and ranged between 0.88 mg CE/g to 1.09 mg CE/g. The flavonoid contents for partly fermented, fully fermented, enriched partly fermented and enriched fully fermented white tef *injeras* were 0.89, 0.88, 1.09 and 1.06 mg CE/g respectively.

In brown tef *injeras,* the highest flavonoid content was noticed in enriched partly fermented *injera* (1.9 mg CE/ g). The lowest content was in fully fermented brown tef *injeras* (1.18 mg CE/ g). The flavonoid content of partly fermented brown tef *injera* and enriched fermented brown tef *injera* were 1.33 mg CE/ g and 1.264 mg CE/g respectively.

Results presented in the Table 6 showed flavonoid contents of red tef *injeras*. It was ranged between 1.64 mg CE/ g and 2.03 mg CE/ g. The results of partly fermented, fully fermented, enriched partly and enriched fully fermented red tef *injeras* were: 1.77, 1.64, 2.03, and 1.87 mg CE/ g respectively.

Flavonoids content of tef *injeras* varieties in comparison with flour is presented in Table 6. From the table it is observed that there was reduction of flavonoids in *injeras*. Processing significantly affect the flavonoid distribution of tef flour varieties (P<0.05). The relative difference of flavonoids content may be attributed due to flavonoids which are present in the tef flour can be destroyed or transformed in to other phytochemicals during heat treatment and processing. Kikuzaki and Nakatani (1993) explain the reduction in the value of flavonoids during heat treatment and processing because transformation of existing flavonoid structure happened in oxidation or may interact with the other compounds.

Table 7 suggested that between unenriched and enriched tef *injeras* with fenugreek, enriched *injeras* showed the highest flavonoid value while unenriched had the lowest value as expected due to enrichment of injeras with fenugreek. As Cowan(1999) reported that fenugreek has been to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory and hypotensive due to the presence of abundant flavonoid contents.

Table 6. **Effects of processing on the phenol and flavonoid contents of tef varieties (white, brown and red) and their *injeras*.**

Variety	Treatment	Phenol (mg GAE/gm)	Flavonoid (mg CE/gm)
White tef	WR	8.28 ± 0.03^{ab}	1.03 ± 0.06^{ab}
	WA	5.81 ± 0.23^{bcd}	0.89 ± 0.02^{a}
	WF	4.03 ± 0.31^{d}	0.87 ± 0.09^{a}
	WEA	6.77 ± 0.77^{bc}	1.09 ± 0.08^{ab}
	WEF	5.59 ± 0.84^{cd}	1.06 ± 0.06^{ab}
	WER	9.41 ± 0.86^{a}	1.24 ± 0.09^{a}
Brown tef	BR	9.73 ± 0.26^{e}	1.78 ± 0.09^{ef}
	BA	7.19 ± 0.29^{fg}	1.32 ± 0.05^{f}
	BF	$5.81 \pm 0\ .976^{g}$	1.18 ± 0.03^{f}
	BEA	8.53 ± 0.64^{ef}	1.90 ± 0.44^{e}
	BEF	7.94 ± 0.92^{efg}	1.26 ± 0.14^{f}
Red tef	RR	11.47 ± 0.84^{x}	2.13 ± 0.01^{x}
	RA	8.75 ± 0.06^{xy}	1.77 ± 0.03^{y}
	RF	7.36 ± 0.89^{y}	1.64 ± 0.06^{z}
	REA	9.12 ± 0.98^{xy}	2.03 ± 0.01^{x}
	REF	8.24 ± 0.97^{xy}	1.872 ± 0.07^{y}

Reported values were mean ±SE (n=3). Means with different letters in the same column are significantly different (P<0.05). NB: WR, WA, WF, WEA & WER were white raw tef, white *aflegna*, white fermented, white enriched *aflegna* and enriched white tef *injeras* respectively) and BR, BA, BF, BEA & BEF represent brown raw tef, *aflegna*, brown enriched *aflegna* and enriched brown fermented *injeras* respectively while RR (raw red tef), RA (*aflegna* red tef *injeras*), RF (fully fermented red tef *injeras*), REA (enriched *aflegna* red tef *injera*) &REF (red enriched fully fermented *injeras*).

Tef varieties *injeras* showed the same trend in their phenol and flavonoid values as the raw tef varieties in the order: the red tef *injeras* > brown tef *injeras* > white tef *injeras* in the same processing parameters. The phenol and flavonoids contents of three tef varieties were significantly affected (P< 0.05) by processing and variety However, variances of analysis of variety * processing was found to be insignificant (P > 0.005).

5. Conclusion and recommendations

5.1. Conclusions

Cereals are the staple foods of an Ethiopian diet, 70 to 80 percent the total energy is provided by cereals. The principal Ethiopian cereal is tef. Tef is the most important food source in Ethiopian diet- used to make *injera*, flat bread. Consumption of cereal grains as part of the diet is now recommended for health reasons because of rich source of antioxidants. Antioxidants have gained interest since it was reported that an increased intake of dietary antioxidants may help maintain an adequate health status. Antioxidants are found in all cereals including tef. However, there are no well organized previous reports on the antioxidant content of tef varieties and their *injera*. Hence, the present investigation was carried out to evaluate the antioxidant content and activity of three tef varieties. Red tef had the most potent of all that could scavenge most free radical and highest reductive potential had the lowest IC_{50} value accompanied by having the highest phenolic and flavonoids contents. While white tef had the highest IC_{50} is the least potent.

The study also showed that processing of tef flour in to partly or fully fermented *injeras* had reduced the total antioxidant contents with respect to raw tef flour ($P<0.05$). The total antioxidants retention capacity of partly fermented *injera* was higher than fully fermented *injeras*. From the study it was also observed that there was enhancement in antioxidant activites of enriched *injeras* with fenugreek at 95:5 ratio of tef flour to fenugreek respectively as compared to unenriched.

5.2. Recommendations

The following recommendations are made based on the study conducted and findings on antioxidants tef varieties and their *injeras*

- Red tef should be getting special attention and emphasis by the consumers due to its good antioxidants capacity and health institutions, should advise people to feed on red tef *injera*.
- Enrichment of fenugreek on tef flour for injera production should also be encouraged since it enhance antioxidant capacity of *injera*
- It also recommended for other researchers to conduct a research on retention of antioxidant activity during home preparation of *injeras*

Recommendation for future line work

- Total antioxidant activities of tef varieties already demonstrated. However, characterizing specific type of antioxidants found in tef, structures and bioavailability that involved in antioxidant activity should also be studied.

- There is considerable variation in the antioxidants levels produced by plants grown under different conditions or farming practices so antioxidants levels of tef varieties should be studied and evaluated in different regional areas.

- Further research should be oriented to the optimisation of antioxidant activity in order to understand the factors controlling the retention of phytochemicals during home preparation of *injeras*. Therefore, the optimum retention of these phytochemicals under various processing parameters and their interaction with other food components should also be studied in future.

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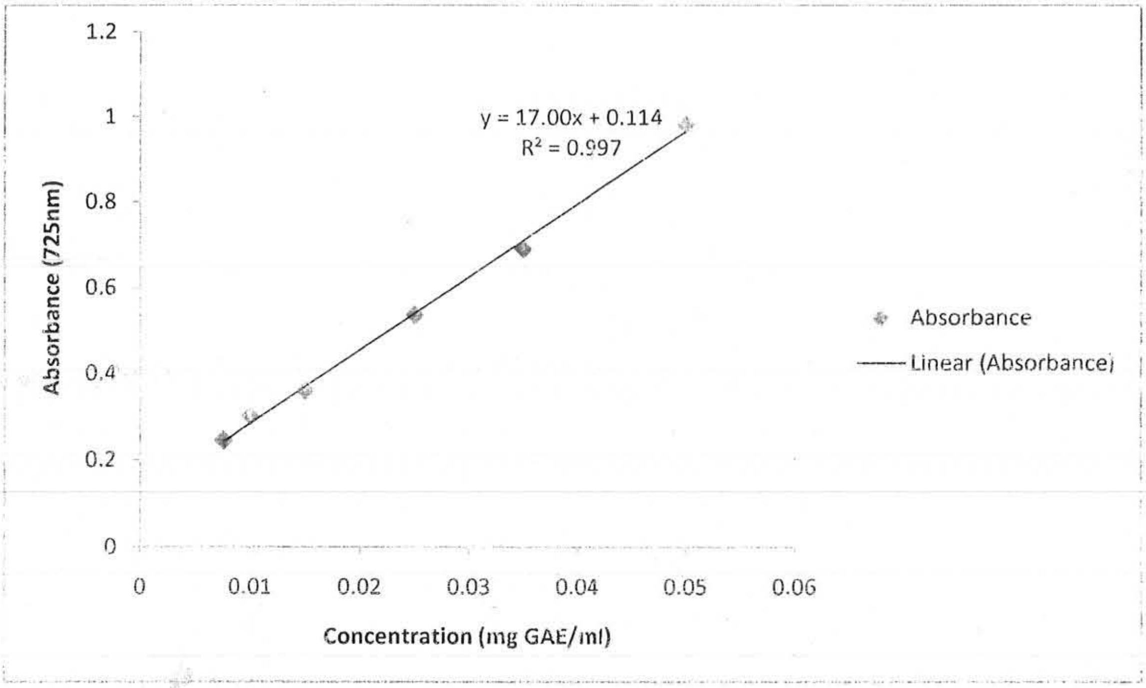
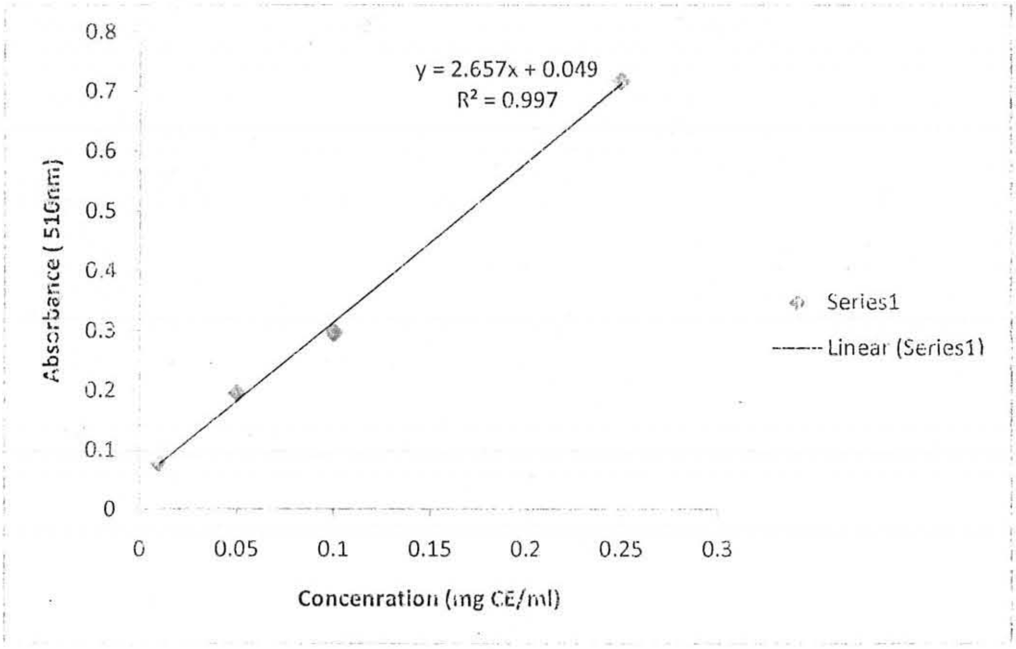
APPENDIX

A.1.Standard curves

A.1.1. Standard curve for total phenolic determination



A.1.2. Standard curve for total flavonoid determination



A.2. Two ways ANOVA table for total phenols and flavonoids of three tef varieties and their processed product, *injeras*.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	phenol	155.840(a)	15	10.389	5.039	.000
	flavanoids	8.064(b)	15	.538	10.547	.000
Intercept	phenol	2883.797	1	2883.797	1398.779	.000
	flavanoids	98.400	1	98.400	1930.348	.000
vareity	phenol	63.526	2	31.763	15.407	.000
	flavanoids	6.064	2	3.032	59.477	.000
processing	phenol	107.986	5	21.597	10.476	.000
	flavanoids	1.531	5	.306	6.005	.001
vareity * processing	phenol	2.982	8	.373	.181	.992
	flavanoids	.488	8	.061	1.197	.332
Error	phenol	65.973	32	2.062		
	flavanoids	1.631	32	.051		
Total	phenol	3105.269	48			
	flavanoids	109.498	48			
Corrected Total	phenol	221.813	47			
	flavanoids	9.696	47			

a R Squared = .703 (Adjusted R Squared = .563)

b R Squared = .832 (Adjusted R Squared = .753)

DECLARATION

I, the undersigned, hereby declare that this thesis is my original work, has not been presented for a degree in any other University and all source of materials used for the study have been correctly acknowledged.

Name: Bemhiretu Boka Guncha

Signature____________________

Place: Addis Ababa University

Date of Submission 04-may-2022

The thesis has been submitted with my approval as supervisors

Name: Gulelat Desse (PhD, Associate professor)

Signature____________________

Date____________________

Mr. Ashagrie Zewdu

Signature____________________

Date____________________