OPTIMIZATION OF PROCESSING CONDITIONS FOR PREPARATION OF GLUTEN FREE BISCUITS FROM TWO TEFF (*Eragrostis tef*) VARIETIES

M.Sc. Thesis

By

Ejigayehu Teshome

June, 2016

Jimma, Ethiopia

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M.Sc. Thesis

Submitted to the School of Graduate Studies Jimma University College of Agriculture and Veterinary Medicine in Partial Fulfillment of the Requirement for the Degree of Master of Science in Post- Harvest Management (Durable crops)

By

Ejigayehu Teshome

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APPROVAL SHEET

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I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted by my advisers. Hence, I hereby kindly request the Department to allow me to present the findings of my work and submit the thesis.

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We, the thesis advisers have evaluated the contents of this thesis and found to be satisfactory, executed according to the approved proposal, written according to the standards and format of the University and is ready to be submitted. Hence, we recommend the thesis to be submitted.

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Decision/suggestion of Department Graduate Council (DGC)

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Chairperson, DGC Signature Date ______________________ _________________

Chairperson, CGS Signature Date

DEDICATION

This thesis manuscript is dedicated to my beloved parents especially for my mother Alem Tekle and my father Teshome Kura and my beloved sisters and brothers for all the sacrifices, wishes and praiseworthy to my success in all my endeavors.

STATEMENT OF THE AUTHOR

I, the undersigned, declare that this Thesis is my work and is not submitted to any institution elsewhere for the award of any academic degree, diploma, or certificate and all sources of materials used for this Thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for M.Sc., degree at Jimma University, College of Agriculture, and Veterinary Medicine and deposited at the University Library to be made available to borrowers under the rules of the library.

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BIOGRAPHICAL SKETCH

The author Ejigayehu Teshome was born on February 1993 from her mother Alem Tekle and her father Teshome Kura in Jimma town. She attended her elementary at Bore primary school up to 2004 and junior education at Hamle 19 School 2005-2006 respectively. Upon completing her junior school education, she attended secondary education at Seto Semero secondary school from 2007 to 2008 after completion of secondary school she attended preparatory at Jimma preparatory school 2009 to 2010. Ejigayehu Teshome started her University level education at University of Gondar in September 2011 and remaining years at Jimma University and graduated with Bachelor of Science degree in Plant Science in July 2013.

After her graduation she directly joined Jimma University College of Agriculture and Veterinary Medicine in September 2014 to pursue her postgraduate study in Post-Harvest Management specializing in Durable crops

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ABBREVIATIONS AND ACRONYMS

AACC	American Association of Cereal Chemists
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
CCRD	Central Composite Rotatable Design
CD	Celiac Disease
CIAFS	Capacity to Improve Agriculture and Food Security
CSA	Central Statistics Authority
DZARC	Debre Zeit Agricultural Research Center
EIAR	Ethiopian Institute of Agricultural Research
EPHI	Ethiopian Public Health Institute
FDA	Food and Drug Administration
GFD	Gluten-Free Diet
HDPE	High Density Polyethylene
IDA	Iron Deficiency Anemia
JUCAVM	Jimma University College of Agriculture and Veterinary medicine
RR	Rehydration Ratio
USAID	United States Agency for International Development
USDA	United States Department of Agriculture

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ABSTRACT

The demand for gluten-free foods is certainly increasing. Because of this, interest in teff has been increasing noticeably due to its very attractive nutritional profile and its gluten-free nature. The limited knowledge of teff processing challenges faced making teff-based food products adapted for international consumers. This study aims to develop teff based biscuits as an alternative food source for gluten intolerant people. Optimization of three independent variables, baking temperature (174,180 and 186^{o}C), baking time (4, 8 and 12 minutes), and biscuit thickness (4.5, 5.5 and 6.5 millimeter) were taken as important factor to determine physical and nutritional quality of biscuits. There were twenty combinations of biscuit baking temperature, time, and thickness from each variety of teff using CCD with the aid of Design-Expert software to get best quality teff biscuit. The major response variables of physicochemical properties at different baking condition were analyzed. The moisture, protein, ether extract, fiber, ash, carbohydrate, and gross energy in the biscuit samples were found in the range of 4.15 to 6.98%, 14.59-18.94%, 13.50-14.80%, 3.72-4.08%, 3.38 to 3.74 %, 51.88-60.25%, and 414.22-422.15Kcal/100g respectively for both tested teff varieties. Results showed that baking temperature and time were the most important factors that significantly affected ($p<0.01$) water rehydration capacity, biscuit hardness, water activity, bulk density, protein, moisture content, carbohydrate and gross energy. Biscuit diameter, thickness, ash, fat, fiber, and mineral were not significantly affected by interaction of baking conditions. Based on all parameters with exception of sensory analysis the best treatment combination of temperature, time, and biscuit thickness; 174^{o}C, 9 min, and 4.5mm thick for both Kuncho and Keyetena based biscuit was selected. Acceptability was also assessed using a 5-point Hedonic scale. Compared with two varieties, biscuits made from Kuncho teff got better overall acceptability but in terms of most of chemical and physical properties almost they are the same except mineral composition. When both teff compared, biscuit made from keyetena teff contains highest calcium and iron content and lower sensory score and Kuncho teff got better sensory acceptability. Generally result of this study confirmed the possibility of production of gluten free teff based biscuit as an alternative food source for gluten intolerant consumers.

Key words: Baking, Celiac disease, Gluten Free Diet, Iron deficiency anemia, Sensory evaluation

1. INTRODUCTION

1.1 Background

Teff (*Eragrostis tef* (*Zuccagni*) *Trotter*) is a tropical cereal that belongs to the family of Poaceae, subfamily Eragrostoidae, tribe Eragrosteae, and genus Eragrostis, which means, "*teffa*" in Amharic means lost due to its small size (Kebede, 2010). It is a major cereal grain in Ethiopia but it was a neglected cereal crop worldwide. It 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 area devoted to cultivation and its productivity is increasing from year to year. It is indigenous cereal crop in Ethiopia with share of area of 22.7 %, 2.4 million hectares, under cereal cultivation and third (i.e. after maize and wheat) in terms of grain production (16.3 %, 24.4 million quintals) (CSA, 2007).

Teff grain flour is widely used in Ethiopia for making *injera* (staples for the majority of Ethiopians, a fermented, pancake-like, soft, sour, circular flatbread), sweet unleavened bread, local spirit, porridges and soups (Laike *et al*., 2010). Teff grain commands premium price among other cereals cultivated in Ethiopia. There is a growing interest on teff grain utilizations because of nutritional merits (whole grain), the protein is essentially free of gluten which is found in wheat (alternative food for consumers allergenic to wheat glutens) (Hopman *et al.,* 2008). Due to its importance in most Ethiopians nutrition, recently there is a growing interest on teff research to explore its potential for nutrition and economic merits such as starch production (Bultosa and Taylor, 2004) production of extruded products (Sirawdink and Ramaswamy, 2011; Kebede *et al*., 2010), as a composite with sorghum for *injera* making (Yetneberk *et al*., 2005) .Teff grain being starch rich cereal (Bultosa, 2007; Bultosa and Taylor, 2004; Gebremariam *et al.,* 2012), could also be potentially used for malting and beer production.

The nutritional value of teff is often referred to as being similar to that of wheat (Dekking *et al*., 2005), but teff is actually more nutritious, as the seeds are so small (between 1 and 1.5 mm long) that they contain a greater proportion of bran and germ. It is as major staple cereals like wheat, rice, oats and barley and even better in some aspects, containing more calcium, zinc, iron, and potassium and being high in dietary fiber. It is a rich source of vitamins and is considered to be an excellent source of essential amino acids with highest levels than wheat and barley (Seyfu, 1993). In addition, precisely because the seeds are so small, teff flour is usually whole-grain. Teff is also quite high in energy, and has an average fat content of about 2.6%. Protein content also tends to be just as high as, or highest than, that of other cereals, ranging from 8% to 15% with an average of 11% (Bultosa, 2007). The iron content seems to play a particularly important role in Ethiopia, as less prevalence of Anemia in the population has been found to correlate with areas of Teff consumption (Piccinin, 2002).

Recent Evidence suggests that glutenin has adverse effects on the intestinal mucosa. *In vitro* and *in vivo* studies indicated that exposure to glutenin leads to immune stimulatory and/or histopathological changes in the tissue of the small intestine of sensitive individuals, or in related cell systems (Vader *et al.,* 2003). Prolamins of wheat, barley, and rye are characterized by high proline content (Haboubi, 2006). These proteins, the main constituents of gluten, contain toxic that can trigger celiac disease. Celiac Disease (CD) is a chronic entheropathy produced by gluten intolerance, more precisely to certain proteins called prolamines, which causes atrophy of intestinal villi, malabsorption and clinical symptoms that can appear in both childhood and adulthood (Bernardo, 2012). These proteins are found in wheat, oat, barley, and rye. The toxic fractions adopt different names, depending on the cereal: gliadin in wheat, avidin in oat, secalin in rye, and horden in barley. Prolamins of wheat, barley, and rye are characterized by high proline content (Vader *et al.,* 2003). These proteins, the main constituents of gluten, contain toxic sequences that can trigger celiac disease.

Most celiac patients under risk of developing an autoimmune disease are diagnosed in the early stages of their life and have a family history of autoimmunity. In these cases, the gluten-free diet has a protective effect. Based on this, the celiac disease can be said to be a genetic-based autoimmune enteropathy characterized by variable degrees of damage in the intestinal mucosa (White *et al.,* 2013). This condition appears in genetically susceptible individuals who have been exposed to gluten consumption. Medical research has produced an extensive list of epitopes intrinsically present in wheat, rye and barley that are recognized by T cells from CD patients. The only treatment for those with CD available to date is to follow a strict gluten-free diet (Gallagher, 2002). Consequently, inadequate intakes of essential nutrients such as folate and vitamin B12 (Hallert *et al*., 2002), calcium, iron, and fiber have been observed in those with CD (Thompson *et al*., 2005) due to fear to eat products of those cereal crops. Also, a highest percentage of energy intakes in such patients were found to be from fat instead of carbohydrates. This has a negative impact on their nutritional status (Bardella *et al*., 2000). Hopman *et al*. (2008) and Dekking *et al*. (2005) investigated the presence or absence of gluten in pepsin and trypsin digests of 14 teff varieties. The digests were analyzed for the presence of T-cell–stimulatory epitopes. In contrast to known gluten containing cereals; no T-cell stimulatory epitopes were detected in the protein digests of all the teff varieties assayed, thus confirming the absence of gluten in teff. Due to this Teff has an increasingly important grain for individuals who suffer from gluten intolerance (Bemihiretu *et al.*, 2013), and hence it is recommended as functional food for celiac patients. Having a gluten free diet not only benefits those with gluten intolerance; new research is showing this type of dieting can also benefit people with other diseases, one of those being autism. A study done recently has shown that children put on a gluten free diet have shown decreased levels of autistic behaviour and an increase in social interaction (Whitely *et al.*, 2010). New gluten free products developing to satisfy consumers other than celiac disease patient's nutritional needs including anaemia, osteoporosis, and diabetes.

Biscuits

The term biscuits as they are called in many parts of the world, refers to a baked product generally containing the three major ingredients; flour, sugar, and fat. Biscuits are one of the low cost processed foods, which are most widely consumed. Biscuit is regarded as a form of confectionery dried to very low moisture content (Sharma *et al.*, 2003) product. Biscuits have been reported to be rich in fat and carbohydrate; hence they can be referred to as energy giving food as well as good sources of protein and minerals (Kure *et al.*, 1998). According to the information provided by various researchers, its moisture content varies widely, because of the variation in its thickness and weight during forming and shaping. Typically, the moisture content of biscuit after baking is below 10%. Therefore, water addition to biscuit dough is lower compared to other bakery products.

1.2. Statement of the problem

In the patients of CD, ingestion of gluten (from wheat, barley and rye) damages the lining of the small intestine and prevents normal digestion and nutrient absorption, thereby leading to chronic disorders of nutrient deficiency diseases like anemia, diarrhea and weight loss (See and Murray, 2006).Recent data indicated that the average worldwide prevalence of celiac patient is estimated as high as 1:266 (one out of 266 individuals). In Ethiopia, Celiac disease kills many children each year, mainly because it usually goes undiagnosed and untreated (Emire and Tiruneh, 2012). The abundance of food products in Ethiopia contains wheat or other gluten-containing cereals due to these gluten sensitive individuals don't consume it because of its gluten content. To alleviate the problem associated with gluten, value added gluten free product diversifications at national and international level are the only opportunity to treat them .The products of Teff grain are becoming popular globally mainly due to the absence of gluten, the cause for CD (Dekking *et al*., 2005; Hopman *et al*., 2008; See and Murray, 2006).

Biscuit produced from teff could be used by celiac disease patients and those people who need to eat gluten free foods. In addition there have been numerous studies on the effects of process conditions such as baking temperature, types of oven used and baking time to the final product qualities of biscuit made from wheat flour. However, so far there is no or limited study conducted to investigate effect of baking conditions on physical and nutritional qualities of teff based biscuits. The control of the baking parameters like temperature and time during baking is the best determinant of final quality of baked goods and helps for successful implementation of commercial flour baking technology (Shittu *et al.*, 2007). The present study was aimed to achieve the following objectives.

1.3. Objectives

1.3.1. General objective

To optimize baking temperature, time and thickness to produce better overall quality teff based biscuit for gluten intolerant people.

1.3.2 Specific objectives

i. To study the influence of baking conditions on certain physical properties of biscuits made out of two teff varieties.

ii. To determine nutritional composition of biscuits made out of two teff varieties.

iii. To select best combination of processing variables based on physical properties and nutritional composition of biscuits made out of two teff varieties.

iv. To determine consumer's sensory acceptability of biscuits made from two teff varieties baked under optimized condition

1.4. Significance of the Study

- Gluten intolerant people will benefit from the result which will enable them to make their own teff based biscuit.
- Information obtained from this study can be used by biscuit industries to diversify their products through production gluten free teff based biscuits.
- Promote the production and use of the grain both in national and international markets.
- Promote teffs contribution to healthy diet for individuals who suffer from different diseases other than celiac and will serve as a source of hard currency through exporting the grain or processed biscuit.

2. LITERATURE REVIEW

2.1. Teff Production

Teff, *Eragrostis tef* (Zucc.) Trotter) is indigenous to Ethiopia in its origin (Kebede *et al.,* 2010). Teff is endemic to Ethiopia; its major diversity is found only in this country. As with several other crops, the exact date, and location for the domestication of teff is unknown. However, there is no doubt that it is a very ancient crop in Ethiopia, where domestication took place far before the birth of Christ (Ketema, 1997). It is believed to have originated in Ethiopia between 4000 and 1000 BC, (Vavilov, 1951). Nowadays, teff is grown in almost all regions of Ethiopia since it is the preferred grain for local consumption and for market because it fetches the highest grain price compared with other cereals (Ketema, 1997). The primary use of teff grain is for grinding into flour to make injera (fermented, pancake-like, soft, sour, circular flatbread), a major food staple in Ethiopia.

Annual teff production has been increasing year after year by about 11%, which has resulted in a 100% increase over seven years. Increased productivity is believed to contribute about 6% of that 11% growth while about 5% was attributed to expansion in area cultivated for teff. During 2009-2010, it was estimated that 3.2 million tons of teff was produced in 2.6 million hectares of land. This is equivalent to 21% and 28% of the total cereal production and acreage in the country, respectively, making teff the leading crop among cereals and other annual crops (Bekabil *et al.,* 2011).

2.2. Types of Teff

There are several varieties of teff, each with characteristics best suited to specific agro ecological conditions. There are few different varieties of teff that vary in color from light to dark (Teffera *et al*., 1995). 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 teff do not have significant variation in their calorie, moisture, protein, carbohydrate, or phosphorus nutrients.

In general there are three main types of teff: white, red, and brown. *White* teff is the preferred type but only grows in certain regions of Ethiopia and does not grow in Eritrea. White teff grows only in the Highlands of Ethiopia, requires the most rigorous growing conditions, and is the most expensive form of teff. White teff was reserved for the wealthiest and most prestigious families in Ethiopia. The prestige associated with consuming white teff, as well as its more stringent growing conditions, contributes to the increased cost of white teff. The shelf life of *injera* is extended with the use of white teff (Stallkneckt, 1997).

Red *teff*, 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 teff is most prevalent, hemoglobin levels were found to be highest with a decreased risk of anemia related to parasitic infection. As studies of the increased health benefits associated with high iron contents in red teff become clarified there is more acceptance of this grain in society (Bultosa, 2007).Today in Ethiopia; red teff is becoming more popular related to its increased iron content.

2.3. Nutritional Composition of Teff

The chemical composition of cereals varies widely and depends on the environmental conditions, soil, variety, and fertilizer. The importance of teff is mainly due to the fact it has attractive nutritional profile and has no gluten found in other common cereals such as wheat, barley, and rye. Teff is similar to wheat in food value; teff has a highest vitamin and mineral content. When wheat is processed, the germ (the embryo of the berry, which contains a high concentration of vitamins) is removed from the endosperm. The endosperm is then milled into flour. In contrast, teff is almost always produced as whole-grain flour. When it is milled, it is difficult to separate the bran and germ because of the relatively small size of the grain. Because there is a greater portion of germ in milled flour, the nutrient content of teff flour is also highest (Piccinin, 2002). According to Lovis, (2003) Teff has an excellent balance of essential amino acid and is a good source of calcium, phosphorus, and iron the finding shows that the teff grain has a good source of vitamin when compared with wheat flour, brown rice, sorghum and white rice flour.

2.3.1. Moisture Content

The moisture content is one of most important and commonly measured properties of different food products. It is measured for a number of reasons including legal and label requirements, economic importance, food quality, better processing operations and storage stability considerations .Moisture content is one of the most important factors affecting the quality of cereals grains, since the amount of dry matter of grains is inversely related to the moisture content (moisture contents has direct economic importance) . Ciferri and Baldrati ,(1939) showed that the moisture content of teff flour range 8.84 – 10.36%, while Lovis, (2003) mentioned that moisture content of teff grain is 11%.

2.3.2. Ash Content

Zeleny, (1971) reported that ash content is directly related to amount of bran in the cereal grains. Ash content also indicates milling performance by indirectly revealing the amount of bran contamination in flour. Some specialized products requiring particularly white flour call for low ash content while other products, such as whole wheat flour, have high ash content. Ciferri and Baldrati, (1939) found that ash content of teff flour ranged between 2.4 and 2.94% as dry matter. Bultosa, (2007) reported that teff grain ash content had ranged from 3.16 to 1.99% with mean of 2.45%.

2.3.3. Protein

The average crude protein content of teff is in the range of 8 to 11 percent, similar to other more common cereals such as wheat. Another important feature of teff is that it has no gluten. This makes teff a valuable ingredient for functional foods destined for celiac patients who are gluten intolerant. The amino acid composition of teff flour is favourable and its protein is easily digestible in comparison to cereals such as maize and sorghum because the main protein fractions such as albumin and globulin are the most digestible types. Bultosa (2007) reported that the grain protein contents of 13 teff varieties are ranged from 8.7–11.1 % with mean10.4 % and also reported the grain protein contents of 13 released teff varieties in the range of12.4–8.7 % with mean 11.0 % and the highest was for DZ-01-99 and the least was for DZ-01-1285.

2.3.4. Fat

Cereals are not the best source of fat, but as they are often consumed in large quantities, cereals can contribute a significant amount of essential fatty acids to the diet (Michaelsen *et al*., 2011). Fatty acids are potentially beneficial to growth, development, and long-term health. Consequently, there has been significant interest in recent years in their inclusion in diets. For instance, increased intake of omega 3 fatty acids (α-linoleic acid) were found to reduce biological markers associated with cardio-vascular disease, cancer, inflammatory and autoimmune diseases among others (Simopoulos, 2001). The crude fat content of teff is highest than that of wheat and rice, but lower than maize and sorghum. The fat contents are mainly of fatty acids. Teff grains are rich in unsaturated fatty acids. The seeds contain 22 % w/v of fixed oil rich in unsaturated fatty acids (72.46 %), among which oleic acid is predominant (32.41 %), followed by linoleic acid (23.83 %) (Elalfy *et al.*, 2011). The unsaturated fatty acids are not only important for our nutrition, especially as some cannot be synthesized by humans (essential fatty acids).

2.3.5. Fiber

The most recent Codex definition further added that dietary fibers should have "proven physiologic effects of benefit to health" (Cummings *et al*., 2009). Some of these physiological effects include faecal bulking (laxation), lowering blood glucose levels after eating, and lowering plasma LDL-cholesterol (Lairon *et al*., 2005). The crude fiber, total and soluble dietary fiber content of teff is several folds highest than that found in wheat, sorghum, rice, and maize. There may be several reasons for this. First, whole grains have highest fiber content than decorticated ones. Second, small grains have a relatively high proportion of bran, which is high in fiber (Bultosa, 2007). Therefore, highest dietary fiber intake and the associated health benefits are expected with increased consumption of teff. Consumption of dietary fiber provides many health benefits. The dietary fiber content of teff (8.0 g/100 g) is high when compared to some fruits, nuts, pulses and cereals such as corn and rice (Saturni *et al.*, 2010). Studies revealed that high fiber diets prevent many human diseases, colon cancer, coronary heart disease, and diabetes (Anderson, 2008).

2.3.6. Carbohydrate

Carbohydrates are the major source of energy for human nutrition and play an important role in metabolism and homeostasis. Based on the molecular size and degree of polymerization, carbohydrates can be classified into sugars, oligosaccharides, starch (amylose, amylopectin), and Non-starch polysaccharides. Complex carbohydrates make up 80 percent of the teff grain. It has a starch content of approximately 73 percent, making teff a starchy cereal. The amylose content of 13 teff varieties tested ranged from 20 to 26 percent, comparable to other grains, such as sorghum (Bultosa, 2007) and National Research Council, (1996) showed that like other cereals teff is predominantly starchy (73 %) and the starch content of teff is highest than that of most other cereals. This makes it to be potential gluten free cereal that replaces wheat and other cereals in their applications as sources of food energy.

Table 1. Nutritional composition of different varieties of Teff flour (per 100 grams).

	White Teff	Red teff	Mixed teff
Energy (kcal/100g)	339	336	336
Moisture (%)	10.4	11.1	10.7
Carbohydrate (%)	73.6	73.1	75.2
Crude protein (%)	11.1	10.5	8.3
Crude fat (%)	2.5	2.7	2.9
Crude fiber (%)	3	3.2	3.6
Ash (%)	2.8	3.1	3

Source: (Gebremariam *et al*., 2012), Agren and Gibson, (1968) and USDA, (2015)

2.3.7. Energy

Calorific value is calculated from fat, carbohydrate, and protein. Teff as mentioned on previous sections it's a good source of energy. According to findings of Bultosa and Taylor, (2004) Values ranges from 336-367 (kcal/100g) respectively for both white and red teff varieties.

2.4. Mineral Composition of Teff

In general, compared to the other cereals, teff is rich in minerals such as calcium, iron, zinc, magnesium, phosphorous and copper (Bultosa and Taylor, 2004). Malabsorption of iron, folate, and calcium is common, as these nutrients are absorbed in the proximal small bowel. In particular, it has been reported that the frequency of iron-deficiency in celiac disease varies from 12 to 69 % (Tikkakoski *et al*., 2007).

The high iron content of teff increases the haemoglobin level of the blood that helps more oxygen to be transmitted (Andrews *et al*., 1999). Studies showed that teff consumers have highest level of haemoglobin in their blood than non-teff consumers, Teff contains an excellent concentration (0.165 %) of calcium, and the level of this mineral in teff is by far highest than other cereals. This leads to conclude that teff is an excellent cereal to prevent the aforementioned health problems associated with less consumption of calcium. The difference in mineral content between and within teff varieties is wide ranging. Red teff has a highest iron and calcium content than mixed or white teff (Abebe *et al*., 2007). On the other hand, white teff has a highest copper content than red and mixed teff (Table 2). Ketema, (1997) analyzed 12 genotypes of teff grown in different agro-ecologic settings and 5 varieties grown in a greenhouse in Great Britain and reported that genetic and environmental factors affect the iron content of teff. This may partly explain the high variability in the mineral content reported in different studies.

Table 2. Mineral content of different varieties of teff grains compared to other cereals, mg/100g

Minerals	**White teff**	**Red teff**	**Mixed teff**	**Maize**	**Sorghum**	**Wheat**	**Rice**
Iron	9.5-37.7	11.6- 150	11.5- 150	3.6-4.8	3.5-4.1	3.7	1.5
Calcium	17-124	18-178	78.8-147	16	5.0-5.8	15.2-39.5	23
Zinc	2.4-6.8	2.3-6.7	3.8-3.9	2.6-4.6	1.4-1.7	1.7	2.2

Sources: Baye *et al*. (2014); Gebremariam *et al*. (2012).

2.5. Health Benefits of Teff and Teff Based Products

2.5.1. Iron deficiency

Iron-deficiency is the most widespread micronutrient deficiency globally, affecting more than 2 billion people (Zimmermann, 2007 and Hurrell, 2010). Growth retardation, impaired mental and psychomotor development, child and maternal morbidity and mortality, and decreased immunity and work performance are among the adverse effects of iron deficiency (Georgieff, 2011). The etiology of iron deficiency includes diseases that induce excessive loss or cause malabsorption of dietary iron, low intakes of bioavailable iron, or increased requirements due to physiological status (e.g. pregnancy, infants and young children) (Pasricha *et al*., 2013).

Inadequate iron intake is common in low and middle-income countries, particularly among infants and young children (Gibson *et al*., 2010) and pregnant women (Clark, 2008). Food fortification and nutritional supplements may constitute effective strategies to prevent iron deficiency. However, these strategies are not without side effects, especially when applied to environments where malaria and infections are prevalent (Sazawal *et al*., 2006; Zimmermann *et* *al*., 2007). Therefore, adjusting iron intakes with iron-rich foods may be preferred. Teff can be a good alternative (Gebremedin, 1999). Alaunyte *et al*. (2012) showed that by supplementing wheat bread with 30 percent teff flour, the iron content of the bread more than doubled. By assuming an average daily consumption of 200g of teff-enriched bread, it is possible to cover between 42 and 81 percent and 72 and 138 percent of daily intake requirements for iron in women and men, respectively.

Bokhari *et al.* (2012) showed that consumption of 30 percent teff-enriched wheat breads can help maintain serum iron levels in pregnant women. The high iron content of teff and its potential contribution to food-based approaches to improve nutrition for further investigations on the iron bioavailability of teff are required. Indeed, if the bioavailability of iron in teff can be confirmed, teff can be a very good ingredient for celiac patients not only due to the absence of gluten, but also for its high iron content.

2.5.2. Celiac disease

Worldwide, 1 to 266 Percent of the population is affected by celiac disease (CD) (Gujral and Thomson, 2012). CD is caused by aberrant T-cell responses to glutens and gluten-like proteins found in wheat, barley, rye, and possibly oats (Vader *et al*., 2003, Arentz et *al*., 2004). The symptoms include diarrhea, abdominal pain, and disturbances in nutrient absorption caused by histological alterations of the small bowel. Extra-intestinal complications such as osteoporosis, infertility, and cancer have also been reported (Alaedini and Green, 2005).

2.5.2.1. Treatment

The only known treatment for CD is a lifelong diet free of the protein fractions glutenin, gliadin, and hordein found in cereals of the family *Triticum* and related grains such as barley, triticale, and rye. Grains, legumes and pseudo cereals that are considered gluten-free and safe for celiac include amaranth, arrowroot, all types of pulses, buckwheat, corn, mesquite, millet, quinoa, rice, sorghum, soy, and teff (Green and Cellier, 2007). Upon first diagnosis, celiac patients typically suffer from iron, folate, and calcium deficiencies due to malabsorption in the small bowel. Secondary lactose intolerance is common in celiac patients due to decreased lactase production from damaged villi in the small intestine. Deficiencies of fat soluble vitamins like A, D, E, and K are also common; however, after treatment with a gluten-free diet damage to the small intestine heals and serological values should return to normal (Niewiński, 2008). Teff, as discussed in previous sections, contains a good amount of minerals and fiber. Compared to gluten free cereals and pseudo cereals such as quinoa, amaranth, buckwheat, maize, brown rice, and sorghum, teff is more nutrient dense (Gebremariam *et al*., 2012). Furthermore, the low glycemic index of teff may help maintain good glycemic control. This is very important given the high incidence of diabetes in those with CD (Viljamaa *et al*., 2005).

2.5.3. Diabetes

The global incidence of diabetes is increasing alarmingly and has become a major public health problem (Danaei *et al*., 2011). In 2010, an estimated 285 million people worldwide were diabetic, a figure projected to rise to 439 million by 2030 (Shaw *et al*., 2010). The socio-economic and health implication of this disease, particularly in low and middle-income countries, are enormous. The onset and progression of diabetes can be prevented by modifying lifestyle factors, of which diet constitutes a great part (Hu, 2011). Several features of teff suggest that its consumption may prevent or control diabetes. Diets high in whole grains have been associated with a 20 to 30 percent reduction in the risk of developing type 2 diabetes (Hu, 2011). Given that teff is consumed as a whole grain, similar effects can be expected from the consumption of teff. Although the mechanism by which whole grains help in the prevention of type 2 diabetes is not clearly clarified, it is thought to be through the synergistic effects of the essential macro and micronutrients, and phyto nutrients (Jonnalagadda *et al.,* 2011). Among macronutrients, the type of carbohydrate and its digestibility play a central role in glucose levels after eating, and hence on the risk to diabetes (Sheard *et al*., 2004).

Relative to wheat, teff has a low glycemic index and thus better suited for diabetic patients (Wolter *et al*., 2013). In addition, the relatively high dietary fiber in teff relative to other common cereals, can decrease fasting blood glucose levels and, thus, contribute to the prevention and management of diabetes (Post *et al.,* 2012). The condition of impaired antioxidant status and inflammation has been linked to the development of insulin resistance and type 2 diabetes (Wellen and Hotamisligil, 2005 and Folli *et al.,* 2011). In this regard, the high phytate and polyphenols content in teff (Abebe *et al*., 2007, Baye *et al*., 2014) and the associated antioxidative property is likely to prevent and control diabetes (Lee *et al*., 2006; Munir *et al*., 2013). However, while studies to evaluate the anti diabetic property of teff consumption are of interest, so far such studies are very limited.

2.6. Gluten Free Diet

Parrish (2006) found that a person with celiac disease may feel entitled to eat anything gluten-free, even if high in fat, sugar, and calories, in an attempt to compensate for the restrictions of a gluten-free diet. Unfortunately, being gluten-free diet does not guarantee nutrition principles recommended to protect the heart. However, Washington (2006) reported that, people with celiac disease can suffer with symptoms for years before being diagnosed. The key to better health is automatically turned when a diagnosis is rendered. However, the key opens the door to a healthy lifestyle only if a gluten-free diet (GFD) is maintained.

Tenorio *et al.* (2011) reported that, celiac disease is an autoimmune disease acquired through genetics and the environment. People with Celiac Disease cannot eat foods with wheat, rye, or barley because of their gluten content. This disease is hard to diagnose considering many of its symptoms are also correlated with other diseases. These symptoms include diarrhea, weight loss, malnutrition, and abdominal distension. Unfortunately, the only treatment of celiac disease is through a strict diet free from gluten Branski (2012) mentioned that, celiac disease is an autoimmune disorder occurring in genetically susceptible subjects. The treatment of CD is a lifelong, strict gluten-free diet.

2.6.1. Gluten free biscuit

The gluten is a protein complex found in the triticeae tribe which provides desirable organoleptic properties (texture and taste) to many bakery and other food products. Influence of gluten-free flour mixes and fat powders on the quality of gluten-free biscuits was studied by Schober *et al.* (2003) and they found that, gluten-free biscuits based on pure starches tend to cause a dry, sandy mouth-feel. As per rule the “Gluten free” is a voluntary term and defined as food containing less than 20 ppm of gluten. Developing gluten-free biscuits from whole grain and pseudo cereal flours like buckwheat, flax, quinoa, brown rice, teff, legumes, sorghum, and nuts would be an ideal option for improving the nutritional quality and the dietary fiber content of gluten-free breads and biscuits (Niewiński, 2008). Teff is increasing in availability with some major companies considering utilizing it in products designed for gluten-free consumers. The gluten-free diet remains until now the only treatment for celiac disease.

2.6.2. Process description for biscuit production

Bakery products include biscuit, muffin, cake, bread, pastries, and pies. They contain significant amount of flours, which are mixed with various other ingredients and ultimately undergo dry-heating process in a baking oven (Cauvain and Young, 2007). In the developed nations, biscuit making is a conventional activity. The most important nutrients they provide are carbohydrates, fat, and B vitamins for energy; calcium for strong bones and teeth; and protein for growth and repair. They also provide minerals, including iron. Biscuits are typically round cakes of bread that are leavened with baking powder, baking soda or sometimes yeast. It may also refer to a biscuit or cracker.

Biscuits represent a fast growing segment of food because of consumer demands for convenient and nutritious food products. The consumers demand has increased for the quality food products with taste, safety, convenience and nutrition (Masoodi *et al.*, 2012).Biscuits are a popular foodstuff consumed by a wide range of population due to their varied taste, long shelf life and relatively low cost. Because of competition in the market and increased demand for healthy, natural, and functional products, attempts are being made to improve the nutritive value of biscuits and functionality by modifying their nutritive composition. By increasing the ratio raw materials other than wheat or different types of dietary fibers in basic recipes with the attempt to increase biscuit's protein and mineral content for quality and availability (Tyagi *et al.*, 2006; Masoodi *et al.*, 2012) or increase dietary fiber content and improve prebiotic characteristics of the final product (Gallagher *et al.*, 2003).

2.7. Major Biscuit Ingredients

Selection of suitable ingredients is an important step in manufacturing the food products. It is, therefore, necessary to have proper idea regarding ingredients, their function, and uses. Biscuit ingredients can be classified as binding or tenderizing materials, depending on their expected effect on the finished product. It includes flour, water, milk solid, egg white, cocoa powder, sugar, shortenings, leaving agents, emulsifier, starch and salt etc (Patel *et al*., 2003).

2.7.1. Flour

Being the basic ingredient, flour requires the greatest attention for the quality testing some specification of different flour use in the preparation of bakery products. Flours other than wheat, rye, barley, and oats lack gluten and therefore, fail to form viscoelastic dough when they are kneaded with water in a conventional bread making process. They form a batter rather than dough. The absence of gluten in these flours makes them suitable for gluten-free products such as biscuits but unsuitable for the production of dough, the product form for which industrial biscuit making process lines were developed. Moreover, the batters tend not to retain carbon dioxide gas during proofing and baking. Thus, the resulting biscuit has a low specific volume (a high density) and does not resemble wheat based biscuits (Schober *et al.*, 2003).

2.7.2. Water

Water gelatinizes starch during baking and serves a solvent for solutes and dispersion medium for the other ingredients. The greater the quality of gluten, the greater is the absorption. The salts in water affect the properties of dough. Hard water containing magnesium and calcium ions may have 'tightening effect' and soft water a 'loosening effect' on dough (Patel *et al*., 2003). The nature of water used in batter is more likely to be of consequence in commercial biscuits production than in non-commercial biscuits making. Water, besides being component of every ingredient, is also directly added into the preparation of most of the products.

In baking, water plays an important role in production by providing the necessary medium for the physical, chemical, biological and biochemical reaction that underlie the conversion of raw material in finished baked foods. In addition, it has decisive influence on the overall quality and palatability of the finished baked products. It leavens the products by converting itself into the vapour during baking. It also assists in the control of dough temperature (Patel *et al*., 2003). Water is added at the mixing stage to nearly all biscuit recipes. It functions as a catalyst because it is almost totally removed during the subsequent baking process. Water is important in determining the nature of the dough, how the dough behaves in the forming processes and ultimately the structure in the baked biscuit.

2.7.3. Fat

The main function of fat is incorporation of air during creaming. When fat entraps the air in the form of minute cells and bubbles the volume increases. The ability of fat to absorb air during mixing is called its creaming quality. Good creaming incorporates about 270% of air when creamed with granulated sugar (Patel *et al*., 2003). Fat has numerous functions in biscuits. Fat act as a stabilizer, when particles of fat are spread in the dough makes it more stable. Fat also contributes to the organoleptic qualities of biscuits such as flavor and aroma. As a result, the soft and more extensible dough will formed. Gupta (1998) observed significant decrease in hardness of biscuits. This was apparently due to softening action of fat on protein, which increased the spread and reduced hardness, compactness of biscuits. Singh *et al.,* (2000) standardized fat (20-35%) and sugar (28-43%) in biscuits and observed that with increasing level of fat, the thickness of biscuits decreased whereas diameter, weight, spread ratio and percent spread factor of Product increased gradually.

2.7.4. Sugar

Sugar is essential component of biscuit. It functions not only as a nutritive agent but also as a texture, colouring agent, and means of controlling spread. Singh *et al.* (2000) standardized the level of sugar in biscuits found that with the increasing level of sugar up to 37% in formulation, attributes such as weight, diameter ratio and percent spread factor of biscuits increase whereas thickness of product decreased. Greater spread at highest level of sugar was attributed to melting of sugar crystal causing spread action (US wheat Associates, 1998).Sugar is most commonly thought of as a sweetener, but in baked goods, it is also involved in several other processes. Sugar undergoes a series of complex browning reactions above160^{o}C, and the products of these form the brown crust of many baked goods. The reactions are known as Millard reactions, and are essentially amino acid catalyzed caramelization reactions in which a sugar aldehyde or ketone is converted to an unsaturated aldehyde or ketone. In non-fermented goods such as biscuits, large quantities of sugar can be added. This improves the keeping quality of the biscuits as well as sweetening those related product (Patel *et al*., 2003).

2.7.5. Whole milk powder

Whole milk powder is used in the biscuit manufacturing because of its extended shelf life and consistent quality as compared to fresh and other forms of milk. It is most important moistening agent. The crust colour and water retention power of biscuits are improved by the milk sugar, i.e. lactose. Milk powder contains casein as a principal protein, it also contain all the essential amino acids hence it improve the nutritional quality of the biscuits (Patel *et al*., 2003).

2.7.6. Salt

The basic function of salt in biscuit is to contribute flavor. Salt also has inhibiting effect on the formation of gluten during mixing. It imparts taste and makes the products appetizing and palatable. It lowers the caramelization temperature of biscuit, thereby, improves the crust colour (Patel *et al*., 2003).

2.8. Major Biscuit Making Principles

2.8.1. Dough-making process

The first stage in dough processing is mixing, in which the development of the dough is established. The mixing process is the crucial operation in bakery industry by which the wheat flour, water, and additional ingredients are changed through the mechanical energy flow to coherent dough (Wilson *et al*., 2001). Mixing is the homogenization of ingredients for uniform dispersion, development of the gluten structure in the dough and incorporation of air bubbles within the dough (Cauvain, 2003). It is a comprehensive series of compressing and stretching (kneading) process of the ingredients to impart the necessary work for formation of extensibility and cohesive strength of the dough for subsequent processing (Gan *et al.*, 1995).

The temperature of the dough is important in terms of the fat used. At high temperatures, fat melts and dough becomes fatty. Dough should be plastic, and the shape given should be maintained (Sumnu and Sahin, 2008).In rotary-molded dough's, the amount of sugar and fat is high, and the amount of water is low (sugar: 20 to 45%, fat: 10 to 40%, water: 5 to 15 for 100% (flour weight basis). This kind of dough can be crumbled easily, the maturity of gluten is undesired, and hence mixing duration is less. There are two steps in rotary-molded dough preparation: Creaming (premix) step and the Addition of flour. In the creaming step, all ingredients except flour are mixed and converted to cream. The creaming step should be prolonged as much as possible, which plays a crucial role in the density of dough. The second step of dough preparation, the addition of flour, takes a short time as formation of a gluten network should be prevented. If the duration of mixing is longer, gluten network formation starts and the dough will gain elasticity. As a consequence, a reduction in volume will be seen in dough during leaving of the molds. In the creaming step, sugar is dissolved and fat is softened, so they surround the protein molecules in flour and this prevents the interaction with water.

Fat is the most important input because it binds the ingredients in rotary-type dough's. Unless the dough is mixed enough, it cannot be formed and exit from the mold will be difficult. Flour strength, product type, dough temperature, mixer speed, and batch size are the most important factors affecting the mixing time for all of the biscuit types. Although dough's that are too cold can cause machining difficulties, high temperature developed during mixing is the usual problem. In any case, uniform temperatures are crucial for making uniform Biscuits as dough temperature affect spread, texture, and surface appearance of the biscuit.

2.8.2. Sheeting gauging and cutting

If biscuits are made by hand the shaping process would be to roll out the dough and use a cutter to cut the biscuits to shape. The scrap dough is then re-rolled and more pieces are cut with the excess being re-rolled and the process repeated until there is insufficient dough to make any more biscuits. It is also possible to shape biscuits by a mechanized system that does the same process. This is called sheeting gauging and cutting. Some biscuits are shaped by extrusion and depositing, while others are wire cut. The dough is fed from a hopper between rollers to produce dough of controlled thickness. The problem with this process is that the rolling works the dough, which causes the gluten to develop. This problem can be minimized by using only one set of rollers. The rollers that determine the thickness of the dough are known as gauge rollers. If the dough is fed to the cutter under tension the dough pieces will shrink during cooking, tending to emerge thicker at the front and back. The answer to this problem is to allow the dough to relax before cutting (Edwards, 2007).

2.8.3. Baking

While biscuits can be and are baked in almost any type of oven, including deck ovens, rack ovens and travelling ovens. In a tunnel oven it is sometimes best to arrange the zones so that the first zone is less hot than the second zone. This prevents the surface of the biscuit becoming too hard too quickly, which could produce a case hardened layer that resists the removal of moisture (Edwards, 2007). Dough pieces undergo physical and chemical changes within the oven. Crust formation, melting of shortening in the dough, conversion of water to steam, gas expansion, and escape of carbon dioxide, other gases, and steam are the physical changes occurring by heat treatment (Sumnu and Sahin, 2008). The first thing that will happen is that any gases, including air and carbon dioxide from leavening agents, will expand, causing the biscuit to expand. Water will be converted into steam; also causing the biscuit to expand this expansion is the oven spring.

Maillard reaction will take place between the proteins and reducing sugars on the surface. As the interior of the biscuit heats up by conduction this reaction will spread to the interior. If the biscuit is excessively alkaline from too much sodium bicarbonate a yellow color will be produced. The proteins present will start to denature and the starch will start to gelatinize. These processes cause the structure to set. Throughout the cooking water is lost from the biscuits. At the end of the cooking process this is the only change that happens. The loss of water will be controlled by the rate of diffusion of water from the middle to the surface. It is at this point that the dockering, i.e. the holes in the biscuit, assist the water removal (Edwards, 2007).

2.8.4. Cooling

The biscuits are cooled on a cloth band after leaving the oven, and they are very hot, very soft, and generally very moist as they emerge from the oven. Hence, even though cooling is a must for Packaging, it may be the least crucial aspect, for so many other things are taking place as the biscuit cools. Moisture loss, temperature decrease, and the changes in the state of the main ingredients affect biscuit dimensions, giving rise to shrinkage and causing stresses to be set up within the biscuit in reaching the set, non molten state. The mentioned stresses may cause cracking of the biscuits to a greater or a lesser degree under adverse conditions. Sudden cooling can be a reason for cracking, as it might firm up the crust and retard the moisture migration rate from the center crumb to the edges. This happens due to the excessive moisture gradient between these areas (Sumnu and Sahin, 2008).

2.8.5. Packaging

The product must be protected from excessive moisture change during its normal storage life as a primary requirement. When the packaging film protects against moisture transfer in an adequate manner, it likely excludes dirt, dust, mold spores, and other foreign particles, and in addition, it gives some protection against the absorption of off-odors. As most biscuits are very susceptible to crushing, mechanical strength should be present in the container if the biscuit is to survive storage and transportation. The package should contribute to the dimensional stability of the biscuit. The packaging material of the biscuits should be appropriate for being formed into the finished package easily and fast by mechanical ways. A fundamental need for packaging films is that the structure heat seal readily. Moreover, the packaging material should not tear, crack, or stretch during the rapid transfers and folding in the wrapping equipment (Sumnu and Sahin, 2008).

2.9. Functional and Physical Properties of Biscuit

The functional properties of flours play important role in the manufacturing of products (Baljeet *et al.,* 2010). Functional properties are refer to those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption as have been defined by (Kinsella, 1976).

2.9.1. Bulk density

Bulk density (BD) gives an indication of the relative volume of packaging material required. Bulk density is a reflection of the load the flour samples can carry if allowed to rest directly on one another. Lower bulk density is desirable for flour because it enhances the greater ease of dispersibility (Udensi and Okoronkwo, 2006).The density of processed products dictate the characteristics of its container or package, product density influences the amount and strength of packaging material, texture or mouth feel (Wilhelm *et al.*, 2004).Baking at highest temperature and relatively longer time causes a product to be denser and such type of product requires stronger or multilayered packaging material.

2.9.2. Water activity

Water Activity (*aw*) indicates the amount of "free water" in a sample. "Free water" refers to the water molecules in a sample that are not chemically or physically bound. Water activity is equivalent to Equilibrium Relative Humidity (ERH), which is the ratio of water vapor pressure above any sample to the water vapor pressure of pure water at the same temperature. Microorganisms require water for survival, too much "free water" in a sample can serve as a medium for microbial reproduction, travel, and contamination. If water within a sample is held with enough force, microorganisms will not be able to exert the energy required to obtain water necessary for their subsistence. Because of this, *aw* is commonly used in the evaluation of quality and safety of foods, drugs and cosmetics (Worobo and Zakour, 1999) Water activity is one of the most important properties of food governing microbial growth. The water activity can be defined as the free or available water in a food product. The requirements for moisture by microorganisms are expressed in terms of aw. Therefore, aw will determine what the lower limit for microbial growth will be in a food product. Food products can be broadly classified into:

1. High *aw* (> 0.8)
2. Intermediate *aw* (0.7 to 0.8)
3. Low *aw* (< 0.7).

Ingredients, e.g. salt and sugars and processing techniques, e.g. baking ,drying, curing, cooking etc. used in the production of food products, will influence the *aw* and therefore the safety and shelf-life of the product. As with pH, the growth range for microorganisms is defined by the minimum, maximum and optimum values of *aw*. Most microorganisms cannot grow below *aw* of 0.60 (i.e. no microbial growth occurs below *aw* of 0.50) with the majority growing at *aw* ≤ 0.90.

2.9.3. Rehydration Ratio

In term water rehydration capacity, water absorption, water binding, and water holding ability are used interchangeably in the literature to denote maximum amount of water that protein material can take up and retain under food formulations. The maximum Water rehydration capacity of protein is very important because it affects the texture and also the ability of protein to bind and retain water enhances the shelf life of bakery products (Hettiarachchy and Kalapathy, (1997).

2.10. Role of Baking in Product Quality

Baking `is a complex process that brings about a series of physical, chemical and biochemical changes in food such as gelatinization of starch, denaturation of protein, and liberation of carbon dioxide from leavening agents, volume expansion, and evaporation of water, crust formation, and browning reactions. It can be described as a simultaneous heat and mass transfer within the product and with the environment inside the oven. Baking test is the final test to judge flour for quality for a particular end product. Flour test baking procedures are chosen in relation to the ultimate purpose for which flour is intended that is bread, cake, and biscuit making. The test is the final criteria by which quality of bread, cake and biscuit flour are judged (Kaur *et al.*, 2004).

Baking involves three major changes to the dough piece in its transformation into a biscuit. These changes are: an increase in thickness (the development of an open internal structure); development of a reddish brown surface coloration (due, principally, to the Mailliard reaction); and a significant reduction in moisture. All of these changes are accomplished by the supply of heat to dough piece. If the heat is not supplied at the optimum rate one or all of desired change will be different from that which is the target (Manley, 1998).The design and control of an oven is principally a matter of heat transfer, but for the baker it is a matter of temperatures and turbulence at specific stages. Heat is transferred much more effectively if the air is moving near the dough piece at a given temperature.

The main nutritional changes during baking occur at the surface of foods, and the ratio of surface area to volume is therefore an important factor in determining the effect on overall nutritional loss. During baking, the physical state of proteins and fats is altered, and starch is gelatinized and hydrolyzed to dextrins and then reducing sugars. However, in each case the nutritional value is not substantially affected. The loss of amino acids and reducing sugars in Maillard browning reactions causes a small reduction in nutritive value. In particular, lysine is lost in Maillard reactions, which slightly reduces the protein quality. The extent of loss is increased by highest temperatures, longer baking times, and larger amounts of reducing sugars. In biscuits, a reduction in dough thickness from 4.9 mm to 3.8 mm each baked at 170ºC for 8 min, produced highest losses of amino acids as follows: tryptophan, from 8% to 44%; methionine, from 15% to

48%; lysine, from 27% to 61%. In maize, lysine loss is increased from 5% to 88% during the manufacture of breakfast cereals, which is corrected by fortification (Fellow, 2000).

2.10.1. Effect of baking temperature and time on sensory qualities of biscuit

The purpose of baking is to alter the sensory properties of foods, to improve palatability and to extend the range of tastes, aromas and textures in foods produced from similar raw materials. Baking also destroys enzymes and micro-organisms and lowers the water activity of the food to some extent thereby preserving the food (Fellow, 2000). During baking sensory and nutritional values of foods are altered positively or negatively. To consumer, the most important quality attributes of biscuit are its sensory characteristics (texture, flavor, aroma, and color) and these characteristics are influenced during baking. Biscuit structure and thickness are developed during baking. The changes are all temperature and times related and involve several aspects of the recipe and formed dough piece. Liberation of gases from leavening chemicals or water vapor are formed which expand and result in a large reduction in the density of the dough. The open porous structure gives a biscuit a pleasant eating texture. The development of the structure is often known as oven spring as it relates to the thickness of the baked biscuit (Manley, 1998). The aromas produced by baking are an important sensory characteristic of baked goods.

The high temperatures and low moisture contents in the surface layers also cause caramelisation of sugars and oxidation of fatty acids to aldehydes, lactones, ketones, alcohols and esters. The Maillard reaction and degradation produce different aromas according to the combination of free amino acids and sugars present in a particular food. Each amino acid produces a characteristic aroma when heated with a given sugar, owing to the production of a specific aldehyde. Different aromas are produced, depending on the type of sugar and the heating conditions used. Further heating degrades some of the volatiles produced by the above mechanisms to produce burnt or smoky aromas, therefore a very large number of component aromas produced during baking. The type of aroma depends on the particular combination of fats, amino acids and sugars present in the surface layers of food, the temperature and moisture content of the food throughout the heating period and the time of heating (Fellow, 2000). Although there is usually a change to a yellow brownish hue during baking, the term color here is used to imply merely a darkening, reduction in reflectance, of the biscuit surface. There a number of reasons for the color changes.

The main one is the Maillard reaction, non-enzymic browning, which involves chemical reaction between reducing sugars in the dough with proteins and produces attractive reddish brown hues. This occurs around 150-160°C and will occur faster in a mildly alkaline situation and only in a moist situation. Color also develops associated with dextrinisation of starch and caramelisation of sugars. At even highest temperatures the biscuit structure chars or burns (Manley, 1998).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) Post-Harvest Management laboratory, Animal nutrition laboratory, and Ethiopian Public Health Institution (EPHI) Addis Ababa between 2014 and 2015. During the experiment the room temperature and relative humidity in postharvest management laboratory was 22-25^{0}C and 56-60% respectively. Average temperatures of Animal nutrition laboratory and Ethiopian Public Health Institute (EPHI) were 24^{0}C and 23^{0}C respectively.

3.2. Experimental Material Collection and Preparation

Teff varieties used for preparation of biscuits were selected based upon their market demand and color "Kuncho" teff variety developed by Ethiopian Institute of Agricultural Research (EIAR) has more than doubled teff productivity: up 137% from 1.6 tons per hectare to 3.8 tons per hectare (USAID and CIAFS, 2012). As a white color teff (V-1), DZ-Cr-387/RIL-355(Kuncho) was selected while Brown color (V-2) DZ-01-1681(Keyetena) was considered as a second variety. The teff grains were brought from Debre Zeit Agricultural Research Center (DZARC). Both varieties were manually cleaned, milled, sieved using 0.5 mm sieve, and stored in High density polyethylene bag till further use. Other ingredients like sugar, milk powder, mayonnaise, salt and baking powder was purchased from Jimma local market based on their shelf life. Fresh egg was obtained from JUCAVM Animal science Department.

Figure 1 Kuncho and keyetena teff used as raw materials in the experiment

3.3. Experimental Plan

Response surface methodology which involves design of experiments, selection of levels of variables in experimental runs, fitting mathematical models and finally selecting variables' levels by optimizing the response (Khuri and Cornell, 1987) was used in the study. A Central Composite Design (CCD) was used to design and conduct the experiments comprising three independent processing parameters (Table 3). According to Dogan (1998) baking condition changed with oven design and operating parameters. These parameters must be well established and controlled for each type of baked products. The optimum baking temperatures and time for the Orlandi electric oven used were determined in the preliminary study to be 170^0C, 180^0C and 190^0C and baking time 5min, 10 min and 15 min, based Cauvain and Young (2006) set for biscuit.

Table 3 Experimental plan used for teff biscuit preparation

Parameter	Amount	Description
Product	1	Biscuit
Ingredients	7	Baking powder ,Sugar ,Milk, Egg, Mayonnaise, Flour and Salt
Teff varieties	2	White Teff (V-1 Kuncho) and Red Teff (V-2 Keyetena)
Baking temperature	2	Ranges from170^0C to 190^0C
Baking time	2	Ranges from 5 to 15 minute
Biscuit thickness	2	Ranges from 4 to 7 mm
Analysis	4	Physical properties, Nutritional composition, mineral and sensory analysis
Packaging material	1	polyethylene bags

3.4. Procedure for Preparation of Teff Biscuits

Biscuits were prepared according to method described by American Association of Cereal Chemists (AACC, 2000, Method No.10.52) with slight modifications. The production process of biscuit involves unit operations like raw materials preparation, dough mixing, laminating, cutting/shaping, baking, cooling, and packaging. All materials except flour were preparatory mixed in a cream mixer .Materials like Teff flour, sugar; salt, mayonnaise, egg, and baking powder weighed accurately. The pre-weighed flour, sugar, salt, and baking powder mixed carefully. Then egg was added and mixed properly to make adequate dough. Mixtures kept at a room temperature for about 10 minutes and allowed for proper resting. In the food industry, bubble formation is used to (23-25 ^{0}C) for 15 minutes to create structure and texture which is primarily responsible for the product attributes (Aguilera and Germain, 2007). The sheet cut according to the desired shape and size of biscuits with a circular cutter of 52 mm diameter and would be baked in mini kitchen oven (model NT 655 China) at different temperature ranges for different time (min). All prepared biscuit baked on the second zone of oven and the oven heated for 10 minutes at 10^{0}C highest than desired temperature until the desired temperature achieved. In addition heat resistant glass thermometer used to check oven temperature before biscuit baking. Baked biscuit would be cooled to ambient temperature and packed with high density polyethylene bags. All combinations followed similar procedure.

Table 4 The proportion of various ingredients for preparation of gluten free biscuits adopted from Mohamed *et al.*, (2004) with minor modification.

Ingredients	**Amount**	**Percentages (%)**
Teff flour (g)	250	52.7
Mayonnaise(g)	45	9.5
Sugar (g)	60	12.7
Salt (g)	2.5	0.56
Baking powder (g)	5	1.08
Milk powder (g)	30	6.38
Whole egg (g)	50	10.6
Deionized Water (ml)	30	6.38

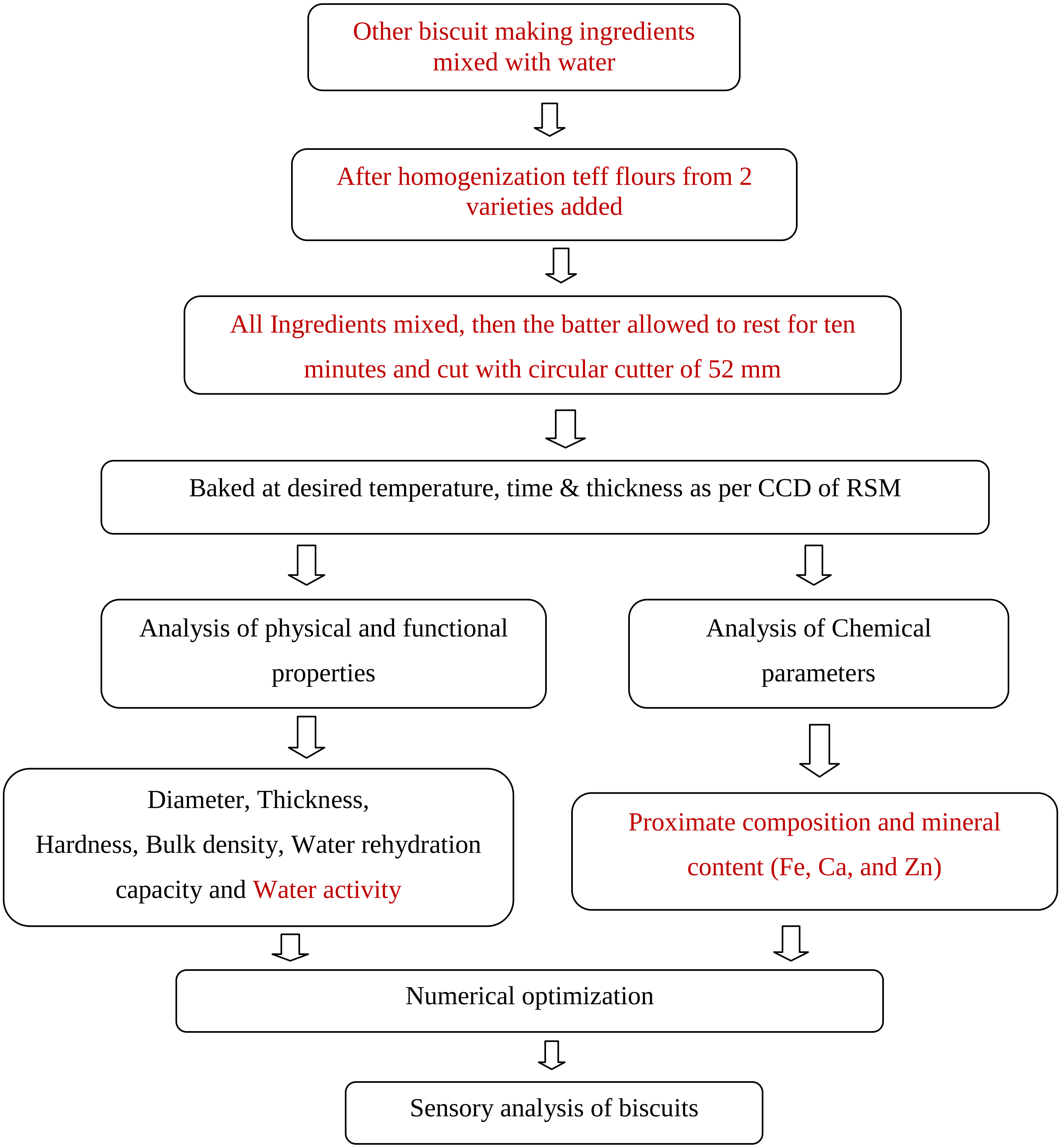


Figure 2. Frame work of the research experiment

3.5. Experimental Design

The study was conducted in two separate phases. In first phase optimization study was done to determine better baking temperature, time and Thickness of teff biscuit. To accomplish this, treatment combination for each Teff variety was designed using A statistical software package (Design-Expert ®, version 6.02, Stat-Ease (Minneapolis USA). Response Surface Methodology specifically Central Composite Design (CCD) used for each variety of teff.

Table 5 Upper and lower limits of baking temperature, time, and thickness of biscuit for two teff varieties.

Teff variety	**Baking temperature(^{o}C)**		**Baking time (min)**		**Biscuit thickness (mm)**	
	Maximum	Minimum	Maximum	Minimum	Maximum	**Minimum**
White (Kuncho)	186	174	12	4	6.5	**4.5**
Red (Keyetena)	186	174	12	4	6.5	**4.5**

Table 6 Treatment combinations developed based upon CCD for both Teff varieties

Stad	**Run**	**Block 1**	**Temperature**	**Time**	**Thickness**
6	1	Block 1	186.0	6.0	6.5
8	2	Block 1	186.0	12.0	6.5
13	3	Block 1	180.0	9.0	3.8
15	4	Block 1	180.0	9.0	5.5
10	5	Block 1	190.1	9.0	5.5
5	6	Block 1	174.0	6.0	6.5
19	7	Block 1	180.0	9.0	5.5
18	8	Block 1	180.0	9.0	5.5
17	9	Block 1	180.0	9.0	5.5
1	10	Block 1	174.0	6.0	4.5
14	11	Block 1	180.0	9.0	7.2
16	12	Block 1	180.0	9.0	5.5
7	13	Block 1	174.0	12.0	6.5
4	14	Block 1	186.0	12.0	4.5
12	15	Block 1	180.0	14.0	5.5
3	16	Block 1	174.0	12.0	4.5
2	17	Block 1	186.0	6.0	4.5
20	18	Block 1	180.0	9.0	5.5
11	19	Block 1	180.0	4.0	5.5
9	20	Block 1	169.9	9.0	5.5

Based up on physical and nutritional analysis of biscuits baking temperature, time and thickness were numerically optimized. Second phase focused on optimum combination of baking conditions were selected and further sensory analysis was conducted for both varieties of teff biscuit.

Table 7 Alpha values for coded factors used in production of biscuits from kuncho and keyetena teff

Code for values	Processing parameters and values		
	Baking temperature (°C)	Baking time (Minutes)	Biscuit thickness (Millimeter)
−1.68	170	4	3.8
−1	174	6	4.5
0	180	9	5.5
+1	186	12	6.5
+1.68	190	14	7.2
No. of experiments	Coded processing parameters		
	X1	X2	X3
8	±1	±1	±1
2	0	0	±1.68
2	0	±1.68	0
2	±1.68	0	0
6	0	0	0

*Code '0' is for centre point of the parameter range investigated, '±1' for factorial points and'±1.682' for augmented points: X1, for Baking temperature; X2, baking time; X3, biscuit thickness having twenty treatment combination.

3.6. Data Collected

3.6.1. Determination of functional and physical properties of biscuits

3.6.1.1. Bulk densities of flour

Bulk density was determined by the method of Adeleke and Odedeji (2010), 50 g flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped several times on a laboratory bench to a content volume. The volume of sample is recorded. Bulk density was calculated from the values obtained as follows.

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of sample}}{\text{Volume of sample after tapping}} \quad \text{..................................Equation 1}$$

3.6.1.2 Rehydration Ratio (RR)

Rehydration ratio (RR) is used to describe the capability of the water absorption in the sample. About 20g broken biscuit samples were weighted (M1) and placed in 500ml of water at 30^{O}C for 15min. The water was drained and the rehydrated samples were weighed (M2). The values kept as percentages. RR is calculated as follows (Eq. 2)

$$RR = \frac{M2 - M1}{M1} \quad \text{..Equation 2}$$

Where M2 = rehydrated samples

M1= broken biscuit samples

3.6.1.3. Diameter

Diameter was measured by two ways that was a digital Venier caliper (Fowler, US) and calibrated ruler as described by Ayo *et al.* (2007). The biscuits were rotated at an angle of 90^{0} for duplicate reading and diameter of the six biscuits was measured with a calibrated ruler divided by six and average diameter was taken in millimeters (AACC, 2000). Difference in diameter calculated by

Biscuit diameter after baking - Biscuit diameter before baking

3.6.1.4. Thickness

Thickness was determined using calibrated ruler as described by Ayo *et al.*, (2007) and using digital Venier caliper (Fowler, US). The measurement was repeated thrice to get an average value and results was reported in mm (AACC, 2000). Difference in thickness calculated by

Biscuit thickness after baking - Biscuit thickness before baking

3.6.1.5. Hardness

Hardness of biscuit was determined with stable micro system Texture Analyzer (TA-XT plus, 2012, UK). Hardness of biscuits is defined as the maximum force (gm, kg, N) which a biscuit can bear before breaking. Hardness of biscuits was measured using (HDP/BSK blade set with knife) probe. Force and height were calibrated and then a test was run by placing sample on the platform of the texture analyzer. The studies were conducted at pre-test speed of 5.0 mm/sec, test speed of 1.0 mm/sec, post test speed of 10 mm/sec, distance 5 mm, Trigger force of 30 g, and load cell of 5 kg. Hardness was measured in terms of major positive peaks (Nath and Chattopadhyaya, 2007). A major peaks obtained in the product during compression. The first peak of the force-distance plot of the texture analyzer was interpreted as hardness of the product, expressed in Newton (N) (Chakraborty *et al.*, 2009).

3.6.2. Determination of water activity

Water activity (*aw*) was measured at 23°C±2°C using Lab Master aw (Novasina AG, CH-8853 Lachen Sprint Switzerland) water activity meter (Hematian Sourki *et al.*, 2010). Prior to the measurement, the sample was carefully homogenized (for 5–10 min) in a mortar. The values of *aw* represent the arithmetic means three independent determinations.

3.6.3. Proximate analysis

Proximate composition for flours and biscuits were done for each teff variety. All the analyses were done in duplicate and average value was taken.

3.6.3.1. Determination of moisture content

Moisture of the sample was determined by air oven method (Model: Leicester, LE67 5FT, England) according to AOAC, (2011) Method 925.10. The petridish was dried at 130^{o}C ± 3^{o}C for 1hr and placed in desiccators and weighed after cooling. About two grams of well-mixed teff biscuit sample was weighed in to dry dish. The dry dish and its contents were placed in the oven for 1 hr provided with opening for ventilation and maintained at 130^{o}C ± 3^{o}C. The dish removed from oven and transferred in to desiccators and weighed soon after reaching room temperature. Then, the moisture content was estimated by the following formula.

Moisture (%) = $\frac{(M_{initial} - M_{dried})}{}$ x 100……………………………………………...Equation 2

$M_{initial}$

Where $M_{initial}$ = sample weight before oven drying

M_{dried} = sample weight after oven drying

3.6.3.2. Determination of total ash

The crucibles used for the analysis were cleaned and dried at 120°C and ignited at 550°C in muffle furnace (Model SX-5-12, China) for 3 hours. Then the crucibles were removed from the furnace and cooled in a desiccators .The mass of the crucible was measured by analytical balance (M1).About two grams of the sample was weighed and put in to crucibles (M2).The sample was dried at 120°C for 1hr in a drying oven. The sample was then placed in furnace at about 550°C until free from carbon and the residues appeared grayish white (about 8 hours). Finally the sample was taken out of the furnace and allowed to cool inside desiccators and weighed (M3), the ash content was determined by official method (AOAC, 2011, 923.03) and applying the following simple formula:-

$$\textbf{Total ash}(\%) = \left(\frac{M3-M1}{M2-M1}\right)\textbf{x100} \quad \text{.....Equation 3}$$

Where M1 = weight of crucible

M2 = weight of crucible and sample

M3 = weight crucible and ash

3.6.3.3. Determination of crude protein

The protein content was calculated from the nitrogen content of the biscuit, which was determined by Kjeldahl method involving digestion, distillation, and titration (AOAC, 2011) method number 988.05. About 0.3gm of sample was measured by analytical balance (Model:ABJ220-4M, WB1151070, Australia) , a gram of catalyst mixture of K_2SO_4 and $CuSO_4$ and five milliliter of sulfuric acid added to each digestion flask (Kjeldahl flask KF250,German) which contained the mixture of sample and catalysts. The solution (0.3gm of sample + 1gm of K_2SO_4 and copper sulfate + 5ml of H_2SO_4) immediately placed in digestion flask at about 420°C for 3-4 hrs, until the solution became clear. The digested sample was then transferred into the distillation apparatus and 25ml of 40% (w/v) NaOH was added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. The mixtures were then steam distilled and the liberated ammonia was collected into a 200ml conical flask containing 25ml of 4% boric acid plus mixed methyl red indicator solution. Next distillation was carried out into the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink colour solution of the receiver flask turned green indicating the presence of ammonia. Distillation was continued until the content of the flask was reaching the required amount. The green color solution was then titrated against 0.1N HCl solutions. At the end point, the green colour turned to red pink colour, which indicated that, all the nitrogen trapped as ammonium borate have been removed as ammonium chloride. The distillate was titrated with standardized 0.1N sulfuric acid to a reddish color. Ultimately the percentage of nitrogen content was estimated using the following formula:

$$\% \boldsymbol{Total\ Nitrogen} = \frac{(V_A - V_B) \times N \times 14.01}{W} \times \mathbf{100}$$ ....Equation 5

Where, VA = volume (ml) of the HCl solution consumed in the sample titration

V_B = volume (ml) of the standard solution used in the sample blank titration

N = Normality of sulfuric acid used (0.1N)

14.01= the molecular weight of nitrogen

W = weight of sample (g)

Crude protein content percent per weight = Total Nitrogen * universal conversion factor

N.B: The % of nitrogen is converted to % of protein by using appropriate conversion factor i.e., (6.25 for biscuits and 5.7 used for teff flour).

3.6.3.4. Determination of ether extract

Ether extract is the term used to refer to the crude mixture of fat-soluble material present in a sample and determined with Soxhlet method of solvent extraction was used during determination of fat (Model:SZC-C fat determinate, China) according to AOAC, (2011) method 45.06 as follows. About 2g of grinded samples were measured in to an extraction thimble lined with fat free cotton. Then the thimbles with the samples were attached to the extraction apparatus. The aluminum cup were placed in the oven at 100^0C for 30 minutes and cooled to room temperature in the desiccators for 30 minutes then the cup weight was measured using a digital balance and recorded as W1 (weight of cup), then 50ml of diethyl ether was added in to each cup, after which set up of the extraction apparatus was done. The samples contained in the thimbles were soaked for about 1 hour by lifting down the thimble in to the cup, started from the apparatus hot plate temperature reached 55^0C, after soaking the thimbles were lifted up and extraction process was taken place for 5h. Then the recycling process made by the diethyl ether was stopped to let the solvent to evaporate from the aluminum cup with the extract, in the process the evaporated solvent was recovered in the apparatus. Then aluminum cup and the content were dried in the oven for 30 min at 100^0C to evaporate the remaining solvent in the cup. After drying it was removed from the oven and cooled in the desiccators for 30 minutes then weighed and recorded as W2 (cup + fat). The percentage fat was calculated using the following formula.

$$\text{Ether Extract } \mathbf{(\%)} = \frac{W_2 - W_1}{W} \times 100 \text{ ....Equation 6}$$

Where:

W_1= Weight of extraction flask before extraction

W_2 = Weight of extraction flask after extraction

W= weight of samples (g)

3.6.3.5. Determination of crude fiber

The determination of crude fiber content of samples was executed as per the procedures stated under (AOAC, 2005, method 962.09). About 1.5 g of sample was weighed in each of 600 ml beaker. A 200 ml of 1.25% sulfuric acid Solution was added to each beaker and allowed to boil for 30 min by rotating and stirring periodically. During boiling the level was kept constant by addition of hot distilled water. After 30 min, 20 ml of 28% potassium hydroxide solution was added in to each beaker and again allowed to boil for another 30 min. The level was still kept constant by addition of hot distilled water. The solution in each beaker was then filtered through crucibles containing sand by placing each of them on Buchner funnel fitted with No.9 rubber stopper. During filtration the sample was washed with hot distilled water. The final residue was washed with 1% sulphuric acid solution, hot distilled water, 1% sodium hydroxide solution and finally with acetone. Each of the crucibles with its contents was dried for 2 hr at 130^0C and cooled in desiccators and weighed (M1). Then again they were ashed for 30 min at 550^0C in furnace (Gallenkamp, Model FSL 340-0100, UK) and were cooled in desiccators. Finally the mass of each crucible was weighed (M2) to subtract Ash from fiber. The crude fiber was calculated from the following equation:

$$\textbf{Crude fiber \%} = \frac{M1-M2}{\text{weight of sample}} \times 100 \text{...Equation 7}$$

Where M1=mass of the crucible, the sand and wet residue,

M2=mass of the crucible, the sand and ash

3.6.3.6. Determination of utilizable carbohydrates

Total percentage carbohydrate was determined by the difference method as reported by Ponka *et al*, (2005). It was determined by subtracting the total percentage values of crude protein, Ether Extract, moisture, crude fiber, and ash constituents of the sample from 100. The value obtained is the percentage carbohydrate constituent of the sample.

Carbohydrate (%) = (100 - (% P+% EE+% M+% F + % A))..Equation 8

Where P= % Protein;

EE = % Ether Extract;

F = % fiber;

A = % ash and

M = % moisture content

3.6.3.7. Gross energy (kcal/100g)

Gross energy of teff flour and biscuit samples were calculated according to the method developed by Osborne and Voogt, (1978).The energy content of each individual variety and biscuit samples were calculated as follow:-

Caloric value = (9 x fat %)+(4 x protein %)+(4 x carbohydrate %)..............................Equation 9

3.6.4. Mineral analyses (calcium, zinc, and iron)

Mineral analyses were accomplished using Atomic Absorption Spectrophotometer (AAS) Auto sampler AA 6800, Japan) method as per the AOAC, (2005**)** method, 985.35. For this purpose, 1.5 g of teff biscuit samples was ashed and weighed. The white ash was treated with 5ml of 6N HCl and dried on the hot plate. Then 15 ml of 3N HCl was added and the crucible was heated on a hot plate until the solution just boiled. The solution was cooled and filtered through a filter paper in to 50 ml graduated flask then make up with distilled water. Then the solution was used to determine Ca, Zn, and Fe. Standard stock solution of iron, zinc, and calcium was made by appropriate dilution. The sample and standard was atomized by using reducing air-acetylene for Ca and oxidizing air-acetylene for zinc and iron as a source of energy for atomization. For Iron content determination absorbance was measured at 248.4 nm and iron was estimated from a standard calibration curve prepared from analytical grade iron with a range of 0, 2, 4, 6, 8, and 10 ml. For zinc concentration determination, absorbance was measured at 213.9 nm and zinc level was estimated from a standard calibration curve prepared from analytical grade zinc with a range of 0, 0.5, 1, 1.5, 2, and 2.5 ml. For Calcium content determination, absorbance was measured at 422.7 nm after addition of 2.5 ml of $LaCl_3$ was added to sample solution and standard to suppress interferences. Calcium content was then estimated from standard solution 0, 2, 4, 6, 8, and 10 ml prepared from $CaCO_3$.

3.6.5. Sensory evaluation

The different biscuits made from two varieties of teff were subjected to sensory evaluation. Biscuit prepared from each twenty, baking conditions optimum were selected based on physical properties and nutritional composition. Biscuit baked under optimized conditions were coded with three digits randomly and allowed for sensory evaluation. Sensory attributes measured were color, taste, aroma, crispness, and overall acceptability using a five point Hedonic scale consisting of 1 (extremely dislike), 2 (dislike moderately), 3 (neither like nor dislike), 4 (like moderately) and 5 (extremely like) (Meilgaard *et al.,* 2007). A total 50 untrained panelists were participated in this study. After tasting each coded sample they rinsed their mouths before moving on to the next sample and use expectorate for spitting. Then the score of all judges for each sample were summed and divided by the number of panelists to find the mean value.

3.7. Data Analysis

A statistical software package (Design-Expert ®, version 6.02, (Minneapolis USA) was used to generate test of factor combination for better quality biscuit. Response surface methodology which involves design of experiments, fitting mathematical models and finally selecting levels of variables by optimizing the response (Khuri and Cornell, 1987) was employed in the study. The combinations were obtained based on a CCD. The statistical significance of the terms in the regression equations was examined by analysis of variance (ANOVA) for each response and the significance test level was set at 5%.

There were twenty experimental runs conducted for the present research work. There were six experiments at center point to calculate the repeatability of the method (Montgomery, 2001). Numerical optimization was used to find a point that maximizes the desirability function and it was carried out to determine the optimum baking temperature, time and thickness of biscuits baked from for both types of teff and to select best combination for baking nutritionally quality biscuit. The processing parameters were kept within range while the responses, diameter, thickness, water rehydration capacity, ash, fat, fiber, protein, carbohydrate, energy, iron, calcium, and zinc were maximized, whereas bulk density, water activity, hardness, and moisture content were minimized

Data obtained from the sensory evaluation of biscuits were analyzed using completely randomized design with three replicates. Minitab version 16.0 statistical software was used to generate test for analysis of means and significant means were separated using Tukey at 5%.

4. RESULTS AND DISCUSSION

4. 1. Effects of Baking Conditions on Physical and Functional Properties

4.1.1. Diameter difference

Diameter ranged from 52.3-53.8 and 52.5-54.0 mm for Kuncho and Keyetena based biscuit. Result shows that the diameter of both teff based biscuits were found not significantly influenced by interaction of baking temperature, time and biscuit thickness ($P<0.05$). However, when baking conditions are considered largest diameter was recorded from biscuit baked at 174^{o}C, 6 min, 6.5 mm thick and lowest at 186^{o}C, 12 min, 4.5 mm thick biscuit baked from Kuncho and Keyetena teff. But relatively highest temperature and longer baking time with lowest thickness had an impact on diameter of biscuit. The principal criterion for good biscuit-making quality is the diameter increases during the baking process so in this finding as diameter of biscuit increased as observed in all baked biscuit. Biscuit baked for short time at low temperature have large diameter this finding was in line to finding of Labuschagne *et al*. (1996) have shown large diameter considered as superior.

4.1.2. Thickness difference

Difference in thickness ranged from -0.1 to 0.6 mm for both Kuncho and Keyetena teff based biscuits as shown on Table 8. Difference in thickness before and after was recorded and the larger difference obtained from biscuit baked at 174^{o}C, 6 min, 6.5 mm thick for biscuit baked from both Kuncho and Keyetena teff. Lower recorded from biscuit baked at 190^{o}C, 9 min, 5.5 mm and 186^{o}C, 12 min, 4.5 mm thickness. Result shows that the thickness of both teff based biscuits were found not significantly influenced by interaction of baking temperature, time and biscuit thickness ($p< 0.05$). Lower and highest results in both cases might be highly associated with temperature of oven on biscuits as the effect of temperature not only found significant on main terms but also in quadratic term. This finding supported by Biniyam, (2010), the average cookie thickness was not significantly reduced but a decrease in thickness of the cookies were observed as the baking temperature and time was increased this could be the reason that as the baking temperature and time increased reduction in moisture content reduce the cookie thickness.

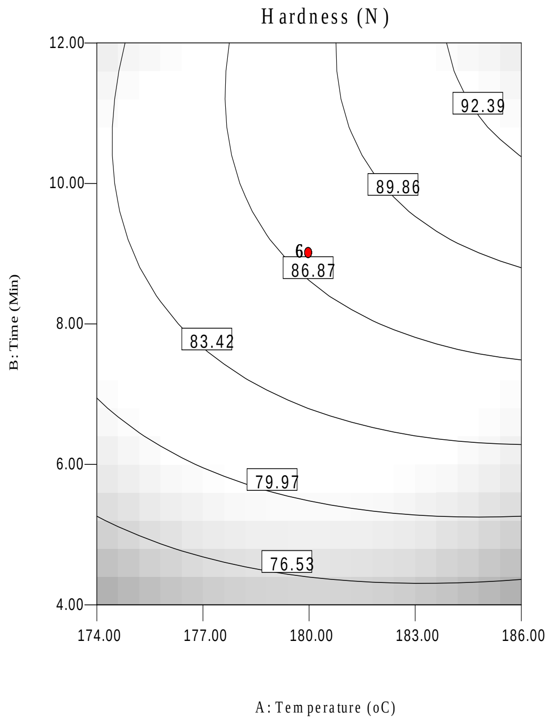
Table 8 Measured Values for Physical and Functional Properties of Teff Biscuit

					Kuncho variety							Keyetena variety						
Std	Run	A	B	C	Diameter (mm)	Thickness (mm)	D/ce in thickness(mm)	Hardness (N)	*aw*	Bulk density (g/ml)	RR (%)	Diameter (mm)	Thickness (mm)	D/ce in thickness(mm)	Hardness (N)	*aw*	Bulk density (g/ml)	RR (%)
6	1	186	6.0	6.5	53.4	6.8	0.3	80.40	0.59	0.72	13.25	53.4	6.8	0.3	85.4	0.60	0.70	12.90
8	2	186	12.0	6.5	52.6	6.5	0.0	90.30	0.43	0.76	15.50	52.7	6.5	0.0	95.3	0.44	0.74	15.40
13	3	180	9.0	3.8	52.8	4.0	0.2	88.10	0.47	0.75	16.05	53.0	4.0	0.2	93.1	0.48	0.74	15.75
15	4	180	9.0	5.5	53.4	5.8	0.3	87.00	0.52	0.71	15.30	53.6	5.9	0.4	92.7	0.52	0.69	15.10
10	5	190	9.0	5.5	52.4	5.4	-0.1	91.00	0.41	0.78	15.10	52.6	5.4	-0.1	96.0	0.42	0.76	15.05
5	6	174	6.0	6.5	53.8	7.1	0.6	75.80	0.63	0.68	12.25	54.0	7.1	0.6	80.8	0.64	0.66	12.10
19	7	180	9.0	5.5	53.3	5.8	0.3	86.20	0.50	0.71	15.50	53.5	5.8	0.3	92.6	0.53	0.69	15.35
18	8	180	9.0	5.5	53.0	5.8	0.3	87.60	0.51	0.71	15.40	53.2	5.8	0.3	92.6	0.52	0.69	15.05
17	9	180	9.0	5.5	53.2	5.7	0.2	87.80	0.50	0.72	15.35	53.4	5.7	0.2	92.6	0.51	0.70	15.20
1	10	174	6.0	4.5	53.6	4.8	0.3	78.80	0.54	0.7	14.20	53.8	4.8	0.3	83.8	0.55	0.67	13.95
14	11	180	9.0	7.2	53.5	7.6	0.4	81.80	0.60	0.72	13.35	53.6	7.7	0.5	86.8	0.61	0.70	13.15
16	12	180	9.0	5.5	53.4	5.8	0.3	87.40	0.51	0.72	15.25	53.5	5.8	0.3	92.6	0.52	0.69	15.10
7	13	174	12.0	6.5	53.2	6.8	0.3	78.80	0.56	0.74	13.65	53.4	6.8	0.3	83.8	0.57	0.72	13.45
4	14	186	12.0	4.5	52.3	4.4	-0.1	95.20	0.40	0.79	16.25	52.5	4.4	-0.1	100.2	0.39	0.77	16.15
12	15	180	14.0	5.5	52.6	5.5	0.0	88.20	0.44	0.78	16.25	52.8	5.5	0.0	92.7	0.42	0.76	16.00
3	16	174	12.0	4.5	53.0	4.7	0.2	83.90	0.45	0.76	15.95	53.2	4.7	0.2	88.9	0.46	0.74	15.55
2	17	186	6.0	4.5	53.2	4.8	0.3	82.90	0.54	0.77	13.85	53.4	4.8	0.3	87.9	0.55	0.75	13.55
20	18	180	9.0	5.5	53.3	5.7	0.2	88.40	0.51	0.71	15.40	53.5	5.7	0.2	92.7	0.52	0.69	15.20
11	19	180	4.0	5.5	53.6	6.0	0.5	74.90	0.62	0.69	12.50	53.8	6.0	0.5	79.9	0.63	0.66	12.30
9	20	170	9.0	5.5	53.7	5.8	0.3	77.90	0.53	0.7	14.00	53.8	5.8	0.3	82.8	0.54	0.68	13.80

Where: A=temperature, B=time and C=thickness RR= Rehydration Ratio *aw* = water activity.

4.1.3. Hardness

Hardness showed highly significant difference in main, quadratic and interaction of model terms at ($p<0.05$). Data for Hardness varied from 75 N to 95 N for Kuncho and 80 N to 100 N for Keyetena based biscuits (Table 8). Biscuit made from the latter variety was a bit harder to break than the former one. This might be associated with the high iron content and relatively highest crude fiber of Keyetena based biscuit as compared to Kuncho (Umeta and Parker, 1996).Peak forces recorded for both kuncho and keyetena based biscuits baked at 186^{o}C, 12 min and 4.5 mm thick and lower value from 180^{o}C, 4 min, 5.5 mm thick. Similar increase in hardness of cookies was also reported by Singh *et al*. (2008) on incorporation of sweet potato flour in wheat and he concluded that an increase in fiber content in the formulation was the reason for



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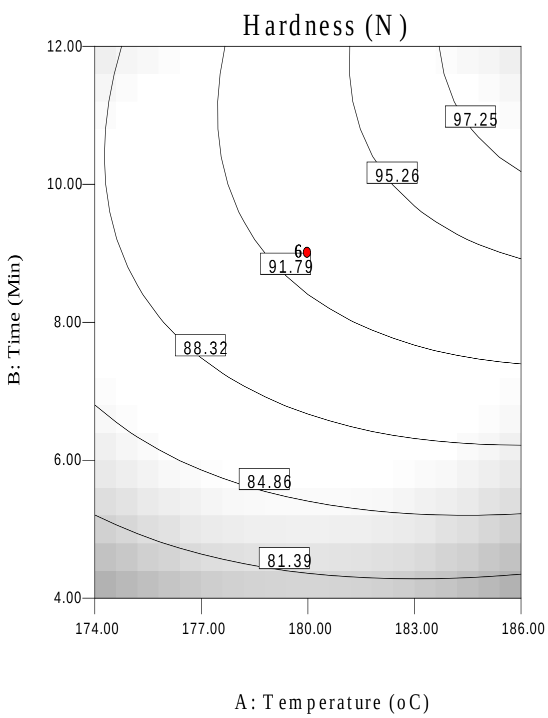


(a) (b)

Figure 3. Effects of baking temperature and time on hardness of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Table 9 Estimated regression coefficients, degree of significance and lack of fit of parameters for physical and functional properties of biscuits model equations.

	Kuncho						keyetena					
Source	D/ce in Diameter (mm)	D/ce in thickness (mm)	Hardness (N)	Water activity	Bulk density (g/cm^3)	Rehydration ratio (%)	D/ce in Diameter (mm)	D/ce in thickness (mm)	Hardness (N)	Water activity	Bulk density(g/cm^3)	Rehydration ratio (%)
Model	***	***	***	***	***	***	***	***	***	***	***	***
Intercept	1.37	0.31	85.89	0.53	0.71	14.98	1.55	0.33	91.12	0.54	0.68	14.78
A	-0.29***	-0.103***	3.33***	-0.03***	0.024***	0.028***	-0.31***	-0.103***	3.92***	-0.026***	0.025***	0.28***
B	-0.40***	-0.189***	7.01***	-0.08***	0.025***	1.71***	-0.40***	-0.180***	3.79***	-0.085***	0.029***	1.74***
C	0.15**	0.065**	-1.72***	0.04****	-0.013***	-0.72***	0.11*	0.078**	-1.92***	0.037***	-0.013***	-0.70***
A^2	-0.07^{ns}	-0.054**	-1.08***	-0.01***	0.010***	-0.32***	-0.08*	-0.063**	-1.16***	-0.012***	0.010***	-0.30***
B^2	-0.09 ^{ns}	-0.002^{ns}	-3.74***	0.02**	0.014***	-0.67***	-0.08^{ns}	-0.018 ^{ns}	-2.27***	0.006*	0.012**	-0.70***
C^2	-0.03 ^{ns}	0.017^{ns}	-0.9***	0.01**	0.008***	-0.26***	-0.04^{ns}	0.025 ^{ns}	-0.96***	0.011***	0.010***	-0.29***
A*B	-0.08 ^{ns}	-0.050 ^{ns}	2.35***	-0.02***	-0.010**	0.25**	-0.07 ^{ns}	-0.05 ^{ns}	1.78***	-0.027***	-0.012**	0.36***
A*C	0.01 ^{ns}	-0.038 ^{ns}	0.088*	-0.02**	-0.005*	0.36***	-0.03 ^{ns}	-0.038 ^{ns}	0.10**	-0.013**	-0.006*	0.32***
B*C	0.02 ^{ns}	-0.017	-0.75**	0.00^{ns}	0.003^{ns}	-0.083^{ns}	0.03 ^{ns}	-0.017 ^{ns}	-0.55***	0.003^{ns}	0.002^{ns}	-0.058^{ns}
Lack-of- fit	0.5372	0.4901	0.9939	0.2054	0.2051	0.0716	0.6468	0.764	0.1816	0.222	0.1152	0.1614
R-Squared	0.9388	0.9556	0.9948	0.9893	0.9791	0.9932	0.9525	0.9363	0.9999	0.9935	0.9847	0.9929
Adjusted R^2	0.8836	0.9157	0.9904	0.9797	0.9604	0.9870	0.9098	0.8790	0.9999	0.9877	0.9710	0.9865
Pred R^2	0.7311	0.7690	0.9902	0.9358	0.8780	0.9561	0.8091	0.7503	0.9995	0.9630	0.8988	0.9581

Where: A= temperature; B= time and C=thickness; ns= non-significant difference P> 0.05; *= P< 0.05; ** = P< 0.01 and *** P< 0.001

The interaction of baking time and baking temperature was highly significant ($p < 0.01$) on the hardness of biscuits. As indicated in Figure 3 (a and b) for Kuncho and Keyetena based biscuits respectively. The increase in the baking time and highest temperature has shown a decrease in moisture loss of breads (Chevallier *et al.*, 2002) and this might be true also for biscuits to have a harder texture due to highest temperature and longer baking time. Biscuits with relatively highest moisture content generally had a softer texture and therefore, there seems to be a good relation between the hardness of the biscuits and their moisture contents. Lower hardness in low temperature and shorter baking time indicates less brittle (but crunchy) biscuits with greater internal cohesiveness and springiness. When the effects of temperature and time were compared, baking time was more effective for increment of biscuit hardness.

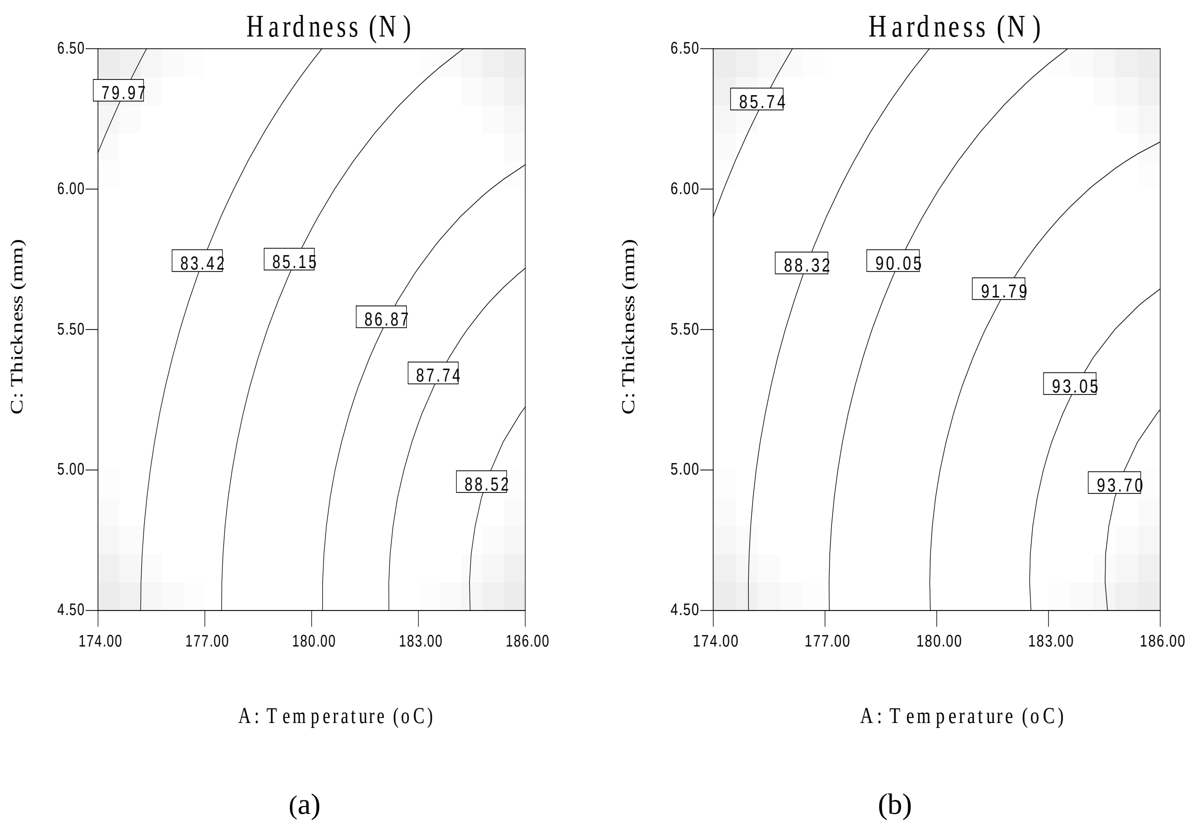
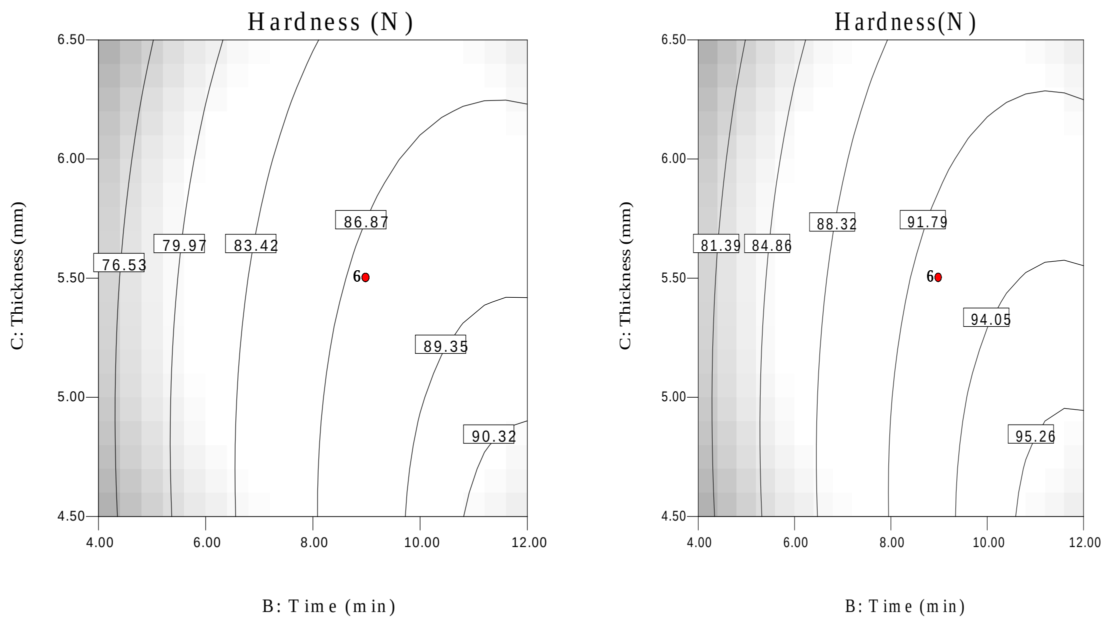


Figure 4. Effects of baking temperature and thickness on hardness of biscuits from (a) Kuncho and (b) Keyetena teff varieties



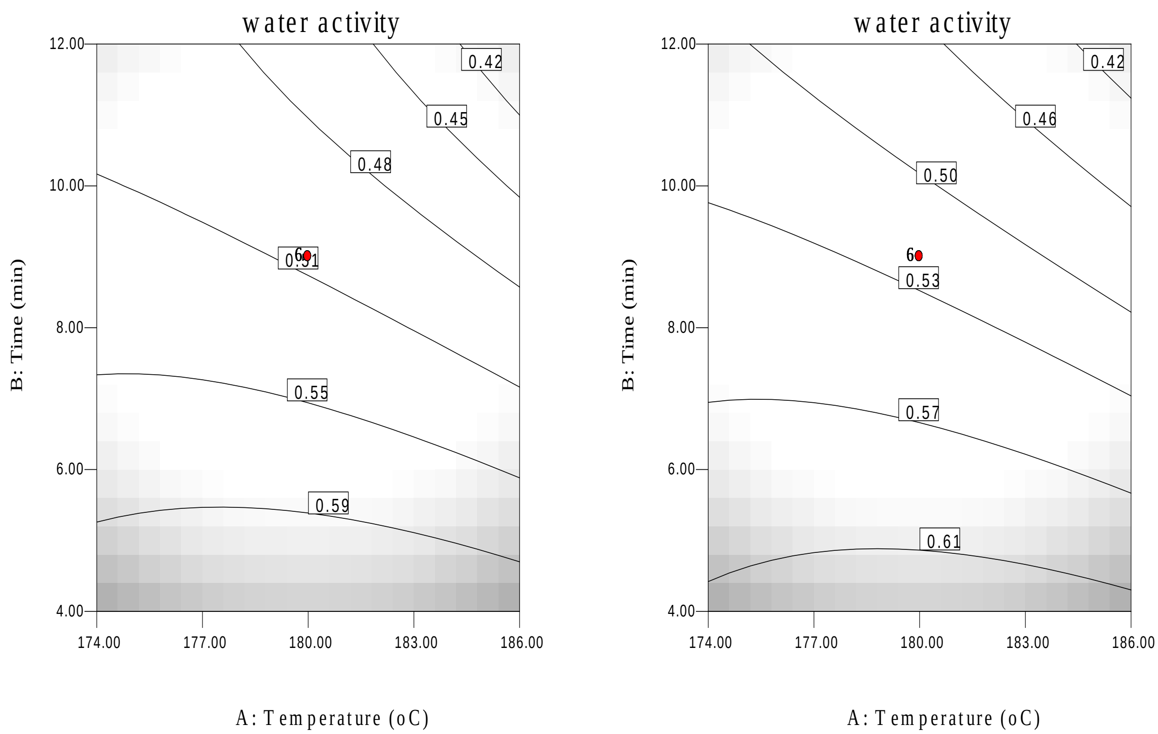
(a) (b)

Figure 5. Effects of baking time and thickness on hardness of biscuits from (a) Kuncho and (b) Keyetena teff varieties

It is apparent that increase in temperature but decrease in thickness, increases hardness values of biscuit from both tested teff based biscuits. The interaction of baking temperature and biscuit thickness was found to have significant ($p < 0.05$) effect on the hardness as indicated Figure 4 (a and b) for Kuncho and Keyetena based biscuit respectively. Increase in time but decrease in thickness increases hardness in both types of teff biscuit. The interaction of baking time and biscuit thickness has highly significant ($p < 0.01$) effect on biscuit hardness.

4.1.4. Water activity

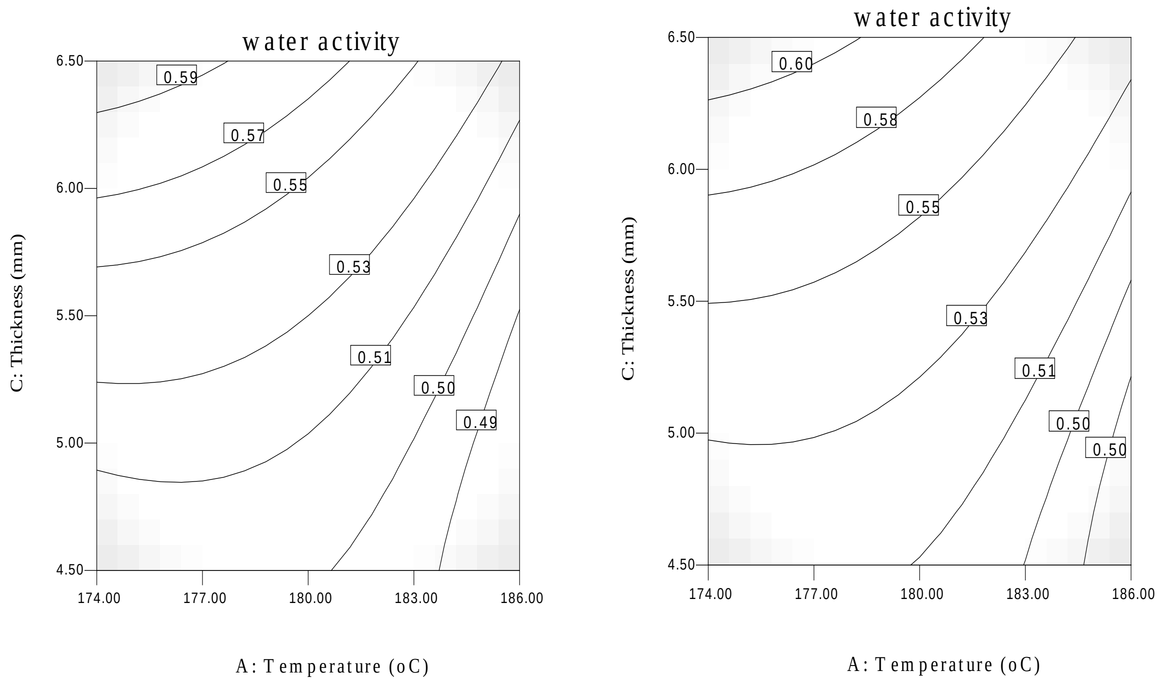
Correlating water activity with shelf-life is of critical importance in work with biocontrol formulations (Connick, 1996). Typical of biscuits is not only low in moisture content but also a rather low value of *aw*. Foods with *aw* <0.60 are considered as microbiologically stable (Young *et al*., 2000). Water activity of biscuits as indicated on table 8 ranged from 0.4 to 0.63 and 0.39 to 0.64 for Kuncho and Keyetena flour based biscuit respectively. From 20 treatment combinations 2 for Kuncho and 3 for Keyetena flour based biscuit were having water activity above 0.6. Water activity was significant in main, quadratic, and interaction of models terms. Highest *aw* was obtained at 174^{o}C for 6 min and 6.5 thick and lower at 186^{o}C for 12 min and 4.5 thick for kuncho and keyetena flour based biscuit respectively. Whenever the interaction effect of time and thickness was not significant (Table 9) but there was decrement of *aw* as baking time increase and biscuit thickness decreases.



(a) (b)

Figure 6. Effects of baking temperature and time on water activity of biscuits from (a) Kuncho and (b) Keyetena teff varieties

The interaction of baking temperature and baking time was highly significant ($P < 0.01$) on water activity of biscuit. As indicated in figure 6 (a and b) as baking time and temperature increases there is a decrement of water activity in both types of teff biscuits. This is from the fact that application of heat results in the evaporation of the water molecules due to the deference in the gravitational force between the water molecule and the vapor form (steam). This results in the raising of the steam upward and detaches from the matrix bond giving low water activity in the biscuit. Similar reports from Bojana, (2013) shows that increase of baking time and temperature on gluten free biscuits enriched with blueberry pomace shows water activity decreased from 0.6 to 0.326. Similar results also reported by Manohar, (1999) indicated that sample of cereals and fruit-containing biscuits showed a decrease in *aw* with an increase in baking temperature.



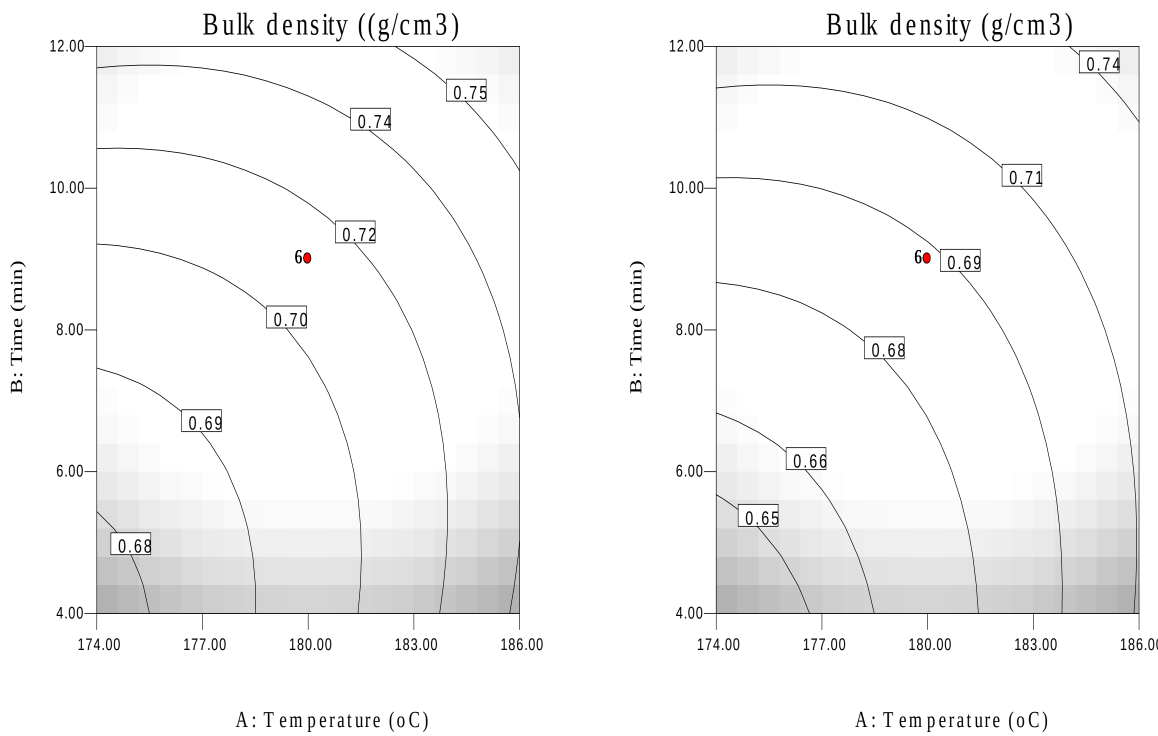
(a) (b)

Figure 7. Effects of baking temperature and biscuit thickness on water activity of biscuits from (a) Kuncho and (b) Keyetena teff varieties

The interaction of baking temperature and biscuit thickness was highly significant ($P < 0.01$) on both types of teff biscuits water activity. As indicated by contour line in figure 7(a and b) water activity in both teff biscuit showed a reduction as baking temperature increases and biscuit thickness decreases.

4.1.5. Bulk density of biscuit

Bulk density of Kuncho and Keyetena teff flour were 0.68 and 0.62 g/ml respectively. The results from biscuit sample ranged from 0.680 g/ml to 0.790 g/ml and 0.660 g/ml to 0.770 g/ ml highest and lower for Kuncho and Keyetena based biscuit respectively. Highest and lower results for bulk density recorded for biscuits baked at 186°C, 12 min, 4.5 mm thick and from 174°C, 6 min, 6.5 mm thickness for both teff based biscuit. Bulk density significantly (P<0.05) affected by interaction of effects temperature and time as well as temperature and thickness, but time and thickness showed non-significance effects for both teff based biscuit (Table 9).

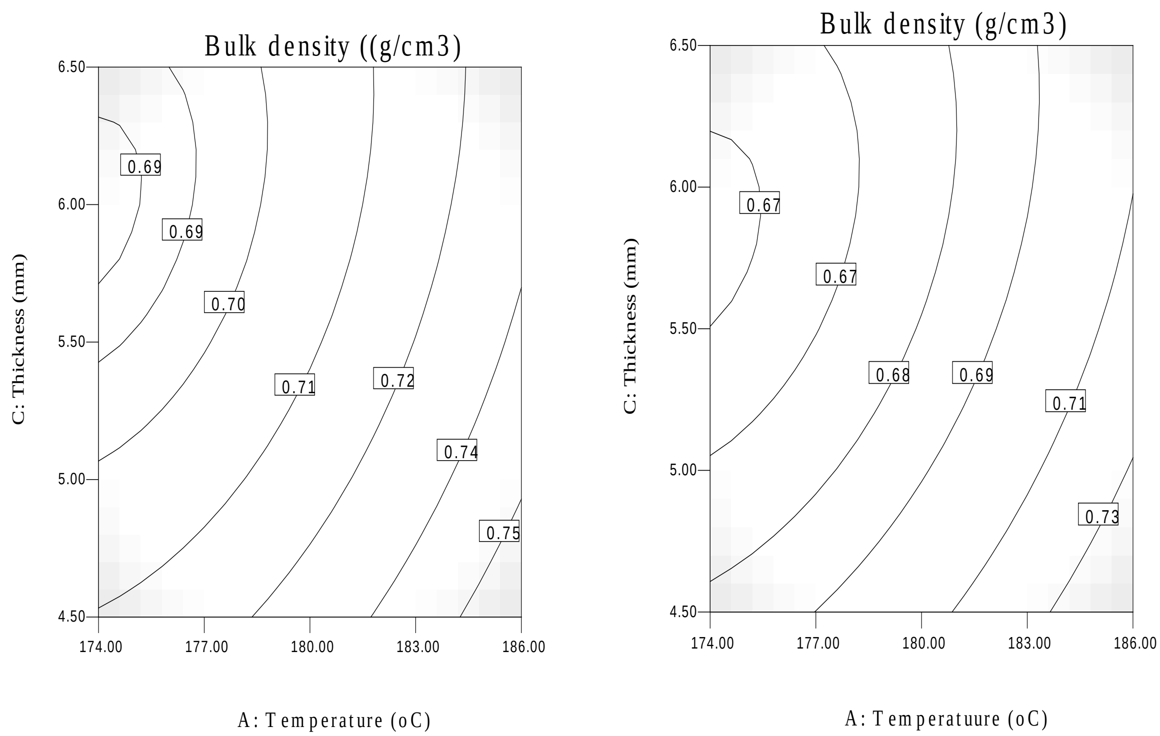


(a) (b)

Figure 8. Effects of baking temperature and time on bulk density of biscuits from (a) Kuncho and (b) Keyetena teff varieties

The interaction of baking temperature and baking time was found to have highly significant (P < 0.01) effect on bulk density of biscuit. As indicated in figure 8 (a and b) as baking time and temperature increases there is an increment of bulk density in both types of teff biscuit. This implies that a denser packaging material may be required for this type product. Bulk density gives information on the porosity of a product and can influence the choice of package and its design (Odedeji and Oyeleke, 2010).

The interaction of baking temperature and thickness was significant ($P < 0.05$) on bulk density of biscuit. As indicated in figure 9 (a and b) as baking temperature increases and biscuit thickness decreases there is an increment of bulk density in both types of teff biscuit. Bulk density increases during high temperature and when biscuit thickness becomes lower.

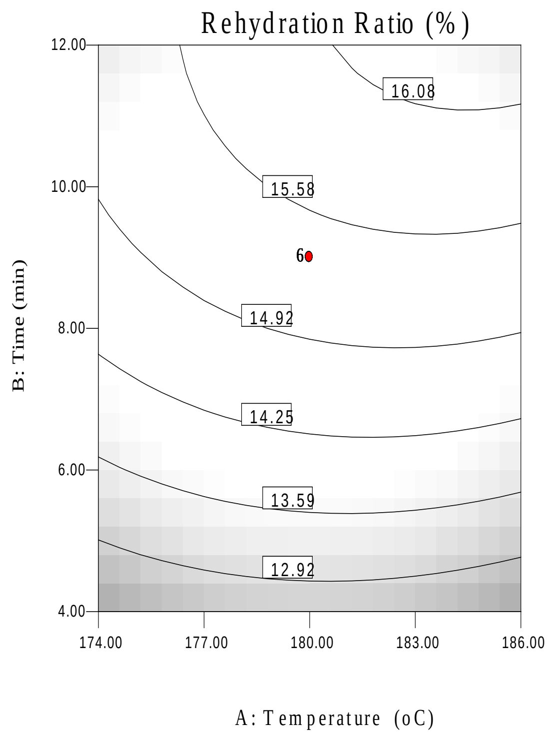


(a) (b)

Figure 9. Effects of baking temperature and biscuit thickness on bulk density of biscuits from (a) Kuncho and (b) Keyetena teff varieties

4.1.6. Rehydration Ratio

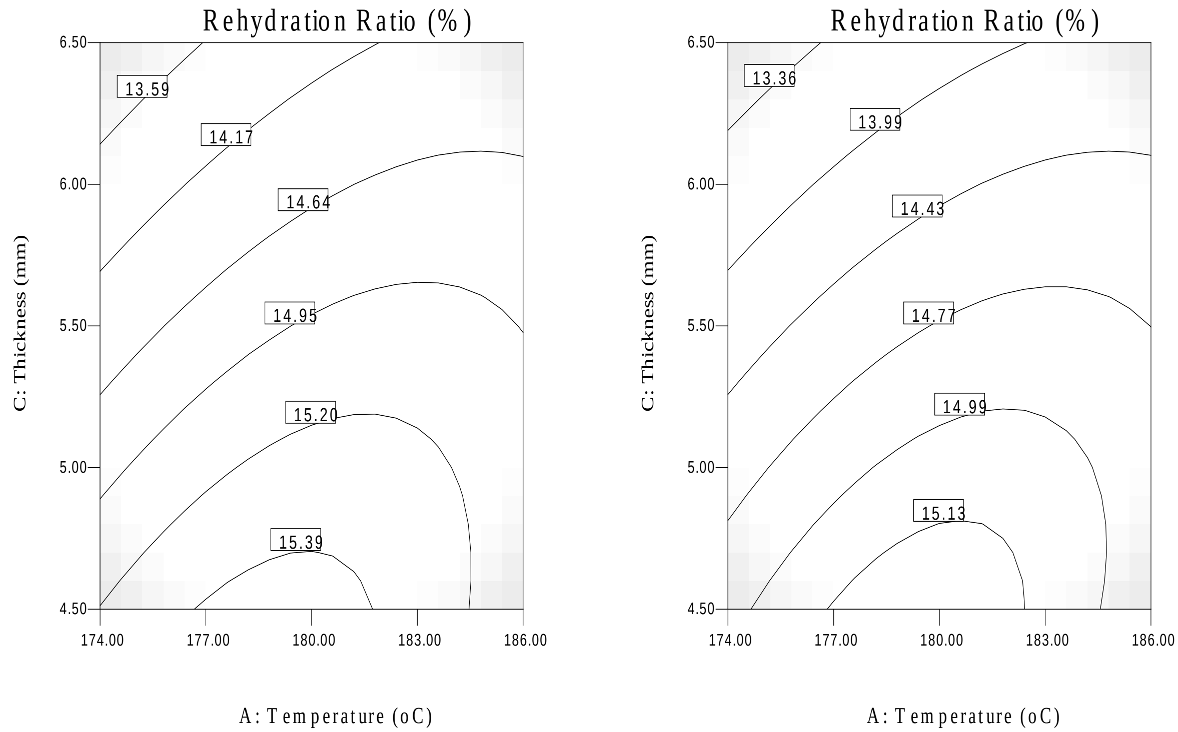
Rehydrated dried foods are increasingly used as components of manufactured foods and their rehydration characteristics depend directly on the drying process (Oliveira and Ilincanu, 1999) or baking condition. Rehydration ratio as indicated on table 8 ranges from 12.25% to 16.25% and 12.10% to 16.15% for biscuits baked from Kuncho and Keyetena based flour respectively. More or less biscuit made from both types of teff have the same rehydration ratios. Highest result was recorded from biscuit baked at 186^{o}C for 12 min at 4.5 mm thick and lowest value was from biscuit baked at 174°C, 6 min, 6.5 mm thickness. Rehydration ratio shows significant difference (P<0.05) in linear, quadratic and interaction of model terms. Effect of time and thickness has not significant effect on rehydration ratio.



(a) (b)

Figure 10. Effects of baking temperature and time on rehydration ratio of biscuits from (a) Kuncho and (b) Keyetena teff varieties

The interaction of baking temperature and time was found to have highly significant (P < 0.01) effect on rehydration ratio of biscuit. As indicated by contour line in figure 10 (a and b) as baking time and temperature increases there is an increment of rehydration ratio in both types of teff biscuit. Rehydration ratio increases during high temperature and longer time of baking. This may be due to the more moisture removed from sample the more increase in water holding capacity. Similar result reported in Mitra *et al.* (2011) when temperature of oven and time increased the rehydration ratio also increases.



(a) (b)

Figure 11. Effects of baking temperature and biscuit thickness on rehydration ratio of biscuits from (a) Kuncho and (b) Keyetena teff varieties

The interaction of baking temperature and thickness was found to have highly significant ($p < 0.01$) effect on rehydration ratio of biscuit. As indicated in figure 11 (a and b) with decrease in thickness and baking temperature close to 180°C increases rehydration ratio in both types of teff biscuit. This might be due the thinner biscuit with optimum baking temperature the change in cell microstructure of the biscuit might be in a position to absorb more water during rehydration process.

4.2. Proximate Composition

Proximate analysis is crucial as one part of quality parameter starting from raw material processing throughout the development process up to final state of product obtained in almost every food product development, production/ process. The proximate composition includes moisture, ash, crude protein, ether extract, crude fiber, and carbohydrate estimation (James, 1995). Proximate composition analysis was made for flours and biscuits, which was made from both teff flours baked under different conditions. The proximate compositions of teff flour indicated on table 10 and for biscuit prepared from Kuncho and Keyetena based biscuit are indicated on Table 12.

4.2.1. Measured values of nutritional composition of teff flour and biscuit

Teff flour from different varieties contains almost similar proximate composition (EHNRI, 1997). However results in this study show that teff flours from two varieties vary in certain parameters as indicated on table10. Highest crude protein, carbohydrate, energy, and zinc obtained from Kuncho teff and highest moisture content, ether extract, crude fiber, iron and calcium content was obtained from Keyetena teff as compared to each other. The values obtained from both teff were close to the finding of Lovis, (2003) and Bultosa, (2007).

Table 10 Chemical composition, energy, and mineral content of teff flour

	Kuncho variety	Keyetena variety
Moisture content (%)	9.30	10.20
Ash (%)	2.80	2.71
Crude Protein (%)	10.30	9.50
Ether Extract (%)	2.80	2.93
Crude Fiber (%)	3.90	4.10
Carbohydrate (%)	70.9	70.56
Energy (K.cal/100g)	350.00	346.61
Iron(mg/100g)	15.60	44.40
Calcium (mg/100g)	96.20	110.95
Zinc(mg/100g)	5.50	3.95

Table 11 Nutritional composition of mayonnaise and milk powder from its label

Nutrient composition	Herman Mayonnaise (Soybean oil based)	Milk Powder (NIDO)
Crude Protein g/100g	1	24
Ether Extract g/100g	71.9	28.2
Crude Fiber g/100g	0	-
Carbohydrate g/100g	3.6	37.4
Energy kcal/100g	653.6	499.4
Calcium mg/100g	16.5	460
Iron mg/100g	0.47	10
Zinc mg/100g	0	4.5

Table 12 Measured values for proximate composition of teff biscuit from Kuncho and Keyetena varieties

Standard	Run	A	B	C	Kuncho Variety							Keyetena Variety						
					M.C (%)	Ash (%)	Crude Protein (%)	Ether extract (%)	Crude fiber (%)	CHO (%)	Energy K.cal/ 100g	M.C (%)	Ash (%)	Crude Protein (%)	Ether extract (%)	Crude fiber (%)	CHO (%)	Energy kcal/ 100g
6	1	186.0	6.0	6.5	6.20	3.64	18.06	14.20	3.84	54.06	416.28	6.40	3.55	17.16	14.33	4.04	54.52	415.69
8	2	186.0	12.0	6.5	4.90	3.54	16.30	13.80	3.77	57.69	420.16	4.95	3.45	15.55	13.93	3.97	58.15	420.17
13	3	180.0	9.0	3.8	5.20	3.52	16.56	13.70	3.76	57.26	418.58	5.30	3.43	15.76	13.83	3.96	57.72	418.39
15	4	180.0	9.0	5.5	5.60	3.62	17.53	14.10	3.85	55.30	418.22	5.70	3.53	16.83	14.23	4.05	55.66	418.03
10	5	190.1	9.0	5.5	4.75	3.54	16.17	13.80	3.77	57.97	420.76	4.80	3.45	15.27	13.91	3.98	58.59	420.63
5	6	174.0	6.0	6.5	6.85	3.74	18.94	14.70	3.89	51.88	415.38	6.93	3.65	18.14	14.80	4.08	52.40	415.36
19	7	180.0	9.0	5.5	5.66	3.63	17.79	14.17	3.85	54.90	418.29	5.80	3.54	16.89	14.29	4.05	55.43	417.89
18	8	180.0	9.0	5.5	5.72	3.64	17.68	14.20	3.84	54.92	418.20	5.80	3.55	17.03	14.33	4.04	55.25	418.09
17	9	180.0	9.0	5.5	5.77	3.63	17.65	14.16	3.85	54.94	417.80	5.85	3.54	16.95	14.28	4.05	55.33	417.64
1	10	174.0	6.0	4.5	5.90	3.67	18.22	14.30	3.86	54.05	417.78	6.00	3.58	17.52	14.42	4.06	54.42	417.54
14	11	180.0	9.0	7.2	6.50	3.62	18.25	14.10	3.84	53.69	414.66	6.65	3.53	17.45	14.22	4.04	54.11	414.22
16	12	180.0	9.0	5.5	5.74	3.64	17.88	14.20	3.83	54.71	418.16	5.82	3.55	17.08	14.34	4.03	55.18	418.10
7	13	174.0	12.0	6.5	6.10	3.60	17.91	14.05	3.81	54.53	416.21	6.15	3.51	17.16	14.18	4.01	54.99	416.22
4	14	186.0	12.0	4.5	4.15	3.47	15.38	13.50	3.73	59.77	422.10	4.20	3.38	14.59	13.63	3.93	60.28	422.15
12	15	180.0	14.0	5.5	4.60	3.52	15.95	13.70	3.76	58.47	420.98	4.67	3.44	15.20	13.82	3.96	58.91	420.82
3	16	174.0	12.0	4.5	5.00	3.55	16.70	13.85	3.78	57.12	419.93	5.10	3.46	15.90	13.98	3.98	57.58	419.74
2	17	186.0	6.0	4.5	5.90	3.62	17.50	14.10	3.82	55.06	417.14	6.00	3.53	16.70	14.23	4.02	55.52	416.95
20	18	180.0	9.0	5.5	5.70	3.64	17.64	14.18	3.84	55.00	418.18	5.74	3.55	16.94	14.30	4.04	55.43	418.18
11	19	180.0	4.0	5.5	6.90	3.70	18.80	14.50	3.88	52.22	414.58	6.98	3.62	18.00	14.60	4.07	52.73	414.32
9	20	169.9	9.0	5.5	5.80	3.67	18.13	14.30	3.85	54.25	418.22	6.00	3.58	17.23	14.43	4.05	54.71	417.63

Where A= temperature B = time C= thickness M.C= moisture content CHO = carbohydrate

4.2.1.1. Moisture content

Moisture is an important parameter in baked foods that significantly affects shelf life and growth of microbial contaminants (ICMSF, 1998). The moisture contents of Kuncho and Keyetena teff flour (i.e. whole-grains) are presented in Table 10. Values for both teff were lower than what indicated in Lovis (2003) reported as 11%. This might vary with pre-history of the grains in harvesting and drying. However, this result is in close agreement with values indicated in Corke *et al.* (2004) for 13 released teff grain varieties which ranged from 9.30-11.22-% with mean of 10.53%. The moisture content of latter variety was a bit highest than that of the former variety flour due to highest fiber content and high water absorption capacity of keyetena teff. This caused by the greater number of hydroxyl existed inside the fiber structures that allow more water interaction through hydrogen bonding. Similarly increase in cookie moisture content is reported by Manoela *et al*. (2006) in their study of blending wheat flour with residue from king palm processing which contains a highest fiber content than wheat flour.

Moisture contents for biscuits baked at different conditions resulted in a range of 4.15 to 6.90% for biscuits baked from kuncho and 4.20 to 6.98% for Keyetena teff. Moisture content showed significant effect ($p<0.05$) in main, quadratic, and interaction of model terms. It is apparent that the moisture content decreases with an increase in baking temperature and time. This makes biscuit harder and more hygroscopic. The moisture content of biscuits baked at 180^{o}C, 4 min and 5.5mm thickness was 6.90 and 6.98 for Kuncho and Keyetena based biscuits respectively. Very low moisture content was measured from biscuit baked at 186^{o}C and 4.5mm thick for 12min was 4.15 and 4.20 for Kuncho and Keyetena teff based biscuits respectively. It shows that low moisture content due to relatively highest baking temperature, long time of baking and thinner biscuit.

Table 13 Estimated regression coefficients, degree of significance, lack of fit and statistical parameters for proximate composition of kuncho teff based biscuit model equations.

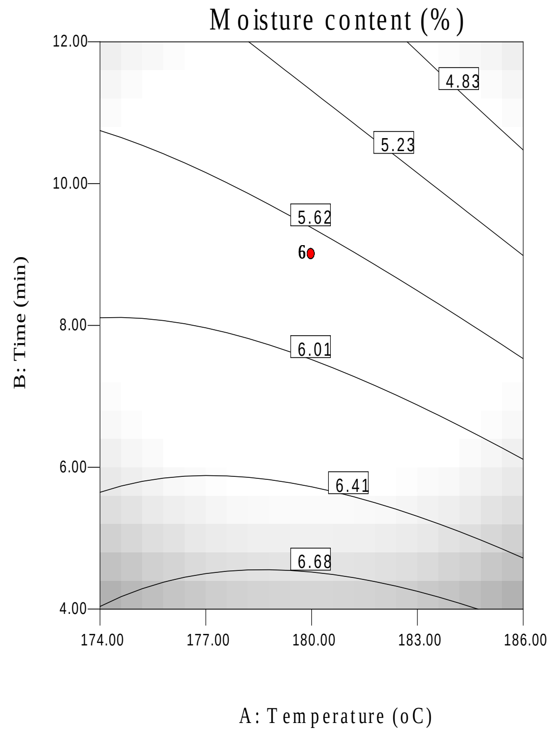
Kuncho Variety							
Source	MC (%)	Ash (%)	Crude Protein (%)	Ether extract (%)	Crude fiber (%)	CHO (%)	EnergyK.cal/ 100g
Model	***	***	***	***	***	***	***
Intercept	5.91	3.65	17.96	14.25	3.85	54.38	417.57
A	-0.27***	-0.038***	-0.52***	-0.16***	-0.023***	1.01***	0.54***
B	-0.85***	-0.074***	-1.01***	-0.33***	-0.044***	2.31***	2.29***
C	0.36***	0.026***	0.42***	0.12***	0.018***	-0.95***	-1.04***
A^2	-0.15***	-0.008ns	-0.17***	-0.03^{ns}	-0.011***	0.37***	0.51***
B^2	0.034^{ns}	-0.011^{ns}	-0.17*	-0.02^{ns}	-0.013*	0.18^{ns}	-0.16^{ns}
C^2	0.055*	-0.020***	-0.08*	-0.08***	-0.014***	0.15*	-0.50***
A*B	-0.23***	0.001^{ns}	-0.22**	0.02^{ns}	0.001^{ns}	0.44***	0.98***
A*C	-0.12**	-0.003^{ns}	-0.06^{ns}	-0.03^{ns}	0.001^{ns}	0.21*	0.41**
B*C	0.10*	0.005^{ns}	0.14*	0.001^{ns}	0.003^{ns}	-0.25*	-0.40*
Lack-of- fit	0.1805	0.0698	0.639	0.0655	0.3855	0.4724	0.0562
R-Squared	0.9936	0.9798	0.9926	0.9748	0.9794	0.9952	0.9891
Adj R^2	0.9878	0.9616	0.9860	0.9522	0.9608	0.9908	0.9792
Pred R^2	0.9612	0.8592	0.9703	0.8161	0.8961	0.9767	0.9285

Where A= temperature B= time C= thickness M.C= moisture content CHO= carbohydrate ns= non-significant difference (p> 0.05) *=P< 0.05 **= p< 0.01 and *** p< 0.001.

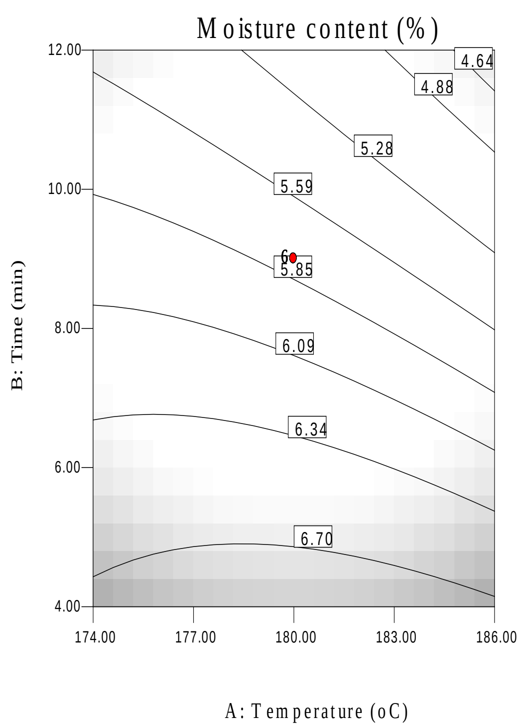
Table 14 Estimated regression coefficients, degree of significance, lack of fit and statistical parameters for proximate composition of keyetena teff based biscuit model equations.

Keyetena Variety							
Source	MC (%)	Ash (%)	Crude Protein (%)	Ether extract (%)	Crude fiber (%)	CHO (%)	EnergyK.cal/ 100g
Model	***	***	***	***	***	***	***
Intercept	6.00	3.56	17.21	14.37	4.050	54.80	417.39
A	-0.275***	-0.038***	-0.54***	-0.16***	-0.02***	1.03***	0.54***
B	-0.871***	-0.07***	-0.99***	-0.3***	-0.04***	2.29***	2.38***
C	0.376***	0.026***	0.40***	0.12***	0.017***	-0.94***	-1.08***
A^2	-0.139***	-0.008*	-0.22***	-0.03^{ns}	-0.0 09**	0.41***	0.47***
B^2	0.020^{ns}	-0.006^{ns}	-0.17**	-0.03^{ns}	-0.017**	0.20*	-0.14^{ns}
C^2	0.064**	-0.02***	-0.10*	-0.08***	-0.015***	0.15*	-0.53***
A*B	-0.262***	0.002^{ns}	-0.19**	0.01^{ns}	-0.002^{ns}	0.44***	1.10***
A*C	-0.104**	-0.004^{ns}	-0.06^{ns}	-0.02^{ns}	0.001^{ns}	0.19*	0.31*
B*C	0.078*	0.005^{ns}	0.19*	0.003^{ns}	0.005^{ns}	-0.28**	-0.34^{ns}
Lack-of- fit	0.2752	0.093	0.1995	0.0699	0.332	0.3406	0.0602
R-Squared	0.9959	0.9822	0.9925	0.9732	0.9755	0.9956	0.9872
Adj R^2	0.9922	0.9663	0.9857	0.9491	0.9534	0.9917	0.9756
Pred R^2	0.9771	0.8778	0.9570	0.8098	0.8735	0.9769	0.9170

Where A= temperature B= time C= thickness M.C= moisture content CHO= carbohydrate ns= non-significant difference (p> 0.05) *=P< 0.05 **= p< 0.01 and *** p<


Moisture content (%)
4.83
5.23
5.62
6
6.01
6.41
6.68
B: Time (min)
A: Temperature (oC)
12.00
10.00
8.00
6.00
4.00
174.00
177.00
180.00
183.00
186.00

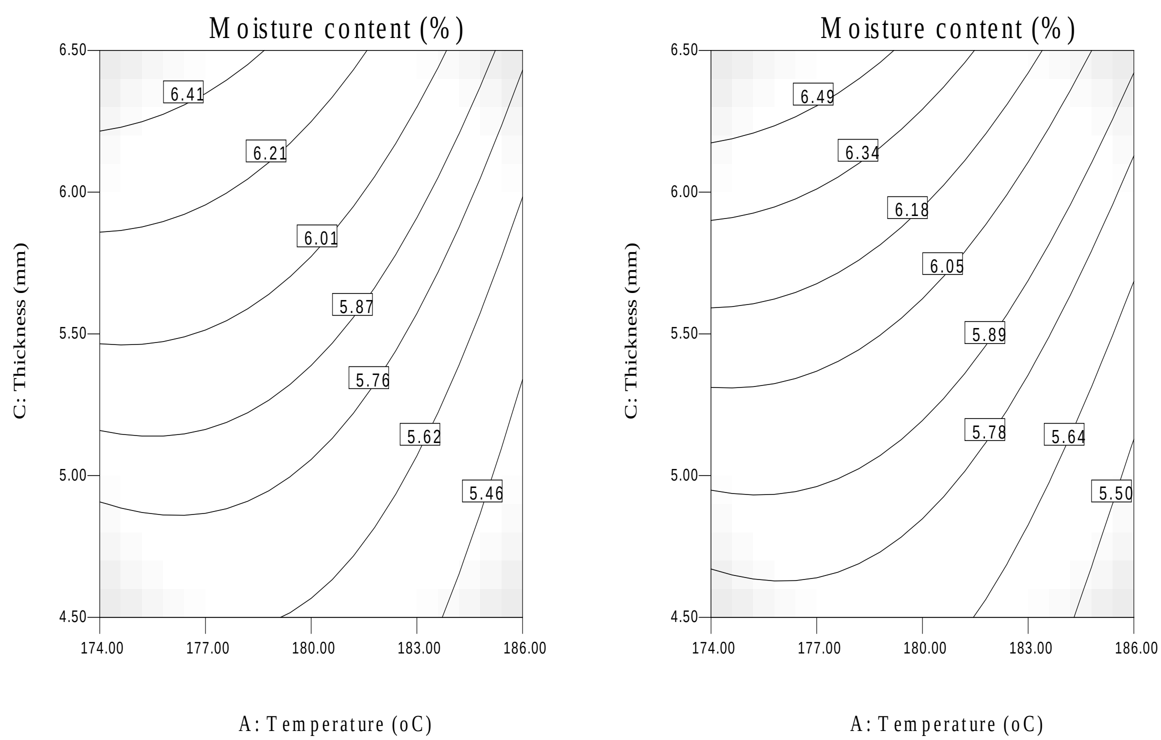
0.001.


Moisture content (%)
4.64
4.88
5.28
5.59
6
5.85
6.09
6.34
6.70
B: Time (min)
A: Temperature (oC)
12.00
10.00
8.00
6.00
4.00
174.00
177.00
180.00
183.00
186.00

(a) (b)

Figure 12. Effects of baking temperature and time on moisture content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

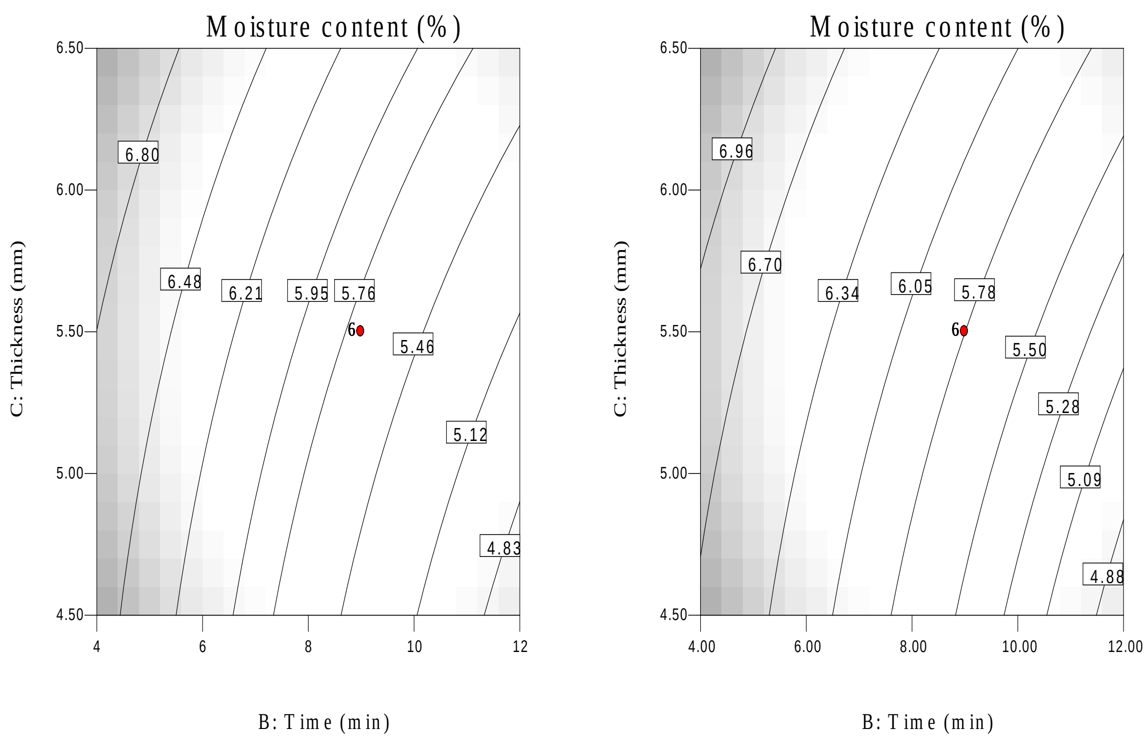
As it can be shown on table 13 and 14 given above the moisture content of the biscuits were significantly influenced by the interaction of baking temperature and time. Baking temperature and time has highly significant ($p < 0.01$) effect on moisture content of biscuit. As indicated by contour line in figure 12 (a and b) as baking time and temperature increases there is a reduction of moisture content in both types of teff biscuit. A similar result was found by Piergiovanni and Farris (2008) in which baking temperature and time was negatively affecting the amaranth cookie moisture content. Bojana (2013) also showed that increase of baking time and temperature on gluten free biscuits enriched with blueberry pomace showed a decrease in moisture content of 10% to 5.2%. Kabirullah *et al.* (1996), analyzed cracker and biscuits and found moisture content in the range of 4.06-4.97% and 4.53-6.37% respectively. Similar result also reported by Patela *et al.* (2005) and Kotoki and Deka (2010) as bread baked at highest time-temperature combinations showed loss more water and the bread become harder and under weight.



(a) (b)

Figure 13. Effects of baking temperature and thickness on moisture content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

As it can be shown on Table 13 and 14, given above the moisture content of the biscuits were significantly ($P < 0.05$) influenced by the interaction of baking temperature and thickness. As indicated in figure 13 (a and b) with an increase in baking temperature and a decrease in biscuit thickness, moisture content of both teff based biscuits decreased. These results were also in agreement with previous results for moisture content of cookies (Ait *et al.*, 2007) and bread.



(a) (b)

Figure 14. Effects of baking time and thickness on moisture content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Baking time and thickness of biscuit have also a significant effect on moisture content. Figure 14 showed as baking time increases and a decrease in biscuit thickness declines in moisture content of biscuit (figure 14a and 14b). This might be associated with biscuits small thickness when exposed to high temperature, rate of heat penetration to biscuit is highest than that of larger thickness. This directly have impact for increase of water vapour from sample so the increase in water vapour decreased moisture content of biscuit.

4.2.1.2. Ash

The ash content is an inorganic residue remaining after the removal of water and organic matter by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals in a food. Ash content of Kuncho and Keyetena teff flours were indicated on table 10. This result is in agreement with Corke *et al.,* (2004). On their review report they indicated that, the ash level in teff varieties varied from 2.66 - 3.00 % with typical value of 2.8%. Apart from the genetics, the ash levels in teff grain are influenced by the agronomic practices as well. Ciferri and Baldrati, (1939) found that ash content of teff flour ranged between 2.4 and 2.94% .The result for ash in this finding was comparable to the values indicated in Bultosa (2007) which were reported as ranged from 1.99% to 3.16 with mean of 2.45%.

Results of ash for baked biscuits at different baking time, temperature, and biscuit thickness are presented in Table 12. Values ranged from 3.47-3.74 for Kuncho and 3.38-3.65 for Keyetena teff based biscuits. However, when biscuits from the two teff compared they almost have the same ash content. Ash content significant main model terms and thickness of biscuit showed highly significant effect on quadratic term this might be associated with the volume of biscuit baked. Ash from both types of teff based biscuit not significantly affected by interaction baking temperature, time, and thickness. Highest ash content was obtained from both types of teff based biscuits as compared to teff flour. Increment in ash content of biscuits associated with presence of additional biscuit making ingredients. According to Fenema (1996) Mineral elements, unlike vitamins and amino acids, cannot be destroyed by exposure to heat for a long time this could be the reason that interaction of baking temperature time and thickness did not influence ash content of the biscuits significantly. This finding also supported by Biniyam, (2010) on ash content of

Cookies from Wheat, Quality Protein Maize, and Carrot Composite Flour ash was found not significantly influenced by baking temperature.

4.2.1.3. Crude protein

The proteins are polymers of amino acids and their amount in a sample represents its quality index. The crude protein is normally determined by measuring the amount of total nitrogen in a sample. Protein content of Kuncho and Keyetena teff flours presented in table 10 was 10.3% and 9.5% respectively. However, this result was lower than results reported in Corke *et al.*, (2004) the crude protein for teff varieties were reviewed in range of 9-13% with mean value 11 %. But this result is comparable to values indicated in Lovis, (2003) who reported the protein content of teff flour to be 9.6%.

The statistical results regarding crude protein content of different biscuits baked at different baking temperature are presented in Table 13 and 14. The protein content of the biscuit showed a significant difference at ($p<0.05$) in main, quadratic and interaction of model terms respectively. Protein content ranged from 15.38 to 18.94% for kuncho and14.59 to18.14% for Keyetena teff based biscuit. An increase in protein content in this case is mainly due to the presence of additional high protein components in biscuit making other than teff flour. However, despite effect of additional biscuit components, highest protein percentage obtained from biscuits baked at 174^{o}C, 6 min, and 6.5 mm thick and lower protein score from biscuit baked at 186^{o}C, 12 min, and 4.5 mm thick for both types of teff. This shows that, the highest the temperature, longer baking time and the thinner the thickness resulted in the reduction of crude protein content of biscuits.

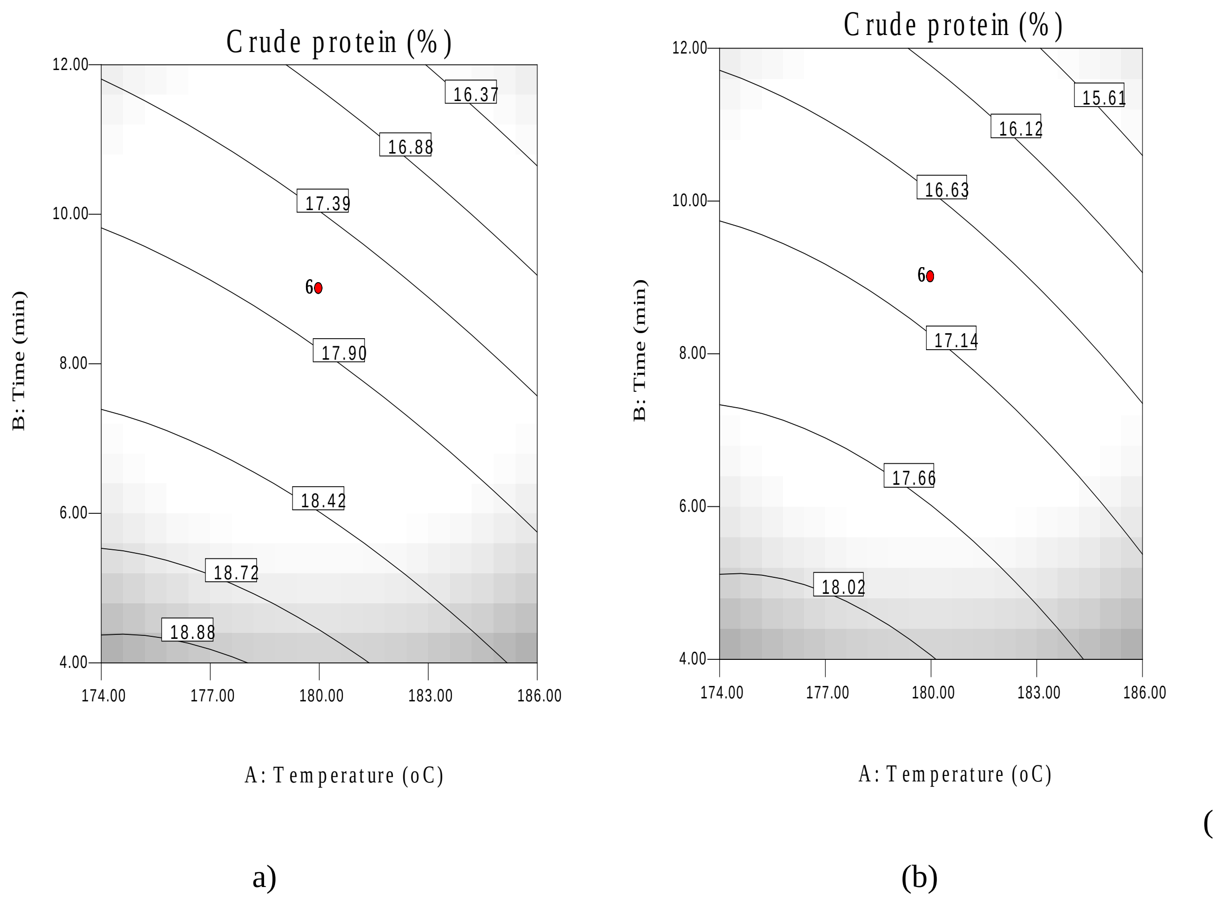
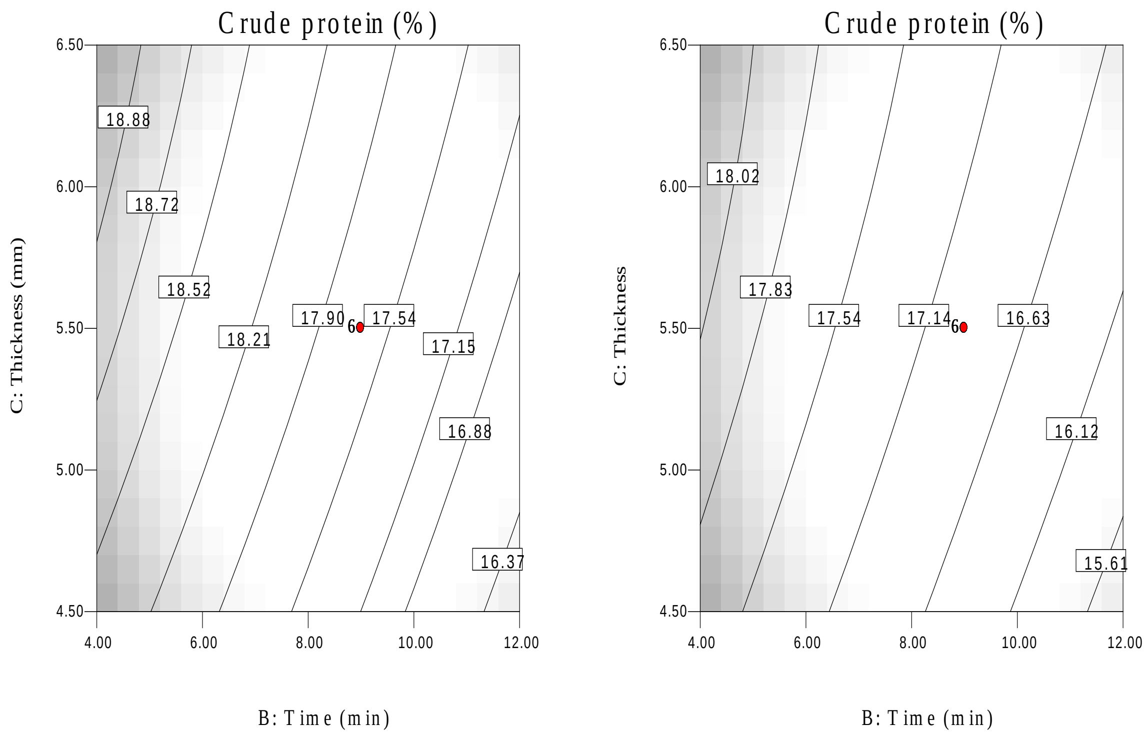


Figure 15. Effects of baking temperature and time on crude protein content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Baking temperature and time has highly significant ($p < 0.01$) effect on crude protein content of biscuit. As indicated by contour line in figure 15 (a and b) as baking time and temperature increases crude protein content decline for both types of teff biscuits. During baking, the physical and chemical properties of protein are altered due to the denaturation of protein where in the hydrogen bonds and non-polar hydrophobic interactions of the secondary and tertiary structures of proteins are disrupted by heat and the soluble amino acids leached out in the baking medium (Fellow, 2000). According to Hui *et al.*, (2006), the Maillard reaction, is mainly a responsible chemical reaction for the loss of protein due to reaction of free amino acids with reducing sugars (glucose and fructose) is favored at temperatures above 120^0C. In Maillard reactions the loss of amino acids, in particular, lysine, and tryptophan is significant, which slightly reduces the protein quality as well (Fellow, 2000).The extent of loss is increased by highest temperatures, longer baking time, and availability of larger amounts of reducing sugars.



(a) (b)

Figure 16. Effects of baking temperature and thickness on crude protein content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

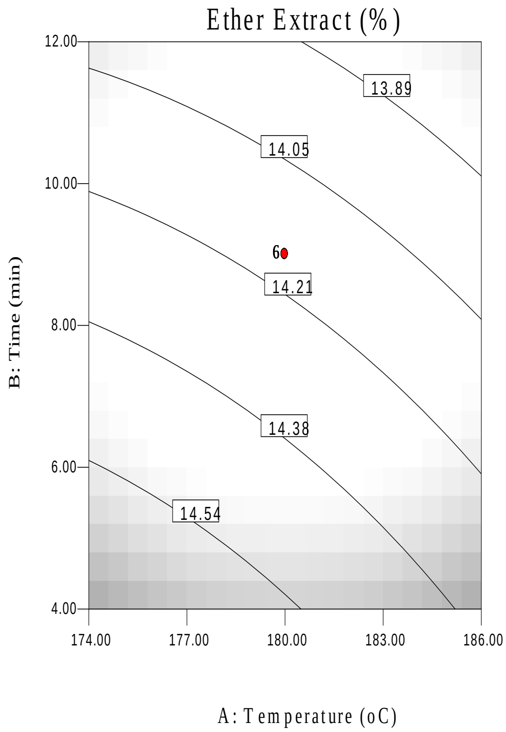
Furthermore, baking time and thickness was found to have a significant ($p < 0.05$) effect on crude protein content of biscuit. As indicated in figure 16 (a and b) as baking time increases and biscuit thickness decreases there is a decrement in crude protein for both types of teff based biscuits.

4.2.1.4. Ether extract

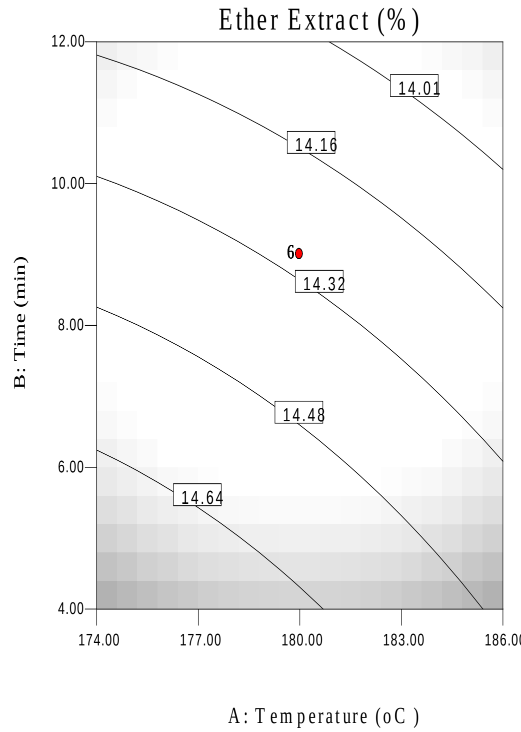
The lipids including fats and oils are one of the major constituents of foods, and are important in our diet for a number of reasons and they are a major source of energy and provide essential lipid nutrients. In many foods the lipid component plays a major role in determining the overall physical characteristics, such as flavor, texture, mouth-feel, and appearance. Ether extract from Kuncho and Keyetena teff flour is illustrated in Table 10. Ether extract values were 2.8 for Kuncho and 2.93% for Keyetena teff. The Ether extract values were comparable with those of Corke *et al.*, (2004) who reported that the crude fat content of teff flour ranged from 2-3 % with mean of 2.3%

Statistical results presented in Table 13 and 14 indicated that the Ether extract content of biscuits were not significantly ($p<0.05$) affected by interaction of model terms but linear terms had significant effect for biscuits from Kuncho and Keyetena based flours. The ether extract content ranged from 13.50 to 14.70 % for Kuncho and 13.63 to 14.80 % for Keyetena teff biscuits. These inflated values for ether extract are mainly due to high fat biscuit making ingredients added. However, with equal proportion of high fat ingredients, highest ether extract was obtained from biscuit baked at 174^{o}C, 6 min having 6.5 thick and lower at 186^{o}C for 12 min having 4.5 thick for both Kuncho and Keyetena flour based biscuit respectively. These results were similar with that of crude protein baking conditions. This finding was in line to Fenema, (1996) high temperature heating trigger polymerization of fat molecules which contributing to its loss during baking, drying, and boiling.

According to Usha, (1995) low, medium, and high fat biscuits should contain 7.5-15%, 15-27%, and more than 27% respectively. According to the author, the fat content of biscuits from this finding is categorized under low fat biscuit.



a)



(

(b)

Figure 17. Effects of baking temperature and time on ether extract content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

There were no significant difference by interaction of time and temperature on ether extract content of both teff based biscuits but as indicated in figure 17 (a and b) when baking temperature becomes highest and biscuit baked for longer time there is decline of ether extract. When the effects of baking temperature and time were compared, baking time was found to be more effective on fat loss. This finding also supported by Biniyam (2010) on fat content of Cookies from wheat, quality protein maize and carrot composite flour ether extract was found not significantly influenced by baking temperature.

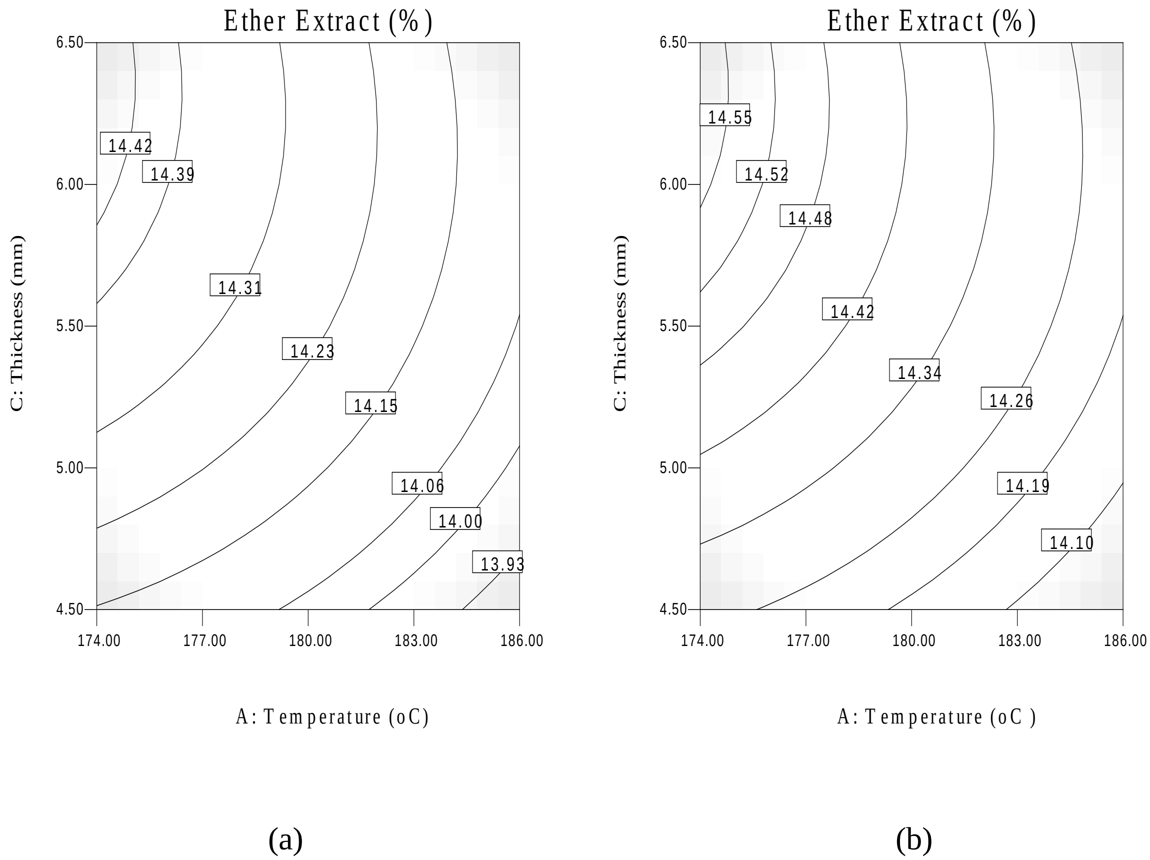


Figure 18. Effects of baking temperature and thickness on ether extract content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Interaction of baking temperature and thickness have not significant impact on ether extract content of both teff based biscuits but as indicated in figure 18 (a and b) when baking temperature becomes highest and thickness was lower there is decline of ether extract. When the effects of baking temperature and thickness were compared, thickness was found to be more effective on fat loss since a significant effect observed in its quadratic terms too this might be due to small thickness allows high heat penetration which have impact on fat loss by melting.

4.2.1.5. Crude fiber

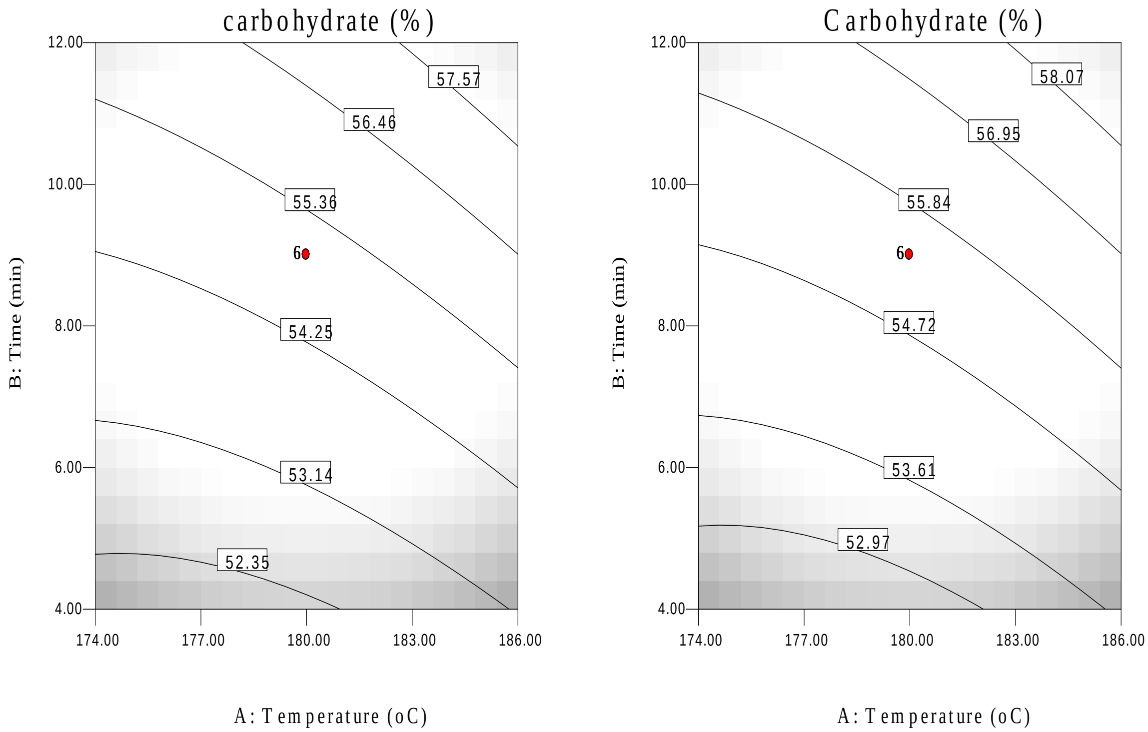
Fiber content in food are believed to prevent or alleviate maladies such as cardiovascular diseases, diabetes, diverticulosis and colon cancer (Azizah and Zainon, 1997). Because it consists of cellulose and lignin, its estimation affords an index for evaluation of dietary fiber (Eddy *et al.*, 2007). Fiber content of the investigated Kuncho and Keyetena teff four presented on Table 10.The result is highest than the values obtained by Lovis, (2003) and Corke *et al.*, (2004) who reported that total dietary fiber of teff grains flour is 3% and 3.8-2.6% respectively.

The crude fiber of the final biscuit varied from 3.73% to 3.89 % for kuncho and 3.93 to 4.08 % for Keyetena based biscuits. Highest fiber content was recorded from biscuit baked at 174^{o}C for 6 min 6.5 thick and low result obtained from biscuit baked at 186^{o}C for 12 min having 4.5 thick for biscuit baked from both types of teff. Fiber content shows significant effect on linear terms but not in interaction of model terms for biscuits from both teff flours. Fiber content significantly affected by baking temperature and biscuit thickness in both linear and quadratic terms indicated in figure 19 shows effect of temperature and thickness.

Lee *et al*., (2006) reported that dietary fiber has a beneficial effect on bowel transit time, affects glucose and lipid metabolism, reduces the risk of colorectal cancer, and stimulates bacterial metabolic activity. There are medical studies about the benefits of total dietary fibers consumption such as falling serum cholesterol concentration, lowering the risk of coronary heart disease, reducing blood pressure, aiding weight control, improving glycemic control, reducing the risk of certain types of cancer and improving gastrointestinal functions (Bonithonp *et al*., 2000; Bingham, 2003). As a result, fibers from different sources and compositions are being obtained, and the fortification of foods with dietary fibers is increasing. It is recommended that 25g/day of fiber. There is no reported data for fiber incorporation on gluten free biscuit.

4.2.1.6. Utilizable carbohydrate

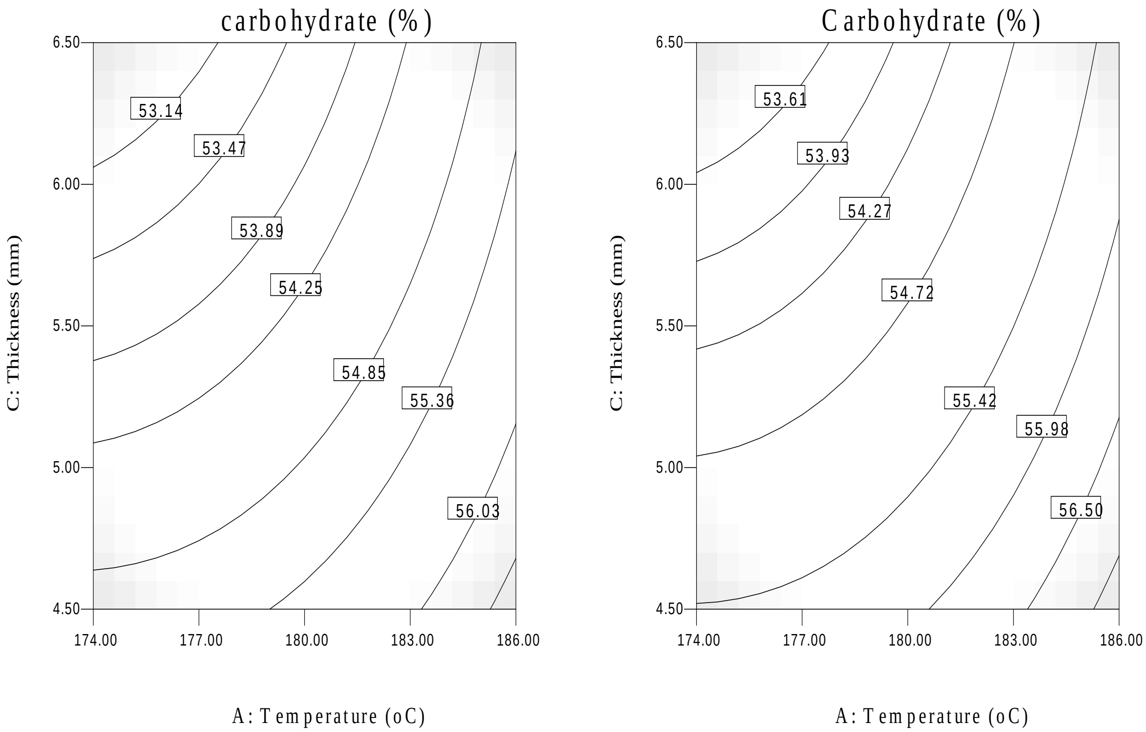
Utilizable carbohydrates content of Kuncho and Keyetena teff flours were presented in table 10, as 70.9 and 70.56 % respectively, almost with the same amount. These results are lower than the values indicated in Lovis, (2003) as 73.0%; they are comparable with the value of 71.4% USDA (2015). Utilizable carbohydrate value for biscuit baked under different conditions varied from 51.88-59.77% for Kuncho and 52.40 to 60.25% for Keyetena teff biscuit. The decrease in carbohydrate could be associated with the addition of other biscuit ingredients which were high in their protein and fat contents. Highest carbohydrate value obtained from biscuit baked at 186^{o}C for 12 min having thickness of 4.5 and lowest value at 174^{o}C for 6 min having 6.5 thicknesses for both Kuncho and Keyetena based biscuits respectively.



(a) (b)

Figure 19. Effects of baking temperature and time on utilizable carbohydrate content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

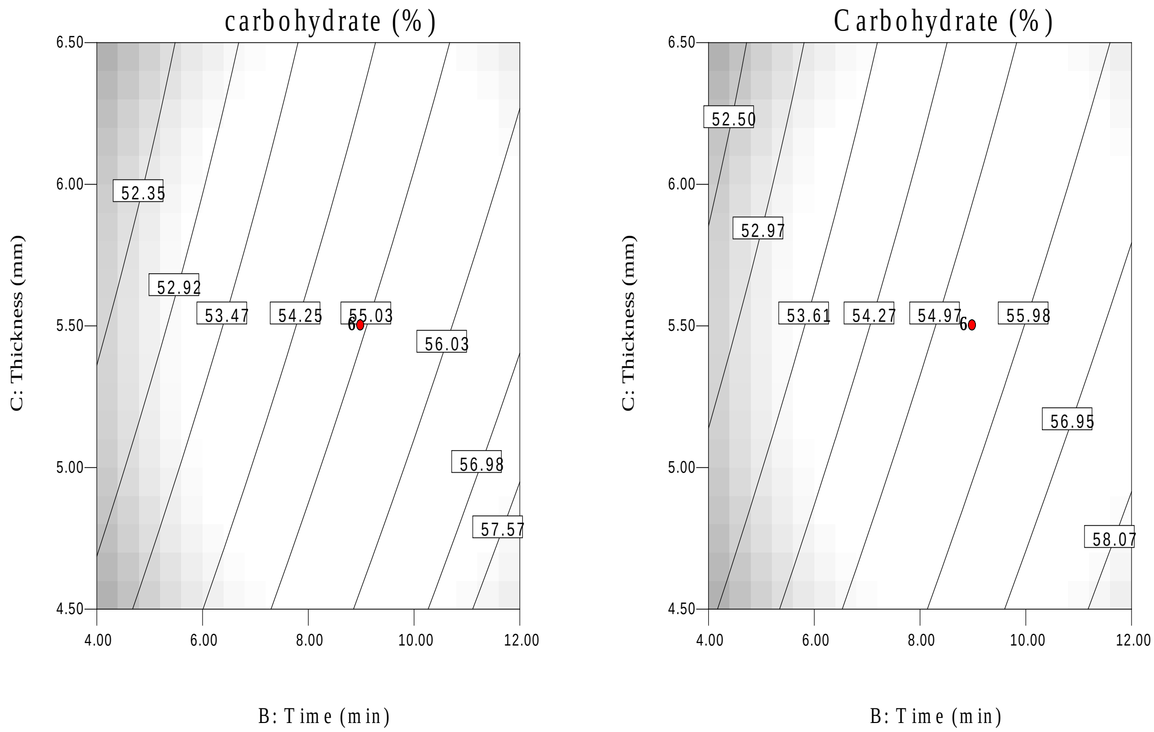
Baking temperature and time was found to have highly significant ($p < 0.01$) effect on carbohydrate content of biscuit. As indicated in figure 20 (a and b) with an increase in baking time and temperature carbohydrate content in both types of teff biscuit increased. These baking conditions are in opposite when compared with conditions for moisture, protein and ether extract. Since utilizable carbohydrate was calculated by difference from other proximate compositions, the highest the other proximate compositions resulted in the lower carbohydrate value in similar baking condition. Furthermore, an increase in carbohydrate might also associate with starch degradation into dextrin and simple sugars (Guy, 2001).



(a) (b)

Figure 20. Effects of baking temperature and thickness on utilizable carbohydrate content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Baking temperature and thickness has significant ($p < 0.05$) effect on carbohydrate content of biscuit. As indicated in figure 21 (a and b) as baking temperature increased and biscuit thickness decreased there is an increase in carbohydrate content for biscuits made from both types of teff flours.



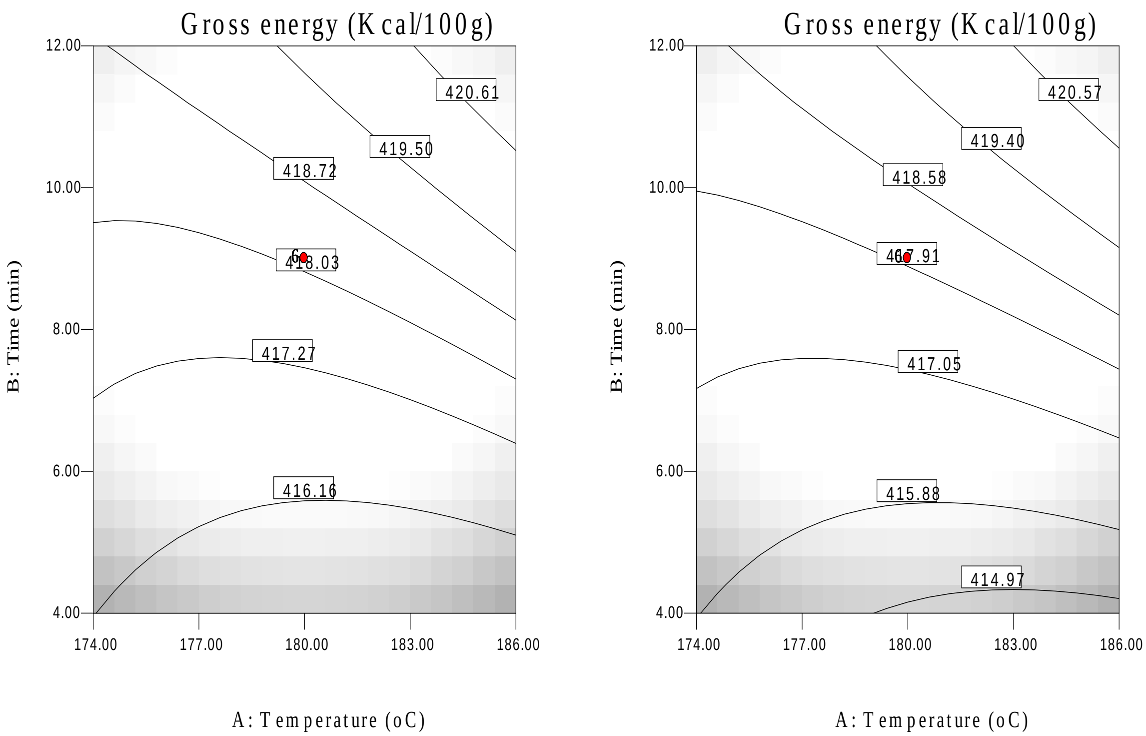
(a) (b)

Figure 21. Effects of baking time and thickness on utilizable carbohydrate content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Baking time and thickness effect was also significant (P < 0.05) on carbohydrate content of biscuit. As indicated in figure 22 (a and b) as baking time increases and biscuit thickness decreases there is increment of carbohydrate content in both types of teff biscuit. This is from the fact that longer time of baking causes other proximate components to degrade and resulted in increment of utilizable carbohydrate.

4.2.1.7. Gross energy

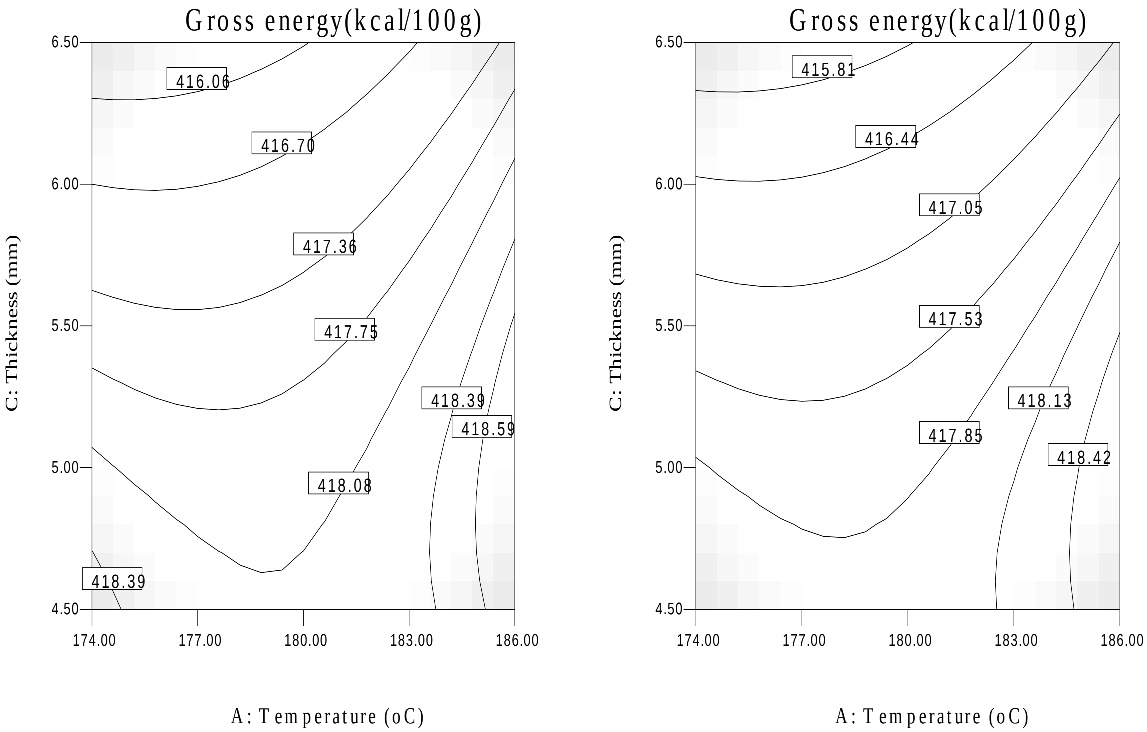
Initially the gross energy was 350 kcal/100g in Kuncho and 346.61 kcal/100g in Keyetena teff flours. But an increase in gross energy was observed from 414.58 to 422.10 kcal/100g in Kuncho based biscuit and 414.22- 422.15 kcal/100g for Keyetena based biscuit. The amount of calorie in the final product showed significant difference (p<0.05) in linear, quadratic and interaction model terms. Highest calorie obtained from biscuit baked at 186^{o}C, 12 min, and 4.5 mm thick for biscuit from both types of teff. Whereas lower value from biscuit baked at 180^{o}C, 4 min and 5.5 mm thick for Kuncho and 180^{o}C , 9 min and 7.2 mm thicknesses for Keyetena respectively.



(a) (b)

Figure 22. Effects of baking temperature and time on gross energy content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Baking temperature and time was found to have highly significant ($P < 0.01$) effect on calorie of biscuit. As indicated by contour line in figure 23 (a and b) as baking time and temperature increases there is increment of energy content in both types of teff biscuit. Since the calorie of biscuit was calculated from value of protein, ether extract and carbohydrate with their conversion factor, highest carbohydrate value played the role for increment of energy content as baking time and temperature increases. With similar justification, with an increase in baking temperature and time relatively highest carbohydrate content of the biscuit was reported which might be associated with starch degradation into dextrin and simple sugars (Guy, 2001).



(a) (b)

Figure 23. Effects of baking temperature and thickness on gross energy content of biscuits from (a) Kuncho and (b) Keyetena teff varieties

Baking temperature and thickness was found to have highly significant (P < 0.01) effect on gross energy content of biscuit. As indicated in figure 24 (a and b) as baking temperature increases and biscuit thickness decreases there is increment of energy content in both types of teff biscuit. This could be explained in line with justification provided in above points.

4.3. Mineral Content

Minerals are chemical constituents used by the body in many ways. They have important roles to play in many activities in the body. The mineral content of all biscuit samples were presented in Table 15. Models for all minerals content were fitted indicated that the lack-of-fit p-values were not significantly different at 5% probability level or (P<0.05).

Table 15 Measured values for mineral composition of biscuit made from both teff varieties (mg/100g)

					Kuncho variety (mg/100 g sample)			Keyetena variety(mg/100 g sample)		
Standard	Block	A	B	C	Iron	Calcium	Zinc	Iron	Calcium	Zinc
6	1	186.0	6.0	6.5	17.78	215.10	7.79	46.58	229.85	6.23
8	2	186.0	12.0	6.5	17.10	214.35	7.12	45.90	229.11	5.57
13	3	180.0	9.0	3.8	17.00	214.65	7.42	45.80	229.39	5.88
15	4	180.0	9.0	5.5	17.40	214.52	7.30	46.15	229.27	5.76
10	5	190.1	9.0	5.5	17.20	214.37	7.19	45.95	229.13	5.64
5	6	174.0	6.0	6.5	18.20	215.97	8.19	46.95	230.72	6.65
19	7	180.0	9.0	5.5	17.53	214.50	7.49	46.28	229.27	5.95
18	8	180.0	9.0	5.5	17.50	214.67	7.47	46.25	229.44	5.94
17	9	180.0	9.0	5.5	17.50	214.57	7.35	46.20	229.33	5.80
1	10	174.0	6.0	4.5	17.97	215.27	8.04	46.67	230.03	6.48
14	11	180.0	9.0	7.2	17.64	215.07	7.88	46.34	229.82	6.33
16	12	180.0	9.0	5.5	17.60	214.82	7.62	46.30	229.57	6.08
7	13	174.0	12.0	6.5	17.45	214.77	7.58	46.15	229.53	6.04
4	14	186.0	12.0	4.5	16.95	214.20	7.06	45.65	228.94	5.51
12	15	180.0	14.0	5.5	17.10	214.23	7.08	45.80	229.00	5.54
3	16	174.0	12.0	4.5	17.39	214.60	7.39	46.17	229.36	5.85
2	17	186.0	6.0	4.5	17.56	214.79	7.58	46.26	229.54	6.04
20	18	180.0	9.0	5.5	17.58	214.60	7.49	46.28	229.37	5.94
11	19	180.0	4.0	5.5	18.10	215.20	8.09	46.80	229.97	6.56
9	20	169.9	9.0	5.5	17.80	215.11	7.90	46.50	229.87	6.35

Where A= temperature B= time and C thickness

4.3.1. Iron content

Iron functions as hemoglobin in the transport of oxygen. The general high nutritional value of teff and its iron content are believed to be the major contributors for high iron content which increases the hemoglobin level of the blood that helps more oxygen to be transmitted (Andrews *et al*., 1999). Iron content obtained from teff flour was 15.6 and 44.4 mg/100g for Kuncho and Keyetena respectively. Keyetena has almost 2.6 times more iron content than Kuncho teff flour. The high levels of iron in red teff were suggested to be due to as in other darker coloured cereal grains, red varieties of teff contained highest levels of iron this has been proposed to be due to highest levels of pigmented material, such as tannins and polyphenols (Umeta and Parker, 1996).

The same is true also for biscuits made from Kuncho and Keyetena teff based flours. Experimentally, the minimum and maximum iron content was (16.95 – 18.20 mg/100g) for Kuncho and (45.65-46.95 mg/100g) for Keyetena based biscuit respectively. Lower values obtained on biscuit baked at186^{o}C, 12 min and 4.5 mm thick for both types of teff based biscuit; the highest iron content was obtained from biscuit baked at 174^{o}C, 6 min, and 6.5 mm thick. As shown on Table 16 interaction effect of temperature, time, and thickness were not significant in both types of teff based biscuits, but there is a small fraction decrement of iron content. Whenever loss of little fraction on iron content obtained from biscuit the values were highest than the iron content of both teff flour because of inclusion of additional ingredients for biscuit making.

This research was in agreement to Fenema, (1996), mineral elements, unlike vitamins and amino acids cannot be destroyed by exposure to heat for a long time this could be the reason that interaction of baking temperature, time and thickness does not influence iron content of the biscuits significantly. Alaunyte *et al*., (2012) showed that by supplementing wheat bread with 30 percent teff flour, the iron content of the bread increased by more than two folds. By assuming an average daily consumption of 200g of teff-enriched bread, it is possible to cover between 42 and 81 percent and 72 and 138 percent of daily intake requirements for iron in women and men, respectively. Baye *et al*., (2014) and Bokhari *et al*., (2012) also showed that consumption of 30 percent teff-enriched wheat breads can help maintain serum iron levels in pregnant women.

Table 16 Estimated regression coefficients, degree of significance and lack of fit and statistical parameters for the mineral content of teff based biscuit model equations.

	Kuncho variety			Keyetena variety		
Source	Iron	Calcium	Zinc	Iron	Calcium	Zinc
Model	***	***	***	***	***	***
Intercept	17.63	214.73	7.56	46.35	229.50	6.02
A	-0.19***	-0.27***	-0.211***	-0.182***	-0.274***	-0.212***
B	-0.46***	-0.52***	-0.445***	-0.453***	-0.524***	-0.446***
C	0.14***	0.18***	0.106**	0.143***	0.179***	0.105**
A^2	0.01^{ns}	0.066^{ns}	0.031^{ns}	0.014^{ns}	0.064^{ns}	0.027^{ns}
B^2	0.08^{ns}	0.10^{ns}	0.080^{ns}	0.071^{ns}	0.105^{ns}	0.082^{ns}
C^2	-0.05^{ns}	0.11*	0.068*	-0.041^{ns}	0.101*	0.066*
A*B	0.007^{ns}	0.088^{ns}	0.012^{ns}	0.002^{ns}	0.087^{ns}	0.008^{ns}
A*C	0.010^{ns}	-0.051^{ns}	-0.009^{ns}	0.039^{ns}	-0.047^{ns}	-0.014^{ns}
B*C	-0.040^{ns}	-0.11^{ns}	-0.018^{ns}	-0.062^{ns}	-0.110^{ns}	-0.018^{ns}
Lack-of- fit	0.1623	0.3288	0.9157	0.1176	0.3178	0.9424
R-Square	0.9601	0.9485	0.9622	0.9683	0.9498	0.9626
Adjusted -R Square	0.9243	0.9022	0.9283	0.9398	0.9045	0.9289
Predicted- R Square	0.7657	0.7273	0.8940	0.8024	0.7311	0.9037

Where A= temperature B= time C= thickness ns= non-significant difference (p> 0.05) *=p< 0.05 ** = p< 0.01 and *** p< 0.001

4.3.2. Calcium content

Calcium is the most common mineral in our body and is crucial for the strength of the skeleton and hardness of teeth. It also plays numerous functions in the body. High calcium diets prevent gaining of weight and fat accumulation (Teegarden, 2003). Calcium content obtained from teff flour was 96.2 and 110.95 mg /100g for Kuncho and Keyetena varieties respectively. Keyetena variety contains highest calcium content as compared to Kuncho also this finding supported by Abebe *et al*., (2007) red teff contains highest calcium and iron content than white teff. Table 15 indicates that experimentally determined Calcium value of biscuits baked under different conditions. The minimum and maximum Calcium content were 214.2 and 215.97 mg/100g for

Kuncho and 228.94 to 230.72 mg/100g for keyetena based biscuits. Highest values were obtained on biscuit baked at174^{o}C for 6 min of 6.5 mm thick and lowest values were for biscuit baked at186^{o}C for 12 min of 4.5 mm thickness. The regression equation describing the effect of the process variable on Calcium content indicated on Table 16 were significant in linear model at ($p<0.05$) for both teff based biscuits. Interaction effects of model terms were not significant. Lower and highest results in both cases might be highly associated with thickness of biscuits as the effect of thickness not only found significant on main terms but also in quadratic term. All biscuit prepared from both teff varieties indicated that the calcium content increased many folds than teff flour this is from the fact that inclusion of additional ingredients for biscuit making.

This finding was in line with Olajide *et al.* (2011), slight reduction in mineral contents during baking of the taro cookies were due to highest temperature above (180°C) which causes mineral degradation as a result of destruction or chemical changes like oxidation. Moreover, as heating continued, for longer time some of the mineral elements such as zinc, iron and calcium may be to lose a few amounts of volatile compounds and contributing to the reduction in the total mineral content.

Epidemiologic evidence (Norat and Riboli 2003) and one intervention study (Holt *et al*., 2001) have shown that highest calcium intake lowers the risk of osteoporosis can be prevented by generous intake of calcium. High consumption of calcium rich foods will help build optimum bone mass during childhood and adolescence and will also slow the rate of bone loss that naturally occurs with aging (Dickinson, 2002).

4.3.3. Zinc content

Zinc plays an important role in normal growth and development (Islam *et al.*, 2009). Zinc is essential part of more than 200 enzymes included in the digestion, metabolism, and reproduction and wound healing. It plays critical role in immune response and is an important antioxidant. Zinc content from teff flour was 5.5 and 3.95 mg for kuncho and keyetena teff. Measured values from biscuit indicated on Table 15. Experimentally, the minimum and maximum Zinc content was 7.06 and 8.19 mg for Kuncho and 5.51-6.65 and for Keyetena based biscuit respectively. Lowest values were obtained on biscuit baked at186^{o}C, 12min, and 4.5 thick for Kuncho and Keyetena teff based biscuit respectively. The highest zinc content was obtained from biscuit baked at 174°C, 6 min and 6.5 thick for Kuncho and Keyetena respectively. The regression equation describing the effect of the process variable on zinc content indicated on Table 16. Zinc content was significant in linear model at ($p \leq 0.05$) for biscuit baked from both varieties. Effect of interaction of model terms was not significant for both types. Values for both teff biscuit were highest than zinc content in both teff flour. The slight increment of zinc content was due to additional biscuit making ingredients.

4.4. Optimization of Processing Variables in Response to Measured Parameters for Biscuit Making

Optimization is the next step involved to determine the best combination of factors that enable us to produce biscuits having better physicochemical properties. The optimization of the baking conditions arises from cooperation among the different responses (Sabanis *et al.*, 2009). Numerical optimization technique of the Design-Expert (6.0.2) software was used for simultaneous optimization of baking conditions. Based up on desired target values of physical and chemical response variables, biscuits baked at 174°C for 9 minute using 4.5 mm thickness was found to be the best baking condition for biscuits made from Kuncho and Keyetena based flours. Optimized values of Kuncho and Keyetena based biscuits for physical parameters like difference in diameter, hardness, water activity, bulk density, and water rehydration capacity varied between 1.48-1.65 mm, 81.3-86.89 N, 0.5-0.52, 0.68-0.7g/m^3 and 2.95-2.99 ml respectively. Optimized proximate composition for moisture, ash, protein, ether extract, fiber, carbohydrate, and energy also varied between 5.7-5.81%, 3.55-3.65%, 17.11-17.8%, 14.2-14.3%, 3.8-4.04%, 54.7-55.15%, and 418.2 kcal/100g. For mineral elements of Kuncho based biscuit with, Iron 17.6, Calcium 215.1, and Zinc 7.8 mg/100g whereas for Keyetena based biscuits with iron 46.22, calcium 230.0 and zinc 6.18 mg/100g contents respectively.

4.5. Sensory Evaluation

Sensory qualities are the main criterion that makes the product to be liked or disliked (Falola *et al.,* 2011). Sensory evaluation was done after overall optimization of physical and chemical parameters then six time, temperature and thickness combination for both varieties selected and mean scores were taken for comparison.

Figure 24 Biscuit samples baked and made ready from Kuncho based flour

Figure 25 Biscuit samples baked and made ready from Keystone based flour

4.5.1. Taste

The mean value of six biscuit samples for taste was found to be in the range from 4.00 to 4.30 (Table 17). Highest value was shown for both Kuncho and Keyetena teff based biscuit baked at174^{o}C for 8 min having 4.5 thickness from three coded samples (like moderately) while the lowest value was given by keyetena teff based biscuit baked at 186^{o}C for 9 minute having 6.5 thicknesses. Taste of biscuit showed that there is no significant difference ($p<0.05$) among Kuncho and Keyetena based biscuit. This might be due to dominant effects of ingredients added (47.3 %) on both teff flours. In addition to that one of the major problems associated with gluten-free products is their inferior taste and/or structure. According to Gallagher *et al*., (2004) teff flour has a good taste than other gluten free cereals and hence allows an opportunity to produce gluten-free product with an attractive taste.

Table 17 Sensory evaluation biscuits baked under selected conditions from both kuncho and keyetena teff based flour

Teff flour source	Temperature (°C)	Time (min)	Thickness (mm)	Taste	Color	Aroma	Crispness	Overall acceptance
Kuncho	174	8	4.5	4.30	4.38	4.22	4.48	4.54
Kuncho	180	8.5	5.5	4.24	4.20	4.10	4.00	4.14
Kuncho	186	9	6.5	4.20	4.10	4.16	4.14	4.00
Keyetena	174	8	4.5	4.26	2.74	4.18	3.95	3.98
Keyetena	180	8.5	5.5	4.10	2.68	4.00	3.88	3.78
Keyetena	186	9	6.5	4.00	2.59	4.10	3.74	3.62

4.5.2. Color

Vision plays a major role in sensory analysis and the appearance of food can have a major effect on its acceptability. The sensory score of six biscuits samples is presented in table (17) and the color preference score ranged between 4.38 and 2.59. The highest value was given for Kuncho teff baked biscuits at 174°C for 8 minutes having 4.5 mm thickness (moderately like) while the

Table 18 Analysis of Variance for sensory evaluation of selected biscuits

Source	Total count	Mean	SE Mean	St Dev	F values	P value	Regression coefficient	Coef Var
Taste	18	4.18	0.0895	0.379	0.20	0.958	75.4	9.08
Color	18	3.44	0.194	0.822	80.29	0.000	97.10	23.86
Aroma	18	4.13	0.0235	0.0996	2.94	0.059	55.02	2.41
Crispness	18	4.19	0.095	0.209	17.02	0.000	87.64	5
Overall	18	4.01	0.0731	0.3102	27.28	0.000	91.91	7.74

Acceptability

Lowest value was given for red teff based biscuit baked at 186^{o}C for 9 minute having 6.5 mm thicknesses (2.59 dislike moderately). Color of biscuit showed that there is highly significant difference ($p<0.05$) among two varieties based biscuits baked under selected condition this might be due to darker colour of keyetena based biscuit it contained darker colour this proposed to be due to highest levels of pigmented material, such as tannins and polyphenols (Umeta and Parker, 1996).

4.5.3. Aroma

The aroma score of six biscuits samples is presented in table (17). The aroma preference ranged between 4 and 4.22. The highest value was given by Kuncho teff baked at174^{o}C for 8 minutes having 4.5 thicknesses (Like Moderately) while the lowest value was given by Keyetena based biscuit baked at 180^{o}C for 8.5 minutes having 5.5 thicknesses. Mean score for aroma of biscuit showed that there is no significant difference ($p<0.05$) among two teff based biscuit. The aroma was mostly influenced by the reaction occurred between cross linking of teff starch gelatinization and protein denaturation. All prepared biscuit liked by sensory panelist this might be due to enhanced distinctive flavor of teff liked by many people and aromas produced during baking.

4.5.4. Crispness

The crispness score of six biscuits samples was presented in table 17. The crispness score ranged between 3.74 and 4.48. The highest value was given for Kuncho based biscuits baked at174^{o}C for 8 minutes and having 4.5 mm thickness (like moderately) while the lowest value was given Keyetena based biscuit baked at 186^{o}C for 9 minutes and having 6.5 mm thickness. The analysis of variance showed highly significant difference in the crispness score ($P<0.05$) among the six biscuit samples. The lowest score from red teff baked at highest temperature and longer time of baking this might be due to its tough texture.

4.5.5. Over all acceptances

Kuncho teff based biscuit baked at 174^{o}C for 7.2 minutes having 4.5 mm thickness gained the highest score of overall acceptance (4.54) as compared to others this may be due to low temperature and smaller thickness of biscuit which might be associated with desired crispness.

While the lowest value was for Keyetena based biscuit baked at 186^{o}C for 9 minute and having 6.5 mm thickness (3.62 neither like nor dislike). Over all acceptability of biscuit showed that there is highly significant difference ($p < 0.05$) between Kuncho and Keyetena based biscuits baked under selected condition. This might be associated with undesired crispness and dark brown color of biscuits.

5. SUMMARY AND CONCLUSION

The only effective treatment for celiac disease is the total lifelong avoidance of gluten ingestion or using a strict gluten-free but nutritious diet. This study was primarily aimed to produce better quality gluten free biscuit as alternative food source for gluten intolerant people and to investigate production of teff biscuit. Secondly it was attempted to determine suitable time-temperature and biscuit thickness combinations for best quality biscuit.

Teff flour is an interesting gluten free raw material and considered as the major ingredient to produce gluten free biscuit. So far no literature data reported that the optimum baking condition to produce teff based gluten free biscuits. Baking condition results showed that highest baking temperature, longer time and thinner biscuit causes a decrease in thickness, water activity, moisture and protein. On the other hand as the baking temperature and time increased rehydration ratio, hardness, bulk density, carbohydrate and energy content all biscuit samples increased. On the contrary biscuit fat, ash, crude fiber and minerals (Fe, Ca, and Zn) were not affected significantly by interaction of baking temperature, time and thickness. In most of physical and chemical parameters measured as such no significant differences were observed on biscuits made from teff flour of both types of teff. However, when baking conditions investigated better physical and chemical qualities were observed for biscuits baked from both sources at 174^{o}C for 8-9 minutes and 4.5 mm thickness were selected as optimum.

Based upon optimized conditions acceptability of the biscuits was assessed through conducting sensorial analysis. Biscuit baked from Kuncho flour got better overall acceptability as compared to Keyetena. This showed that, color and crispness significantly makes an influence on consumers than other sensorial properties in terms of choosing biscuit types. Lower baking temperature, baking time of 9 with 4.5 thickness are preferred conditions to produce better quality gluten free biscuits. However the sensory score for keyetena teff based biscuit is lower it is nutritionally outstanding than kuncho teff based biscuit in addition to highest composition of calcium and iron content so it is recommended for nutrient sensitive individuals. In other way Kuncho teff based biscuit got better overall acceptability in addition to other sensorial parameters and it is recommended for nutrient insensitive individuals. Using teff flour as an ingredient is an interesting alternative raw material for manufacturing nutritionally improved gluten free foods. This avoids efforts and costs associated to remove gluten from wheat flour.

The out comes from this study would be used to generate baseline information for subsequent use and studies to produce nutrient enriched gluten free products. Many gluten-free products may not meet the recommended daily intake for fiber and minerals. However, gluten free biscuit made from teff based flour under recommended baking condition will mitigate such limitations and provide alternative snack food source for gluten intolerant individuals.

6. FUTURE LINE OF WORKS

During the process of undergoing this research work, there had been some constraints. Based on this, the following recommendations are made.

i. Optimum baking conditions determined in this study were made under laboratory scale. For further scale up and commercial use as per the interest of the industry further validation study need to be conducted using generated models as an input to initiate the test.

ii. Since teff is gluten free grain and hence biscuits made using teff as a major ingredient may not attain the desired properties of wheat based biscuits. Therefore further study needs to be made to develop desired properties of gluten in teff based biscuits.

iii. On this study only two teff varieties included for gluten free biscuit making, further study might be needed on different varieties of teff.

iv. Color score for keyetena teff is lower, further study to be conducted to improve its color by addition of colorants.

v. Even though biscuits baked in this study are intermediate moisture products with better shelf stability, further study might necessary to study change in quality of baked biscuits under different storage conditions and packaging materials.

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8. APPENDECIES

Appendix 1

Appendix Table 1 ANOVA for teff biscuit physical and functional properties

	Kuncho						Keyetena					
Source	Diameter Difference (mm)	D/ce in thickness (mm)	Hardness (N)	Water activity	Bulk density (g/cm^3)	RR	Diameter Differenc e(mm)	D/ce in thickness (mm)	Hardness (N)	Water activity	Bulk density(g/cm^3)	RR
Model	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Intercept	1.37	0.31	85.89	0.53	0.71	3.00	1.55	0.33	91.12	0.54	0.68	2.96
A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C	0.0068	0.0016	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0154	0.0024	< 0.0001	< 0.0001	< 0.0001	< 0.0001
A^2	0.1172	0.0028	< 0.0001	0.0006	0.0002	< 0.0001	0.04	0.0043	< 0.0001	0.0001	< 0.0001	< 0.0001
B^2	0.2366	0.9513	< 0.0001	0.0054	0.0008	< 0.0001	0.2216	0.564	< 0.0001	0.1143	0.0017	< 0.0001
C^2	0.4402	0.2471	< 0.0001	0.0017	0.0008	< 0.0001	0.2216	0.1754	< 0.0001	0.0004	< 0.0001	< 0.0001
A*B	0.2579	0.0684	< 0.0001	0.0004	0.0087	0.0037	0.292	0.1354	< 0.0001	< 0.0001	0.0018	0.0004
A*C	0.8152	0.0684	0.6613	0.0012	0.0553	< 0.0001	0.5903	0.1354	0.0042	0.0011	0.0134	< 0.0001
B*C	0.8152	0.5115	0.0158	1.000	0.3036	0.2377	0.5903	0.6001	< 0.0001	0.3879	0.5623	0.4115
Lack-of- fit	0.5372	0.4901	0.9939	0.2054	0.2051	0.0716	0.6468	0.7640	0.1816	0.2222	0.1152	0.1614
R-Squared	0.9388	0.9556	0.9948	0.9893	0.9791	0.9932	0.9525	0.9363	0.9999	0.9935	0.9847	0.9929
Adj R^2	0.8836	0.9157	0.9904	0.9797	0.9604	0.9870	0.9098	0.8790	0.9999	0.9877	0.9710	0.9865
Pred R^2	0.7311	0.7690	0.9902	0.9358	0.8780	0.9561	0.8091	0.7503	0.9995	0.9630	0.8988	0.9581

Appendix 2

Appendix Table 2 ANOVA for teff biscuit (kuncho variety) nutritional composition

Source	MC (%)	Ash (%)	Crude Protein (%)	Ether extract (%)	Crude fiber (%)	CHO (%)	EnergyK.cal/ 100g
Model	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Intercept	5.91	3.65	17.96	14.25	3.85	54.38	417.58
A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001
B	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
A^2	< 0.0001	0.0518	0.0002	0.0972	0.0009	< 0.0001	< 0.0001
B^2	0.3787	0.12	0.0105	0.4651	0.0103	0.0791	0.262
C^2	0.0257	0.0002	0.0193	0.0005	0.0001	0.0174	< 0.0001
A*B	< 0.0001	0.7944	0.0021	0.5853	1	0.0008	< 0.0001
A*C	0.0012	0.4405	0.193	0.2858	1	0.0126	0.0025
B*C	0.0231	0.4405	0.0249	1	0.4396	0.0219	0.0162
Lack-of- fit	0.1805	0.0697	0.6390	0.0655	0.3855	0.4724	0.0562
R-Squared	0.9936	0.9798	0.9926	0.9748	0.9794	0.9952	0.9890
Adj R-Square	0.9878	0.9616	0.9860	0.9522	0.9608	0.9908	0.9792
Pred R-square	0.9612	0.8592	0.9703	0.8161	0.8961	0.9767	0.9285

Where A=Temperature, B=Time and C= Thickness, R^2=determination coefficient M.C= moisture content CHO= carbohydrate K.cal = Kilo calorie

Appendix 3

Appendix Table 3 ANOVA for teff biscuit (keyetena variety) nutritional composition

Source	MC (%)	Ash (%)	Crude Protein (%)	Ether extract (%)	Crude fiber (%)	CHO (%)	EnergyK.cal / 100g
Model	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Intercept	6.00	3.56	17.21	14.37	4.05	54.80	417.39
A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002
B	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001
A^2	< 0.0001	0.0273	< 0.0001	0.0936	0.0031	< 0.0001	0.0003
B^2	0.5257	0.361	0.0098	0.3365	0.0031	0.0422	0.384
C^2	0.0037	< 0.0001	0.0109	0.0006	0.0001	0.0132	0.0001
A*B	< 0.0001	0.7811	0.0065	0.7458	0.707	0.0006	< 0.0001
A*C	0.0011	0.4118	0.1897	0.3409	0.707	0.0193	0.0241
B*C	0.0279	0.4118	0.0058	0.9137	0.2728	0.0095	0.0504
Lack-of- fit	0.2752	0.093	0.1995	0.0699	0.332	0.3406	0.0602
R-Squared	0.9959	0.9822	0.9925	0.9732	0.9755	0.9956	0.9872
Adj R-Square	0.9922	0.9663	0.9857	0.9491	0.9534	0.9917	0.9756
Pred R-square	0.9771	0.8778	0.9570	0.8098	0.8735	0.9769	0.9170

Where A=Temperature, B=Time and C= Thickness, R^2= determination coefficient M.C = moisture content CHO = carbohydrate K.cal = Kilo calorie

Appendix 4

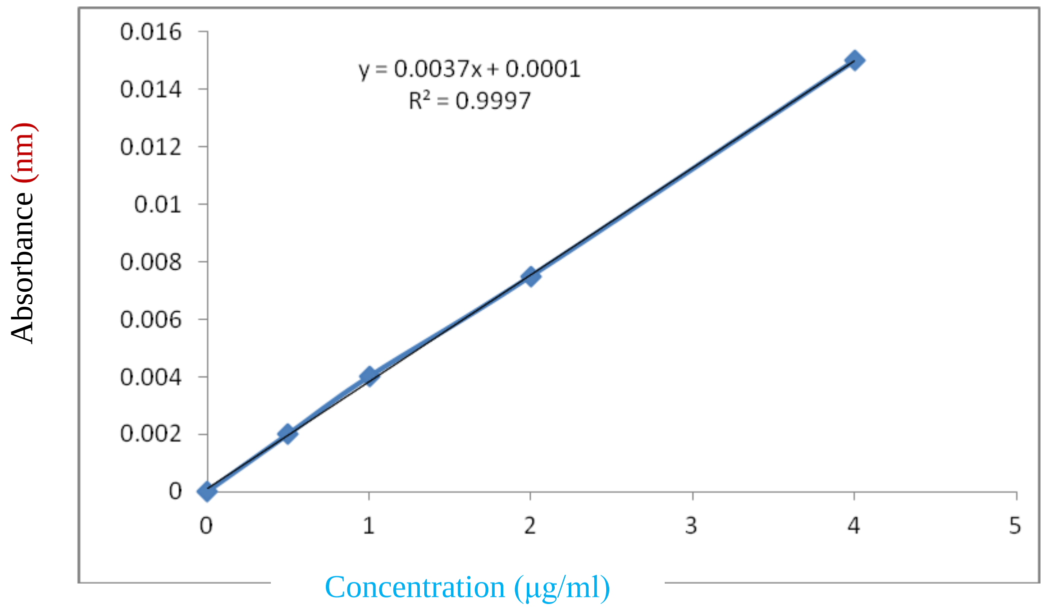
Appendix Table 4 ANOVA for both Teff biscuit Mineral Compositions

	Kuncho variety			**Keyetena variety**		
Source	Iron	Calcium	Zinc	Iron	Calcium	Zinc
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Intercept	17.63	214.73	7.56	46.35	229.50	6.02
A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C	0.0006	0.0011	0.0027	0.0002	0.0009	0.0028
A^2	0.6899	0.0881	0.227	0.5435	0.0905	0.2834
B^2	0.098	0.1328	0.0895	0.0946	0.1163	0.0793
C^2	0.0586	0.0111	0.0174	0.0886	0.0144	0.0197
A*B	0.8845	0.1872	0.7902	0.9667	0.189	0.8484
A*C	0.7718	0.2991	0.7902	0.2146	0.3272	0.6751
B*C	0.3924	0.0951	0.6766	0.1448	0.1039	0.6751
Lack-of- fit	0.1623	0.3288	0.9157	0.1176	0.3178	0.9424
R-Square	0.9601	0.9485	0.9622	0.9683	0.9498	0.9626
Adjusted R^2	0.9243	0.9022	0.9283	0.9398	0.9045	0.9289
Predicted R^2	0.7657	0.7273	0.8940	0.8024	0.7311	0.9037

Where A=Temperature, B=Time and C= Thickness, R^2=determination coefficient

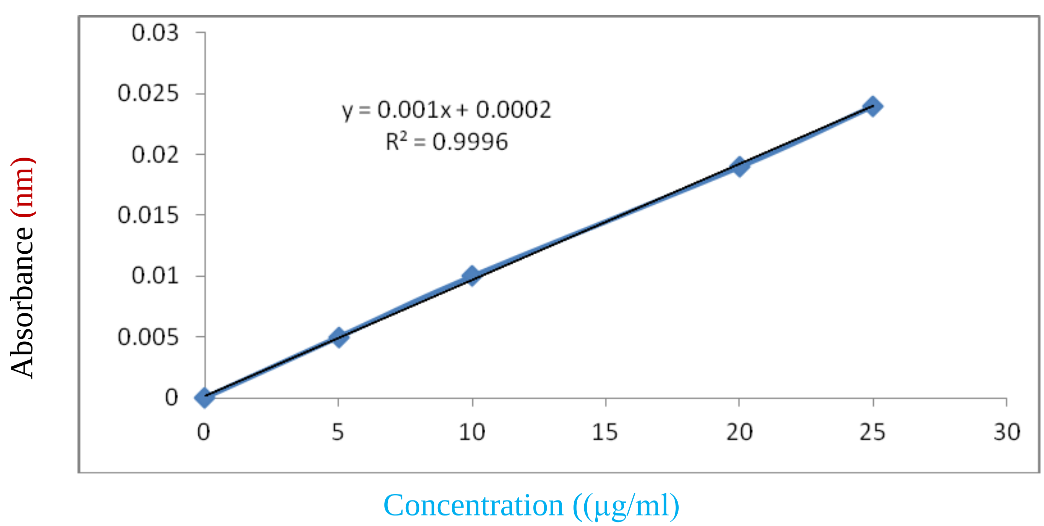
Appendix 5: Calibration curve of Iron, Zinc, and Calcium

I. Iron calibration curve

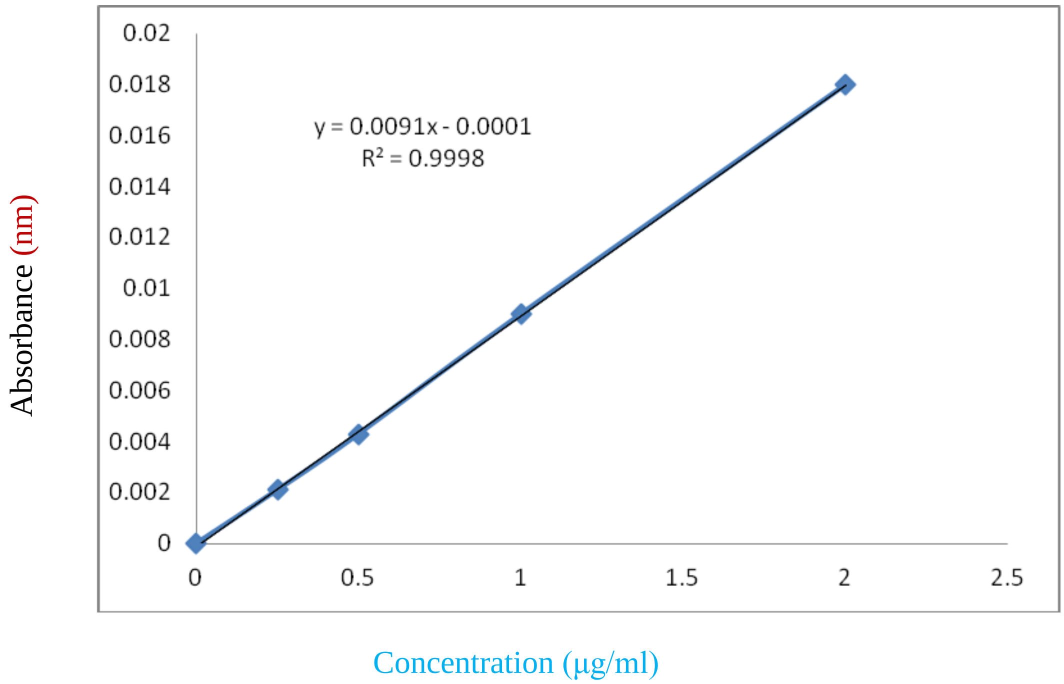


Abs=conc+0.003 y=0.998

II Calcium calibration curve



III Zinc calibration curve



Appendix 6

Ballot for sensory evaluation of biscuit using a five point hedonic scales. Please look at and taste each sample of biscuit in order from left to right or right to left as shown on the ballot. Indicate how much you like or dislike each sample by checking the appropriate phrase of category which is listed below and mark your choice with the number that corresponds to your preference on each parameter.

1. Extremely dislike
2. Dislike moderately
3. Neither Like Nor Dislike
4. Like Moderately
5. Extremely Like

Appendix Table 5 Sensory Evaluation Form

Sample Code	Taste	Color	Aroma	Crispness	Overall Acceptability