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SCHOOL OF GRADUATE STUDIES
CENTER FOR FOOD SCIENCE AND NUTRITION

THE EFFECT OF TRADITIONAL SOURDOUGH STARTER CULTURE AND INVOLVED MICROORGANISMS ON SENSORY AND NUTRITIONAL QUALITY OF WHOLE WHEAT BREAD

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List of acronyms

AAU	Addis Ababa University
AOAC	Association of Official Analytical Chemist
CFU	Colony Forming Unit
EPHI	Ethiopian Public Health Institute
EPS	Exopolysaccharides
FDSN	Food Science and Nutrition
Lb	Lactobacillus
LAB	Lactic acid bacteria
NSLAB	Non starter lactic acid bacteria
PA	Phytic acid

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Abstract

Consumers wish to have a wide range of foods that are nutritious and flavorful and have long shelf life without added preservatives. The objective of this work was to study the effect produced on whole wheat bread by using natural sourdough starter culture. pH and acidity of natural sourdough starter culture were measured after 72 hrs of fermentation and sourdough after 24 hrs of fermentation pH and acidity of sourdough bread and commercial yeast bread were analyzed. Sensory characteristics and nutritional value of wheat sourdough bread and commercial yeast bread were analyzed. Microfilora of natural sourdough starter culture and sourdough throughout fermentation were also analyzed. Proximate composition of wheat flour was analyzed. Traditional sourdough and commercial yeast bread were evaluated for their acceptability by semi-trained panelists using 9-point hedonic scale. Decreased pH of sourdough to /4.0/ leads to phytate phytate degradation and as a result phytate content in wheat flour which is 20.1% Phytic acid in mg/100g was reduced to 6% Phytic acid in mg/100gsourdough bread. The traditional sourdough bread did not show mould growth after four days storage at room temperature while commercial yeast bread showed. Among the sensory attributes tested by panelists, aroma is significantly ($p < 0.05$) differed from that of commercial yeast bread. The use of natural sourdough starter and fermentation had a number of beneficial effects that include prolonged shelf life, improved bread flavor and good nutritional value. Sourdough also improves sensory characteristics such as color, aroma, taste, and texture of breads.

Keywords: Whole wheat, bread, fermentation, traditional sourdough, microfilora, sensory characteristics.

1. Introduction

1.1. Background

Cereals are those members of the grass family, the Poaceae grown for their characteristic fruit, the caryopsis, which have been the most important sources of world's food for the last 10,000 years (Ismailia, et al., 2010).

The role of cereals to modern society is related to its importance as food crop throughout the world. In most parts of Asia and Africa, cereals products comprise 80% or more of the average diet, in central and western Europe, as much as 50% and in the United State, between 20 - 25% (Onwueme and Sinha, 1991). Cereals are consumed in a variety of forms, including pastes, noodles, cakes, breads, drinks etc. depending on the ethnic or religious affiliation. The bran, husk, plant parts and other residues (after processing) are useful as animal feeds and in the culture of micro-organism. Wax syrup and gum are extracted from cereals for industrial purposes (Ismaila, et al., 2010).

Cereals are grown over 73% of the total world harvested area and contribute over 60% to the world food production providing dietary fiber, proteins, energy, minerals and vitamins required for human health . However, the nutritional quality of cereals and the sensorial properties of their products are sometimes inferior or poor in comparison with milk and dairy products. The reasons behind these are the lower protein content, deficiency of certain essential amino acids, the presence of antinutrients (phytic acid, tannins and polyphenols) and the coarse nature (Blandino et al., 2003).

Due to increasing consumer demand for more natural, tastier and healthier food, the traditional process of sourdough bread production has enjoyed renewed success in recent years. Sourdough is employed in the manufacture of a number of baked products, such as breads, cakes and crackers. It is reported that the application of sourdough to wheat breads produced several effects, including leavening, acidification, improvement of the dough properties, and flavor of the bread, texture, delayed firmness and staling, increased resistance to microbial spoilage and

improved nutrient availability of cereals. It was suggested that all these benefits were attributable to the lactic acid bacteria (LAB) and yeasts naturally present in sourdough (Aplevicz, et al., 2013). Moreover, it has been noted that when sourdough is added, there are changes in the fundamental rheological properties of wheat dough, making it soft, less elastic and therefore easily extendable (Clarke et al., 2002). The impact of changes in the rheology of dough must be considered in order to choose an appropriate fermentation time and obtain good quality bread. The dough must contain a large volume of gas and also reserve gas retention for oven rise (Golshan Tafti, et al.,2014). However, there are other factors to be taken into account, such as the type of flour, the sourdough fermentation conditions (pH and temperature) and the selection of starter cultures with specific and desirable metabolic properties (Aplevicz,et al.,2013).

Bread baking using sourdough is a common practice and has the advantage of improving the nutritional value and sensory qualities of breads, achieving the baking ability of dough for wheat bread production, and increasing the shelf life of breads by delaying the germination of bacterial and mould spores. The microbiota of sourdoughs consists of specifically adapted lactic acid bacteria (LAB), mostly lactobacilli, as well as yeasts (Yousif and Safaa, 2014). Its composition is affected by the endogenous ecological factors which in turn are determined by the flour and process (exogenous) factors.

1.2. Statement of the problem

Fermented plant products are among the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for many people in the developing world. However, due to the lower protein content and deficiency of certain essential amino acids, the nutritional and sensory qualities of these products are considered poor in comparison with foods of animal origin. Attempts to improve nutritional qualities of cereal products include genetic improvement and amino acid supplementation with protein concentrates or other protein-rich sources such as grain legumes. Additionally, several processing technologies, which include cooking, sprouting, milling and fermentation, have been put into practice to improve the nutritional properties of cereals. Fermenting microorganisms can synthesize certain amino acids and improve protein quality and availability of B group vitamins. Fermentation also results in reduction in phytate, which may increase the amount of soluble iron, zinc and calcium several folds (Blandino, et al.,

2003). Even though all the above described advantages are mostly related to fermentation and byproducts of involved microorganisms it is less known about the microbial effect during fermentation stage of wheat sourdough and how to use traditional sourdough starter for production of good quality bread with improved shelf-life and enhanced nutritional quality with compared to commercial yeast wheat bread.

1.3. Hypothesis of the study

Traditional sourdough starter has positive effect on sensory characteristics, microfilora, nutritional and sensory quality of whole wheat bread.

1.4. Objectives of the study

1.4.1. General objective

The objective of this research work is to investigate the effect of traditional sourdough starter culture on the sensory acceptance, microfilora, nutritional quality and shelf life whole wheat bread.

1.4.2. Specific objectives

- To analyze the proximate analysis of kekeba variety wheat flour.
- To analyze mineral content and phytate mineral molar ratio of whole wheat flour, sourdough bread and commercial yeast bread for prediction of mineral bioavailability.
- To determine the pH change and titratable acidity of sourdough starter culture, sourdough and bread and.
- To enumerate involved fungi and lactic acid bacteria in the process of traditional sourdough fermentation.
- To evaluate sensory quality/acceptance of both the commercial yeast bread and traditional sourdough bread

2. Literature review

2.1. History and evolutionary processes of bread wheat

The evolutionary process, which began some ten thousand years ago, involved the following major steps. Wild einkorn *T. urartu* crossed spontaneously with *Aegilops speltoides* (Goat grass) to produce Wild Emmer *T. dicoccoides*; further hybridizations with another *Aegilops* (*A. taushi*), gave rise to Spelt (*T. spelta*) and early forms of durum wheat (cultivated emmer); Bread wheat finally evolved through years of cultivation in the southern Caspian plains. This evolution was accelerated by an expanding geographical range of cultivation and by human selection, and had produced bread wheat as early as the sixth millennium BC. Modern varieties are selections caused by natural mutation starting with emmer wheat up to husk less modern wheat (Feldmann, 2001)

2.1.1. Nutritional aspects and production of wheat worldwide.

Wheat (*Triticum aestivum L.*) is one of the important grain crops produced worldwide. According to the FAO, 2005 report, about 620 million metric tons of wheat was produced from 217 million hectares in the year 2005/06 with an average yield of 2.85 metric tons per hectare. Wheat is grown on larger area than any other crop and its world trade is greater than for all other crops combined. It is easily stored and transported (Slafer and Satorre, 1999)

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

The nutritional value of wheat is addressed through its macronutrient and micronutrient components. These groups consist of carbohydrate, proteins and lipids, for macronutrients, and vitamins, minerals and phytochemicals for micronutrients. Grains consist of approximately 75% carbohydrate and therefore many believe that the importance of carbohydrate and fiber within

wheat takes precedence over their concentrations of vitamins, minerals, and phytochemicals (McKevith, 2004).

Carbohydrates are categorized into “good carbs” and “bad carbs,” the difference between them being rates of fiber digestion. “Good carbs” are unrefined complex carbohydrates, such as whole grains, and have a very slow rate of fiber digestion, delivering a slow, steady rate of glucose to the blood, creating a feeling of full-ness. Refined complex carbohydrates, such as white flour and white pasta, are known as “bad carbs.” These have a very fast rate of fiber digestion, which leads to a feeling of hunger soon after a meal. This is where the idea of “low-carb” diets originated, believing that eating no carbs would help to eliminate the amount of “bad carbs” being consumed (McKevith, 2004).

The second major macronutrient within wheat is protein, the most concentrated being gliadins and glutenins. However, these proteins are still in relatively low amounts and therefore, essential amino acids must be supplied from another source of the diet. Lastly, lipids are a very minor component, only consisting of about 1-3% within wheat. Unlike macronutrients, micronutrients are not as concentrated within wheat and therefore cannot be compared within their percentages. They do, however, serve an extremely important purpose to the human body, specifically in their roles of promoting health and preventing disease. The most significant vitamins found within wheat are B-vitamin, specifically thiamin, riboflavin and niacin, and vitamin E. (College Seminar 253, 2008).

Wheat grains are generally oval shaped, although different wheat have grains that range from almost spherical to long, narrow and flattened shapes. The grain is usually between 5 and 9mm in length, weighs between 35 and 50mg and has a crease down one side where it was originally connected to the wheat flour. The wheat grain contains 2-3% germ, 13-17% bran and 80-85% mealy endosperm (all constituents converted to a dry matter basis) (Šramková, et al., 2009).

Wheat is the dominant crop in temperate countries being used for human food and livestock feed. Since wheat is considered good source of protein, minerals, B-group vitamins and dietary fiber, thus, it has become the principal cereal, being more widely used for the making of bread than any other cereal because of the quality and quantity of its characteristic protein called gluten. Wheat is the most important stable food crop for more than one third of the world population and

contributes more calories and proteins to the world diet than any other cereal crops. It is nutritious, easy to store and transport and can be processed into various types of food. (Kumar et al., 2011)

2.1.2. Wheat production and consumption in Ethiopia

2.1.2.1. Production

Ethiopia is the second largest wheat producing country in Africa behind South Africa. Wheat is mainly grown in the central and south eastern highlands during the main (*Meher*) rainy season (June to September) and harvested in October-November. Arsi, Bale, and parts of Shoa are considered the wheat growing belt. Bread wheat is the major variety of wheat grown in Ethiopia. However, farmers grow durum and bread wheat (mixed together) in some parts of the country. Wheat is produced on large state-owned farms covering around 124,000 ha of land in the Arsi and Bale regions. The remaining ninety two percent (1,390,000 ha) of production is from small farms (Tefera, 2013)

2.1.2.2. Consumption

The wheat consumption trend in Ethiopia is gradually increasing in urban areas due to high population growth (about 2.6 percent a year), migration of people to urban areas, and changes in life styles. Because of the price escalation of teff compared to wheat and of the ease of preparation of wheat, most middle and lower class populations are shifting to greater wheat consumption (Tefera, 2013).

2.2. Bread

Bread is the oldest of all the foods manufactured by man. It also ranks as the most widely eaten, and is often called the staff of life. For thousands of years, people throughout the world have eaten bread in its many different forms. The earliest breads were hard and flat. They were made from a mixture of ground grain and water, and baked in the sun or on hot rocks. Most bread today are leavened, or raised with yeast, baking powder or baking soda. Bread is a consumer product that integrates the table of most homes, either to accompany meals or as the only food.

The bread making in mold is a complex process in which the dough may consist of flour, water, yeast, sugar, salt and fat, and then it is fermented and baked (Bot et al., 2014). Quality of bread is dependent on the method of baking. Application of sourdough is a traditional way to improve the taste, texture, shelf-life, and nutritional value of bread. It is a good source of nutrients, such as macronutrients (carbohydrates, protein, and fat) and micronutrients (minerals and vitamins) that are essential for human health (Malomo, et al., 2012).

2.3. Fermentation

The term “fermentation” comes from latin word *fermentum* (to ferment); the historical definition describes fermentation as the process in which chemical changes an organic substrate occur as a result of action of microbial enzymes. Fermentation can be described as respiration without oxygen. Fermentation is the oldest known form of food biotechnology, which has been practiced for thousands of years by the ancient man as the potent tool for imparting longevity to foods and beverages. It is a process by which useful products are made by the application of microorganisms. The product may be one which is synthesized by the organism(s), the organism themselves or a combination of two. It is well established that the process of fermentation enhances the nutritional quality of any product by enhancing the amount of vitamins and protein solubility and thus fermented food products act as nutraceutical agents to impart beneficial health effects (Sharma, et al., 2013).

Fermented foods are produced world-wide using various manufacturing techniques, raw materials and microorganisms. However, there are only four main fermentation processes: alcoholic, lactic acid, acetic acid and alkali fermentation. Alcohol fermentation results in the production of ethanol, and yeasts are the predominant organisms (e.g. wines and beers).

Lactic acid fermentation (e.g. fermented milks and cereals) is mainly carried out by lactic acid bacteria. *Acetobacter* convert alcohol to acetic acid in aerobic conditions as in the production of vinegar. Alkali fermentation is basically a proteolytic type and often takes place during the fermentation of fish and seeds rich in proteins (Ashenafi, 2006).

2.3.1. Purpose and benefits of fermentation

As new preservation techniques have been developed, the importance of fermentation processes for food preservation has declined (Guizani and Mothershaw, 2007) and nowadays the main purpose of food fermentation is not just to preserve but also to produce a wide variety of fermentation products with specific taste, flavor, aroma and texture.

The primary benefit of fermentation is the conversion of sugars and other carbohydrates to usable end products. According to Steinkraus (1995), the traditional fermentation of foods serves several functions, which includes: enhancement of diet through development of flavour, aroma, and texture in food substrates, preservation and shelf-life extension through lactic acid, alcohol, acetic acid and alkaline fermentation, enhancement of food quality with protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient availability, detoxification of anti-nutrient through food fermentation processes, and a decrease in cooking time and fuel requirement.

2.3.2. Nutritional benefits

Fermentation can produce important nutrients or eliminate anti-nutrients. Food can be preserved by fermentation, since fermentation uses up food energy and creates conditions unsuitable for spoilage microorganisms. For instance, in pickling, the acid produced by the dominant organism inhibits the growth of all other microorganisms. Fermenting makes foods more edible by changing chemical compounds, or predigesting, the foods for us.

Reduction in anti-nutritional and toxic components in plant foods by fermentation was observed in a research which showed " Cereals, legumes, and tubers that are used for the production of fermented foods may contain significant amounts of antinutritional or toxic components such as phytates, tannins, cyanogenic glycosides, oxalates, saponins, lectins, and inhibitors of enzymes such as alpha-amylase, trypsin, and chymotrypsin. These substances reduce the nutritional value of foods by interfering with the mineral bioavailability and digestibility of proteins and carbohydrates. In natural or pure mixed-culture fermentations of plant foods by yeasts, molds,

and bacteria, antinutritional components (e.g. phytate in whole wheat breads) can be reduced by up to 50%; toxic components (Larsson and Sandberg, 1991).

2.4. Sourdough

Sourdough is a mixture of flour and water in which fermentation has been occurred by the LAB. Spontaneous fermentation occurs in the dough, made from flour and water when left for 1-2 days at ambient temperature, due to the naturally occurring microorganisms in the flour. The LAB derived from the cereals or flours depend on the flour preparation and sourdough production technology. The traditional process for sourdough depends on the use of mother dough that are continuously maintained over a long periods of time that may extend to several decades or even longer. The mother dough represents the natural microbial inoculums for the subsequent doughs. Spontaneous fermentation was optimized through back slopping, i.e., inoculation of the raw material with a small quantity of a previously performed successful fermentation, which is dominance of the best adapted strains.

The production of fermented foods and beverages through spontaneous fermentation and back slopping represents a cheap and reliable preservation method in the under-developed countries. During the fermentation there is a favoring of the gram-positive LAB from the flour at the expense of gram-negative bacteria that dominate the microflora of the flour. The sourdoughs are still started by the addition of a piece of ripe-sourdough from the day before to a mixture of flour and water. Many standardized, commercial sourdoughs are also available containing defined strains of LAB and sourdough yeasts with specific properties (Vogel, 1999)

2.4.1 Classification of sourdough

Sourdoughs are classified into three types on the base of procedures during their production.

1. Type I sourdoughs or traditional sourdoughs
2. Type II sourdoughs or accelerated sourdoughs
3. Type III sourdoughs or dried sourdoughs

2.4.1.1. Type I sourdoughs or traditional sourdoughs

Type I sourdoughs are produced by traditional techniques, and are characterized by continuous (daily) refreshments to keep the microorganisms in an active state. Type I sourdough is indicated by high metabolic activity, with regard to leavening, i.e. gas production. The process is conducted at room temperature (20–30 °C) and the final pH is approximately 4.0. Traditional, type I sourdough encompass pure culture, pasty sourdough starter preparations from different origin , spontaneously developed, mixed culture sourdoughs made from wheat and rye or mixture thereof and prepared through multiple stage fermentation processes. Pure culture sourdoughs are derived from natural sourdough fermentations (De Vuyst and neysens, 2005).

2.4.1.2. Type II sourdough or accelerated sourdoughs

Type II sourdough is prepared in semi-fluid silo condition. Those bakery preproducts serve mainly as dough acidifiers. Several modified, accelerated processes with continuous propagation and long-term one-step fermentations are common. They guarantee more production reliability and flexibility. A recent trend of industrial bakeries exists in the installment of continuous sourdough fermentation plants. Type II process last for 2-5 days and are often carried out at increased fermentation temperature (usually > 30 °C) to speed up the process. Those sourdoughs exhibit a high acid content at a pH of <3.5 after 24 hours of fermentation. The microorganisms are commonly in the late stationary phase and therefore exhibit restricted metabolic activity. The high dough yields of these preparations permit pumping of the dough. They are frequently used in local bakeries. Those sourdoughs are stored fresh until use (up to one week), they can be produced in large quantities. In industry, they are applied for the production of dried sourdough products as well (De Vuyst and neysens, 2005).

2.4.1.3. Type III sourdough or dried sourdoughs

Type III sourdoughs are dried dough in powder form, which are initiated by defined starter cultures. They are used as acidifier supplements and aroma carries during bread making. They

mostly contain LAB that are resistant to drying and are able to survive in that form, e.g. heterofermentative *Lb. brevis*, facultative heterofermentative *P. pentosaceus* and *Lb. plantarum* strains. The drying process (spray-drying or drum-drying) also leads to an increased shelf-life of the sourdough and turns it into a stock product until further use. Dried sourdoughs are convenient, simple in use, and result in standardized end products. They can be distinguished in color, aroma and acid content (Aplevicz et al., 2013).

2.5. Most dominant microorganisms in sourdough

LAB and yeasts are engaged in fundamental interactions of sourdough ecosystems. LAB are the predominant organisms with significant numbers of yeast cells (Yann and Pauline, 2014). LAB is responsible for the acidification of the sourdough, whereas the yeasts are very important for the production of a well balanced flavor in combination with the acids. A sponge should contain metabolically active lactic acid bacteria at 10^8 - 10^9 CFU/g responsible for acid production and yeasts at 10^6 - 10^7 CFU/g responsible for leavening action of dough, respectively. However, the LAB may either originate from natural flour contaminant, a fermented dairy product or from a commercial starter culture containing characterized strains of LAB in sourdoughs.

LAB are a group of gram-positive bacteria, which are catalase-negative, non-motile, non-spore forming rods or cocci that produce lactic acid as the major end product during the fermentation. They are strictly fermentative, microaerophile, acidophilic, salt-tolerant and have complex nutritional requirements for carbohydrates, amino acids, peptides, fatty acids, salts, nucleic acids derivatives and vitamins. Lactobacilli may be homofermentative (producing more than 85% lactic acid) or heterofermentative (producing lactic acid, carbon dioxide, ethanol, and/or acetic acid in equimolar amounts). In wheat sourdough, mixed cultures were estimated as: *Lb. Sanfranciscensis* - 20%; *Lb. Alimentarius* - 14%; *Lb. Brevis* - 12%; *Leuconostoc citreum* - 7%; *Lb. Plantarum* - 6%; *Lactococcus lactis* subsp. *lactis* - 4%; *Lb. fermentum*, *Lb. acidophilus* and *Weissella confusa* - each 2%; *Lb. delbrueckii* subsp. *Delbrueckii* (Corsetti et al., 2001).

The mixed cultures of LAB and yeasts were different in composition in sourdough sponges. The use of mixed cultures has a number of advantages, such as improved flavor and texture and retained freshness for longer period as compared to baker's yeast bread. The microflora of the

fluid sourdoughs is dominated by *Lb. pontis*, *Lb. reutri* and *Lb. sanfranciscensis* and the development of sourdough yeast is poor in fluid sourdoughs, therefore, it is necessary to add baker's yeast to the bread dough for optimal leavening. More than 20 species of yeasts are found in sourdoughs. The most abundant yeast is *S. cerevisiae* added through baker's yeast. The yeasts which are associated with LAB in sourdoughs are *S. exiguus*, *Candida humilis* (*C. humilis*) and *Issatchenkia orientalis* (*Candida krusei*). The variation in the species is related to several factors like cereal type, leavening temperature and dough yield. Wild-type LAB dominate the microflora of the traditional fermented foods that may come from the raw material, the process apparatus, or the environment, and that is responsible for the fermentation process when the commercial starter is not available. The activities of non-starter lactic acid bacteria (NSLAB) may add flavor to many traditional fermented products (Hellström, et al., 2010).

2.6. Sensory characteristics and nutritional quality of sourdough bread.

When choosing a food, sensory perception is very important, so that the manufacturer always tries to meet the needs of consumers. Fermented products have particular sensory characteristics (taste, odor, texture) owing to the combined and complementary actions of the great diversity of microorganisms. The sensory profile of sourdough bread partly depends on lactic (produced by homofermentative lactobacilli) and acetic acids (only produced by heterofermentative lactobacilli) concentrations. Acids modify the gluten structure, which can be rather elastic (if lactic acid is favoured) or brittle (acetic acid), and thus the consistency of the bread. Corsetti and Settanni (2007) reported that, a majority of homofermentative LAB led to an increased production of diacetyl and aldehydes in the sourdough.

A majority of heterofermentative bacteria led to the production of ethyl and hexyl acetate. Specific flavors are generated by secondary proteolysis, a bacterial catabolism which leads to the release of free amino acids from macropeptides. Amino acids can be aromatic compounds in themselves *i.e.* sulphur amino acid or at the root of aromatic compounds (keto acids, alcohols, esters, etc.) (Corsetti and Settanni, 2007).

The association of bacteria and yeast helps to increase the aromatic diversity of breads. Sourdough breads have a longer shelf-life than bread made only with yeasts. Indeed, it goes stale less rapidly due to the presence of exopolysaccharides (EPS) produced by LAB. *Lb sanfranciscensis* is well-known for its ability to synthesize high quantities of EPS. EPS enhance water retention and play the role of hydrocolloids. Therefore, the inside of the bread keeps its sensory quality over a longer period of time; in particular, it is more elastic compared with yeast bread (Rehman et al., 2007).

2.7. Health and nutritional benefit of sourdough bread.

2.7.1 Nutritional benefits

The fermented quality of naturally leavened bread has several healthful advantages over yeasted breads. Yeasted breads are raised very quickly by a refined yeast strain that has been isolated in a laboratory under controlled conditions. In the process of making sourdough bread, during the rising time (called proofing), bran in the flour is broken down, releasing nutrients into the dough. In particular, the phytic acid in grain needs to be 90% neutralized in order for the minerals, concentrated in the bran, to be absorbed by the human body. Phytic acid is an organic compound occurring in many cereal grains, oilseeds and legumes. The salt form of PA, phytate, is an anhydrous form of phosphate with an affinity for divalent food minerals such as Fe, Zn and Ca. The resulting phytate-mineral complexes are generally insoluble at physiological pH, which obstructs mineral uptake in the human intestine and leads to mineral deficiencies, such as iron deficiency anaemia. (Kumral, 2015).

Phytic acid is hydrolyzed into inositol and phosphates or phosphoric acid by enzymatic activities (phytases) or non-enzymatic breakdown. Fermentation and microorganisms with phytase activity are effective together with several other methods (germination, cooking, soaking, and autolysis) on decreasing the negative effect of phytic acid on mineral absorption (Corsetti and Settanni, 2007).

2.7.2. Health benefit of sourdough bread

In a study comparing the effects of sourdough bread with yeasted bread, researchers reported that sourdough bread significantly lowered serum glucose and insulin responses and gave greater satisfaction than the other bread. Sourdough baking and other fermentation processes may improve the nutritional features of starch. Sourdough bread rates a 68 on the glycemic index as opposed to the rating of 100 by other breads. Sourdough bread have lower incidence of diseases and unhealthy conditions that run rampant in our culture such as diabetes. Undigested gluten risen bread can seriously weaken the intestinal wall. By damaging the villi in the small intestine led to decrease the absorption of the nutrients. This greatly contributes to the widespread incidence in our society of people with problems of mal-absorption and who are missing out on vital nutrients caused by the effect of gluten produces a protective mucus coating over the intestinal wall and this makes it still more difficult for nutrients to pass through the intestinal wall (De Vuyst;neysens,2005). *Lactobacillus bulgaricus* is effective to inhibit spoilage microbial and increasing the shelf life stability of bread. (Golshan Tafti, et al., 2014).

2.8. Shelf life of traditional sourdough bread

The preservative activity of LAB has been observed in some fermented products such as cereals. The lowering the pH to below 4 through acid production, inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease. For example, LAB bacteria have antifungal activities. By doing this, the shelf life of fermented food is prolonged. This is because the sheer overgrowth of desirable edible bacteria in food outcompetes the other non-desirable food spoilage bacteria. Thus LAB fermented foods have lactic acid as the main preservative since lactic acid bacterial growth is accompanied by the production of lactic and acetic acids with decrease in pH and increase in titratable acidity. The process of fermentation usually takes 4-5 days. The bacterial population stabilizes at around 48 hours (Chelule et al., 2010).

The use of sourdough in the preparation of bread has a positive effect on bread volume, crumb structure, flavor, and mold-free shelf life (Corsetti et al., 1998). Sourdough bread is the oldest and the most original form of leavened bread. It is reported that sourdough has been used in

bread making since 3000 BC. In today's bread making technology, the aim of sourdough usage is to improve texture and aroma characteristics and to lengthen the shelf life of breads. Katina (2005) reported that the rate of starch retro gradation is lower for sourdough breads.

It is well known that bread firmness increases or bread softness decreases during storage. It was reported that the acidification by sourdough lactic acid bacteria and their activities, including proteolysis of gluten and hydrolysis of starch, had a positive effect in delaying both bread firmness and staling (Corsetti et al., 1998). Acidification was related to the improved softness, as the pH of sourdough correlated positively with firmness values (Katina et al., 2006). Singh et al. (2002) stated that acetic and lactic acids are used in bread formulation to improve the shelf life of bread. Historically, fermentation products were food products, but in recent years an increased interest has been observed towards the production of food additives (e.g. flavor modifiers). Yet, fermentation is a relatively efficient, low energy, inexpensive preservation process, which increases the shelf life and decreases the need for refrigeration or other forms of food preservation technology. Therefore, it remains a very appropriate method for use in developing countries and rural communities with limited facilities. In addition, the non-dependence of fermentation on the use of chemical additives to the food appeals to the "more aware" consumer market (Battcock, 1998). Fermented foods are popular throughout the world and in some regions make a significant contribution to the diet of millions of individuals. (Battcock, 1998)

3. Materials and Methods

3.1. Sample collection and flour preparation

Kekeba variety wheat was obtained from Melkassa Agricultural Research Center Eastern Oromia, Ethiopia. The grain was made into flour using a flour miller and sieved through 0.5 mm sieve and stored in air tight plastic container for further analysis.

3.1.1. Study site

Practical activities were done in the laboratories of Food Science and Nutrition Center (FDSN), Entoto Poly Technique College, baking technique room and the Ethiopian Public Health Institute (EPHI). Entoto Poly Technique College was preferred for baking activity since they have baking technique practical room and all necessary facilities.

3.2. Methods

3.2.1. Processing Methods

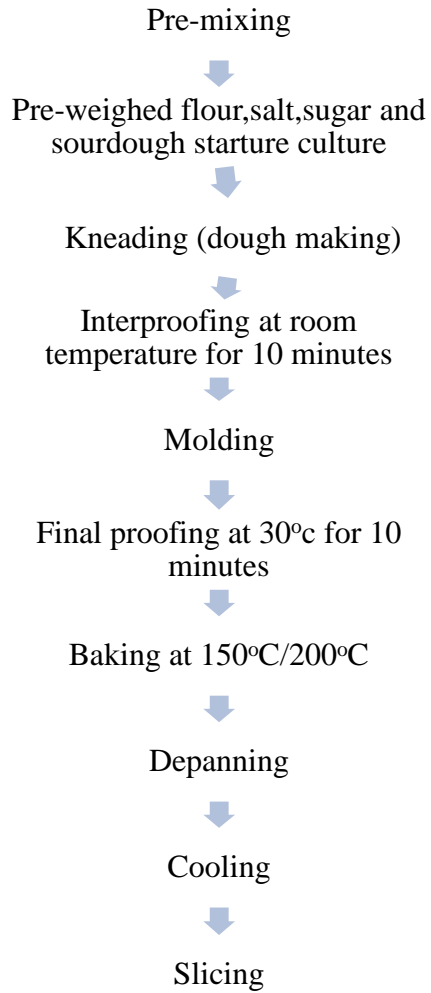
3.2.2. Homemade starter culture preparation

Sourdough starter culture was prepared by mixing 150g of wheat flour with proportional luke warm tap water and left for 72 hrs until it become ready to be used as a starter.

3.2.3. Bread making

Two types of breads were baked; one with traditionally prepared sourdough starter culture and the other with commercial baker's yeast as control. For traditional sourdough bread ,1.5kg wheat flour, 10g salt, 150g traditional sourdough starter culture which was spontaneously fermented and 200 ml oil were added and mixed well, while for the bread using commercial yeast all recipes were the same except leavening agent which were commercial baker's yeast .In the mixing step the formula(recipe)were incorporated into a homogenous mixture. This was done in

two stage processes. First: flour, water, salt, oil and leavening were mixed together and water was added gradually. After 60 % (900 ml) of water on flour base was added, the second stage of gluten formation (kneading) begins. The resulting dough was allowed to rest for 10 minutes in a cabinet at room temperature (inter-proofing) and afterwards, the dough was molded and put into baking tins and proofed at 30°C for another 10 minutes the loaves were baked at 150°C /200°C in an electric oven for 30 minutes and then cooled at room temperature and ready for further sensory and proximate analysis. Both breads were baked at similar conditions. However, the fermentation period for both types of breads was different. The traditional sourdough was ready for baking after 48 hrs of fermentation time but the yeasted dough was fermented only for 1 hr before baking.



weighed using a digital analytical balance to the nearest milligram (W_1). About 5 g of fresh sample was weighed (W_2) in dried and pre-weighed drying dishes. The dishes and their contents were then placed in drying oven and dried for 3 hours at 105°C. The dishes and their contents were cooled in desiccators to room temperature and weighed (W_3). The procedure was repeated until a constant weight was attained (AOAC, 2000).

$$\text{Moisture content (\%)} = \frac{w_2 - w_3}{w_2 - w_1} \times 100\%$$

Where, W_1 = weight of the crucible

W_2 = Weight of crucible and fresh sample

W_3 = Weight of the crucible and dry sample

3.3.2. Determination of total ash content of flour

Ash was quantified by (AOAC 923.03) as the inorganic residue present after incineration at 550 C for 5 hours until loss of organic matter. Porcelain crucible were cleaned and dried in a muffle furnace for 30 min at 105⁰ C. Crucibles were cooled in a desiccator (with granular silica gel) for about 30 minutes or more at room temperature and weighed as (W_1). About 2.5 g of fresh sample was weighed to an accuracy of 2 decimal places in the dish and weighed as (W_2). The sample was burned on a hot plate under a fume hood and slowly increased the temperature until smoking becomes over. Then, ashed in the muffle furnace at 550⁰C for 5 hrs. The ash looked clean and has white appearance. Finally, the crucibles were cooled to room temperature and reweighed (W_3) each crucible with ash.

$$\text{Total ash (\%)} = \frac{w_3 - w_1}{w_2 - w_1} \times 100\%$$

Where, W_1 = weight in grams of the dried dish

W_2 = weight in grams of the dish and the sample

W_3 = weight in grams of the dish and ash

3.3.3. Determination of crude fat of flour

Crude fat was determined by AOAC 45.01, following appropriate procedure. Extraction cylinders were washed with hot water and impurities were removed and then dried in drying

oven at 105⁰c for 1 hr and cooled in a desiccator. The masses of the cooled extraction cylinders were measured by analytical balance and recorded as W₁. The bottom of the extraction thimbles were covered with the layer of fat free cotton. About 2 g of the powdered sample was weighed and added into each thimble lined with cotton at their bottom and covered with layer of fat free cotton and thimbles were putted into extraction chamber. The thimbles with their samples content were placed into the Soxhlet extraction apparatus. Then 50 ml of diethyl ether was added in to the extraction cylinder and moved in to the heating plank and the extraction last for four hours. Then, the extraction cylinder was disconnected and putted in the drying oven at 70⁰c for 30 minute. Finally, the extraction cylinders were cooled in the desiccator and weighed W₂.

$$\text{Crude fat\% by weight} = \frac{w_2 - w_1}{w} \times 100\%$$

Where;

W₁=weight of dried extraction flask

W₂= weight of extraction flask and dried crude fat

W= weight of the sample

3.3.4. Determination of crude protein of flour

Crude protein of the flour was determined by the Kjeldahl method (AOAC979.09) in a Kjeltac system with acid (sulfuric acid) digestion of the sample and then an alkaline (sodium hydroxide) distillation using a nitrogen-to-protein conversion factor of 6.25.

Wheat flour sample; 0.5 g was measured in a tecator tube and placed in the tecator rack. A 6 ml of concentrated sulphuric acid was added from glass pipette and immediately the sample and the acid was carefully shaken. A 3 g of the catalyst mixture was added and step by step 3.5 ml of hydrogen peroxide was added. Violent reaction was observed and the tube was shaken a few times manually and placed back to the rack.

3.3.4.1. Digestion

The temperature of the digester was adjusted to 370 °C and lowered the tubes in the rack into the digester. The digestion was continued until a clear solution was obtained which takes about 3-4 hrs. Then, the tube was transferred in the rack into the fume hood for cooling.

3.3.4.2. Distillation

Distillation was done by adding 25 ml of 40% sodium hydroxide solution into the digested and diluted solution. A 250 ml conical flask containing 25 ml of boric acid, 25 ml of distilled water and indicator solution was placed under the condenser of the distiller with its tip immersed into the solution. The distillation was continued until a total volume become between 200 and 250 ml. The tip was rinsed with a few ml of distilled water before the receiver was removed.

3.3.4.3. Titration:

Titrate was done with 0.1 N hydrochloric acids to a reddish color

Calculation:

$$\text{Nitrogen (\%)} = \frac{V_{\text{HCl in L}} \times N_{\text{HCl (N 0.1)}} \times 14.00 \times 100}{w_0}$$

Where, V- volume of HCl in L consumed to the end point of titration

N- The normality of HCL (0.1 N)

W₀- weight of sample

14.00- the molar weight of nitrogen

Conversion:

$$\text{Protein (\%)} = 6.25 \times \% \text{ nitrogen}$$

3.3.5. Determination of crude fiber of flours

Crude fiber was determined by the method of AOAC 962.09, as the combustible and insoluble organic residue was obtained after the samples were subjected to acid digestion and then alkaline distillation. Clean crucible was dried with 1g celite in the oven at 105°C for 1 hour and placed in the desiccator to cool. A 1gram of wheat flour sample was measured in the dried crucible using analytical balance. A 200 ml of 1.25% (R1) H₂SO₄ solution was added to each beaker and allowed to boil for 37 minutes. The temperature was seted between 6-8. After 37 minutes, the acid was drained using vacuum pump and the sample was cooled for 5 minutes and then, the sample was washed three times using distilled water. For the second step, 1.25%NaOH solution (R₂) was added to each column and the same step was followed as previous. Crucibles containing residue was dried at 130 °C for 2 hrs by drying oven and cooled in the desiccator and crucibles were weighted (W₂).Crucibles were transferred to muffle furnace for 3 hrs at 525°C and cooled down to 250 °C before removing them from the furnace. Finally, crucibles were cooled in the desiccator and weighed (W3).

Calculation was:

$$\text{Crude fiber (g/100g)} = \frac{w_2 - w_3}{w_1} \times 100\%$$

Where:

W₂= mass of the crucible,

W₃= mass of the crucible and the sand

W₁ = Weight of sample

Carbohydrate content was determined by difference which will be calculated as:

$$\% \text{ Carbohydrates} = 100 - (\% \text{ moist} + \% \text{ ashes} + \% \text{ Proteins} + \% \text{ fats} + \% \text{ total dietary fiber})$$

3.4 Mineral analysis

3.4.1. Calcium, iron and zinc determination

Calcium, iron and zinc content of kekeba variety wheat flour, traditional sourdough bread and commercial yeast bread were determined according to the standard method of AOAC (2005) using atomic absorption spectrophotometer (AAS). Ashing of the samples was followed by digestion and absorption. Crucibles and glass wares were washed with 10% nitric acid and placed in the oven at 105^oC for 1 hour. Crucibles were cooled in the desiccator for 30 minutes and accurately 2.5 g samples were taken and charred on the hot plate under the hood until the smoke was completed. Then, the samples were ashed in the muffle furnace at 550 ^oC for 5 hours and taken out from the furnace and cooled in the desiccator and the total ash weight was measured. Some drops of deionized water was added to moisten it and evaporated on the hot plate and some drops of concentrated nitric acid were added and evaporated again on the hot plate and ashed once more for 30 minutes to ensure its complete ashing.

Dissolution of the ash was started by treating the ash with 7 ml of 6N HCl to wet it completely and carefully taken to the dryness on a lower temperature hot plate. Then, 15 ml of 3N HCl was added on each dish and heated on the hot plate until the solution just boils. Then the solution was cooled and filtered through the filter paper into a 50 ml graduated flask. Again, 10 ml of 3N HCl was added to the crucible and heated until the solution just boiled. Then the solution was cooled and filtered into the graduated flask. Crucibles were washed with de-ionized water three times and the washing was filtered into the flask. The filter paper was washed thoroughly with de-ionized water and the washing was collected in to the flask. A 2.5 ml of lanthanum chloride solution was added per 50 ml of solution. Finally, the contents of the flask was diluted and marked to 50 ml with de-ionized water. The sample solutions were transferred to the urine cap bottle. The blank was prepared by taking the same amount of the reagents following the same instruction used for the sample.

Calculation:

$$\text{mg/100g of metal content} = \frac{[C_s - C_b * V]}{10 * W}$$

Where,

C_s : concentration of sample in ppm

C_b : Concentration of blank in ppm

V: Volume (ml) of the extract

W: weight (g) of sample

3.4.1. Standard reagent preparation

10ppm Zn: 1ml of zinc was added from 1000ppm in 100ml of volumetric flask with de-ionized water. For a series of standard of Zn prepared: 0.5, 1.0, 1.5, 2.0 and 2.5 ppm was taken respectively in 10 ml flask containing 2.5 3N HCl and marked up with de-ionized water.

20ppm Fe: 2ml of Fe was added from 1000 ppm in 100ml of de-ionized water. For a series of standard of Fe prepared 0.5, 1.0, 1.5 and 2 ppm was taken respectively in 10 ml flask containing 2.5 3N HCl and marked up with de-ionized water.

10ppm Ca: 1ml of Ca was added from 1000 ppm in 100ml of de-ionized water. For a series of standard of Ca prepared: 2, 4, 6, 8 and 10 3ppm was taken respectively in 10 ml flask containing 2.5 3N HCl, 0.5ml 10% LaCl_3 and marked up with de-ionized water.

3.4.2. Phytate mineral molar ratio calculation

The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals. (Norhaizan, & Norfaizadatul, 2009)

3.5. Determination of antinutritional content of the wheat flour and the baked bread

3.5.1. Phytate content analysis of the wheat flour and the baked bread

Phytate content was determined using the method described by Vaintraub and Lapteva (1988). About 0.1gram of each samples were weighed separately in centrifuging test tube and the samples were extracted with 10 ml of 0.2 N HCl for 1hr at room temperature and centrifuged at

3000 rpm for 30 minutes. Clear supernatant was used for the phytate determination. A 2 ml of wade reagent was added to 3 ml of the supernatant sample solution. Then homogenized and centrifuged at 3000 rpm for 10 seconds. The absorbance at 500 nm was measured using UV-Vis spectrophotometer. The phytate concentration was calculated from the difference between the absorbance of the blank (3 ml of 0.2 N HCl + 2 ml of wade reagent) and that of assayed sample. The amount of phytic acid was calculated using phytate acid standard curve and the result was expressed as phytate in mg/100 g fresh weight.

$$\text{Phytic acid in mg/100 g} = \frac{[Ab - As - \text{intercept}] \times 10}{\text{slope} \times w \times 3}$$

Where A_s = sample absorbance

A_b = blank absorbance

W = weigh of sample

3.5.2. Standard solution preparation

A series of standard solution was prepared containing 40 mg/100 g phytic acid in 0.2N HCl. A 3 ml of each standard was pipetted into 15 ml centrifuge tubes and 3ml of 0.2 N HCl to the blank. A 2ml of wade reagent was added to each tube and the solution was mixed on vortex mixer for seconds. The supernatant and the standard absorbance were read at 500 nm by using distilled water to make the spectrometer zero. Using x-cell the calibration curve was plotted and the slope and intercept was found.

3.6. pH and titratable acidity determination

3.6.1. Determination of pH

Starter culture, sourdough and bread samples; a 10 g of each placed in a beaker containing 90 ml of distilled water, mixed homogeneously, and then left for 30 min at room temperature. The resultant supernatant was measured with a pH meter (Hanna Instrument-pH 301) after calibrating the instrument using pH 4.0 and 6.86 buffer. (Katina, K. 2005).

3.6.2. Determination of titratable acidity

Starter, sourdough and bread; 10 g of each samples were mixed with 30 ml distilled water as one part of the flour to three parts of the water (w/v) ratio in beaker and left for 30 minutes. A 10 ml of distilled water was used for further dilution in order to hydrolyze all the acids in the samples. Before the titration of the sample the water that had been used for dilution purpose was titrated and used as blank. Three drops of 1% alcoholic phenolphthalein indicator was added into water extract of the sample (dispersion). Then the dispersion was titrated with standard base (0.1N NaOH) to phenolphthalein end point. The result of determination was reported as percentage acid consuming definite volume of 0.1N NaOH. The end point of the titration was reached after the white dispersion changed from a clear white solution to a faint violet colored turbid solution. Duplicate determinations were made in all cases (Katina, K. 2005).

The amount of the acid in the samples was determined by the relation: -

$$\% \text{acid (wt/wt)} = \frac{N \times V \times \text{Eq.wt} \times 100}{W \times 1000}$$

Where: N=normality of titrat, usually NaOH (mEq/ml)

V=volume of titrant (ml)

Eq.wt. =Equevalent weight of predominant acid (mg/mEq)

W=mass of samples (g)

1000=factor relating mg to grams (mg/g)

3.7. Microbial analysis

3.7.1. Enumeration of LAB

One g of each samples of traditional sourdough and commercial yeast dough were mixed separately with 9 ml sterile distilled water in a sterile flask. One ml of the mixture was taken and serially diluted in test tubes each containing 9 ml sterile distilled water and aliquots of 0.1 ml from appropriate dilutions (10^{-1} - 10^{-5}) were spread-plated in duplicate on pre-dried agar plates of MRS, Colonies of LAB were counted on MRS agar plates after anaerobic incubation in Gas Pak jars (GasPak System,) incubated at 32°C for 48 hrs. (Randazzo, et al., 2005).

3.7.2. Dominant yeast enumeration

3.7.2.1 Media preparation

Yeast extract peptone dextrose (YPD) agar was prepared by combining (yeast extract, 10g; peptone, 20 g; glucose, 20 g; agar 20 g; tap water 1,000 ml) containing 0.01g chloramphenicol/ml to inhibit the growth of bacteria. These ingredients were mixed in conical flask, boiled and autoclaved for 15 minutes. Thenafter the media was poured into petridish.

3.7.2.3 Making dilution

One g of each samples traditional sourdough and yeasted dough were mixed separately with 9ml sterile distilled water in a sterile flask. One ml of the mixture was taken and serially diluted in test tubes each containing 9ml sterile distilled water. This is followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1} - 10^{-5}) on YPD agar plates which were prepared in the presence chloramphenicol of. All the plates were incubated at 28°C for 5 days. The colonies were counted by colony counter and recorded accordingly (Pons, et al, 1986).

3.8. Shelf life of traditional sourdough and commercial yeast bread.

Both breads were stored at room temperature in the same condition and visual observation was done at 12 hrs interval for physical change and mould growth and the change was recorded.

3.9. Sensory evaluation

Ten panelists (2 females and 8 male, ranging in age between 24 and 40), who were M.Sc student at Addis Ababa University, Center for Food Science and Nutrition were selected. Hedonic evaluation of the sensory attributes of traditional sourdough and yeast bread samples were done using a nine-point hedonic scale valued as (1 = extremely dislike, 9 = extremely like). In the test, participants were provided with the two different breads coded with three random digit numbers and were asked about their liking or disliking concerning experimental traditional sourdough and yeasted bread. The samples were randomly presented to the panelists.

The panelists were given their informed consent to participate in the study and the scorecard sheets were prepared based on five attributes. Descriptive terms (color/appearance, taste /flavor, texture/consistency, aroma/smell and overall acceptability) were provided to the panelists and they were asked to rank all products on nine hedonic scale. Mean values of the scores from all panelists for each of the attributes was computed and analyzed (Agiriga Anna Ngozi 2014).

3.10. Statistical analysis

The raw data were analyzed using SPSS version 20 and. The mean comparison was done by least significant difference (LSD). One way ANOVA was used to find the mean difference between the groups. A $p < 0.05$ was considered as significant.

4. Results and discussions

4.1. Proximate composition of the Kekeba variety wheat flour

Proximate composition of the sample; Kekeba wheat variety was determined before using for experimental activities and the results were presented in Table 1.

Table 1: Proximate composition of Kekeba variety wheat flour

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Total Carbohydrate (%)
Wheat flour	2.00±0.00	10.15±0.25	1.25± 0.35	1.35±0.22	3.91±0.00	81.34±0.00

Values are expressed as mean ±standard deviation

The moisture content shows very less result followed by protein but high ash, fat and fiber compared to Rehman et al.,(2007). This high ash content indicates that the wheat flour contains high mineral.

4.2. Mineral and phytate content analysis of Kekeba variety wheat flour, sourdough bread and commercial yeast bread

Table 2 shows the Fe, Ca, Zn and phytate content of whole-wheat flour, sourdough bread and yeasted bread. The phytate content of the flour (20 mg/100g) was the highest followed by yeasted bread; bread baked with traditional sourdough had the lowest level (6 mg/100g). This is due to long time fermentation and phytase enzyme in cereals and some LAB. The values for Iron, calcium and zinc content in whole wheat flour, sourdough bread and commercial yeast bread did not show significant ($p>0.05$) difference. Lopez *et al.* (2001) found that the phytate content was more efficiently reduced in wheat sourdough bread (62%) as compared to yeast fermented bread (38%) and the results of this study shows better improvement in phytate reduction since phytate content in natural sourdough and commercial yeast dough bread were reduced by 70% and 30% respectively. The values for Iron, Calcium and Zinc content in wheat flour, sourdough and commercial yeast bread did not show significant ($p>0.05$) difference.

Table 2: Mineral and phytate composition (mg/100g) of whole wheat flour, sourdough bread and commercial yeast bread

Samples	Phytate (mg/100g)	Iron	Calcium	Zinc
WWF	20.1±0.46 ^a	2.78±0.00 ^a	48±0.00 ^a	1.95±0.00 ^a
SDB	6±1.13 ^c	2.77±0.37 ^a	48.49±2.04 ^a	1.987±0.08 ^a
YB	14.6±0.45 ^b	2.75 ±0.16 ^a	46.89±3.65 ^a	1.90±0.03 ^a

Values are reported as mean ± standard deviation SDB= sourdough bread, YB=yeast bread, WWF= whole wheat flour; Means with different superscripts in the same column are significantly different (p<0.05)

The calculated [phytate]:[Fe] ,[Ca]:[phytate], [phytate]:[Zn]and [Ca]:[phytate]/[Zn] molar ratios are presented in Table 3. The [Phytate]:[Fe] ratio was highest in whole wheat flour (0.6) followed by the commercial yeast bread. The sourdough bread had the lowest level (0.185) of.

Table 3: Calculated phytate/Fe, Ca/phytate and (Ca): (phytate)/(Zn) molar ratios of whole-wheat flour, sourdough bread and commercial yeast bread(mg/100g)

Samples	[Phytate]:[Fe]	[Phytate]:[Ca]	[Phytate]: [Zn]	[Phytate]:[Ca]/[Zn]
WWF	0.60±0.00 ^a	0.025±0.00 ^a	1.02±0.00 ^a	0.011±0.00 ^a
SDB	0.185±0.003 ^c	0.0075±0.00 ^c	0.300±0.01 ^c	0.00 ±0.00 ^b
YB	0.45±0.002 ^b	0.019±0.00 ^b	0.754±0.01 ^b	0.00 ±0.00 ^b

Values are reported as mean ± standard deviation SDB= sourdough bread, YB=yeast bread, WWF= whole wheat flour; Means with different superscripts in the same column are significantly different (p<0.05)

The mean values of phytate: Iron, molar ratio in present the study were 0.60, 0.45 and 0.185, for whole wheat flour, yeast and sourdough bread, respectively Table 3. For all samples phytate:Fe, phytate:Zn and phytate: Ca molar ratios were calculated and their results were found to be lower than their reported critical values This indicates that absorption of iron, zinc and calcium were not inhibited by phytate and as a result these minerals in all samples are bioavailable. The [Phytate]: [Ca]/ [Zn] molar ratios for all samples also show the result below the 0.5 mol/kg

critical value, thus predicting the bioavailability of Zn. Diets with a phytate-zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate-zinc molar ratio between 5 and 15 have medium zinc bioavailability and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability (Walingo, 2009).

4.3. Change in pH during fermentation and after baking

4.3.1. pH and TA of starter culture before used for dough leavening.

At the beginning of fermentation time; 0 hr, the pH of sourdough starter culture shows the value 6.475 and after 72 hrs fermentation the pH had decreased to 5.253 (Table 4). Following a decrease in pH, TA value increased significantly ($p < 0.05$) from 0.199 at 0 hr to 0.355 after three days fermentation and this shows that there was production of organic acids by naturally occurring LAB and yeasts in spontaneously fermented starter culture.

Table 4: Changes in pH and titratable acidity of Kekeba variety wheat sourdough starter culture during fermentation

Fermentation time	starter culture	pH change	Titratable acidity
0 hrs		6.475 \pm 0.007 ^a	0.199 \pm 0.015 ^b
72 hrs		5.253 \pm 0.007 ^b	0.355 \pm 0.007 ^a

Reported values are mean \pm standard deviation. Means with different superscripts in the same column are significantly different ($p < 0.05$)

Decrease in pH of starter culture is less compared to sourdough fermented for 24 hrs which might be due to the absence of sugar in preparation of starter since yeast need sugar as a source of glucose to produce acetic acid as by product and scarcity of nutrients due to long fermentation time.

4.3.2. Change in pH during fermentation of traditional sourdough and commercial yeast dough.

The changes in pH during fermentation of wheat dough treated with traditional sourdough and baker’s yeast were presented in Table 5. At initial point of fermentation, pH value of traditionally fermented dough and baker’s yeast fermented dough were 6.2 and 5.8, respectively. During the fermentation period (from 0 hr to 48 hrs) of traditional sourdough and (from 0 hr to 1 hr) yeast dough acidification increased and resulted in a pH drop from an initial value of 6.2 to a final value of 4.00 and from 5.8 to 5.8, respectively.

Table 5: Changes in pH during fermentation of Kekeba variety wheat dough treated with traditional starter culture and baker’s yeast.

Fermentation time	TSD	Fermentation time	YD
0 hrs	6.2±0.014 ^a	0 hr	5.865 ±0.007 ^a
24 hrs	4.33±0.021 ^b	30 min	5.860±0.00 ^a
48 hrs	4.000±0.144 ^c	1 hr	5.830±0.00 ^a

Reported values are the mean±standard deviation (n=2).Means with different superscripts in the same column are significantly different (p<0.05 TSD: - traditional sourdough YD: - yeast dough

The drop in pH was high in the traditionally treated sourdough than in yeast dough as indicated in Table 5.The lowest value of pH for traditionally fermented sourdough is 4.00 while 5.830 is for yeasted dough. Long fermentation decreased the pH of the traditional sourdough significantly (p<0.05) with time but not in yeast dough.

The results agreed with the fact that effect of fermentation on pH was significantly influenced by the presence of LAB in traditional sourdough since they can produce many organic acids such as lactic, acetic and propionic acids as end products of fermentation and provide an acidic environment unfavorable for the growth of many pathogenic and spoilage microorganisms (Ross, et al., 2002).The lowest pH /4.00/ recorded in traditional sourdough after 48hrs fermentation was therefore due to the production of the organic acids by lactic acid bacteria.

4.3.3. Change in pH and TA of sourdough bread and commercial yeast bread.

The pH of fermented products was 5.2 and 5.48, respectively; Table 6. Sourdough bread scored less pH compared to yeasted bread and this value is probably due to long fermentation time and activities of LAB in cooperation with yeasts in traditional sourdough bread. The TA for both breads showed significant ($p < 0.05$) difference, but less increase was observed as pH decrease.

Table 6: The pH and titratable acidity of sourdough bread and commercial yeast bread.

Sample types	pH value	Titratable acidity
SDB	5.2 ± 0.014^b	0.775 ± 0.127^a
YB	5.48 ± 0.00^a	0.666 ± 0.0124^b

Reported values are the mean \pm standard deviation ($n=2$). Means with different superscripts in the same column are significantly different ($p < 0.05$), TSDB=traditional sourdough bread, YB=yeast bread.

4.4. Change in titratable acidity of sourdough and commercial yeast dough

The changes in titratable acidity (TA) during fermentation of traditional sourdough and yeasted dough were presented in Table 6. At the beginning of fermentation (0 hr) TA values ranged from /0.274%/ to /0.360% /for traditional sourdough (TSD) and yeasted dough (YD), respectively. At the end of fermentation time (48hrs), the TA for traditional sourdough (TSD) increased significantly ($p < 0.05$) from 0.274% to 0.783%. But there was a drop in TA from 0.360% to 0.324% during fermentation (from 0 hr to 1 hr) for yeasted dough that might be because of the absence of LAB metabolic activities to produce lactic acid as a result of short fermentation time.

Table 7: The changes in titratable acidity during fermentation of wheat dough treated with traditional starter culture and baker’s yeast.

Titratable acidity (% of lactic acid)			
Fermentation time	TSD	Fermentation time	YD
0 hrs	0.274±0.006 ^c	0 hr	0.360 ±0.013 ^b
24 hrs	0.590±0.019 ^b	30 min	0.337±0.006 ^{ab}
48 hrs	0.738±0.006 ^a	1 hr	0.324±0.00 ^a

Reported values are the mean±standard deviation (n=2). Means with different superscripts in the same column are significantly different (p<0.05). Sample codes are as stated in Table 4.

From the results of the pH and TA of traditional sourdough, it could be observed that as pH decrease the TA of the dough increased. This can hinder or inhibit the growth of food borne pathogens (Ross, et al., 2002).

4.6. Microbial analysis

4.6.1. Lactic acid bacterial counts

The data on the lactic acid bacterial colony counts in traditional sourdough and yeasted dough at different fermentation times are given in Table 9. Lactic acid bacteria counts on MRS agar shows an exponential phase significantly (p<0.05) from 5.990 Log cfu/ml to 6.454 Log cfu/ml at 0 hr and 24hrs fermentation time but decreased to 6.005 Log cfu/ml at 48 hrs fermentation time. This result is in agreement with the finding of Emils et al., (2008). These authors reported that at the first stage of fermentation time which is from 0 hr to 24 hrs, LAB showed high counts although at the end of first stage sourdough fermentation LAB count started to decrease caused by limitations of nutrients.

Table 8: Lactic acid bacterial count during fermentation of traditional sourdough and commercial yeast dough.

LAB count (Log cfu/ml)			
Fermentation time	TSD	Fermentation time	YD
0 hr	5.990±0.224 ^b	0 hr	ND
24 hrs	6.454± 0.021 ^a	30 min	ND
48 hrs	6.005 ±0.057 ^{ab}	1 hr	4.770±0.000 ^a

Reported values are the mean±standard deviation (n=2). Means with different superscripts in the same column are significantly different (p<0.05). Sample codes are as stated in Table 4.

ND=Not detected

The colony counts of LAB in baker's yeast dough shows no growth at both 0 hrs and 30 min fermentation time but 4.770 Log cfu/ml were recorded after 1hr fermentation time and this result show that LAB needs long fermentation time to be in exponential phase(growth phase) (Emils et al., 2008).

4.6.2. Yeast counts

The results of yeast colony count for traditional sourdough and yeasted bread were as indicated in Table 10. Yeast growth in traditional sourdough at 0 hr fermentation time was 3.776 Log cfu/ml but not detectable for 24 hrs and 48 hrs sourdough fermentation time that is due to dominance of LAB which are naturally present in traditional sourdough starter which was used to start fermentation instead of baker's yeast.

Table 9: Yeast count during fermentation of traditional sourdough and yeast dough.

Yeast count (Log cfu/ml)			
Fermentation time	TSD	Fermentation time	YD
0hr	3.776±0.509 ^a	0 hr	6.332±0.011 ^a
24 hrs	ND	30 min	6.389 ±0.013 ^a
48 hrs	ND	1 hr	6.283±0.123 ^a

Reported values are the mean±standard deviation. Sample codes are as stated in Table 4. ND=Not detected

Similarly as that of LAB in traditional sourdough which shows different growth patterns, yeasts colony count also show an increase in number from 6.332 Log cfu/ml to 6.389 Log cfu/ml at 0 hr and 30 min fermentation time but decreased to 6.283 Log cfu/ml at 1hr fermentation time that is due to limited nutrients as stated by Emils et al., 2008.

4.7. Shelf life determination

After four days storage at room temperature in the same environmental conditions, mould growth appeared highly on the yeast bread, while the traditional sourdough bread showed a little growth of moulds. The softness of the bread with traditional sourdough is also better than yeast bread. This observation is due to use of sourdough in the preparation of bread as it has a positive effect on mold-free shelf life (Corsetti et al., 1998).



4.8.2. Aroma

The mean score of aroma for traditional sourdough and yeasted bread were 7.60 and 6.7, respectively. Even though both products show score above acceptable range, traditional bread had significantly ($p < 0.05$) higher mean score in aroma 7.60 while the yeasted bread shows less mean score 6.70. These results are in agreement with results of Salim-ur-Rehman *et al.*, (2000). These authors stated that the sourdough fermentation is central to acceptability in flavor, as chemically acidified breads prepared with pure commercial starter cultures are not well scored in sensory preference assessments. Chelule *et al.*, (2010) also reported that sourdough fermentation makes the food palatable by enhancing its aroma and flavor.

4.8.3. Color

The mean score for the color of traditional sourdough and yeasted bread were 6.8 and 6.5 respectively. These results show that the color of both product were liked by panelists and no significant ($p > 0.05$) difference were observed among the two types of breads.

Table 10: Sensory characteristics of traditional sourdough bread and baker's yeast bread.

	Taste	Color	Aroma	Texture	Acceptability
Code					
TSB	7.1±1.449 ^a	6.8±0.918 ^a	7.6±1.075 ^a	6.8±1.398 ^a	7.1±0.1.197 ^a
BYB	6.4±1.173 ^a	6.5±1.080 ^a	6.7±1.059 ^b	6.5±1.354 ^a	6.5±1.170 ^a

Reported values are the mean ± standard deviation (n=2). Means with different superscripts in the same column are significantly different ($p < 0.05$). TSB:-Traditional sourdough bread
BYB:-Baker's yeast bread.

4.8.3. Texture

Texture is another important sensory attributes of food. The outcome of sensory evaluation of texture showed the same mean scores as scored in color for both traditional and yeast bread with no significant($p>0.05$) difference between the two products but the traditional one show larger mean score as compared to yeast bread. This result is in agreement with Yann & Pauline (2014) who stated that exopolysaccharides produced by LAB enhances water retention and plays the role of hydrocolloids. Therefore, the inside of the bread keeps its sensory quality over a longer period of time; in particular, it is more elastic compared with yeast bread.

4.8.4. over all acceptability

Generally, the average over all acceptability of traditional sourdough bread and yeast bread mean score were lasted with the acceptance of both products by the panelists in spite of their difference in degree of acceptance in which traditionally baked bread showed higher liking by panelist with mean score of 7.1 for traditional bread and 6.5 for yeast bread.

5. Conclusion and recommendation

5.1. Conclusion

The phytate mineral molar ratio results show the bioavailability of Ca, Zn and Fe which was as a result of phytate degradation during fermentation. This study suggests that baking bread with spontaneously fermented starter culture and long time fermentation further reduced phytate compared to baker's yeast. Hence using sourdough improves Zn and Ca bioavailability which is nutritionally beneficial.

Traditional sourdough increased the nutritional quality of the product by significantly decreasing phytic acid, enhances mineral bioavailability and improved nutritional status of the products. The use of sourdough is useful for making bread products with an increased level of flavor compounds, ultimately increasing consumer satisfaction. Overall sensory acceptance of traditional sourdough bread for this study showed higher mean score than control bread and was highly accepted by panelists compared to control bread. Thus sourdough could be useful in serving mankind with wholesome, tasty, and convenient foods

5.2. Recommendation.

- Since traditional way of preparing food products using natural starter culture and long time fermentation had effect on acceptance of sensory characteristics, improving nutritional quality, decreasing antinutritional factors (phytate) , more emphasis should be given to advocate / advertise the importance of baking bread using traditional sourdough at home to mothers of Ethiopia and to everyone concerned.

- Improving processing techniques of sourdough and scaling up the sourdough use to industrial level is necessary.

- Additional investigation should be encouraged on
 - ✓ Appropriate processing methods of traditional sourdough bread,
 - ✓ Development of sourdough starter culture with Specific strains lactic acid bacteria.
 - ✓ Appropriate ingredient combination to get best bread.

6. References

- Aplevicz, K. S., Ogliari, P. J., & Sant'Anna, E. S. (2013). Influence of fermentation time on characteristics of sourdough bread. *Brazilian Journal of Pharmaceutical Sciences*, 49(2), 233-239.
- Ashenafi, M. (2006). A review on the microbiology of indigenous fermented foods and beverages of Ethiopia. *Ethiop. J Biol. Sci*, 5(2), 189-245.
- Battcock, M. (1998). *Fermented fruits and vegetables: a global perspective* (No. 134). Food & Agriculture Organisation
- Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., & Webb, C. (2003). Cereal-based fermented foods and beverages. *Food research international*, 36(6), 527-543.
- Bot, B., Sánchez, H., de la Torre, M., & Osella, C. (2014). Mother dough in bread making. *Journal of Food and Nutrition Sciences*, 2(2), 24-29.
- Clarke, C. I., Schober, T. J., & Arendt, E. K. (2002). Effect of single strain and traditional mixed strain starter cultures on rheological properties of wheat dough and on bread quality. *Cereal Chemistry*, 79(5), 640.
- Chelule, P. K., Mokoena, M. P., & Gqaleni, N. (2010). Advantages of traditional lactic acid bacteria fermentation of food in Africa. *Current research, technology and education topics in applied microbiology and microbial biotechnology*, 2, 1160-1167.
- Corsetti, A., Gobbetti, M., Balestrieri, F., Paoletti, F., Russi, L., & Rossi, J. (1998). Sourdough lactic acid bacteria effects on bread firmness and stalin. *Journal of Food Science*, 63(2), 347-351.

Corsetti, A., & Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Research International*, 40(5), 539-558.

De Vuyst, L., & Vancanneyt, M. (2007). Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiology*, 24(2), 120-127.

De Vuyst, L., Vrancken, G., Ravyts, F., Rimaux, T., & Weckx, S. (2009). Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiology*, 26(7), 666-675.

De Vuyst, L., & Neysens, P. (2005). The sourdough microflora: biodiversity and metabolic interactions. *Trends in Food Science & Technology*, 16(1), 43-56.

Golshan Tafti, A., Peighambaroust, S. H., Hejazi, M. A., & Moosavy, M. H. (2014). Diversity of Lactobacillus Strains in Iranian Traditional Wheat Sourdough. *Journal of food quality and hazards control*, 1(2), 41-45.

Guizani, N., & Mothershaw, A. (2007). Fermentation as a method for food preservation. *Handbook of food preservation*, 216-236.

Hellström, A. M., Vázquez-Juárez, R., Svanberg, U., & Andlid, T. A. (2010). Biodiversity and phytase capacity of yeasts isolated from Tanzanian togwa. *International journal of food microbiology*, 136(3), 352-358.

In college Seminar 253. 2008. Food for Thought: The Science, Culture, & Politics of Food Spring

Katina, K. (2005). *Sourdough: a tool for the improved flavor, texture and shelf-life of wheat bread*. VTT.

Ismaila, U., Gana, A. S., Tswanya, N. M., & Dogara, D. (2010). Cereals production in Nigeria: Problems, constraints and opportunities for betterment. *African Journal of Agricultural Research*, 5(12), 1341-1350.

Kaminski, E., Przybilski, R., & Gruchala, L. (1981). Thermal degradation of precursors and formation of flavour compounds during heating of cereal products. Part I. Changes of amino acids and sugars. *Food/Nahrung*, 25(6), 507-518.

Karovi ová, z. k. j. (2007). Fermentation of cereals for specific purpose. *Journal of Food and Nutrition Research*, 46(2), 51-57.

Katina, K., Arendt, E., Liukkonen, K. H., Autio, K., Flander, L., & Poutanen, K. (2005). Potential of sourdough for healthier cereal products. *Trends in Food Science & Technology*, 16(1), 104-112.

Katina, K., Heiniö, R. L., Autio, K., & Poutanen, K. (2006). Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT-Food Science and Technology*, 39(10), 1189-1202.

Khalid, K. (2011). An overview of lactic acid bacteria. *International Journal of Biosciences* 1(3), 1-13.

Komleni , D. K., Sla anac, V., & Juki , M. (2010). Influence of acidification on dough rheological properties. *Rheology. InTech Publishers, Rijeka, Croatia*, 265-292.

Kozlinskis, E., Skudra, L., Klava, D., Kunkulberga, D., Karklina, D., Venskutonis, P. R. & Rukshan, L. (2008). Characterization of rye sourdough microflora. *FOODBALT-2008*, 89.

Kumar, P., Yadava, R. K., Gollen, B., Kumar, S., Verma, R. K., & Yadav, S. (2011). Nutritional contents and medicinal properties of wheat: A review. *Life Science Medical Research*, 22, 1-10.

Kumral, A. (2015). Nutritional, chemical and microbiological changes during fermentation of tarhana formulated with different flours. *Chemistry Central Journal*, 9(1), 16.

Lacaze, G., Wick, M., & Cappelle, S. (2007). Emerging fermentation technologies: development of novel sourdoughs. *Food microbiology*, 24(2), 155-160.

Larsson, M., & Sandberg, A. S. (1991). Phytate reduction in bread containing oat flour, oat bran or rye bran. *Journal of cereal Science*, 14(2), 141-149.

Leenhardt, F., Levrat-Verny, M. A., Chanliaud, E., & Rémésy, C. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *Journal of Agricultural and Food Chemistry*, 53(1), 98-102.

Lopez, H. W., Krespine, V., Guy, C., Messenger, A., Demigne, C., & Remesy, C. (2001). Prolonged fermentation of whole wheat sourdough reduces phytate level and increases soluble magnesium. *Journal of Agricultural and Food Chemistry*, 49(5), 2657-2662.

Lopez, H. W., Duclos, V., Coudray, C., Krespine, V., Feillet-Coudray, C., Messenger, A., ... & Rémésy, C. (2003). Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. *Nutrition*, 19(6), 524-530.

Malomo, O., Ogunmoyela, O. A. B., Oluwajoba, S. O., Dudu, O. E., & Odeyemi, O. A. (2012). Microbiological and nutritional quality of warankashi enriched bread. *The Journal of Microbiology, Biotechnology and Food Sciences*, 2(1), 42-68.

Murtaza, M. A., & Ahmad, M. S. (2007). Effect of sourdough bacteria on the quality and shelf life of bread. *Pakistan Journal of Nutrition*, 6(6), 562-565.

McKevith, B. (2004). Nutritional aspects of cereals. *Nutrition Bulletin*, 29(2), 111-142.

Ngozi, A. A. (2014). Effect of whole wheat flour on the quality of wheat-baked bread. *Global Journal of Food Science and Technology*, 2(3), 127-133.

Norhaizan, M. E., & Norfaizadatul, A. A. (2009). Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malaysia Journal of Nutrition*, 15(2), 213-222.

Nyanzi, R., & Jooste, P. J. (2012). *Cereal-based functional foods*. INTECH Open Access Publisher.

Onwueme, I. C., and T. D. Sinha (1991) *Field crop production in tropical Africa: principles and practice*. CTA. 22-40.

Oyetayo, F. L., & Oyetayo, V. O. (2007). Dynamics of Phytate-Zinc/calcium balance of processed African Breadfruit (*Treculia africana* Decne) seeds. *Food Global Science Books*, 1(1), 88-90.

Oyewole, O. B. (1997). Lactic fermented foods in Africa and their benefits. *Food control*, 8(5), 289-297.

Peryam, D. R., & Girardot, N. F. (1952). Advanced taste-test method. *Food Engineering*, 24(7), 58-61.

Pons, M. N., Rajab, A., & Engasser, J. M. (1986). Influence of acetate on growth kinetics and production control of *Saccharomyces cerevisiae* on glucose and ethanol. *Applied microbiology and biotechnology*, 24(3), 193-198.

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food microbiology*, 26(7), 693-699.

Prikhodko, D., & Rybchynsky, R. (2009). *Agribusiness Handbook: Wheat Flour. Agribusiness Handbooks. Rome, Italy: FAO.*

Rahman, M. S. (Ed.). (2007). *Handbook of food preservation.* CRC press.

Randazzo, C. L., Heilig, H., Restuccia, C., Giudici, P., & Caggia, C. (2005). Bacterial population in traditional sourdough evaluated by molecular methods. *Journal of applied microbiology*, 99(2), 251-258.

Reddy, G., Altaf, M. D., Naveena, B. J., Venkateshwar, M., & Kumar, E. V. (2008). Amyolytic bacterial lactic acid fermentation—a review. *Biotechnology advances*, 26(1), 22-34.

Rehman, S. U., Paterson, A., & Piggott, J. R. (2007). Optimisation of flours for chapatti preparation using a mixture design. *Journal of the Science of Food and Agriculture*, 87(3), 425-430.

Rossi, J. (1996). The yeasts in sourdough. *Advances in food sciences*, 18(5-6), 201-211.

Ross, R. P., Morgan, S., & Hill, C. (2002). Preservation and fermentation: past, present and future. *International journal of food microbiology*, 79(1), 3-16.

Rouzaud, O., & Martínez-Anaya, M. A. (1997). Relationships between biochemical and quality-related characteristics of breads, resulting from the interaction of flour, microbial starter and the type of process. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, 204(4), 321-326.

Ryan, L. A., Zannini, E., Dal Bello, F., Pawlowska, A., Koehler, P., & Arendt, E. K. (2011). *Lactobacillus amylovorus* DSM 19280 as a novel food-grade antifungal agent for bakery products. *International journal of food microbiology*, 146(3), 276-283.

Sabir, D. A., & Sharef, P. H. (2013). Effect of Using Sourdough on the Rheological Properties, Sensory and Shelf Life Stability of Loaf Bread. *Journal of Agricultural Science and Technology. B*, 3(4B), 306.

Sharma, & Gupta, (2013). Comprehensive study of different traditional fermented foods/beverages of himachal pradesh to evaluate their nutrition impact on health and rich biodiversity of fermenting microorganisms. *International Journal of Research in Applied Natural and Social Sciences*, 1, 3, 19-28.

Shewry, P. R. (2009). Wheat. *Journal of experimental botany*, 60(6), 1537-1553.

SIM I , M. (2009). Phytate degradation during breadmaking: the influence of flour type and breadmaking procedures. *Czech J. Food Sci* , 27(1), 29-38.

Singh, N., GUJRAL, H. S., & SINGH, J. (2002). Effect of baking ingredients and mixing duration on dough development, gas release and bread making properties. *Journal of food quality*, 25(4), 305-315.

Slafer, G. A., & Satorre, E. H. (1999). An introduction to the physiological–ecological analysis of wheat yield. *Wheat: Ecology and physiology of yield determination*, 3-12.

Šramková, Z., Gregová, E., & Šturdík, E. (2009). Chemical composition and nutritional quality of wheat grain. *Acta Chimica Slovaca*, 2(1), 115-138.

Steinkraus, K. (1995). *Handbook of Indigenous Fermented Foods, revised and expanded*. CRC Press.

S.-ur-Rehman et al. / Trends in Food Science & Technology 17 (2006) 557e566

Tefera, A. (2013) grain and annual feed report GAIN Report Nuber:ET-1301,date 3/25/2015

Thiele, C., Gänzle, M. G., & Vogel, R. F. (2002). Contribution of sourdough lactobacilli, yeast, and cereal enzymes to the generation of amino acids in dough relevant for bread flavor. *Cereal Chemistry*, 79(1), 45-51.

Urbano, G., Lopez-Jurado, M., Aranda, P., Vidal-Valverde, C., Tenorio, E., & Porres, J. (2000). The role of phytic acid in legumes: antinutrient or beneficial function?. *Journal of physiology and biochemistry*, 56(3), 283-294.

Vaintraub, I. A., & Lapteva, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Analytical biochemistry*, 175(1), 227-230.

Vogel, R. F., Knorr, R., Müller, M. R., Steudel, U., Gänzle, M. G., & Ehrmann, M. A. (1999). Non-dairy lactic fermentations: the cereal world. In *Lactic Acid Bacteria: Genetics, Metabolism and Applications* (pp. 403-411). Springer Netherlands.

Walingo, M. K. (2009). Indigenous food processing methods that improve zinc absorption and bioavailability of plant diets consumed by the Kenyan population. *African Journal of Food, Agriculture, Nutrition and Development*, 9(1), 523-535.

Yann, D., & Pauline, G. (2014). Usefulness of Natural Starters in Food Industry: The Example of Cheeses and Bread. *Food and Nutrition Sciences*, 5, 1679-1691

Yousif, M. R. G., & Safaa, M. (2014). Effect of Using Different Types of Yeasts on the Quality of Egyptian Balady Bread *Journal of American Science*; 10(2)

APPENDICES

APPENDIX I: A SCORE SHEET FOR ACCEPTANCE (HEDONIC) TEST

Panelist code: -----Date: -----

Please taste the breads in the following order: Use the water provided to cleanse your palate before testing each sample:

101 102

Indicate how much you like the sample by ticking the numbers indicated below:

- | | |
|-----------------------------|--------------------|
| 1. Dislike extremely | 6. Like slightly |
| 2. Dislike very much | 7. Like moderately |
| 3. Dislike moderately | 8. Like very much |
| 4. Dislike slightly | 9. Like extremely |
| 5. Neither like nor dislike | |

Attributes	Sample codes	
	101	102
Taste		
Color		
Aroma		
Texture		
Over all acceptability		

Comments _____

Abso

