# OPTIMIZATION OF OKARA, WHEAT AND RED TEFF BLENDING RATIOS FOR BETTER PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF COOKIES

**M.Sc. THESIS** 

BY

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JUNE, 2016

JIMMA, UNIVERSITY

## OPTIMIZATION OF OKARA, WHEAT AND RED TEFF BLENDING RATIOS FOR BETTER PHYSICO-CHEMICAL, AND SENSORY PROPERTIES OF COOKIES

M.Sc. Thesis

# Submitted to the School of Graduate Studies Jimma University College of Agriculture and Veterinary Medicine

In Partial Fulfillment of the Requirements for the Degree of Master of Science

In Post-Harvest Management (Durable)

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June, 2016

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### **DEDICATION**

This thesis is dedicated to the soul of my beloved Family who blessed me to achieve this work with my respect and love. I forward this work to who gave me maximum and endless help and support. Thank you for everything you have done for me. I will always treasure my memories of you. I wish you could have seen me graduate, but I think this is the next best thing for you thank you all.

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#### **BIOGRAPHICAL SKETCH**

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#### ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful Alhamdulillah, all praises to Allah SWT for His blessings and strength given to me in completing this study he gave me health, the courage and the strength to accomplish my mission. I am particularly very grateful to my advisors. **Dr. Neela Satheesh** and **Mr.Kumela Dibaba** for their meticulous guidance, encouragement, willingness to supervise my research and valuable comments from early stage of proposing the research to the final thesis manuscript write- up. I have learnt a lot from my association with them for which I am deeply indebted.

I would like to express my heartfelt gratitude to Post Harvest Management Department and Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) for giving me an opportunity to pursue my M.Sc Study in post-harvest management through the PHMIL project funded by CIDA. Also I would like to acknowledge JUCAVM Animal Nutrition laboratory, Post-Harvest Management laboratory and Ethiopian Health and Nutrition Research Institute for their all rounded facilitation and support to execute the experimental work timely.

My special appreciation goes to Jimma Agricultural research center and Holeta Agricultural Research Center for their giving the raw the experimental materials samples for the thesis work. My deep acknowledge also goes to JUCAVM CASCAPE Project for their financial support; especially Dr. Gezahagn Berecha for his facilitation and support during my thesis work.

Special thanks go to Prof.essor Tessema Astatike who helped me in interpreting and analyzing my data analysis and interpretation and sharing ideas by spending his precious time. I am extremely lucky to have favorite's brothers and Sisters who have special place in my heart for their good will and assistance contributed to my study you have encouraged me in every way possible and inspired me to believe in my life. Lastly to my lovely husband Dr. Mohammed Merga (surgery specialist) for understanding and sharing my dreams, believing in my ability to support and sacrifice to enable me achieve, words on a page not enough me to express your importance to me. Thank you for never allowing me to give up on my dreams and being there to support me each step of the way.Thank you all.

# LIST OF ABBREVIATIONS AND ACCRONYMS

ALA	α-Linoleic acid	
CD	Celiac Disease	
GAE	Gallic Acid Equivalents	
GI	Glycemic index	
IDA	Iron-deficiency anemia	
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine	
LA	Linoleic acid	
OF	Okara flour	
SCR	Soybean curd residue	
SEM	Scanning electron microscope	
SR	Spread ratio	
UVS	Ultra violet spectroscopy	
WAC	Water Absorption Capacity	
WF	Wheat Flour	

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#### ABSTRACT

Okara is a by-product of soy milk processing and it is often underutilized because of knowledge gap and undesired sensory quality related with soyabean products. Inline with this, the present study was initiated to assess the potential of incorporating okara in bakery products to produce value added product. The cookies were made with mixture of red teff, wheat and okara flours blended in different proportions. Sixteen composite flours were prepared using D-optimal mixture Design with three ingredients in the ranges of 20-40%, 10-20%, and 40-50% for red teff, wheat and okara flours. Standard procedures were used to analyze proximate compositions, anti-nutritional factors (phytate and tannin), mineral composition, physical properties and sensory quality of cookies made from the flours. High ratio of okara in the blend increased fat, fiber, protein, gross energy, mineral contents of cookies. But an increse in wheat and red teff flour increased the carbohydrate value. The breaking strength, diameter and thickness of cookies were significantly (p < 0.05) different as compared with control. The red teff and wheat, wheat and okara, and red teff with okara were showed highly significant effect on spread ratio. The higher proportion of okara flour in the blend reduced the physical characteristics of the cookies due to the dilution of the gluten network. The mineral contents (Zn, Ca, and Fe) were significantly (p < 0.05) different and cookies prepared from more portion of okara and red teff have good mineral content than the control. Interms of anti- nutritional contents cookies made with high concentration of okara flour significantly different in tannin content but cookies made from different composite flour were non significance in phytate content. Control cokkies samples were superior in terms of colour, aroma, taste and crispness but none significantly different in terms of overall acceptability of cookies made from teff 30-40%, wheat 17-20% and okara 43-50%. Optimized mix ratios were 35-40%, 15-18% and 47-50% for Teff, Wheat and Okara flours respectively. At this optimum mix the ranges for protein were 15.4-18.56%, carbohydrate 45.11-57.45%, fat 14.8-18.2%, energy 409.88-416.2 kcal/100gm, fiber 7.4-8.9 %, Iron 6.66-7.3 mg/100g, Calcium16.6-18mg/100gm, Zinc 2.66-2.88 and overall acceptance received hedonic ratings 3.32-4.55 were obtained. The results from this study revealed addition of okara improved the nutrient content and other functional properties than discarding as a waste from soya milk processing.

Key words: Cookies, Okara, Red teff, Wheat, Composite flour, Nutrient composition

## **1. INTRODUCTION**

The soybean is a legume, one of the richest and cheapest sources of plant protein that can be used to improve the diet of millions of people, especially the poor and low income earners in developing countries (LIU, 2000). Also are high in unsaturated fatty acids and contain no cholesterol (Singh et al., 2007). The insoluble residue by-product obtained during processing of soybean soymilk is known as okara and is underutilized.

Okara, the pulpy by-product of the soy milk besides its application as an environmental waste and animal feed or sent to landfills for disposal, which creates an ecological problem, not fully utilized as human food consumption in world wide recipes because of knowledge gap (Murayama K., 2006). Recently in Ethiopia okara produced yearly pose a significant disposal problem, not utilized for foods and ishighly susceptible to putrefaction in the environment. It is generally considered as a waste product with strong beany smel, making it unacceptable to all (Rinaldi *et al.*, 2000).

Raw Okara is high content of 75% of moisture, 25% protein, 10-15% oil on wet basis and bulk amount of crude fiber (Van Der Riet, 1989., Wang, 1994). According to Travaglini et al., (1980) the amino acid profile of Okara is slightly superior to that of soymilk itself. Similarly Bowles and Demiate (2006) also showed that approximately 1/3 of the isoflavone present in the soybean remains in the Okara. The presence of 95% of the grain solid components in Okara makes it a very high nutritional value resource (Smith *et al.*, 1978).

Never the less no industrial product made of okara based product is currently available commercially. However, a possible use of okara in develop baked products as it has a large amount of protein is important to value added of okara based food. Okara can be utilized in foods specially formulated with high fibre content (Cheng Y., *et al.*, 2005). So that to value addition the the Formulation of composite flour is vital for development of value-added products with optimal functionality (Rehman *et al.*, 2010). The concept of using composite flours is not new and has been the subject of numerous studies (Vieira et al., 2007). It indicates that the Okara protein is of extremely high quality suggesting that it is a good, low cost source of income and can be utilized as food for human consumption by mixing with other cereals.Hence, blending of this food

ingredients through optimum amount can be assumed as a good strategy for improving the nutritional and sensoryquality of cookies using simple technology.

Teff (*Eragrostis tef*) is an ancient tropical cereal thathasits centerof origin and diversity in the northern Ethiopian highlands from where it is believed to have been domesticated (Ketema 1997; Demissie 2001). Teff has been cultivated and used for human consumption in Ethiopia for centuries. However, teff's global use for human consumption has been restrained partly due to limited knowledge about its nutrient composition and the processing challenges faced in making teff-based food products. Wheat is one of the most important staple foods for humans (Akhtar *et al.*, 2008). The importance of wheat is mainly due to the fact that its seed can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as Pastas, and thus it presents the main source of nutrients to the most of the world population.

Composite flour is a mixture of several flours obtained from cereal and legume, with or without the addition of wheat flour (Adeyemi and Ogazi, 1985). This idea has also resulted in the use of local raw materials that could be combined for optimum nutritive value with good processing characteristics. This enables created to satisfy specific functional characteristics and nutrient requirements. The use of composite flours from cereals and legumes for cookies production is therefore expected to enhance the utilization of cereal grains as raw material and improve the nutritive quality of cookies. It have thus advantageous in the sense that inherent deficiencies are supplemented from other sources and it plays a vital role to complement the lack of essential nutrients with this possible to save hard currency; promote high yielding local plant species and enhances overall use of domestic food (Khan 2005). Composite flours have been used extensively and successfully in the production of baked foods. The bakery products produced using composite flour were of good quality, though the texture and the properties of the composite flour bakery products were different from those made from wheat flour, with an increased nutritional value and other properties

Usually, the aim of producing composite flour is to get a product that is better in certain features than the individual components. Better may mean improved properties or performances, or in some cases, improved economies, or nutrition. For instance, composite flours produced from cereals and legumes have the advantage of improving the overall nutrition (FAO 1995). Thus

much research involving the use of non-wheat flour has been carried out to substitute wheat in baked products in developing countries (UNECA, 1985).

Teff is a minor cereal crop worldwide, whereas in Ethiopia, it is a major food grain, mainly used to make injera, a traditional fermented Ethiopian pancake (Yetneberk *et al.*, 2005). Relative to more common cereals red teff has a higher iron and calcium content (Abebe *et al.* 2007). Inadequate iron intake is common in low and middle income countries like Ethiopia, particularlyamong 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 (Stoltzfus, 2011). Therefore, adjusting iron intakes with iron-rich foods may be preferred and red *teff* can be a good alternative. However, over the past decade, the red *teff*, recognized as gluten-free grain has spurred global research interest by nutritionists and food scientists. Consequently, studies on the nutritional composition of red *teff* and its processing qualities have grown, and the development of new red *teff*-based products has accelerated to produce good baked products because of high iron content.

Wheat is the whole grain milled to leave just the endosperm for white flour, while the byproducts of bran and germ are discarded. It has been shown that the whole grain is a concentrated source of essential nutritional components such as minerals, protein, fat and fibre while the refined grain is mostly starch (Potter, 2006). Wheat therefore, is perhaps the most popular energy grain for the production of confectionary products, because of the unique properties of gluten which combines strength and elasticity required to produce cookies of desirable texture and flavor (Potter 2006, Akhtar *et al.*, 2008).

Among ready-to-eat snacks cookies are popular and widely consumed all over the world by all ages especially by children to provide energy (Pratima and Yadava, 2000) are very popular in daily diet of almost all profiles of consumers (Belgrade, 2007). Not only this, they are appetizing because of sweetness, due to their simple manufacturing process, long shelf life and potential for containing high nutrient components (Noor Aziah et al., 2012). Commercially available cookies are prepared from white flour that is nutritionally inferior to whole wheat flour (Elahi, 1997).

Generally, the shortage of energy, protein and iron deficiency are amongst the major problems of human nutrition in developing and under developed countries including Ethiopia. Soybean production is popularizing in the southwestern part of Ethiopia especially in Jimma and Illu Abbora zones. Different governmental and non-goveremental organizations are supporting the community on populazing soybean production, processing and comsumption. On the other hand, small scale soymilk processing enterprizes are emerging. As a result, huge amount of okara is produced every year which is either used as animal feed or discarded as a waste.Causing environmental problem. However, there is no information about the usage of okara as a formulation in a food product to improve the protein deficiency.

Utilization of food wastes like okara is not only providing nutritional benefits but also minimizes the environmental pollution and puplic health problems due to the wastes accumulation. Moreover, to enhance the utilization of okara in the daily diet of people, it is highly desirable to develop novel and value added food products of nutritional status from okara of the world specially Ethiopia. This is an alternative option to reduce protein, energy and iron deficiency problem by blending with iron enriched red teff flour. Therefore, another alternative that can be taken as part of problem solving of this thesis issues and the aim of this research work is to produce cookies by utilizingand value added waste product of soya milk okara, blending with red teff and wheat which would ensure food security, enhance sublic health, combat malnutrition problems, economic activities leading to wealth creation, and reduce environmental problem.

#### 1.3. Objectives

#### 1.3.1. General objectives

• To produce better quality cookies from Okara, red *teff* and wheat flours blend.

#### 1.3.2. Specific objectives

- To investigate the effect of blending ratios of composited flours on nutritional contents and selected anti-nutritional factors of cookies.
- To determine the effect of blending ratios of okara, what and teff flour on physical and functional properties of cookies.
- To determine the effect of blending ratio on sensory acceptability of cookies.
- To determine optimal ratio of okara, wheat and teff flour for better overall quality cookies. .

#### **2. LITERATURE REVIEW**

# 2.1. Production, consumption and Nutritional composition of Soybean okara, Red Teff and Wheat

Soy bean curd residue (SCR) or okara is a relatively inexpensive source of protein that is widely recognized for its high nutritional and excellent functional properties (Cheng Y., *et al* 2005). Teff (*Eragrostis tef*) is an ancient tropical cereal that has its center of origin and diversity in the northern Ethiopian highlands from where it is believed to have been domesticated (Ketema, 1997; Demissie, 2001). Wheat is one of the most important staple foods for humans (Akhtar et al., 2008). The kernel consists of the wheat germ and the endosperm, which is full of starch and protein (Mannay and Shadaksharaswany, 2005).

#### 2.1.1. Okara Production and Consumption Trends

Okara, the pulpy by-product of the soy milk and tofu processing industry, is a potentially nutritious product that is high in fiber, protein, vitamins, minerals, and fat. Besides its application as an animal feed, Okara is used directly in food dishes such as soups and salads, or fermented into products such as tempeh in Asian countries known to consume more soy products. America and other Western countries have not utilized okara for foods as it is generally considered a waste product with strong beany flavor, making it unacceptable to U.S. Consumers (WHO, 2002).

In America, okara is largely an agro-waste and is either used in animal feeds or sent to landfills for disposal, which creates an ecological problem due to the increase in sales of the soymilk and tofu industry in the U.S. Presently, small scale production of okara is observed in home cooking where fresh soymilk is produced in household kitchen. Direct incorporation of freshly produced okara into food dishes appears to increase due in part to popular online recipes. However, no industrial product made of soy okara is currently available commercially (Travaglini *et al.*, 1980). Still now there are a lot of gap about okara lack of knowledge all over the world.

A possible use for okara is reported in baked goods as it has a large amount of fiber and protein. Even greater value can be added to okara if it can be properly introduced into gluten-free formulations, a market that is not only growing (as the incidence of celiac disease is increasing), but also lacks a successful gluten-free all-purpose flour. Therefore, gluten free all-purpose flour formulated with okara as the main ingredient offers a value added application for this agro waste while increasing the nutritional value of gluten-free baked goods (Ohno, A.*et al.*, 1993).

#### 2.1.2. Okara Composition

The proximate composition of okara will depend on the amount of water phase extracted from soybeans, and whether further water is added to extract residual extractable components. With a moisture content of 84.50%, the proximate composition of wet okara is 4.73% protein, 1.5% lipid, 7.0% sugars, 1.5% fiber and 0.4% ash at pH 6.7. Okara has scope to use as an ingredient in cookies, cakes, and other confectionery, as well as in traditional side dishes. Okara is also rich in, fat, fiber and protein. Carbohydrate percentages in soybeans are rich but low in okara because during the processing of okara it may be reduced. This soybean carbohydrate also contributes greatly to intestinal health. The abundant oligosaccharides contained in soybean carbohydrates nourish the friendly bacteria in the gut. Soybeans are high in protein, fat, and fiber. After soymilk extraction, much of that fat, fiber, and protein remains in okara (O'Toole, 1999; Riaz, 2006).

Okara, soy bran and soy isolate are the three basic types of soy fibers. "Dietary plant fiber" is now considered an essential part of a well-balanced daily diet. It is a good dietary fiber, which cannot be digested in the small intestine but can be fermented by microbes in the large intestine. It is reported that dietary fiber in okara can reduce blood fat and blood pressure, lower the level of cholesterol in the blood, protect against coronary heart disease, and prevent the occurrence of constipation and colon cancer. It also regulates diabetics' blood sugar levels. Due to these effects, dietary fiber is called the seventh nutrient by nutritionists. Especially, dietary fiber is known to provide important health benefits to the body, with regard to the maintenance of normal bowel function (Periago *et al.*, 1997). The monosaccharaides units are formed from the polysaccharides in the insoluble fiber component include glucose, arabinose, and galactose when it is enzymatically digested. In addition, there is some soluble dietary fiber in okara. Okara is mainly composed of insoluble fiber, ranging from 40.2 to 43.6% on a dry basis. The fiber components were reported by Guermani *et al.*, (1992) as around  $12.1 \pm 0.2\%$  hemicellulose, 5.6  $\pm 0.9\%$  cellulose,  $11.7 \pm 1.4\%$  lignin and  $0.16 \pm 0.07\%$  phytic acid.

According to reports in the literature, the amount of soluble dietary fiber in okara treated by high hydrostatic pressure increased more than 8-fold (Mateos-Aparicio, C. *et al.*, 2010). Furthermore, soluble dietary fiber plays an important role in the reduction of cholesterol levels in some hyper

lipidemic individuals (Reynolds, A. *et al.*, 2006) and it can also be used to improve glucose tolerance in diabetics (Jenkins, C. *et al.*, 2002). The soluble fiber in okara treated by high hydrostatic pressure has anti-inflammatory and anti-carcinogenic effects on the digestive tract in the other hand; insoluble dietary fiber increases faecal bulk and reduces gastrointestinal transit time. Moreover, it reported that fiber from okara to have a positive effect on diarrhea and constipation and as a treatment for irritable bowel (Bosaeus, 2004). Generally, dietary fiber in foods has been established to improve gastrointestinal functions (Anderson et al., 1990; Kay, 1987).

Okara has high quality protein, especially essential amino acids. It is well-documented that SCR contains about 27% proteins (dry basis) with good nutritional quality and a superior protein efficiency ratio. The protein in okara is of better quality than from other soy products; for example, the protein efficiency ratio of okara is 2.71 compared with 2.11 for soymilk, but the ratio of essential amino acids to total amino acids is similar to tofu and soymilk. However, the low solubility of okara protein in water, it is showing detrimental effects on its extraction. Accordingly, many researchers focused on how to increase protein extraction from okara. The related literature illustrates that solubility of protein in okara treated by acid increased markedly with other functional properties such as emulsifying and foaming properties being improved (Chan and Ma., 1999).

Protein isolated from okara had a lower solubility than commercial soy protein isolate considering both acidic and alkaline pH levels, probably due to protein aggregation. A related report suggests that the protein recovery is relevant to the particle size, and the maximum protein recovery of 93.4% was achieved with okara flour from its fine fractions (<75µm) using a three - step-sequential extraction process (Vishwanathan, V. *et al.*, 2011). The functional properties of protein from okara, including emulsification, water and fat binding, and foaming properties, were found to be comparable to the commercial soy isolate. Moreover, the protein in okara after fermentation produces free amino acid and soy peptide (Chae, In, and Kim, 1997). Okara has a higher quality protein as calculated by the Protein Efficiency Ratio (PER) (okara = 2.71, milk = 2.86) than any other soybean fraction (Shurtleff et al., 1979), largely due to its high content of limiting essential amino acids, cysteine and methionine, which have been proven to be similar to the FAO scoring pattern, and high (80%) *in vitro* protein digestibility (Ma et al., 1997; Khare et

al., 1993). Therefore the development of protein resources from soybean residue (Okara) has great potential.

The mineral content in okara is relatively well documented. Okara contains macro and micro elements that increase its nutritional value. Mateos Aparicio *et al.*, (2010) emphasized that within the group of macro elements potassium is the main mineral, whereas iron is the predominant micro element in okara. In the literature there are no data on the contents of other mineral in okara, although the amounts of these microelements in foods are strictly limited. Khare, (1995) specified that mineral composition, along with unspecified monosaccharaides and oligosaccharides, can be found in the okara.

#### 2.1.3 Health benefit of okara

Epidemiologically investigations have established that elevated levels of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are major risk factors for the development of cardio-vascular diseases, and a low ratio of TC to High density cholestrol (HDL-C) are protective against them. Cholesterol abundance in the human body is governed by the levels of okara its dietary intake and internal production.

Therefore, are being made to find a use for this potentially beneficial extract, and many trials have been reported on okara's fermentation, (Mizumoto *et al.*, 2006) extraction (Quitain *et al.*, 2006) and digestion (Kasai, *et al.*, 2004). However, the use of okara itself has yet to be examined in detail. An increasing prevalence of obesity has recently been reported worldwide in both developed (Bendixen, *et al.*, 2004) and developing (Salazar-Martinez., et al 2006) countries. Obesity can induce many serious health problems and appears to lessen life expects-any, (Fontaine, et al 2003) and its prevention should therefore be a worldwide priority by okara. Okara contains a large amount of crude fiber composed of cellulose, hemicellulose, and lignin. A dietary fiber intake in okara is generally known to result in body fat loss, (Slavin, J. L 2005) and soy protein is also known to be effective in preventing obesity, Torres., *et al.*, (2006) suggesting that okara would also exert a beneficial effect on preventing obesity and/or lipid disorders. Dietary fiber's health benefits include lowering the frequency of chronic illness, improving bowel health and possibly preventing some types of cancer. Furthermore, soluble dietary fiber plays an important role in the reduction cholesterol levels in some hyperlipidemic individuals (Reynolds, A., *et al* 2006) and it can also be used to improve glucose tolerancein diabetics

(Jenkins *et al.*, 2002). Therefore, okara is to investigate the possible effects of the prevention of obesity and protein malnutrition problem.

#### 2.2. Red teff production, Composition and Consumption trends

Teff is mainly grown in Ethiopia and Eritrea, it has superior nutritional quality. It contains 11% protein, 80% complex carbohydrate and 3% fat. It is an excellent source of essential amino acids, especially lysine, the amino acid that is most often deficient in grain foods. Teff contains more lysine than barley, millet, and wheat and slightly less than rice and oats. Teff is also an excellent source of fiber, iron, calcium content, and high content of phosphorus, copper, and thiamine and other essential minerals found in an equal amount of other grains. Teff is nearly gluten-free, and is gaining popularity in the whole food and Health food industry as an alternative grain for persons with gluten sensitivity. Teff is very useful for persons with Celiac Disease (Doris, 2002).

Teff is possibly the smallest cereal grain with an average length of ~ 1 mm (Adebowale *et al.*, 2011). The average thousand kernel weights of 12 teff varieties tested by Bultosa (2007) and reported as 0.264 g. The minuteness of *teff* grains has nutritional and technological implications. For instance, as teff grains are difficult to decorticate, the cereal is consumed as a wholegrain, improving nutrient intake for consumers. In Ethiopia, three major categories of teff can be identified: white (*nech*), red (*quey*) and mixed (*sergegna*). It is also common for wholesalers to further sub-divide white *teff* into very white (*magna*) and white (*nech*).

However, given that these classifications are imprecise and subjective, what may be referred as *magna* by some may be considered as *nech* by others. However, in recent years, red *teff*, which is believed to be more nutritious, is also gaining popularity among health conscious consumers in Ethiopia and others in world.

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 higher 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 elucidated, there is more acceptance of this grain in society. Today in Ethiopia, red teff is becoming more popular related to its increased iron content. The data composition tables available were not able to differentiate the iron content between red and white teff.

#### 2.2.1 Nutritional composition of red teff

#### 2.2.1.1. Carbohydrates

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%, making teff a starchy cereal. The amylose content of red teff varieties tested ranged from 20 to 26 %, comparable to other grains, such as sorghum (Bultosa, 2007). The extent to which carbohydrate is digested and absorbed in the small intestine determines its health effect. Rapidly digested and absorbed carbohydrates (Glycemic carbohydrates) have greater impact on blood glucose levels, as they lead to greater metabolic perturbation (Lafiandra et al., 2014). Such perturbations have been associated with metabolic diseases such as type-2 diabetes and cardiovascular diseases (Ludwig, 2002). Hence, from a health standpoint, slowly digesting carbohydrates are preferred over rapidly digesting ones. The rate of carbohydrate digestion of a food can be characterized by its Glycemic index (GI) (Harris and Geor, 2009). The GI of a food depends on endogenous factors of the food matrix such as starch susceptibility to α-amylase, protein and lipid content, and the macroscopic structure of the food (Fardet *et al.*, 2006). Starch susceptibility to  $\alpha$ -amylase is in turn determined by its structure, encapsulation, crystal structure, degree of gelatinization, the proportion of damaged granules as well as the retrogradation of the starch granules (Fardet et al., 2006). Using a scanning electron microscope (SEM), the size of teff starch was found to be 2-6 µm (Bultosaet al., 2002; Wolter et al., 2013).

This makes teff starch granules smaller than those of wheat (A type 20-35  $\mu$ m), sorghum (20  $\mu$ m) and maize (20  $\mu$ m) (Delcour *et al.*, 2010). Given their larger surface area, smaller starch granules are more susceptible to enzymatic attack (Tester *et al.*, 2004). This some what lower GI for teff than expected may be explained by its amylose content, lower starch damage, and the possible formation of amylose-lipid complexes that can hinder enzymatic access and thus starch digestibility (Wolter *et al.*, 2013). In addition, the high (68-80 °C) gelatinization temperature of teff (Bultosa, 2007; Wolter *et al.*, 2013) can hinder gelatinization and thus decrease susceptibility to enzymatic attack by  $\alpha$ -amylase (Fardet *et al.*, 2006).

#### 2.2.1.2. 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 Teff's fractional protein composition suggests that glutelins (45 percent) and albumins (37 percent) are the major protein storages, while prolamins are a minor constituent (~12 percent) (Bekele *et al.*, 1995; Tatham *et al.*, 1996). In contrast, more recent studies report that proclaims is the major protein storages in teff (Adebowale *et al.*, 2011).

Red Teff's amino acid composition is well-balanced. A relatively high concentration of lysine, a major limiting amino acid in cereals, is found in teff. Similarly, compared to other cereals, higher contents of isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine are found in teff.

Another important feature of red teff is that it has no gluten (Hopman *et al.* 2008; Spaenij-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. This makes teff a valuable ingredient for functional foods destined for celiac patients who are gluten intolerant.

#### 2.2.1.3. 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 n-3 fatty acids ( $\alpha$ -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 of that of grain teff is around 2% (dry base) (Nyakabau et al., 2013). Crude fat (petroleum ether extract) consist mostly of non-starch lipids i.e., it is not endogenous to the starch. The low crude fat content in teff starch is most probably related to the low crude fat content of the grain. Bultosa (2002) found that the teff total starch lipid was higher than that of pearl millet (5.0 mg/g) and slightly higher than that of rice (7.6 mg/g).

#### 2.2.1.4. Fiber

The American Association of Cereal Chemists defines dietary fiber as the "edible parts of plant or analogous carbohydrates resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine" (DeVries, 2003). 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 (Champ *et al.*, 2003).

The crude fiber of red teff is 3.1%, total and soluble dietary fiber content of red teff is several folds higher than that found in wheat, sorghum, rice and maize. There may be several reasons for this. First, whole grains have higher fiber content than decorticated ones. Second, small grains have a relatively high proportion of bran, which is high in fiber (Bultosa, 2007). Therefore, higher dietary fiber intake and the associated health benefits are expected with increased consumption of teff based baked products.

#### 2.2.1.5. Minerals

Red teff has a higher iron and calcium content than mixed or white teff (Abebe *et al.*, 2007). On the other hand, white teff has a higher copper content than red and mixed teff. 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.

Teff has a higher iron, calcium and copper content than other common cereals (Mengesha, 1966). The zinc content of red teff is also higher than that of sorghum and wheat. However, the very high mineral (i.e. iron) content of red teff has been contested and in many instances attributed to soil contamination (Ketema, 1997; Abebe *et al.*, 2007). For example, Hallberg and Björn-Rasmussen (1981) reported that teff's iron content is not different from other cereals by showing that iron content drops from 39.7 mg/100 g to 3.5 mg/ 100 g when grains are washed with dilute hydrochloric acid.

However, washing with acid is likely to lead to loss of acid-labile intrinsic iron and thus may underestimate the iron content. For instance, Areda et al., (1993) reported that acid washing of teff grains led to a 50 percent greater loss of iron than washings with de-ionized water. Comparing uncontaminated teff to barley, wheat, maize and sorghum, Mengesha (1966) reported that teff is superior in its mineral content, particularly in calcium and iron. More recently, Baye *et al.*, (2014) examined the content of iron, zinc and calcium in teff, barley, wheat, and sorghum before and after washing with de-ionized water. Mengesha (1966) found by washing the grain, that this significantly decreased the iron content as well as the variability between replicates. Despite this decrease, the variability between replicates for teff remained relatively high suggesting that soil contamination in teff is relatively high compared to other cereals. The mineral contamination of red teff is probably due to its small size, and suggests increased contact with soil over a larger area (Baye *et al.*, 2014).

The contamination of cereal grains in Ethiopia, particularly in teff, has often been associated with traditional methods of threshing grain under the hooves of cattle (Bezwoda *et al.*, 1979). More recently, Ambaw (2013) compared the iron content of the same teff variety after laboratory (manually) and traditional threshing. The iron content of the laboratory threshed teff was 16 mg/100g which was still higher than what is found in many cereals. This suggests that although the intrinsic iron content of red teff may not be as high as previously thought, teff is still a better source of iron than other cereals like wheat, barley, sorghum, and maize. In contrast to iron, Bave *et al.*, (2014) showed that under the same conditions, the values reported for calcium andzinc care consistent and are less affected by washing. This suggests that soil contamination contributes little to the content of these minerals in teff.

#### 2.2.2. Health benefits of red teff and red teff-based products

#### 2.2.2.1. To avoid Iron deficiency

Iron-deficiency is the most widespread micronutrient deficiency globally, affecting more than 2 billion people (Zimmermann and Hurrell, 2007). 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 mal absorption

of dietary iron, low in-takes of bio available iron, or increased requirements due to physiologic status (e.g. pregnancy, infants and young children) (Pasricha *et al.*, 2013).

Inadequate iron intake is common in lowand 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 (Stoltzfus, 2011). 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.*, 2010). Therefore, adjusting iron intakes with iron-rich foods may be preferred. Teff can be a good alternative (Gebre-Medhin *et al.*, 1976; Adish *et al.*, 1999). Alaunyte *et al.*, (2012), showed that by supplementing wheat bread with 30 percent teff flour leads to 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 (Alaunyte *et al.*, 2012).

The bioavailability of iron in teff is likely to vary depending on processing. For instance, during injera fermentation, significant decreases in phytate content results in an ideal phytate to iron molar ratio (Umeta *et al.*, 2005, Baye *et al.*, 2014). Given that part of the iron in teff has been attributed to soil contamination, to what extent molar ratios accurately predict iron bioavailability has been questioned (Baye *et al.*, 2014). However, Bokhari *et al.*, (2012) showed that consumption of 30 percent teff-enriched wheat breads can help maintain serum iron levels in pregnant women. The study also suggested that degradation of phytates may lead to better iron bioavailability. Given the high iron content of teff and its potential contribution to food-based approaches to improve nutrition, 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.

#### 2.2.2.2. To cure Celiac disease

Worldwide, 0.6-1.0 % of the population is affected by celiac disease (CD) (Gujral*et al.*, 2012). The prevalence of the disease in populations at risk of CD is as follows: 3 to 6 percent in type 1 diabetic patients, up to 20 % in first-degree relatives, 10 to 15 % in those with symptomatic iron-

deficiency anemia (IDA), 3 to 6 percent in those with asymptomatic IDA, and 1 to 3 percent in individuals with osteoporosis (Dubé *et al.*, 2005). 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-Hansen *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). The only treatment for those with CD available to date is to follow a strict gluten-free diet (Fasano and Catassi, 2001). This in practice is difficult given the abundance of food products containing wheat or other gluten-containing cereals. 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).

Also, a higher 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). Therefore, nutrient dense gluten-free alternatives are needed. Teff, as discussed in previous sections, contains a good amount of minerals, fiber, and phyto chemicals. Compared to gluten-free cereals and pseudo cereals such as quinoa, amaranth, buckwheat, maize, brown rice, and sorghum, teff is more nutrient-dense (Alvarez-Jubete, Arendt, and Gallagher 2010, Gebremariam *et al.*, 2012). Furthermore, the low glycemic index of teff may help maintain good glycaemiccontrol. This is very important given the high incidence of diabetes in those with CD (Viljamaa *et al.*, 2005).

#### 2.2.2.3 To protect from 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, Sicree *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 elucidated, it is thought to be through the synergistic affects of the essential macro- and micronutrients, and phytonutrients (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 conditions of impaired antioxidant status and inflammation have been linked to the development of insulin resistance and type-2 diabetes (Wellen and Hotamisligil 2005, Folli *et al.*, 2011). In this regard, the high phytate and polyphenol content in teff (Abebe *et al.*, 2007, Baye *et al.*, 2014) and the associated anti-oxidative property is likely to prevent and control diabetes (Lee *et al.*, 2006). However, while studies to evaluate the anti-diabetic property of teff consumption are of interest, so far such studies are very limited.

#### 2.3. Wheat production, Composition and Consumption trends

Wheat (*Triticum aestivum* L.) is one of the most important cereals cultivated in Ethiopia for the purpose of bread production. It ranks fourth after Teff (*Eragrostis tef*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) in area coverage and third in totalproduction (CSA, 2007). Although most wheat is consumed within the country where it is produced, roughly one-fifth of the annual crop is exported (FAO, 2002). Wheat production in Ethiopia in previous year was 3077 thousand tones whereas previous five years average production was 2800 thousand tones which show an increase in production but there was still a demand of importation 1000 thousand tones (FAO/GIEWS, 2012). By complementing wheat flour with other legumes, the nutritional values of baked goods can be improved (Dora et al., 2010).

The nutritional content of wheat is illustrated it is the most important stable food crop for more than one third of the world population. It contributes more calories and proteins to the world diet than any other cereal crops (Adams *et al.*, 2002; Shewry, 2009). Some wheat varieties (e.g. *Triticum aestivum*) are suitable for bread making while others (e.g. *Triticum durum*) are suitable for bread making while others (e.g. *Triticum durum*) are suitable for biscuits and cooking making (Sapirstein *et al.*, 2007). The major factor for the suitability of

wheat varieties for making different types of bakery products is the ability to form gluten network. Gluten, the protein component of flour which gives the dough elasticity and strength, can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water soluble constituents (Wieser, 2007; Kaushik, *et al.*, 2013).

In wheat products such as bread, gluten network formation is desirable for gas retention and better volume of product, while in products such as biscuits, cookies extensibility is required, so gluten formation is undesirable. Getting the desired quality of wheat flour for making specific kinds of bakery products is a challenging task for bakery industries.

#### 2.3.1. Nutritional Composition of wheat

An alternative for the management of wheat flour is the modification of the gluten protein by different agents (Liu *et al.*, 1996). Gluten-modifying agents such an oxidizing and reducing agents are used in controlling the rheological properties of wheat flour (Sandhu *et al.*, 2011). Reducing agents cleave the intermolecular and intra molecular disulfide bonds in the gluten proteins. This cleavage results in reduced molecular weight for the proteins, and the extensibility of the dough is increased (Stauffer, 1994). The incorporation of reducing agents, such as L-cysteine hydrochloride (L-cysteine HCl), reduced the water absorption capacity (WAC) and stability of medium-strong wheat flour as well as of weak wheat flour (Ravi *et al.*, 2000).

Wheat is one of the most important staple foods for humans (Akhtar *et al.*, 2008). The kernel consists of the wheat germ and the endosperm, which is full of starch and protein (Mannay and Shadaksharaswany, 2005). Usually the whole grain is milled to leave just the endosperm for white flour, while the by-products of bran and germ are discarded. It has been shown that the whole grain is a concentrated source of essential nutritional components such as vitamins, minerals, protein, fat and fibre while the refined grain is mostly starch (Potter and Hotchkiss, 2006; Bakke and Vickers, 2007). Wheat therefore, is perhaps the most popular energy grain for the production of confectionary products, because of the unique properties of its protein (gluten) which combines strength and elasticity required to produce bread, cookies, cakes and pastries such as spaghetti, macaroni and noodles of desirable texture and flavour (Potter and Hotchkiss, 2006; Akhtar *et al.*, 2008).

Although most wheat is grown for human food (for production of cakes, cookies, bread, pancake etc.) and about is retained for seed and industry (for production of starch, paste, malt, dextrose, gluten). Wheat grain contains all essential nutrients; kernel contains about 12% water, including carbohydrates (60-80% mainly as carbohydrate), proteins (8-15%) containing adequate amounts of all essential amino acids (except lysine, tryptophan and methionine), fats (1.5-2%), minerals (1.5-2%), vitamins (such as B complex, vitamin E) and 2.2% crude fibers12 to 13% moisture, 2.5% sugar (Nachit, 1992 and Oberoi *et al.*, 2007). And also wheat flour is main ingredients used in the manufacturing of noodles and characteristics of wheat used for milling are very important. Soft wheat is used in cakes, pastries, cookies, crackers and oriental noodles where as hard wheat is used in breads.

#### 2.3.2. Health Benefits of whole wheat

The many benefits of whole wheat products are being recognized more and more by consumers. Even though many health-conscious individuals have been cutting back on their intake of total carbs and refined wheat products the demand for whole wheat products has actually increased during that same time period. This trend fits in well with a diet approach to health, which looks to lower overall carbs but higher whole grains, including whole wheat Liu S (2003).

The benefits of wheat's bran portion don't stop here; it has also been shown to function as an anti-cancer agent. Wheat bran is thought to accelerate the metabolism of estrogen that is a known promoter of breast cancer. In one study, pre-menopausal women, ages twenty to fifty, who ate three to four high fiber muffins per day made with wheat bran, decreased their blood estrogen levels by 17 percent after two months. The fact that only wheat bran, and not corn or oat bran, is beneficial in preventing cancer-promoting changes in the colon, provides additional clues that wheat bran contains something special that makes it a true cancer fighter. Only the bran from wheat has been shown to reduce the concentration of bile acids and bacterial enzymes in the stool that are believed to promote colon cancer Lopez HW (2003).

The whole grain wheat is very low in fat. A regular diet with whole grain wheat associated with physical exercises can have good effects on weight control. Wheat fibre slows the digestive process and decreases the absorption of fat by the body from the food. It makes us feel full which reduces the need for larger portions Mc Keown (2004).

The importance of choosing whole wheat rather than refined wheat is to maintain a healthy body weight. Weight gain was inversely associated with the intake of high-fiber, whole-grain foods, such as whole wheat, but positively related to the intake of refined-grain foods, such as products made from whole wheat. Who consumed more whole grains consistently weigh less than those who ate less of these fiber-rich foods, but those consuming the most dietary fiber from whole grains were 49% less likely to gain weight compared to those eating foods made from refined grains (Suzuki R 2008).

#### 2.4. Limitations associated with cereal based diets and need of improvement

The low-level ingestion of micronutrients affects three billion people around the world. One of the reasons is the consumption of poor-quality diets, which are based on high amounts of staple foods and low amounts of complementary food products. Numerous microelements and vitamins are considered essential to human nutrition and even minor deficiencies markedly increase the risk of serious diseases and even death. A lack of iron (Fe), protein and zinc (Zn) nutrients are currently the most important deficiencies for human health in the developing world. Therefore, the bio formulation of crops is being proposed as an alternative to combat malnutrition.

#### 2.5. Gaps associated with use of okara and teff to enhance nutrient content of foods

Cereals and legumes are good sources of protein, which complement each other with respect to their amino acid profile (Ihekoronye 1985). The low protein content of wheat flour, which is the most important ingredient used for the production of different kinds of baked goods has been of major concern in its utilization but it is low nutritional content when we compare to other cereals and legumes .

Therefore, cereal and legume proteins have a supplementary effect on each other, when appropriately blended (Nnam, 2001). Promoting consumption of locally available vitamin A-rich foods that can be grown in home gardens holds particular promise in many countries, due to its technical feasibility and cost-effectiveness.

Relative to more common cereals with technological limitations in processing teff, has for long restricted its more widespread consumption from its center of origin, Ethiopia. The limited information available to the general public and the lack of global interest in teff has prolonged thinking by Ethiopians that their grain is of inferior nutritional quality. However, over the past

decade, the recognition that teff is gluten-free has spurred global research interest by nutritionists and food scientists. Consequently, studies on the nutritional composition of teff and its processing qualities have grown, and the development of new teff-based products has accelerated.

The soybeana grain legume is one of the richest and cheapest sources of plant protein that can be used to improve the diet of millions of people, especially the poor and low income earners indeveloping countries because it produces the greatest amount of protein used as food by man (LIU, 2000). In this approach, Okara can be a very suitable product .Hence, blending of this food ingredients through optimum amount can be assumed as a good option for improving the nutritional quality of cookies using simple technology.

The by-product obtained during processing of soybean is either underutilized or unutilized. Okara (insoluble residue from tofu or soymilk) and whey are two major by-products of the tofu making process. These can be put in a number of uses which can serve as potential sources of income and utilize their nutrients.

soymilk residue which is a byproduct and known as Okara is used as a food material for its high content of protein and fiber. Okara is the pulp fiber residue generated as a byproduct in large quantities from the soymilk production process. Raw Okara contains about75% of moisture Okara makes it a very high nutritional value (Smith *et al.*, 1978) and may be utilized as an ingredient in a variety of processed foods (O'Toole, 1999) because it reduces calorie intake and increases dietary fiber. On the other hand, SCR is a relatively inexpensive source of protein that is widely recognized for its high nutritional and excellent functional properties (Y. Cheng.,*et al* 2005)

The enrichment or fortification of cookies and other bakery products with other protein sources such as cereals and legumes has received considerable attention. Legume proteins should complement the protein in cereal grains since the chemical and nutritional characteristics of legumes make them natural complements to cereal-based diets (Altschull, A.M.1994).

#### 2.6. Importance of food formulation

The low-level ingestion of micronutrients affects three billion people around the world. One of the reasons is the consumption of poor-quality diets, which are based on high amounts of staple foods (wheat, corn and rice) and low amounts of complementary food products (fruits, legumes, vegetables, animal products and fish). The latter two are especially high in bioavailable minerals and vitamins (Dibb *et al.*, 2005). Numerous microelements and vitamins are considered essential to human nutrition and even minor deficiencies markedly increase the risk of serious diseases and even death. A lack of iron (Fe), iodine (I), vitamin A, and zinc (Zn) nutrients are currently the most important deficiencies for human health in the developing world. Therefore, the bio formulation of crops is being proposed as an alternative to combat malnutrition. Moreover, legume-based flours have the potential to increase the nutritional value when compared to other types of flour and are of interest in the production of food products for all age groups especially for children, who constitute the most vulnerable population in regards to a lack of minerals, such as Zn and Fe.

The increased use of legume-based flours indifferent formulations of food products has raised great interest among researchers looking for high-quality sources of vegetable protein due to the limited availability and high cost of animal-based proteins in developing countries (Kohajdová *et al.*, 2013; Raya-Pérez *et al.*, 2012). The addition of legumes to cereal-based products could be an efficient way to increase the consumption of these products. Furthermore, legume proteins are rich in lysine but deficient in sulfur-containing amino acids; whereas, cereal proteins are deficient in lysine but have adequate levels of sulfur containing amino acids. Thus, the combination of cereal and legume proteins would provide a more complete spectrum of essential amino acids, which is of great importance in a balanced diet (Kohajdová *et al.*, 2013).

Dendy (1992) concluded that the composite flour prepared from wheat flour and non-wheat flour including cereals, root-tuber, legumes raw material and fiber sources is important for both nutrition and economic aspects food fortification has been identified as one of most effective and sustainable ways of controlling iron deficiency in populations (INACG, 1977). Thus, the enrichment of cookies processed with these ingredients leads to the improvement of their nutritional qualities. Moreover, food formulation can be an economical, flexible, and socially

acceptable way to improve the nutrient intake of groups at risk in order to ensure nutritional adequacy of the diet (Hoffpauer; Wright, 1994) being an option by which people have access to milled or processed food (Mason *et al.*, 2001). Okara can be processed into high-quality flour for use as partial substitute for wheat flour and other flours, such as teff, maize and rice etc., and it can be used in the formulation of food products, such as cookies, bread, biscuit, pasta, cake mixes, pastries, and in flour mixes for creams and soups. Furthermore, the addition of legumes to cereal-based products could be an efficient way to increase the nutritional contentPreparation of value added cookies.

The demand for food and agricultural products is changing in unprecedented ways. The nature and extent of the changing structure of agri- food demand offer extra ordinary opportunities for diversification and value addition in agriculture, particularly in developing countries. The prospects for continued growth in demand for value-added food and agricultural products constitute an incentive for increased attention to agro industries development within the context of economic growth, food security and poverty- fighting strategies. Agro- industries, here understood as a component of the manufacturing sector where value is added to agricultural raw materials through processing and handling operations are known to be efficient engines of growth and development. With their forward and backward linkages, agro-industries have high multiplier effects in terms of job creation and value addition (Carlos and Doyle, 2009).

According to New Brunswick, Canada Value-added Food Sector Strategy 2012-2016, any step in the production process that improves the product for the customer and results in a high Ernest worth called value addition. Value-added food sector encompasses companies producing agriculture and seafood-based products, beverages and other food made from both local and imported resources. The sector includes live, fresh, frozen, packaged, processed and preserved food products whose value and profitability has been increased by making them more appealing and valuable to the buyer. In Ethiopia, the food-processing sector is by far the largest manufacturing industry and accounts for 39% of the gross value of production in large. Generally the importance of this work arises from the possibility of utilizing flours from locally grown crops other than wheat to produce cookies of acceptable nutritional, sensory, and physical quality. The use of wheat, red Teff, and okara flour blends in cookies production can greatly enhance the protein content, without compromising consumer acceptance.

## 2.7. Importance of Composite Flour

Composite flour technology refers to the process of mixing various flours to make use of local raw material to produce high quality food products in an economical way. Formulation of composite flour is vital for development of value-added products with optimal functionality (Rehman et al., 2010). The concept of using composite flours is not new and has been the subject of numerous studies (Vieira et al., 2007). The use of composite flours from cereals and legumes for cookies production is therefore expected to enhance the utilization of local crops as raw materials and improve the nutritive quality of cookies. Composite fours are thus advantageous in the sense that inherent deficiencies are supplemented from other sources and it plays a vital role to complement the deficiency of essential nutrients and it saves hard currency; promote high yielding local plant species and enhances overall use of domestic food (Khan 2005). Usually, the aim of producing composite flour is to get a product that is better than the individual components. Better may mean improved properties or performances, or in some cases, improved economies, and nutritionally.For instance, composite flours produced from cereals and legumes have the advantage of improving the overall nutrition (FAO 1995). This reason has also resulted in the use of local raw materials that could combine optimum nutritive value with good processing characteristics. Moreover, food formulation can be an economical, flexible, and socially acceptable way to improve the nutrient intake of groups at risk in order to ensure nutritional adequacy of the diet (Hoffpaurer: Wright, 1994)

#### 2.8. Importance of cookies

Among ready-to-eat snacks, Cookies are products made from different flour that are characterized by a formula high in sugar and shortening and relatively low in the water. Cookies have become one of the most desirable snack for both youth and elderly people due to their low manufacturing lost, more convenience, long shelf-life and ability to serve as a vehicle for important nutrients (Akubor, 2003; Hooda and Jood, 2005). It represents the largest category of snack item among baked food products throughout the world (Pratima and Yadava, 2000). Cookies are not considered as staple food as in bread, but may be feasible fibre carriers because of their long shelf life and thus enable large scale production and widespread distribution (Vratania and Zabik, 1978). In many countries, cookies are prepared with fortified or composite flour to increase its nutritive value (Gonzalez-Galan et al., 1991). It has become quite popular, particularly in fat-food establishment (Hoseney, 1986).Cookies are highly sought after snacks,

for children as well as for adults, not only because they are appetizing, but also due to their simple manufacturing process, long shelf life and potential for containing high nutrient components (Noor Aziah *et al.*, 2012). Therefore, they can be used as nutritional supplements. The rich compositions of whole and fractioned grains, together with their high content of dietary fiber, have motivated numerous nutritional interventions focused on exploiting their potential in order to obtain healthier and more nutritious food products for human health.

### 2.9. Importance of Anti-nutritional factors of cookies

Anti-nutritional factors (ANFS) are defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms which exert effects contrary to optimum nutrition (Kumar, 1992). Although soybean okara, wheat and red teff are rich in many nutrients, they contain anti-nutritional compounds that have to be removed. Some of these chemicals are tannins and phytic acid (Khattab and Arntfield, 2009).

For minerals to be used for normal metabolic functions (bioavailable), they need to be absorbed through the small intestine (Fairweather-Tait, 2002). The bioavailability of minerals depends on subject/host and dietary factors (Hurrell and Egli, 2010). Among dietary factors, phytochemicals, such as tannin and phytates, constitute major mineral absorption inhibitors and hence were, for a long time, referred to as anti-nutritional factors. However, in recent years, the recognition of their health promoting effects including anti-diabetic, anti-cancer and antioxidative (Shamsuddin, 1995) properties made the term anti-nutritional factor obsolete (Schlemmer *et al.*, 2009).

#### Phytates

Phytates are a common constituent of cereals and legumes (Schlemmer *et al.*, 2009). Phytate are the principal storage form of phosphorus and are particularly abundant in cereals and legumes (Reddy *et al.*, 1989). Phytate accumulates in the seeds during the ripening period and is the main storage form of both phosphate and inositol in plant seeds and grains (Mohamed *et al.*, 2011). The mineral content of legumes like soybean is generally high, but the bioavailability is poor due to the presence of phytate, which is a main inhibitor of iron, calcium and zinc absorption. Phytate not only decreases the bioavailability of essential minerals, it also decreases the bioavailability of proteins by forming insoluble phytate-mineral and phytate protein complexes (Tajoddin *et al.*, *a*).

2011). During processing, phytate-protein complexes are formed and precipitated to bind metal ions. The ultimate solution to solve this problem is to reduce phytate in cereals and soybeans. In order to effectively evaluate the relationship between phytic acid and mineral availability one must be able to accurately measure both (Thompson and Erdman, 1982).

It is the primary form of phosphorus storage in seeds and accounts for 60-90 percent of the total phosphorus. It can constitute as much as 1.5% of the dry weight of cereals (Loewus 2002; Bohn *et al.*, 2008). Teff contains high amounts of phytate with a wide range of variability, probably due to differences in varieties and growing conditions. Teff phytate content is comparable to values reported for wholegrain cereals (Schlemmer *et al.*, 2009). Such high values in phytate are likely to impair the absorption of iron and zinc (Hurrell & Egli, 2010). The mechanism by which phytate inhibits mineral absorption is based on the formation of insoluble phytate-mineral or peptide-mineral-phytate complexes in the gastrointestinal tract (Weaver and Kannan, 2002).

Furthermore, phytates form complexes with endogenously secreted minerals such as zinc (Sandström, 1997; Manary et al., 2002) and calcium (Morris and Ellis, 1985), making these minerals unavailable for reabsorption into the body. Phytate can be degraded by endogenous phytates which can be activated by food processing techniques like soaking, fermentation, and germination and to a lesser extent during cooking. For instance, fermentation of injera has been shown to result in phytate degradation through the activation of endogenous phytases (Umeta *et al.*, 2005; Baye *et al.*, 2013, Baye *et al.*, 2014).

However, the application of exogenous commercial enzymes can be more effective in degrading phytates (Troesch *et al.*, 2009; Baye *et al.*, 2013). On a positive side, phytates have been shown to prevent kidney stones by serving as crystallization inhibitor of calcium salts in biological fluids (Curhan *et al.*, 2004). They also have glucose lowering (Lee *et al.*, 2005, 2006) and anticancer properties (Singh *et al.*, 2003). Given these positive effects of phytates, it remains unknown as to whether there is an optimal concentration of phytate where the beneficial effects can be appreciated with little or no compromise to mineral bioavailability. Further investigations are needed to find more conclusive results.

#### Tannins

Tannins are predominantly located in the pericarp and/ or testa, particularly of pigmented cultivars of legumes and millets (Deshpande *et al.*, 1982). Food tannins are poly phenolic compounds that are widely distributed in plants they are readily form indigestible complexes with proteins and other macro-molecules under specific environmental conditions. Tannins are known to be present in food legumes and to inhibit the activities of trypsin, chemo trypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption (De Lumen and Salamat, 1980). It has been reported that tannins affect protein digestibility and adversely influencing the bioavailability of non-haem iron leading to poor iron and calcium absorption. The carbohydrate is also affected by tannins leading to reduced energy value of a diet containing tannins (Adeparusi, 2001). Hydrolysable tannins (HT) are made up of a carbohydrate core whose hydroxyl groups are esterifies with phenolic acids (mainly Gallic and hex hydroxyl diaphonic acid). The condensed tannins (CT), are non-branched polymers of flavonoids units (flavan-3-ol, flavan-3, 4-diol), and usually have a higher molecular weight than the hydrolysable tannins (Frutos *et al.*, 2004).

#### 2.10. Importance of Functional and Physical properties of cookies

The main quality factors that account for the superior performance of cookies are physical parameters. And the main criteria in evaluating baking potential of these physical properties are diameter; thickness, spread ratio and hardness(breaking strength). This attributes based foods are important to consumers and to manufacturers (Mc Manuis 2001). Texture evaluation is an important step in developing a new food product or optimizing process variables (Meullenet *et al.*, 1998). Breaking strength is one of the textural properties of a food products and it is an important determinant of the food quality (Lewis, 1990). Breaking strength measurements can be very valuable for the quality control and process optimization as well as for the development of new products with desirable properties and characteristics (Bourne, M.C., 1978 & Matz 1962).

Spread ratio (diameter/thickness) is one of the most important properties in evaluating the quality of cookies. Greater spread ratios are desirable and indicate a better cookies quality (Seker *et al.*, 2010). Also described the texture of cookies as a combined function of the size and shape of the crumb structure, the strength with in individual pockets of mostly discrete dough masses, the

strength of the contiguous boundaries between those pockets, the moisture content and gradients, and the internal stresses produced during baking and cooling. Many variables probably affect the hardness of cookies. Although cookies physical parameter affects consumer acceptance and repeat sales, the evaluation of cookies physical is often too expensive and time-consuming to be routinely included in quality assurance.

The functional properties of flours play important role in the manufacturing of bakery products (Baljeet *et al.*, 2010). Functional properties are reffer to those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption (Kinsella, 1976). Blending non wheat flours with wheat might result in technological difficulties and impairment of baking quality. For the purpose, determining potential use of composite flour blends in food formulations, information regarding functional properties of blends are essential (Akubor and Ukwuru, 2003). The functional properties such as water absorption capacity, and bulk density of flour are important to determine either the flour would be useful in development of food that affect processing applications, formulation, food quality, and acceptance of food product (Mahajan and Dua 2002; Wu *et al.*, 2009).

Functional properties are the fundamental physico-chemical properties that reflect the complex interaction between the composition, structure, molecular conformation and physico-chemical properties of food components together with the nature of environment in which these are associated and measured (Kinsella, 1976; Kaur and Singh, 2006; Siddiq *et al.*, 2009). Functional characteristics are required to evaluate and possibly help to predict how new proteins, fat, fibre and carbohydrates may behave in specific systems as well as demonstrate whether or not such protein can be used to stimulate or replace conventional protein (Mattil, 1971; Kaur and Singh)

#### Water absorption capacity (WAC)

The water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain and also it represent the ability of the product to associate with water under conditions where water is limiting (Edema *et al.*, 2005; Ojukwu *et al.*, 2012). Water absorption capacity is an important functional property required in food formulations especially those involving dough handling. Increase in water absorption capacity implies increase in digestibility of the starches. And also respect to, the microbial activities of food products with low water absorption capacity wouldbe reduced (Ijarotimi and Keshinro, 2012).

#### **Bulk density**

Bulk density is a measure of heaviness of flour and in packaging (Appiah *et al.*, 2011). The lower loose bulk density implies that less quantity of the food samples would be packaged inconstant volume thereby ensuring an economical packaging. However, the packed bulk densities would ensure more quantities of the food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with immature digestive system (Ijarotimi and Keshinro, 2012). According to Nelson-Quartey *et al*, (2007), low bulk density flours are desirable in infant food preparation. 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).

#### **2.11. Importance of Mineral contents in human diet**

Minerals are chemical constituents used by the body in many ways, although they yield no energy and impotent in many activities of the body (Eruvbetine, 2003). Minerals are present in foods at low but variable concentrations and in multiple chemical forms. The role of minerals in food is to provide a reliable source of essential nutrients in a balanced and bio-available form. Iron is necessary for red blood cells formation and required for oxygen transport throughout the body and aerobic energy transfer (Mc Ardle *et al.*, 2010). The nutritional characterization of the fortified cookies based on the mixed flour indicated their high quality, considering that the percentage of Fe has been described as a reliable indicator of its availability in food products (Sanz, 2012). 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 as an essential nutrient for human health; zinc also plays an important role in normal growth and development (Gardner *et al.*, 2005; Islam *et al.*, 2009). Calcium is also a mineral required by the body for a variety of physiological functions essential for developing and maintaining for healthy bones, teeth, assists in blood clotting, muscle contraction, nerve transmission, oxygen transport and the maintenance of bone tissues throughout life (Broadus, 1996).

## 2.12 Bioavailability of cereal and legumes based product

Bioavailability is a general term that refers as the proportion of an ingested nutrient in food that is absorbed and utilized through normal metabolic pathways and used by the body (Hurrell, 2002). It can be affected by many factors such as the presence of anti-nutrients, for example, phytates, and tannins in foods, a person's need, fibre, competition with other nutrients and acidity of intestinal environment (Paul, Turner & Ross, 2004). It is influenced by both host- and diet-related factors. Food preparation and processing methods to reduce the phytate content of cereal- and/or legume-based complementary foods in the household include soaking, germination, fermentation, and pounding.

Cereal- and/or legume-based complementary foods can also be fortified with micronutrients, including minerals, in an effort to close the gap that may still remain between the level of absorbed minerals in high phytate cereal- and/or legume-based complementary foods and the WHO recommendations. Minerals, classified as micronutrients are needed by our body in small amounts. Deficiency in minerals, however, can have a major impact on health such as anemia and osteoporosis that commonly occur in both developed and developing countries. This study focused only on iron (Fe), zinc (Zn) and calcium (Ca). Presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phytate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption (Walter *et al.*, 2002).

Phytates are generally found in food high in fibre especially in wheat bran, whole grains and legumes (Lori, Thava & James, 2001). Due to evidence showing that fibre-rich foods protect against diseases such as cardiovascular disease (CVD), colon and breast cancer, more have now started adopting a dietary pattern containing high fibre foods. This has indirectly caused increased consumption of phytates, hence possibly reducing bioavailability of minerals.

Content item	Red Teff( A)	Wheat whole(B)	Dry Okara(C)
Food energy (cal.)	336	339	323
Moisture (%)	11.1	10.8	11.80
Protein (g)	10.5	10.3	25.4-28.4

Table1. Nutritional composition of Teff, Wheat, okara per 100g

Fat (g)	2.7	1.9	6.9-22.2
Carbohydrate(g)	73.1	71.9	3.8-5.3
Fibre (g)	3.1	3.0	9.1-18.6
Ash (g)	3.1	1.6	2.90
Calcium (mg)	157.0	49.0	21
Phosphorus(mg)	348.0	276.0	0.0
Iron (mg)	58.9	7.5	1.3

**Source:** (A) Agren and Gibson (1968), (B) USDA, 2008, (C) Van der Riet *et al.*, (1989) Bourne*et al.*, (1976)

## 2.13. Major ingredients of cookies production

## Flour

Flour, are the structural ingredients in baking for cookies making soft wheat with high extensibility and low elasticity and (9 - 9.5%) protein content is used generally (NCFM, 2003), the water is added as a toughened (Matz, 1968). cookies not only vary in looks and taste but also in the type of flour needed to produce a desirable product Sugar and syrup: Sweetener is an important component for cookie formula; it affects the flour, texture and appearance. It is either added as granulated or powder to cookie mixture (Matz, 1968).

### Water

Water is the second main ingredient on the label, and the control of it is important. The main function of water is hydration, to moisten the flour protein to form gluten structure and is necessary in all recipes and also during heating promotes the gelatinization of starch. Water can dissolve certain constituents, such as sugars, salts and baking powder. Water also contributes to dough consistency and helps to control the temperature of the dough or batter (Bennion, 1980; Stevenson and Miller, 1960; and Kordylas, 1991).

Water is vital throughout the cookies making processes for dissolution of salt and sugar, assisting the dispersion of yeast cell, starch and sucrose hydrolysis, activation of enzymes to form new bonds between the macromolecules in the flour and consequently alters the rheological properties of the dough (Giannou *et al.*, 2003). The optimum water level is crucial in determining the dough properties and subsequently the final quality of bread. The aqueous phase is necessary

for dissolving the soluble flour components and providing the medium for various reactions to take place throughout the bread making process (Gan *et al.*, 1995). Adjustments of water levels during dough mixing were in accordance to the water absorption capacity of the flour to compensate for the changes of the dough consistency (Cauvain, 1998).

#### Shortening

Shortening is used in cookies production to provide overall lubrication. It is often added to the dough to obtain a softer crumb, improvement in loaf volume and to act as ant staling effect, which may extend the shelf life of loaf. Shortening is a term used in the baking industries to describe fats, oils and their derivatives to improve the cookies quality. Addition of shortening allows the weaker flour to be used in the formulation by aiding the increment of the dough strength and stability, and gas retention (Stampfli and Nersten, 1995). Partial substitution of non-wheat flour into the cookies formulation required higher level of shortening due to the disruption of gas cell network in the dough (William and Pullen, 1998; Cauvain, 2003).

#### Milk powder

The dried milk is more preferred because of convenience of use and their strong stability and also addition of milk helps the product to be brown during baking, also adds nutritive value to the product, color improvement, water absorbing, spread control properties and flavor (Kordylas, 1991).

#### Salt

According to Kordylas (1999), and Stevenson and Miller (1960), salt is used to improve the flavor. It is used in a little amount about 1% and it affects the texture and taste. Addition of salt at optimum level helps in conditioning the dough by improving its tolerance to mixing process, subsequently producing a more stable and stiff dough by affecting the dough rheological properties. Salt has an inhibiting effects on the formation of gluten during mixing (Cauvain, 2003) and further restrict the gas expansion by yeast conversion in the dough system (Mondal and Datta, 2008). Hence, salt in cookies formulation consequently strengthened the dough through protein interactions, presumably shielding charges on the dough gluten protein network by retaining the  $CO_2$  from the leavening agent (Lombard *et al.*, 2000).

#### Sugar

Sugar, particularly sucrose provides the characteristics of sweetness of the cookies. The common practice of sugar level added in the cookies is up to 4% of total flour. Sugar normally used as the fermentable carbohydrate for the yeast during initiation of fermentation (Belderok, 2000).

Sugar also acts as anti-plasticizers by retarding pasting of native starch or functions as anti staling ingredients through inhibition of starch recrystallization. Addition of sucrose liberates competition for water between starch and sucrose, which consequently alters the swelling of the native starch in the presence of sucrose. In certain cases, the sugar level was being increased to produce more gas production and to improve the crust color through the caramelization and Maillard reactions during baking process (Giannou *et al.*, 2003). Fermentation of sugars by yeast generated a large number of volatile compounds that is responsible for the distinctive characteristics associated with bread flavor (Martinez, 1996).

#### Eggs

Eggs provide leavening; add colour, texture, flavor and richness to the batter. They are important in helping to bind all the other ingredients together. Beaten eggs are a leavening agent as they incorporate air into the batter, which will expand in the oven and cause the cookies to rise. They help to distribute shortening in the mixture by means of their power to emulsifying. Owing to their fat content, eggs also add some shortening to mixture, no substitute has been found for the desirable flavor of eggs in flour products. Fresh egg Yolk adds tenderness and color to baked goods (Bennion, 1980; and Kordylas, 1991). Also eggs add some nutritive value to the baked product. The egg white is mostly protein. The institute of Home Economics defines a serving of eggs as 2 medium eggs; provide a quantity of protein equal to that obtained from 3 ounce of cooked meat. The protein content of whole eggs is consistently 12.8% (Stevenson and Miller, 1960). In fact, egg proteins are often used as standard for measuring protein quality. Furthermore, eggs are good source of essential fatty acids, a high amount of unsaturated fatty acids, all vitamins except vitamin C and most of the minerals needed in our diets (Froning, 1994). The egg yolk is rich in fat (11.5%), vitamins and minerals (Stevenson and Miller, 1960).Cold-storage of eggs of good quality and frozen eggs yield desirable baked products. Eggs help to retard evaporation from baked products, and prevent them from quickly going stale (B ennio, 1980; and Koedylas, 1991).

#### **Baking soda**

It is the most popular leavening agent because 1) it has a relatively low cost, 2) it is nontoxic, 3) it is easy to handle, 4) it gives a relatively tasteless end product, and 5) the commercial product is of high purity (Hoseney, 1986). Sodium bicarbonate is quite soluble in water and dissolves rapidly when the dough or batter is mixed. That of course, raises the pH of the batter or dough to the point where no carbon dioxide is released. To obtain significant amounts of gas, the dough or batter must contain an acid (Hoseney, 1986). Kordylas (1991) reported that many ingredients used in baking are sources of acid, e.g. vinegar, lemon juice, sour cream, yogurt, butter milk, chocolate, honey, molasses (also brown sugar), fruits. When baking soda is mixed with an acid found in the recipe, it releases the carbon dioxide gas: Baking soda or sodium bicarbonate (alkali) exerts a weakening effect on the flour proteins (gluten) and helps to promote spreading. Baking soda can be added alone or as component of baking powder. Baking powder is a is a combination of one or more acid salts with baking soda. When baking powder is mixed in a batter with the wet ingredients, the dry acid and the baking soda can then react together and release carbon dioxide (Meyer, 1960). By law, a baking powder must yield not less than 12% available carbon dioxide (Hoseney, 1986).

# **3. MATERIALS AND METHODS**

# 3.1. Description of the Study Area

This experimental work was conducted at Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) and Ethiopian Health and Nutrition Research Institute (EHNRI). JUCAVM is geographically located at south western part of Ethiopia at 346 km from Addis Abeba, at about 7033'N latitude and 36057' E longitudes at an altitude of 1710 meters above sea level. The two laboratories room temperature while the experiment was performed wasin the rage of 23-25 °C and 22-25 °C for post-harvest management and EHNRI respectively.

#### 3.2. Raw materials collection

Soy bean variety Avgat was obtained from Jimma Agricultural Research Center for the production of okara. Red teff variety H-01 99 and Wheat variety Digalu were collected from Holeta Agricultural Research Center. Milk powder, Baking powder, Table salt, sugar were collected from local market. Egg was obtained from poultry farm and distilled water and baking material from post-harvestlaboratory.

## 3.2.1. Sample Preparation

## 3.2.1.1. Preparation of Red teff flour

The Red teff was cleaned manually by winnowing and hand sorted to remove husks, stones, dust, light materials, glumes, stalks, and other extraneous materials. It was then ground into flour by miller and sieved through a 0.5 mm sieve and packed in airtight polythene plastic bags for further processing.

## 3.2.1.2. Preparation of okara flour

Soybeans were soaked in water at 1:5 soybean water ratios (W/V) which contains 0.5 % sodium bicarbonate (NaHCO<sub>3</sub>) solution for 12 hours at ambient temperature. The wet beans were dehulled manually and rinsed with clean water until the soybeans were enough clean. Cleaned soybeans were blanched in 0.5 % of NaHCO<sub>3</sub> solution at 100  $^{\circ}$ C for 10 minutes and washed again to clean them more. Grinding was done by electric blender machine (BL-767, China) forfour minutes at high speed with addition of water (1:3 W/V) to extract soymilk and the

mashes filtered through double cheese cloth to separate the soymilk and residue (okara) (Howrah, 2012).

## 3.2.1.3. Preparation of wheat flour

The whole wheat grain was collected from Holeta research center and it is cleaned from dirt and contaminants such as soil paticles, sands, sticks, leaves and other impurities and was later washed and dried. Dried whole wheat grain was milled using attrition mill and sieved into fine flour of uniform particle size using 0.5 mm sieve. Then, the wheat waswas milled by miller (KARLKOLB D-6072 DreichWest Germany) and sieved using 0.5 mm sieve.

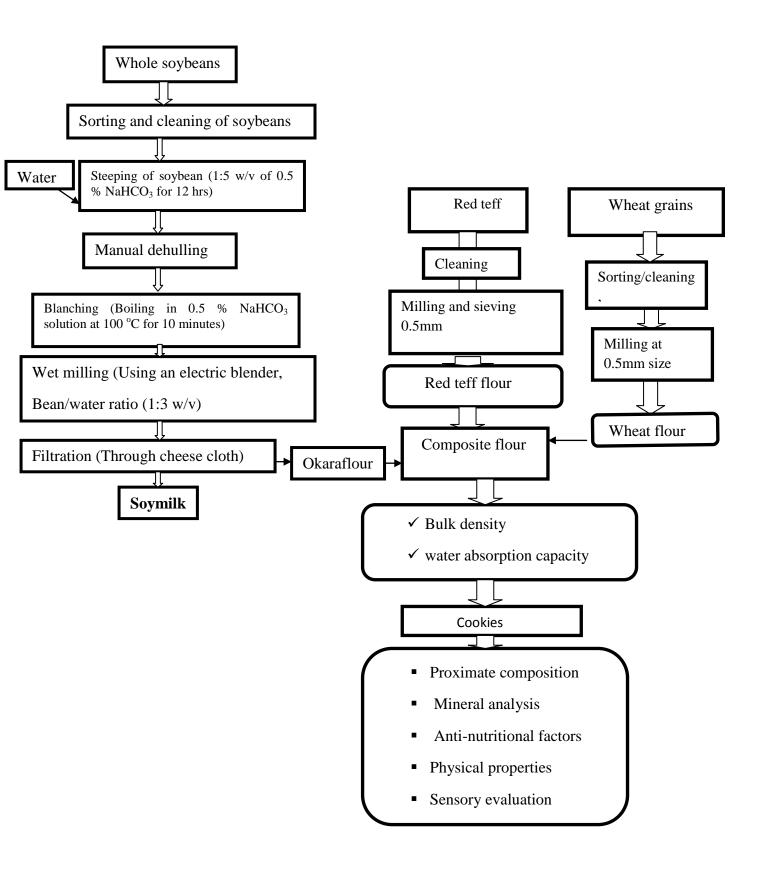


Figure 1.The flow diagrams of the flours (Okara, Wheat and red teff) preparation and cookies preparation and analysis (Howrah, 2012, Onabanjo *et al*, 2009)

# 3.3. Experimental design and formulation of composite flour

# **3.3.1. Experimental design**

This study was carried out to find the appropriate ratio of Red teff, Wheat and Okara flour, tro produce better qualitycookies. The ratio of flours of the three basic ingredients's were formulated using computer generating ratio Design-Expert®, Stat-Ease software Version 8, and Stat-Ease software. D-optimal mixture design with a total formulation of 16 (Table 3) was obtained by considering the range of teff 20-40%, wheat 10-20% and okara 40-50% was in (Table 2).

The range of these ingredients was determined based on different literatures (Mahgoub, 1999; Esmat *et al.*, 2010; Aziah *et al.*, 2012; Adebayo-Oyetoro *et al.*, 2012; Hefnawy *et al.*, 2012). The simplex design plot for the three components of the mixture formulation is presented in Figure 2

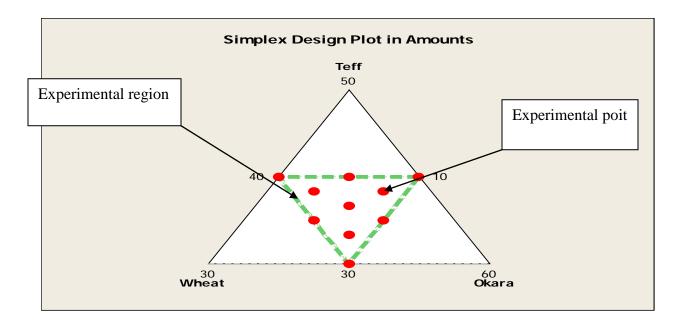


Figure 2. Simplex design plots of the three-component mixture formulations

	Lower limit	Upper limit	
Teff	20	40	
Wheat	10	20	
Okara	40	50	

Table 2. Lower and upper limits of ingredients used in the cookies preparatio

# 3.4. Blending of flour for cookies preparation

		Blending Ratio	0			
Run order	Red Teff %	Wheat %	Okara%			
1	35.0	15.0	50.0			
2	40.0	20.0	40.0			
3	30.0	20.0	50.0			
4	30.0	20.0	50.0			
5	37.0	16.0	47.0			
6	40.0	10.0	50.0			
7	34.0	20.0	46.0			
8	35.0	18.0	47.0			
9	40.0	17.0	43.0			
10	38.0	18.0	44.0			
11	40.0	15.0	45.0			
12	40.0	10.0	50.0			
13	34.0	20.0	46.0			
14	35.0	15.0	50.0			
15	40.0	20.0	40.0			
16	33.0	18.0	49			
17	0	100	0			

Table 3. Proportion of different ratios of red teff, wheat, okara in preparation of composite flour

### 3.5. Preparation of Cookies

Cookies were prepared with some modification according to the method reported by AACC 2000, Okaka and Isieh (1990). The flour was taken used for each batch with the different flour blends according to the design 1kg of composited flours. Whole egg (60g), powdered milk (20g), baking powder (0.1g), salt (1g), sugar (200g) was added and the ingredients were mixed in a

bowl by a mixer for 3 minutes until a stiff paste (batter) was obtained. Then creaming of shortening (60gr) and water 250 mlwas done in a mixer till foaming occurred. The flour was added to the creamy mass and mixed for 10 minutes at medium speed in a laboratory mixer. The dough was then rested for 30 minutes followed by cutting the dough to a desired shape of uniform thickness. The dough was rolled on a floured board using rolling pin to a thickness of 0.3 Cm. The rolled dough was cut into shapes and arranged on a greased plate and baked at 120<sup>o</sup>Cfor 15 minutes the time and temperature was determined during preliminary work. The cookies were cooled to ambient temperature, packed in low density polyethylene bags and kept in airtight containers prior to subsequent analysis (nutritional analysis, physical properties, mineral composition, anti nutritional properties) and sensory evaluation for further analysis.

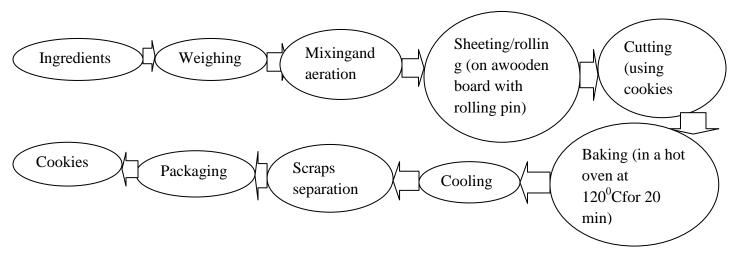


Figure 3: Flow Chart for the Production of cookies (Source: Ihekoronye, and Ngoddy 1999), **N.B**: Ingredients mixed (flours, milk powder, sugar, salt, egg, baking powder, water)

## **3.6. Data collection (Laboratory Analysis)**

Different response variables were measured and data were collected at different parameters to analyse by experiment. Data were collected on proximate composition, physical properties, mineral content, anti-nutritional factors, functional properties and sensory evaluation of cookies

## 3.6.1. Proximate composition

Proximate composition of cookies prepared from different levels of the mixed flour was determined. All analyses were performed in triplicated and analyzed of their average.

#### **3.6.1.1.** Determination of moisture content (%)

Moisture content of the prepared cookies samples were determined by dry air oven method according to AOAC (2005) method 925.10. The moisture dish was dried at  $130 \pm 3^{\circ}$ C for 1 hr and placed in desiccator and initial weight was recorded after cooling. About 2 g of cookies flour was weighed by analytical balance and put in dry dish and moisture dish with the sample was placed in hot air oven (M200CF) and maintained at  $130^{\circ}$ C  $\pm 3^{\circ}$ C for 1 hr. The sample containing dish was transferred to a desiccator and weighed soon after reaching room temperature, the same process was repeated to get constant weight and considered as final weigh. Then, the moisture content was estimated by the following formula

$$Moisture (\%) = \frac{(M_{intial} - M_{dried})}{M_{intial}} * 100\%$$
.....Equation 1

#### 3.6.1.2. Determination of total ash (%)

Ash content of the cookies samples were determined by (AOAC, 2005) method 923.03. The crucibles were cleaned and dried at 120°C and ignited at 550°C in furnace (5x-5-12) for 30 min. The crucibles were removed from the furnace and were placed in desiccators to cool down to room temperature. The initial masses of crucibles were measure by analytical balance and the reading was recorded as (M1). About 3 g of the cookies flour samples were weighed in to crucibles (M2). The sample was dried at 120°C for 1 hr in drying oven (M200CF). The sample was placed in furnace at 550°C until free from carbon and the residues appear grayish white in color (for about 8 hrs). The sample was removed from the furnace and placed in the desiccator and the final weight of the crucible was recorded.

$$Total ash(\%) = \left(\frac{M_3 - M_1}{M_2 - M_1}\right) * 100$$
.....Equation 2

## 3.6.1.3. Determination of crude protein (%)

The crude protein content of cookies was analyzed by Kjeldahl method of nitrogen analysis according to AOAC (2000) method 979.09. To a digestion flask containing about 0.5 g of sample, 6 mL of acid mixture (Conc. Sulfuric acid) and about 3 g of catalyst mixture ( $K_2SO_4$  and Selenium) were added and digested (Kjeldahl flask KF250, Technical Glass Products, Inc., USA)

at about 370°C until the solution becomes clear. The digested sample, distilled water (30 mL) was added and then, ammonia was distilled off after adding 25 mL of NaOH (40%) into receiving flask (25 mL of boric acid with 10 drops of indicator solution). Finally, the distillate was titrated with standardized 0.1N sulfuric acid to end point as reddish color. Simultaneously blank was conducted with test sample. The crude protein content was estimated using the following equation:-

**Digestion**: Food sample 0.3 g weigh was added into the digestion flask or larger test tube for digestion by addition of small volume (3-5ml) of concentrated  $H_2SO_4$  (an oxidizing agents which digests the food), anhydrous  $Na_2SO_4$  or  $K_2SO_4$  that speed up the reaction by raising the boiling points of  $H_2SO_4$  and a catalyst (CuSO<sub>4</sub>, selenium, titanium or mercury) to speed the reaction. One gram of catalyst mixture was made of  $Na_2SO_4$  or  $K_2SO_4$  with anhydrous CuSO<sub>4</sub> in the ratio of 10:1 was used. During digestion any nitrogen in the food (other than that which is in the form of nitrates or nitrites) was converted into ammonia and other organic matter to  $CO_2$  and  $H_2O$ .

**Distillation**: After digestion is completed, the content in the flask was diluted by water and a concentrated NaOH (40%) solution using distiller (model KDN-F-China). It was added to make the solution slightly alkaline and to liberate ammonia gas. The ammonia was then distilled into receiving flask that consist a standardized strong acid such as solution of excess boric acid (4%) or sulfuric acid for reaction with ammonia or sulfuric acid. The total nitrogen and percentage of protein content in sample was estimated using.

Where:

T=Volume in ml of the standard acid solution used in the titration for the test material

- B = Volume in ml of the standard acid solution used in the titration for the blank determination
- N = Normality of standard sulphuric acid
- W = Weight in grams of the test material

14.007= the molecular weight of nitrogen Crude protein = 6.25 \* total nitrogen

## 3.6.1.4. Determination of crude fat (%)

Crude fat of cookies was determined by Soxhlet extraction according to AOAC (2005) method, 2003.06. About 2 g of sample was weighed and put into a thimble. The thimble and its contents was placed in to a 50 mL beaker and dried in an oven (Memmert 854, West Germen) for 2 hrs at  $102\pm2^{\circ}$ C. The thimble contents were transferred in to extraction unit. The sample contained in the thimble was extracted with the solvent diethyl ether in a Soxhlet extraction apparatus (Foss Tecator, Sweden) for 6 hrs. After the extraction completed, the extraction thimble was dried in the oven (Memmert 854 schwabach, West Germen) for 30 mins at  $102^{\circ}$ C ±  $2^{\circ}$ C to remove moisture. Then it was removed from the oven and cooled in a desiccator. The cup and its contents were weighed. The crude fat was determined by the following formula:-

Crude 
$$fat(\%) = \frac{W_2 - W_1}{Weight of sample} \times 100\%$$

Where:

W1= Weight in grams of the extraction flask.

W2= Weight in grams of the extraction flask plus the dried crude fat.

## 3.6.1.5. Determination of crude fiber (%)

The crude fiber was determined by the non-enzymatic gravimetric method (AOAC, 2000, 920.168). Well defatted 2 g food sample was placed into 600 ml beaker and 200 ml of 1.25%  $H_2SO_4$ . Then the beaker was placed on digestion apparatus and was boiled exactly for 30 min., while shaking at every 5 min. intervals. The solution was passed through screen sieve and the digested sample was decanted. The digestion beaker was washed with 3 x 50 ml portion of near boiling point water and each was transferred into the screen for filtration. The residue left on the screen was transferred into 600 ml digestion flask by washing the screen with 200 ml (50mlx4) 1 % NaOH. It was then placed on digestion apparatus and boiled for 30 min. while shaking at every 5 min interval. The digested sample was filtered in coarse porosity (75 $\mu$ m) crucible in apparatus at a vacuum of about 25mm. The residue was dried at 130°C for 2 hrs. and cooled in

desiccators and weighed (M1). The dried residue was igniting for 2 hrs at  $600\pm15^{\circ}$ C until washing was complete and then cooled in desiccators and was reweighed (M2).

Where:

M1 = mass of crucible with wet residue

M2 = mass of crucible with ash residue

## 3.6.1.6. Determination of utilizable carbohydrates (%)

The utilizable carbohydrate content of the cookies samples was determined by difference method using the following mathematical expression

Total carbohydrates (CHO %) was estimated by difference method: (Monro and Burlingame, 1996).

## 3.6.1.7. Gross energy

Gross energy was calculated according to the method developed by Osborne and Voogt (1978).

The gross energy content in raw and processed sample was calculated as follow:

## 3.6.2. Determination of anti-nutritional factors

## 3.6.2.1. Phytate determination

Phytate was determined by using the procedure of Latta and Eskin (1980) and later modified by Vaintraub and Lapteva (1988). About 5g of dried sample was extracted with 100 ml 2.4 % HCl for an hour at an ambient temperature and centrifuged at 3000 rpm for 30 min. The clearsupernatant was collected and 3ml of sample solution was mixed with 1ml of wade reagent (0.03% solution of FeCl<sub>3</sub>.6H<sub>2</sub>O containing 0.3% sulfosalicylic acid in water) followed

bycentrifugation. The absorbance at 500 nm was measured using UV-Vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA).

**Standard solution preparation**: A series of standard solution was prepared containing 4-40  $\mu$ g/ml phytic acid in 0.2N HCl. standard was pipette in to 15 ml centrifuge tubes with 3ml of water used as a zero level (blank). Then 2 ml of the Wade reagent was added to each tube, and the solution was mixed on a vortex mixer for 5 seconds. The mixture was centrifuged for (3000 rpm for 10minutes) and the supernatant was read at 500 nm by using water to make zero the spectrophotometer. Using SPSS plot the calibration curve was prepared (absorbance with concentration) and the slope and intercept was found out from the graph equation.

 $phytic \ acid \ in \frac{\mu g}{g} = \frac{Absorvance-Intercept}{Slope \times Density \times weight \ of \ sample} \, .$ 

.....Equation 8

## 3.6.2.2. Condensed tannin determination

Condensed tannins were determined by the procedure given by Maxson and Rooney (1972). About 1 g of weighed sample was extracted with 10 mL of 1% HCl in methanol for 24 hrs at room temperature by mechanical shaking (Edmund Buhler, USA) then centrifuged at 3000 rpm for 5 min. One milliliter of the supernatant was mixed with 5 mL of vanillin HCl reagent which was prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol while waiting for 20 min until the reaction is completed. Finally, the absorbance was read at 500 nm after 20 min using UV-Vis Spectrophotometer (CE1021, England). A stock catechin solution was used as the standard (20 mg catechin dissolved in 100 mL 1% HCl in methanol) and value of tannin was expressed in mg of catechin in gram of sample. Calibration curve was constructed by using a series of 0, 0.2, 0.4, 0.6, 0.8, and 1 mLof stock solution were taken in test tubes and the volume of each test tube was adjusted to 1 mL with 1% HCl in methanol. 5 mL of Vanillin-HCl reagent was added into each test tube.

**Standard solution preparation**: D-Catechin was used as the standard value of tannin in mg D-Catechin per g of sample. 40 mg of D-Catechin was weighed and dissolved in 100ml of 1% HCl in methanol (stock) then was taken 0, 0.2, 0.4, 0.6, 0.8 and 1ml of stock solution in a test tube and adjusted volume of each tube to 1ml with 1% HCl in methanol. In each tube 5ml of vanillin-HCl reagent was added and allowed for 20 minutes to complete the reaction. The absorbance was

read at 500nm. Reference curve was prepared from the series of standard solution. The amount of tannin was calculated using tannin standard curve and result was expressed as tannin in mg/g fresh weightcalculation.

 $Tannin \ in \frac{mg}{g} = \frac{Absorvance-Intercept}{Slope \times Density \times weight of sample}$ 

.....Equation 9

## 3. 6. 3. Mineral analyses

The mineral analyses were determined by Flame Atomic Absorption Spectrophotometer (AAS) (Auto sampler AA 6800, Japan) as per the AOAC, (2005) method, 985.35. One gm of cookies flour samples were ashed and weighed. The white ash was treated with 5 mL of 6N HCl and dried on the hot plate. Added 15 mL of 3N HCl and heated the crucible on the 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 dilutions. The sample and standard was atomized by using reducing air-acetylene for Ca and oxidizing airacetylene for zinc and iron as a source of energy for atomization (AACC, 2000). For Iron content 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, 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 LaCl3 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<sub>3</sub>.

#### 3.6.4. Determination of molar ratio of phytate/mineral:

Phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660 g/mol; Fe: 56 g/mol; Zn: 65 g/mol; Ca: 40 g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals (Norhaizan, 2009)

## 3.6.5. Functional properties

## 3.6.5.1. Bulk densities of the cookies flour

Bulk density was determined by the method of Adeleke and Odedeji (2010). Fifty gram of cookies flour was placed 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:

Bulk density 
$$\binom{g}{Cm^3} = \frac{\text{weight of sample}}{\text{volume of sample after tapping}}$$
......Equation 10

## 3.6.4.2. Water absorption capacity of composited flour

The WAC was determined using the method developed by Beuchat (1977). One gram sample was mixed with 10 mL of distilled water for 30 seconds in a mix (set on fast speed). The samples were allowed to stand at room temperature for 30 min, centrifuged at 2000 rpm (Centrifuge model 800-1) for 30 min and the volume of the supernatant was measured in a 10 mL graduated cylinder.

$$\% WAC = \frac{Weight of water bound \times 100\%}{Weight of sample (dry basis)}$$
Equation 11

## 3.6.5. Physical properties

## 3.6.5.1 Breking strength determination

Determination of the breaking strength of cookies was made following the method described by Singhet al., (2008) using a texture analyzer (Model TA-PLUS, LLOYD instrument, England). The distancebetween the two beams was 40 mm. Another identical beam was brought down from above at a Pre-Test Speed: 2.0 mm/s, Test Speed: 0.5 mm/s, Post-Test Speed: 10.0 mm/s Distance: 5 mm to contactthe cookie. The downward movement was continued till the cookie breaks. The peak force wasreported as braking or fracture strength.

## 3.6.5.2. Determination of thickness

The thickness was measured with a calibrated ruler as described by Ayo et al (2007). The total thickness was measured in millimeters with the help of caliper after baked. This process was repeated thrice to get an average value and results was reported in mm (AACC, 2000).

## 3.6.5.3. Diameter

To determine the diameter it is measuring by caliper. The total diameter of the six cookies was measured in mm by using a ruler. This was repeated once more and average diameter was taken in millimeters (AACC, 2000

## 3.6.5.4. Spread ratio

Spread ratio was calculated as diameter (length) to thickness ratio (Shrestha and Noomhorm 2002).

 $Spread Ratio = \frac{Diameter}{Thickness}$ Equation 12

## 3.6.6. Sensory evaluation

Prepared cookies samples were coded randomly with three digits and were allowed for sensory evaluation. Sensory attributes measured were color, aroma, taste, crispness and overall acceptability using a five point hedonic scale, where 1= dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like extremely (Muhimbula *et al.*, 2011). A total 50 untrained panelists were participated in this study. After tasting each coded sample panelists are instructed to palate cleaning with water before moving to the next.

Then the score of all judges for each sample were summed up and divided by the number of panelists to find the mean value.

## 3.7 Data Analysis

The data collected from the lab experiment was analyzed by using Minitab 16 software package. The statistical significance of the terms in the regression equations were examined by analysis of variance (ANOVA) for each response and the significance test level was set at 5% (P < 0.05). All data normality distribution was checked. The fitted models for all the parameters were generated two-dimensional contour plots. Graphical optimization was carried out to determine the optimum formula of okara based cookies by substituting with some level of red teff and wheat in terms of nutrient composition and sensory attributes.

# **4. RESULT AND DISCUSSION**

In this chapter discussed on the data which collected on proximate compositions (moisture content, ash, protein, crude fiber, fat, carbohydrate, total energy, Anti nutritional factors (phytate and tannin), functional properties (water absorption capacity, bulk density) mineral composition (Fe, Ca, Zn) and sensory properties are presented follows

## 4.1. Proximate analysis

The proximate composition analyzed in this study includes moisture, ash, crude protein, crude fat, crude fiber and carbohydrate estimation (James, 1995). The proximate compositions of cookies prepared from different mixtures of composite flour are presented in Table 4. Analysis of variance results are summarized in Appendix 1.

### 4.1.1. Moisture analysis

As indicated in Table 4, moisture content of cookies was within the range of 6.25 to 8.00%. The highest moisture content was obtained from 40% red teff, 10% wheat, and 50% of okara. While the lowest result was obtained from 40% red teff, 20% wheat and 40% okara made cookies. The moisture content increase with the increase in supplementation of okara flours this could be due to the fact that soya can absorb moisture in baked product (Neha and Ramesh, 2012). Moisture content results were almost similar with result reported by Ndife *et al.*, (2014) which is about 7.24%, for cookies prepared from 50% soy flour supplemented with wheat flour. The present result was not significantly different in both linear and quadratic terms.But Significant different was observed in the blend of wheat with okara. The experimental results of  $R^2$  values indicated that the models were satisfactory and lack of fit value showed non-significant (Appendix A). The contour plot in figure 4 indicates that the proportion of okara flour increase the result of Moisture content increase

*MC* (%) *y*= 4.1*A* +45.4*B*+27.9*C*-36.0*AB*-29.3*AC*-101.0*BC*...... Equaltion 13

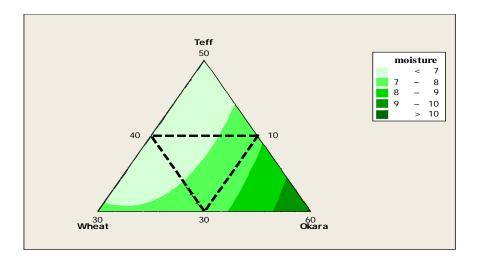


Figure 4.Blending effect on mixed flours cookies moisture content indicated by contour plot

#### 4.1.2. Ash content

The ash content of the cookies showed significance difference in quadratic model. Significance variation was observed that highly significant difference (P < 0.01) was observed between red teff and okara and also significantly different (P < 0.05) in red teff with wheat. The ash content of the cookies sample varied between 1.25-2.4%. The highest ash content was recorded for 35% red teff, 15% wheat and 50% okara. Whereas the lowest results were recorded for cookies prepared from 40% red teff, 20% wheat and 40% okara. The result of this sudy indicted that the ash content of the blends was increased steadily with increasing okara flour (OF). This might be due to the better ash content of soya bean than others cereals. Legumes have been reported to be good sources of ash (Pyke, 1981). Similar result was reported by Ndife et al., (2014) for cookies prepared from 50% soy flour supplemented with wheat flour. The present study was dis agreement as reported by a study done by Okoye et al. (2008) found out that the ash content of flour blends increased from 0.86-4.62 % as the amount of soybean increases from 0% to 100% in the biscuit prepared from wheat and soybean composite flour. The contour plot in figure 5 indicates that as the proportion of okara flour increase the Ash content increase. The regression model for ash content was represented in (Eq. 14) as indicated in guadratic model with three variables. (A= Red teff, B=Wheat, C=okara)

Ash (%) =32.5A+11.1B+25.6C-67AB.8-101.8AC-30.9BC...... (Equation 14)

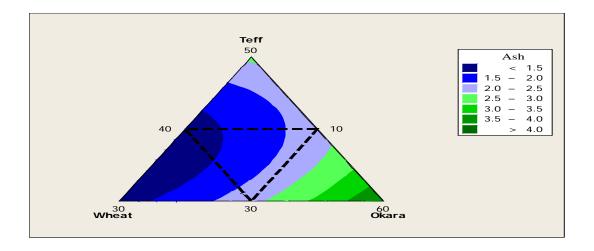


Figure 5.Blending effect on cookies Ash content indicated bycontour plot

Cookies components (%)					Proximate composition				
Teff	Wheat	Okara	M.C%	Ash%	Fat%	Protein%	Fiber%	CHO%	Enegry*
35	15	50	7.56	2.00	17.80	18.25	8.65	45.74	415.80
40	20	40	6.30	1.25	13.25	15.35	7.25	56.6	407.05
30	20	50	7.65	2.40	18.20	18.56	8.67	44.52	416.12
30	20	50	7.60	2.35	18.00	18.30	8.50	45.30	416.20
37	16	47	7.30	1.88	16.00	16.80	8.35	49.67	409.88
40	10	50	8.00	2.20	17.79	18.00	8.90	45.11	412.55
34	20	46	7.00	1.62	14.67	16.34	7.66	52.71	409.80
35	18	47	7.15	1.85	15.80	16.40	8.00	50.80	409.50
40	17	43	6.40	1.55	13.45	15.40	7.35	55.85	406.05
38	18	44	6.60	1.57	14.00	15.65	7.40	54.78	407.72
40	15	45	6.76	1.60	14.37	15.75	7.43	54.09	408.69
40	10	50	7.60	2.40	17.50	17.85	8.85	45.80	412.10
34	20	46	6.90	1.65	15.60	16.00	7.60	52.30	412.90
35	15	50	7.54	2.26	17.00	17.56	8.78	46.86	410.68
40	20	40	6.25	1.30	13.00	15.00	7.00	57.45	406.80
33	18	49	7.45	2.00	16.57	17.00	8.54	48.44	410.89
0	100	0	5.5	1.20	6.5	10.46	2.4	73.94	396.10

Regression	Moisture	Ash	Protein	Fat	Fiber	СНО	G.E
Linear	0.198	0.648	0.391	0.263	0.028	0.110	0.812
Quadratic	0.166	0.22	0.005	0.017	0.031	0.002	0.245
Teff*Wheat	0.398	0.210	0.242	0.249	0.394	0.210	0.225
Teff*Okara	0.498	0.002	0.002	0.021	0.047	0.002	0.259
Wheat *Okara	0.048	0.005	0.022	0.018	0.023	0.005	0.186
Lack of fit	0.553	0.192	0.490	0.663	0.100	0.192	0.799

**M.C=** Moisture content; **CHO**= Carbohydrates; \* (**Kcal/100g**) =Kilo calorie per hundred gram Table 5. Analysis of variance (ANOVA) p-values of proximate parameters and energy

M.C= Moisture content; G.E= gross energy

#### 4.1.3. Crude protein

The results of protein content were varied from 15% -18.56%. The highest protein content was found in blending of 30% red teff, 20% wheat and 50% okara. This might be due to the high amount of okaraflour in the product. It was due to that high protein content in soybean flour (Fabiyi, 2006).Whereas the lowest protein contents was found in cookies sample produced from blending of 40% red teff, 20% wheat and 40% okara. The results of the present studyis supported by the results of Yaseen *et al.*, (2010) who reported protein content of 17.8 % for biscuitsprepared from soybean and wheat flour. The protein values have high comparability with the different reported studies (Edema *et al.*, 2005; Olaoye *et al.*, 2006; Adeyemi and Beckly, 1986; Adeyemi *et al.*, 1987; Osungbaro *et al.*, 2009) who prepared agiddi from maize and soy bean flour. Result of the blended cookies had highly significantly more protein contents than the control (cookies made with wheat). The result contained was also highly significant difference in quadratic models.

Similar result was reported by Yewelsew *etal.* (2000) who reported combination of cereals with legume proteins would provide better overall essential amino acid balance, helping to overcome the world protein calorie malnutrition problem. It is a potential way to increase the nutritional value of traditional cookies prepared from wheat flour. The contour plot in figure below

indicates that as the proportion of red okara flour increase the Protein content increaseThe regression model of protein content is presented below equation 15, (A= Red teff, B=Wheat, C=okara)

Protein (%)=100.7A+74.4B+126.1C-112.7AB-374.4AC-268.7BC...... (Equation 15)

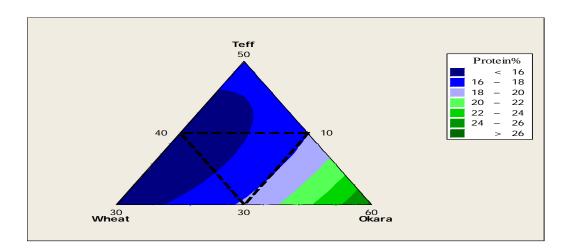
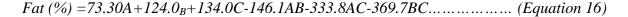


Figure 6. Blending effect on protein percentage of cookies showed in mixture contour plot

## 4.1.5. Crude fat

The crude fat content of all 16 cookies samples from red teff, wheat, okara and control (100% wheat) was shown in Table 4. Significant difference (P<0.05) was observed in the fat content in quadratic models (Appendix Table 1). The fat content of all cookies samples was varied between 13-18. 2%. The result was observed that significantly difference between red teff with okara and also significantly difference in wheat with okara (Appendix Table 1). The highest value of fat content was obtained from cookies prepared from 30% red teff, 20% wheat and 50% okara, whereas the lowest value of fat content recorded for thecookies prepared from 40% red teff, 20% wheat, and 40% okara. This might be because of relatively higher amount of fat found in soybean okara. This result is in agreement withresults reported by Akubor and Ukwuru (2005) who reported the fat content of the biscuits prepared from wheat and soy bean increased from 14.6 to 24.0% with increase in soybean flour. Similarly Iwe andOnuh (1992) also concluded that soybeans have been reported to be a good source of the fat values also closer to the results of present study. Furthermore Sanful *et al.* (2010) also reported addition of soybean flour increases the content of fat in fortified cookies while the amount of carbohydrate is reduced. Generally the

soybean have been reported to contain an appreciable quantity of minerals and fat (Onyeka and Dibia, 2002; Plahar *et al.*, 2003).The present study was dis agreement as reported by Eneche (1999), the Fat values for millet and pigeon pea flour compared non favorably with 1.8% and 4.8% The contour plot in figure below indicates that as the proportion of okara flour increase the fatcontent increase.The regression equation of crude fat is presented below equation 16. The regression model forfat content was represented in quadratic model with three variables. (A= Red teff, B=Wheat, C=okara)



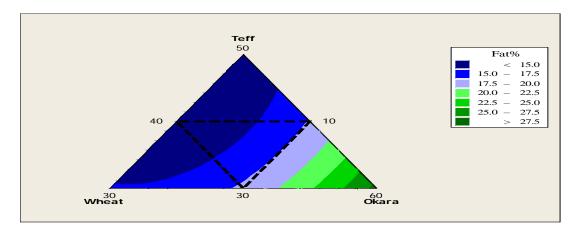


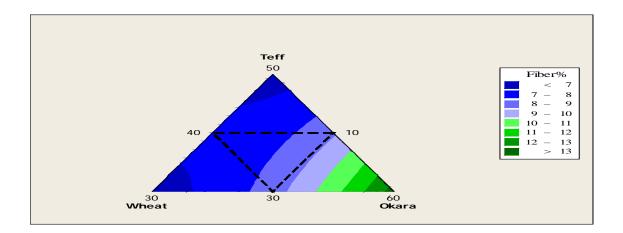
Figure 7. Blending effect of mixed flours on cookies fat content indicated by contour plot

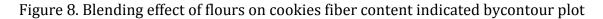
## 4.1.4. Crude fiber

The crude fiber content of Red teff, wheat and okara flour cookies including control sample (100% wheat) was shown in Table 4. The fiber content of 16 cookies samples prepared fromcomposite flours was varied between 7-8.9 %. Sample prepared from blends of 40% red teff, 10% wheat and 50% okara was showed the highest value, whereas, blends of 40% red teff, 20% wheat 40% okara were showed the lowest fiber content than other blended samples. Significant difference (P<0.05) was observed for both the linear and quadratic models. Significance difference was also observed for red teff with okara and wheat with okara (Appendix Table1). The result of the present study was in agreement with study reported by Ndife *et al.*, (2014) who reported 5.73% of crude fibre in cookies prepared from 50% soy flour supplemented with wheat flour. The increased fibre content of cookies have several health benefits, as it will aid digestion often associated with products from refined grain flours (Slavin, 2005; Elleuch *et al.*, 2011). Other studies have also showed that combining okara with soft wheat

flour resulted an increased dietary fiber, contents as compared to with the use of soft wheat flour alone (Rinaldi *et al.*, 2000). The contour plot in figure below indicates that as the proportion of okara flour increase the Fiber content increase. The regression model for fiber content was represented in quadratic model with three variables in equation 17. (A= Red teff, B=Wheat, C=okara)

```
Fiber (%) =16.7A+20.5B+58.4C+45.1AB-117.2AC-148.6BC...... (Equation 17)
```





## 4.1.6. Utilized Carbohydrate

Carbohydrate content of cookies made from red teff, wheat, and okara were highly significant difference in quadratic model and also highly significant difference between red teff with okara and wheat with okara interaction and not significant difference on alla interaction and both of models(Appendix Table 1). The Total carbohydrate content of cookies sample varied between 44.52 to 57.45%. The highest carbohydrate content was recorded in cookies sample from 40% red teff, 20% wheat 30% red teff, 20% wheat, and 50% okara. The results of this study showed that soybeans are not good sources of carbohydrate when compared to wheat and teff (Salunkhe *et al.*, 1992). The carbohydrate contents decreased with the increase in the proportion of okara in the composite flour supplemented cookies.

Generally, the reason of reduction in carbohydrate content of the cookie could be an increasing in moisture, fat, ash and fiber content of the cookies as the proportion of okara and red teff flour in the formulation was increased which leads are duction in carbohydrate content since carbohydrate is calculated by difference. This trend was supported by claim of Akpapunam (1997). The decreased in carbohydrate could be due to the low content of carbohydrate in the added mixed okara compare to wheat flour. This result was agreed with the finding of Iwe (2004) who reported that soybean is poor sources of carbohydrate.

The decreased carbohydrate content of the cookies with addition of red teff and okara flours would be useful to people that need low carbohydrate foods leading to enhanced health for overweight and obese persons. However, cookies which made from control (100% wheat) had reported the highest carbohydrates content; this is due to higher amount of carbohydrate found in wheat. Although, cookies carbohydrate value resulted inthis study is still lower than the regular value of wheat-based cookies thus, it could be possible to develop low-carbohydrate cookies using fresh okara. The contour plot in figure below indicates that as the proportion of red teff and wheat flour increase the total carbohydrate content increasecarbohydrate content increase. The regression model for Carbohydrate content was represented in quadratic model with three variables showed in Eq 18. (A= Red teff, B=Wheat, C=okara)

*CH* (%) = -127.4*A*-172.2*B*-272.8*C*+310.5*AB*+958.7*AC*+917.4*BC*......(*Equation* 18)

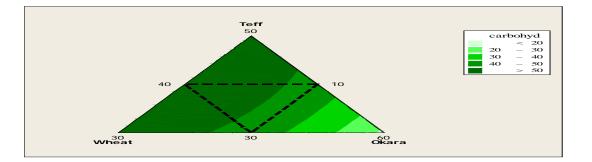


Figure 9.Blending effect of mixed flours on cookiescarbohydrate content indicated bycontour plot

### 4.1.7. Gross Energy

The result was not Significant difference (P<0.05) was observed in the gross energy content of mixed flours cookies sample between among all interaction composite flour but, none significance between both linear and quadratic models. The gross energy value of blended cookies and control cookies was shown in Table 4. The gross energy of cookies samples were varied from 406.06 to 416.20 kcal/100g. The higher gross energy value content was observed in cookies sample prepared from a formulation of red teff 30%, wheat 10% and Okara 50%

whilethe lower gross energy value was observed in cookies sample prepared from a formulation of red teff 40%, wheat 17% and Okara 43%. This observation may be attributed to the high energy content of cookies sample prepared from a formula consisting of high proportion of soy bean okara flour. Onabanjo *et al*, (2009) reported that the high content of legume and oil crops flour further increased the energy density of the products developed from different formulations. This result was in agreement with the other findings obtained by Aleem *et al.*, (2012) who reported that blending of soybean flour in biscuit preparation with wheat showed increment of total energy up to 462.3 k cal/100g. The contour plot in figure below indicates that as the proportion of okara flour increase the persent gross energy content increase.

The regression model for gross energy was shown in quadratic model with three variables in the following equation 19. (Where A= Red teff, B=Wheat, C=okara)

G.E (Kcal/100gm)=552.1A+804.7B+607.0C-664.5AB-628.5AAC-799.9BC....... (Equation 19)

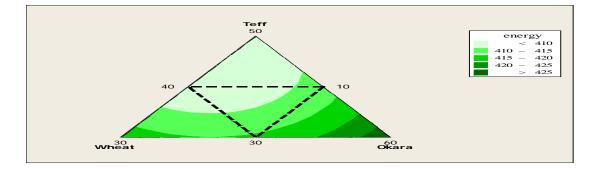


Figure 10. Blending effect of mixed flours on cookies gross energy indicated by contour plot

## 4.2. Anti-nutritional factors

The anti-nutritional factors (tannin and phytate) of cookies sample were analyzed and the value was summarized in Table 6. Models for anti-nutritional factors fitted were indicated that the lack-of-fit, p-values were significantly different at 5% probability level or (P<0.05). Diagnostic tools like normality plot of residuals indicated that the residuals of all the response variables were normally distributed

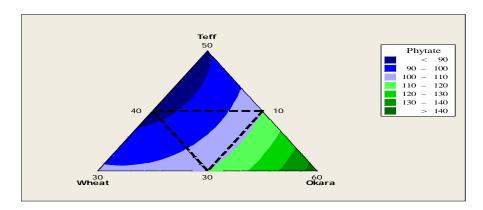
## 4.1.2.2. Phytate

Phytate content of the formulations were ranged from 85 - 115 mg/100g. The high and low amount of phytate was recorded for the proportion of 40% red teff, 10% wheat and 50% okara

and 40% red teff, 17% wheat, 43% okara respectively. This might be due to the high amount of phytate content found in okara of soybeans (Liener, 2000) flour than wheat and red teff. Phytate content was not significantly influenced by the proportion of red teff, wheat and okara in both models. Previous reports by Tajoddin *et al.*, (2011) showed that, phytate content is high in legumes and decreases the bioavailability of essential minerals and bioavailability of proteins by forming insoluble phytate-mineral and phytate-protein complexes. Thephytate amount increased as the amount soybean flour increased in the cookies. This is because of the high amount of phytate found in soybeans (Liener, 2000). Phytic acid naturally occurs in soybeans (and most soybean products) and can make up to 1-1.5 g per 100 g of the dry weight (Liener, 1994). The findings of the present study are in agreement to that of Maqbool *et al.* (1987) who concluded phytic acid content of wheat rotis increased by the increasing supplementation level of soy flour. The contour plot in figure below indicates that as the proportion of okara flour increase the persent phytate content.

The regression model for Tannin and phytate was shown in quadratic model with three variables in following equation 20. (A= Red teff, B=Wheat, C=okara)

*Phytate*=172A+663B+439C-812 AB722 AC3-1369 BC..... (Equation 20)



Where A=Red teff B=Wheat C=Okara

Figure 11 Contour plot of displaying Phytate content of Cookies

okies co	mponents (%)	3	nti-nutritional fa	ctors (mg/100g)
Teff	Wheat	Okara	Phytate	Tannin
35	15	50	108.90	17.30
40	20	40	85.00	13.75
30	20	50	110.00	18.68
30	20	50	109.90	18.79
37	16	47	96.00	16.57
40	10	50	111.00	17.65
34	20	46	100.20	15.75
35	18	47	102.40	17.00
40	17	43	92.00	14.36
38	18	44	95.00	14.77
40	15	45	95.00	14.77
40	10	50	108.60	17.52
34	20	46	97.70	15.35
35	15	50	107.40	17.75
40	20	40	89.00	13.50
33	18	49	106.30	17.49
0.0	100	0.0	15.50	13.35

 Table 6. Anti-nutritional factors (phytate and tannin) of prepared cookies

 Table 7.
 Analysis of variance p-value of anti-nutritional factors

Regression	Phytate	Tannin
Linear	0.374	0.493
Quadratic	0.137	0.120
Teff*Wheat	0.215	0.373
Teff*okara	0.277	0.040
Wheat*Okara	0.069	0.281
Lack of fit	0.342	0.034

### 4.1.2.1. Tannin

Tannins form insoluble complexes with proteins thereby decreasing the digestibility of proteins (Uzoechina, 2007). Tannins also decrease palatability, cause damage to intestinal tract, andenhance carcinogenesis (Makkar and Becker, 1996). The tannin amount increased from 13.5 – 18.79 mg/100g 9.7 to. Table 9 indicated that although there was not significantly affected by the proportion of the mixture components (red teff, Wheat and okara) used for cookies preparation in both linear and quadratic models. The significantly difference cookies tannin content was observed from the interaction of red teff with okara. The amount of tannin in the cookies increased as the amount of okara flour increased. This result occurred because soybean contains high amount of tannin (Folake *et al.*, 2012). Thefindings of the present study are in agreement with that of Maqbool *et al.* (1987) who concluded tannin content of wheat rotis increased by the increasing supplementation level of soy flour.

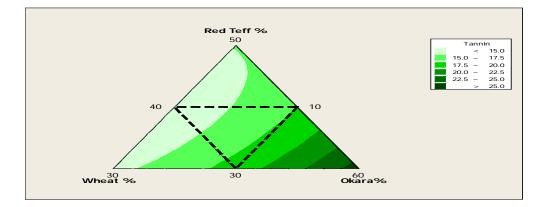


Figure 12. Blending effect of mixed flours on cookies Tannin indicated by contour plot

## 4.1.5. Mineral contents analysis of (Calcium, Iron, and Zinc)

All 16 cookies and control samples were analyzed for mineral content (calcium, zinc, and iron) as Shownin Table 7.

Table 8. Selective minerals (Fe, Zn, Ca) Concentration in cookies

Compositi	Compositions Minerals (mg/100gm)					
Red teff	Wheat	Okara	Iron	Zinc	Calcium	
35	15	50	6.95	2.70	17.85	
40	20	40	6.89	2.73	17.25	
30	20	50	6.40	2.78	17.50	
30	20	50	6.35	2.70	17.45	
37	16	47	6.30	2.73	16.75	
40	10	50	6.90	2.85	18.00	
34	20	46	6.20	2.67	16.60	
35	18	47	6.50	2.70	16.64	
40	17	43	6.85	2.75	16.73	
38	18	44	6.66	2.70	16.68	
40	15	45	7.02	2.80	17.52	
40	10	50	7.24	2.88	17.89	
34	20	46	6.44	2.66	16.65	
35	15	50	6.50	2.70	17.20	
40	10	40	7.30	2.80	17.15	
33	18	49	6.84	2.75	16.70	
0	100	0	5.00	2.30	10.35	

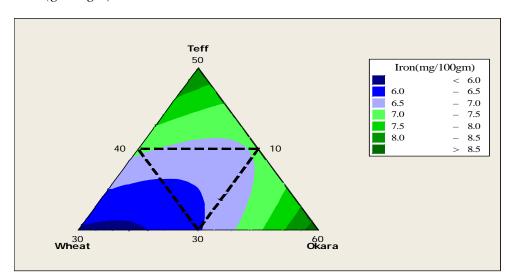
Table 9. Analysis of variance p-value of Mineral concentration
--

Regression	Iron	Zinc	Calcium
Linear	0.746	0.057	0.602
Quadratic	0.194	0.012	0.007
Teff*Wheat	0.976	0.009	0.129
Teff*Okara	0.060	0.150	0.003
Wheat*Okara	0.300	0.468	0.078
Lack of fit	0.509	0.597	0.213

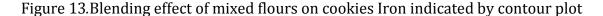
## 4.1.5.1. Iron

The iron content of sixteen cookies samples was varied between 6.20 to 7.30 mg/100gm. The highest result of Fe content determined in the cookies from 40% red teff, 10% wheat and 40% okara, while the lowest result value of Fe was reported for cookies from 34 % red teff 20 %

wheat and 46% okara. This might be due to an increase in proportion of red teff flour and to some extent with the level of okara. Similarly, Abebe *et al.*, (2007) reported that Teff grain is particularly high in iron content. The compositions of Fe showed none significance different (P>0.01) in both linear, and quadratic model and all possible interactions of mixed samples of red teff, wheat and okara. A similar result has also been reported by Rawat *et al.*, (1994) as soy fortified chapattis contained higher iron, than whole wheat flour chapattis. The Regression model of iron, content is presented below equations from 22 where A= Red teff, B=Wheat, C=okara).The contour plot in figure below indicates that as the proportion of okara and red teff flour increase the iron content of cookies increase



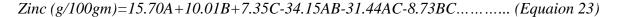
*Iron* (g/100gm) =50.4A+8.9B+45.1C-2.3AB-160.1AC-88.6BC...... (Equation 22)



#### 4.1.5.2. Zinc

In the present study the zinc content of sixteen cookies samples was varied from 2.66 to 2.88 mg/100 g. The highest result of present study was obtained from 40% red teff, 10% wheat and 50% okara, while the lowest value was identified in cookies from 34% of red teff, 20% of wheat and 46% of okara blended cookies. As the amount of red teff and some extent of okara increase the zinc content also increase. Jan *et al.*, (2000) reported that oilseeds flour contained appreciable quantity of mineral which resulted in increase in mineral contents of mixed flours. The zinc content of the 16 blended cookies was higher than values of control. The zinc content showed significant difference (P<0.01) in quadratic model between the interaction of red teff with okara

and shown highly significant difference in red teff with wheat other interaction andbut not significantly in linear model. Hence, using red teff and okara flour in cookies formulation improve the zinc content of the mixed flour. This can be evident from the fact that the zinc content in wheat flour was low as compared to the zinc content of red teff and okara. So, in this study red teff and okara was a good source of zinc in mixed cookies flour than wheat. The contour plot in figure below indicates that as the proportion of red teff flour increase the Zinc content of cookies increaseThe Regression model of, zincontent is presented below equations from equation 23 where A= Red teff, B=Wheat, C=okara).



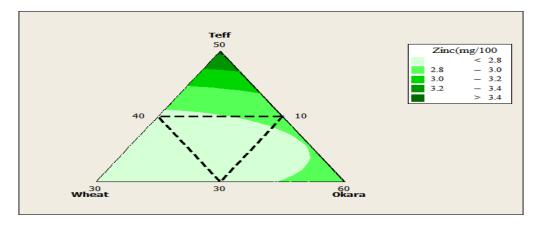


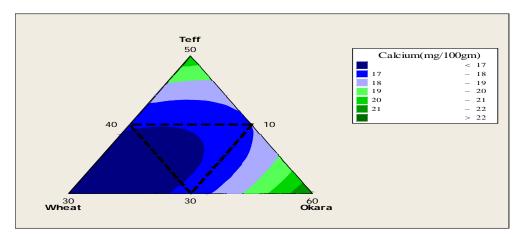
Figure 14. Blending effect of mixed flours on cookies Zinc indicated by contour plot

## 4.1.5.3. Calcium

The calcium content of blended sixteen cookies samples were varied between 16.60 to 18.00 mg per 100g. The highest result was obtained from 35% red teff, 15% wheat, and 50% okara, while the lowest identified in cookies from 34% red teff, 20% wheat and 46% okara cookies. The calcium content of cookies was increase with the increment of some red teff and okara proportion in the mixed flour cookies sample.

Calcium content was found highly significantly (P<0.05) in quadratic model and in the interaction of red teff with okara. The results also revealed that an increased Ca contents were observed when there were high concentration of okara and red teff flour in the cookies. Higher mineral content in the present study found in different cookies may be attributed to higher concentration of calcium in the soybean okara and red teff (Asiedu, 1989; Tinsley, 2009). A similar result has also been reported by Rawat *et al.*, (1994) as soy fortified chapattis contained

higher calcium than whole wheat flour chapattis. The contour plot in figure below indicates that as the proportion of okara and red teff flour increase the calcium content of cookies increase .The results of this study showed that the cookies samples would contribute substantially to the recommended dietary requirements for minerals (Wardlaw, 2004). The Regression model of iron, zincand calcium content is presented below equations from 24 where A= Red teff, B=Wheat, C=okara



*Calcium* (*g*/100*gm*)=118.6A+60.4B+95.4C-139.6AB-342.6AC-181.3BC..... (Equation 24)

Figure 15 Blending effect of mixed flours on cookies Calcium indicated by contour plot

## 4.1.5.4 Molar ratios and bioavailability of minerals

The molar ratios for calcium, zinc, Iron and phytate were calculated to evaluate the effects of elevated levels of phytate in the bioavailability of dietary minerals. Bioavailability is the ability of the body to digest and absorb the mineral in the food consumed (Fekadu *et al.*, 2013). The calculated values are also compared with the reported critical point values for these ratios. The calculated Phy Fe: Phy Zn: and Phy Ca: molar ratios of Blended cookies accessions are shown in

Red Teff%	Wheat %	Okara %	Phytate :Fe	Phytate :Zn	Phytate :Ca	Phytate
35	15	50	0.75	0.25	2.70	6.06
40	20	40	0.95	0.32	3.35	7.76
30	20	50	0.68	0.25	2.63	6.00

Table 10. Calculated molar ratios of Phy: Fe, Phy: Zn and Phy: Ca of cookies from okara, red teff and wheat blends (mol/kg)

30	20	50	0.68	0.24	2.62	6.01
37	16	47	0.77	0.28	2.88	6.87
40	10	50	0.73	0.25	2.67	5.94
34	20	46	0.72	0.26	2.74	6.59
35	18	47	0.74	0.26	2.68	6.44
40	17	43	0.87	0.30	3.00	7.17
38	18	44	0.86	0.28	2.90	6.95
40	15	45	0.89	0.29	3.04	6.95
40	10	50	0.78	0.26	2.72	6.08
34	20	46	0.77	0.27	2.81	6.75
35	15	50	0.71	0.25	2.64	6.14
40	20	40	0.96	0.31	3.17	7.42
33	18	49	0.75	0.26	2.66	6.21
0	100	0	3.78	1.49	11.03	42.6

### Phy: Fe

Phytate is known to be the main inhibitor of iron absorption in plant-based foods. To remove the adverse effect of phytates on iron absorption, phytate contents have to be reduced to concentrations of less than 1g/100g. In cereal-based foods, it has been shown that the phytate/iron molar ratio has to be lower than 1, and preferably 0.4, to obtain a significant increase in absorption (Hurell, 2004), with this regard, most of cookies samples reported the phy: Fe molar ratios lower than 1 as indicated in (Table 9). This indicates iron from these cookies would be bioavailable and is good for human body. The molar ratios of phytate to minerals in the present study were ranged from 0.68-0.95. Most of cookies samples show lower values of phytate: Fe molar ratio when okara flour increase than wheat and teff in the blend this might be correlated to leaching out of phytic acid in soybean seed during soaking and washing in water.

### Phy: Zn

Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH (Oberleas, 1983). The formation of the chelates depends on relative levels of both zinc and phytic acid (Davies, 1979). Oberleas *et al.*, (1983) showed that foods with a molar ratio of Phy: Zn less than 10 showed adequate availability of Zn and problems were encountered when the value was greater than 15. As Table 9 indicated, all the Cookies samples analyzed had low Phy: Zn values which is less than the critical value 15. This means that Zn obtained from these cookies would be bioavailable for the human metabolisam. The values in this study of value added cookies were in the range of 0.24-0.31 and lower in all accessions than the reported critical molar ratios of Phytate to Calcium, indicating that absorption of calcium not adversely affected by phytate in all the accessions. Among all blended cookies samples the lowest phytate: Zn molar ratio was obtained when okara flour increase in the composite cookies flour this might be due to decrease in phytate during soaking of soybean seeds. The values of value added cookies accessions were lower than the critical molar ratios of Phy: Zn, which indicates the availability of zinc, is good.

#### Phy: Ca

Phytic acids markedly decrease Ca bioavailability and the Phy: Ca molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of Phy: Ca is reported to be 0.24 (Morris, 1985). The values in the present study were ranged from 2.62-3.65. The molar ratios of Phy: Ca obtained for cookies samples (Table 9) were all greater than the critical values indicating that lower absorption of calcium and to be adversely affected by phytate in these cookies samples.

## 4.1.4. Functional properties

The functional properties determine the application and use of food materials for various purposes. The mean values of functional properties for cookies such as water absorption capacity (WAC), is summarized in Table 10. Analysis of variance results is also summarized in Appendix Table 2. Models for functional properties were fitted indicated that the lack-of-fit; p-values were significantly different at 5% probability level Diagnostic tools like normal

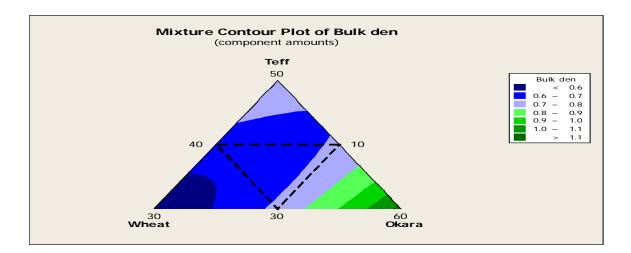
distribution plot of residuals indicated that the residuals of all the response variables are normally distributed.

## **Bulk Density**

The bulk density of the mixed flours of cookies was varied between 0.62-0.75 g/cm<sup>3</sup>. Therewas statistically significant difference ( $\mathbb{P} \ge 0.05$ ) between teff and okara mixed flours of cookiesand control. The result showed that the models were satisfactory and good fitting (lack-of-fit, P<0.05) to experimental results in both linear and quadratic models (Appendix A). Masood and Rizwana, (2010) indicated that the bulk density was increased in the compositeflour with soybean protein content. Similar results reported by Malomo et al., (2011) whoindicated that bulk density of composite flour of yam with soybean increased as the amount ofsoybean increased. The low bulk density of the blends is an advantage in the formulation of complementary foods (Akubor and Ukwuru, 2003). Increase in bulk density is desirable; in that it offers greater packaging advantage as greater quantity may be packed within constant volume (Molina et al. 1983) The bulk density of a food material is important in relation to its packaging (Bello and Okezie 1982). A good quality complementary diet shouldhaveappropriate nutrient density, bulk density, viscosity, texture and consistency that allow easyconsumption (WHO, 2003). The regression equation

Bulk density=6.74A-0.06B+6.59<sub>C</sub>-1.80AB-23.68AC-9.2BC

Where A=Red teff BWheat C=Okara



Teff	wheat	Okara	B.D density( $g/cm^3$ )	WAC (%)
35	15	50	0.75	135
40	20	40	0.68	133
30	20	50	0.74	132
30	20	50	0.72	130
37	16	47	0.68	134
40	10	50	0.73	140
34	20	46	0.65	129
35	18	47	0.66	127
40	17	43	0.62	136
38	18	44	0.62	126
40	15	45	0.69	137
40	10	50	0.70	138
34	20	46	0.64	124
35	15	50	0.70	134
40	20	40	0.64	131
33	18	49	0.67	136
0	100	0	0.6	110

Table 11. Functional propert ies of cookies from different compositions

# WAC=Water Absorbance Capacity

Table 12. Analysis of variance p-value of Functional properties

Regression	B.D	WA
Linear	10.448	0.105
Quadratic	0.068	0.082
Teff*Wheat	0.826	0.915
Teff*Okara	0.014	0.014
Wheat *okara	0.334	0.694

## WAC=Water Absorved Capacity

#### **4.1.4.2.** Water absorption capacity

Water absorption capacity is the ability of a product to associate with water under limiting conditions (Singh 2001). Water absorption capacity is the ability of flour to absorb water and swell for improved consistency in food. It is desirable in food systems to improve yield and consistency and give body to the food (Osundahunsi et al., 2003). And also Water absorption capacity is important in bulking and consistency of product as well as inbaking applications (Lorenz and Collins, 1990). The major chemical compositions that enhance the water absorption capacity of flours are proteins and carbohydrates, since these constituents contain hydrophilic parts, such as polar or charged side chains (Lawal and Adebowale, 2004). The water absorption capacity of the composite flour increased as the proportion of okara and red teff flour increased in the mixture this was important for the quality of cookies preparation. The water absorption capacity (WAC) of the present study was varied between 123-140 % (Table 11) and the WAC was shown a significant difference at (P<0.01) in red teff with okara interaction (Appendix table 2). This may be attributed to increase in protein content of the various cookies samples and in agreement with the work of Kinsella (1976) whoreported that the ability of food materials to absorb water is sometimes attributed to the protein content. Simon (1987) stated that the flour with increased water absorption would result infavorable characteristics of final products, because the products might remain soft for a longer period with improvement in texture and reduced cost According to Kaur and Sing (2005), flours with high WAC have more hydrophilic constituents, such as polysaccharides. The present results are similar to previous researches done by different authors. The contour plot in figure below indicates that as the proportion of okara and red teff flour increase the WAC of flour increase. The regression equation of water absorvability is presented in following equaltion 25 where A= Red teff, B=Wheat, C=okara.

W. A (%) =987A-578B +629C-95AB-2639AC + 38BC...... (Equaion 25)

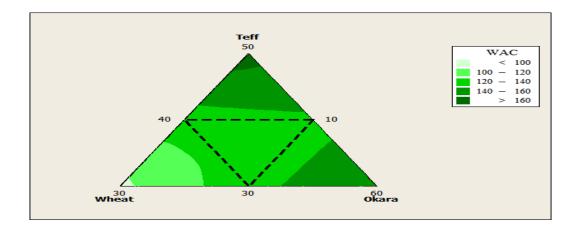


Figure 17. Blending effect of mixed flours on cookies Water Absorvability indicated by contour plot

## 4.1.3. Physical properties of cookies

The results of physical properties of cookies(Breaking strength,diameter, thickness, spread ratio,) produced from red teff, wheat and okara blends as well as 100% wheat flour cookies blends are presented in Table 13. The Breaking strength, diameter, thickness and spread ratio of cookies ranged from 64-71N, 6-7.4 cm, 3.2-3.7 cm and 16.84-20.67 g/cm<sup>3</sup>, respectively.

## 4.1.3.1. Breaking strengthAnalysis of cookies

Breaking strength is one of the textural properties of a food products and it is an important determinant of the food quality (Lewis, 1990). As can be seen from the result given in Table 13, the breaking strength of the cookies was found significantly difference (p<0.05) among the interaction of red teff with okara samples. However, the internal crumb of the cookies which were made form incorporation of okara and red teff flour showed a compacted structure. This could be the reason that as the proportion of okara and red teff flour increased in the formulation, the gluten content of the flour which is contributed by the wheat is decreased hence when the released gas from the baking powder and the water vapor has nothing to hold them, hence they can escape easily and no pores structure would be formed that is the reason for a compacted structure was observed as the proportion of okara and red teff flour increased in the formulation and it is obvious that acompacted matter requires a larger force to be broken, in this way an increased in the formulation. The highest in hardness value was 71 N this was recorded of cookies prepared from blended of 40% red teff 10% wheat and50% okara flour, The cookies

containing blended required the maximum cutting force to break the cookie whereas the lowest value (23N) was obtained from the control (100 % wheat flour) cookie it was softer than of blended cookies. This might be resulted from incorporation of protein rich flour which need more water to obtain good cookies dough. Hoseney and Rogers reported that hardness of the cookies was caused by the interaction of proteins and starch by hydrogen bonding (Hoseney & Rogers, 1994).Thus, refined flour (low gluten flour) used in the present study for cookies preparation might contribute to the insignificant development of gluten network.

The other reason for increasing in cookies breaking strength could be the higher the water absorption capacity of the blend, which could limit the spread ability of the cookies this was observed because of an increasing in fiber content as the okara and red teff flour increasing in the formulation. The cookies prepared from high-absorption dough tend tobe extremely hard (Hoojjat and Zabik, 1984). Similar finding was reported by Lee and Beuchat (1991) that more strength was needed to break cookies incorporated with legumes flour. In conclusion, increment of hardness and adhesiveness may be due to adding red teff and okara powder because of their water absorvability. The regression equation model for breaking strength, is presented in follow equations 26, Where (A= Red teff, B=Wheat, C=okara). The contour plot in figure below indicates that as the proportion of okaraand red teff flour increase the hardness of cookies texture increase.

Breaking strength (N) = 297.8A + 180.0B + 257.9C + 300.6AB + 791.6AC + 506.3BC.....(Equation26)

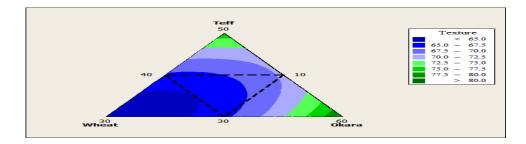


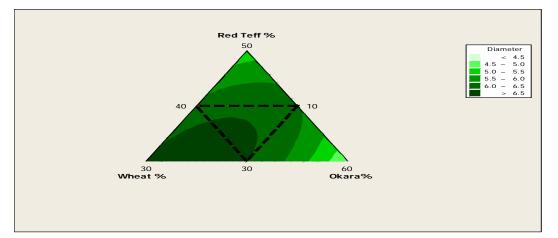
Figure 18.Blending effect of mixed flours on cookies Texture indicated by contour plot

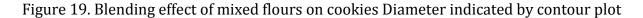
## 4.1.3.2. Diameter

The data on the physical properties of cookies are shown in the table 13. There was asignificant difference (p < 0.05) in quadratic model and also significant difference between the values

obtained for the cookie diameter supplemented teff with wheat and teff with okara composite cookies and 100% wheat cookies but non-significant difference on linear model. The diameters the blended cookies was varied from 6 to 6.9 mm and also the cookies which was made from the control (100% wheat flour) showed a larger diameter (7 cm), The highest result was obtained from 34% red teff, 20% wheat, and 46% okara, while the lowest identified in cookies from 35% red teff, 18% wheat and 47% okara cookies. And the diameter of control cookies was 7cm from 100% wheat flour and increase as compared to the other blend proportion. This decreased in diameter in the composited cookies could be the reason that an increased in fiber content due to the proportion of okara flour and red teff increased in the formulation, this might be due to the spreading ability of fat and glutein contents of composite flour. A similar decreased in diameter was also reported for cookies prepared with Wheat and sweet potato flour (Singh et al., 2008). In which both red teff flour and residue from soybean processing (okara) contains higher fiber content than wheat, and as their proportion increased in the formulation a gradual decreased in diameter was found. The regression equation model for Diameter, is presented in follow equations 27, Where (A = Red teff, B=Wheat, C=okara). The contour plot in figure below indicates that as the proportion of wheat flour increase the Diameter of cookies increase.

Diameter (Cm) =-81.82A-77.69B-41.35C+221.93AB+250.94AC+162.51 BC..... (Equation 27)





## 4.1.3.3. Thickness

The thickness of blended sixteen cookies samples were varied between 3.2 to 3.7cm. The highest result was obtained from 34% red teff, 20% wheat, and 46% okara, while the lowest identified in cookies from 40% red teff, 10% wheat and 50% okara cookies. And the thickness of control

cookies was 12cm from 100% wheat flour and increased as compared to the other blend proportion .The thickness of the cookies from 100% wheat flour were significantly different (P<0.05) from the cookies made from composite flours of wheat, red teff and okara.The thickness result of cookies was highly significant (P<0.05) different in linear model and interaction of both red teff and wheat. However, the thickness of the cookies blended were thicker than (Control) cookies (100% wheat).

However a decreased in the average thickness of the cookies was observed as the proportion of redteff and okara flour was increased in the formulation. This report might be due to the gluten content of wheat cookies and it would be due to the addition of baking powder which increases the dough thickness during baking. Similar results were observed by Ryanand Brewer (2006) they reported that soy flour-added cookies was thicker and smaller in thickness compared to wheat cookies due to the extreme water absorptive properties of soy flour. The high water absorption characteristic of fiber (insoluble fiber of pitaya peel; 56.50%) (Jamilah *et al.*, 2011) can attract more water, thus, the dough viscosity decreased leading to decreased thickness.

The regression equation model for Thickness, is presented in follow equations 28, Where (A= Red teff, B=Wheat, C=okara). The contour plot in figure below indicates that as the proportion of wheat flour increase the Thickness of cookies increase.

*Thickness* (*Cm*) =0.78A+30.96B-3.10C-43.97AB+19.6AC-15.33BC...... (Equation 28)

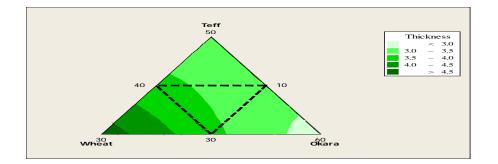


Figure 20.Blending effect of mixed flours on cookies Thickness indicated by contour plot

Table 13. Physical properties of cookies

Red teff	Wheat	Okara	B.strength(N)	Diameter(cm)	Thickness(cm)	S.ratio(cm)
35	15	50	67.88	6.4	3.32	19.270

40204068.006.23.6816.84030205067.006.33.7017.02030205067.506.73.6018.10037164766.606.53.4520.28040105070.006.13.3018.18034204665.006.63.7017.83035184767.506.03.5820.67040174367.006.53.5018.57038184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250							
30205067.506.73.6018.10037164766.606.53.4520.28040105070.006.13.3018.18034204665.006.63.7017.83035184767.506.03.5820.67040174367.006.53.5018.57038184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	40	20	40	68.00	6.2	3.68	16.840
37164766.606.53.4520.28040105070.006.13.3018.18034204665.006.63.7017.83035184767.506.03.5820.67040174367.006.53.5018.57038184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	30	20	50	67.00	6.3	3.70	17.020
40105070.006.13.3018.18034204665.006.63.7017.83035184767.506.03.5820.67040174367.006.53.5018.57038184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	30	20	50	67.50	6.7	3.60	18.100
34204665.006.63.7017.83035184767.506.03.5820.67040174367.006.53.5018.57038184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	37	16	47	66.60	6.5	3.45	20.280
35184767.506.03.5820.67040174367.006.53.5018.57038184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	40	10	50	70.00	6.1	3.30	18.180
40174367.006.53.5018.57038184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	34	20	46	65.00	6.6	3.70	17.830
38184467.256.83.5619.01040154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	35	18	47	67.50	6.0	3.58	20.670
40154566.706.23.4018.23040105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	40	17	43	67.00	6.5	3.50	18.570
40105071.006.13.2019.06534204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	38	18	44	67.25	6.8	3.56	19.010
34204664.006.93.6718.80035155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	40	15	45	66.70	6.2	3.40	18.230
35155068.006.43.3520.00040204066.006.03.6316.52033184965.006.73.4819.250	40	10	50	71.00	6.1	3.20	19.065
40204066.006.03.6316.52033184965.006.73.4819.250	34	20	46	64.00	6.9	3.67	18.800
33 18 49 65.00 6.7 3.48 19.250	35	15	50	68.00	6.4	3.35	20.000
	40	20	40	66.00	6.0	3.63	16.520
	33	18	49	65.00	6.7	3.48	19.250
0 100 0 23.00 7.0 12.0 5.4160	0	100	0	23.00	7.0	12.0	5.4160

Table 14. Analysis of variance p-value of physical properties

Regression	B.S	Diameter	Thickness	Spread ratio
Linear	0.888	0.229	0.003	0.049
Quadratic	0.113	0.016	0.016	0.003
Teff*wheat	0.392	0.027	0.005	0.004
Teff*okara	0.040	0.017	0.155	0.011
Wheat*okara	0.200	0.114	0.287	0.043
Lack of fit	0.161	0.423	0.916	0.335

Where BS=Breking strengthSR =spread ratio

4.1.3.4. Spread ratio

The spread ratio is an indicator of cookies quality. There was statistically significant difference (P<0.05) with both linear and quadratic models and red teff with okara and wheat with okara, where as highly significance difference in both red teff with wheat. The spread ratio of the mixed flours of cookies was varied between 16.52-20.67cm. The highest results was reported from the cookies prepared from 35% of red teff, 18 of wheat, and 47% okara flour while the lowest results were obtained from 40% of red teff, 20% of wheat, and 40% of okara flour. The increase in the spread ratio could be attributed to the increased number of hydrophilic sites in the dough mixture leading to increased water absorption and swelling index. Various workers in contrast have reported decreased spread ratio with usage of composite flours for cookie production (Sathe et al., 1981) A decrease in spread ratio was reported by many workers including (Singh et al., 2008) and (Laura et al., 2012) who worked on the blends of germinated pigeon pea, fermented sorghum and cocoyam flours.

Cookies from wheat had the lowest spread ratio and this indicates suggests that the starches in wheat were very hydrophilic (Yahya 2004). Cookies having higher spread ratios are considered the most desirable (Kirssel and Prentice 1979). In the present study the result was high spread ratio were recorded for cookies from blended flour than that of control sample because of low gluten content of red teff and okara and also low content of wheat. Chinma and Gernah (2007) observed a similar trend in cookies produced from cassava /soyabean/mango composite flours. The spread factor is increased by increased protein (Singh & Mohamed, 2007) .This was in agreement with earlier studies by Mc Watters (1978), Singh *et al.*, (1993) and Guttieri *et al.*, (2004) who also reported decrease in spread ratio of cookies with increase in protein so this suggestion is similar to the present result because of high protein content of okara. The increased spread ratio observed in okara flour substituted cookies samples was due to the difference in the particle sizes and the characteristics of the constituent flours of soya, wheat and teff (Agu *et al.*, 2007).

The contour plot in figure below indicates that as the proportion of okara flour increase the spread ratio of cookies increase. The regression equation model for Spread ratio is presented in follow equations 30, Where (A= Red teff, B=Wheat, C=okara)

S.R (cm) =-226.5A-326.2B-101.2C+785.6AB+670.9AC+535.0BC..... (Equation 30)

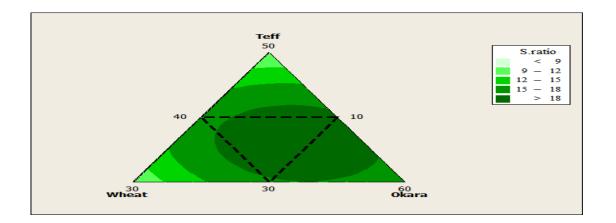


Figure 21. Contour plot of displaying Spread ratio of cookies

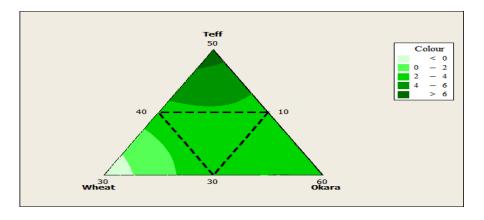
#### 4.1.6. Sensory acceptability Analysis

The results of sensory evaluation are presented in Table 15. Cookies made with wheat had the highest ratings for all the sensory parameters tested. With the exception of over all acceptability, cookies made from red teff, okara and wheat flour blend were not significantly different (P>0.05) from the control. The sensory analysis of variance result was summarized in the (Appendix Table 4). The data were presented on the mean panelists' acceptance of the cookies prepared from red teff, wheat and okara mixed flour for color, aroma, taste, crispness and overall acceptability. All the cookies were at least slightly liked for the parameters studied. This result implies that the evaluated sensory properties of 100% wheat cookies are not different from the cookies developedfrom blended flour.

## 4.1.6.1 Color acceptability

Color is one of the most important sensory qualities of food product. As can be noted from the table 15 given below the cookies color made from different blend proportion was found none significantly affected by both quadratic and linear models and also all possible interaction of mixed samples (p< 0.05). The mean value of the present study was ranged from 2 to 4.5. Among the entire mean values of color, the highest color value was recorded for the cookies made from 40% red teff, 10% wheat and 50% okara, whereas the lowest result was recorded for cookies prepared from 38% red teff, 18% wheat and 44% okara blend. The color of the cookies gets darker which might be due to the high level of red teff present in the product.On the other hand it was found that blended proportion did not significantly affect cookies color however all the blend proportions score lower than the control cookies this is because of the non beautiful dark

color imparted by the red teff flour, but as the proportion of red teff flour increased the score on cookies color decreased this could be because of the deep dark color formed. The contour plot in figure below indicates that as the proportion of red teff and okara flour increase the color of cookies increase. The regression model for color was presented below equations from 31 where A= Red teff, B=Wheat, C=okara.



*Color*=81.1A-145.7B+26.7C+54.0AB-206.1AC+223.0BC......(Equation 31)

Figure 22. Blending effect of mixed flours on cookies Color indicated by contour plot

#### 4.1.6.1. Aroma acceptability

Aroma is imparted by volatile compounds and perceived by the odor receptor sites of the smell organs. The aroma of the cookies samples showed significance difference in linear model and non-significant difference (P>0.05) in quadratic model and in the interaction of all possible sample mixture. The mean value of aroma reported in this study is in the range of 1.65 to 3.89. Among the entire mean values of aroma, the highest acceptability of aroma was recorded from 40% red teff, 20% wheat and 40% okara, whereas the lowest results were recorded for cookies prepared from 40% red teff, 10% wheat and 50% okara. This might be due to that the beany flavor of okara. Similarly, Watters Mc. (1978) indicated that the beany flavor in legumes flour could reduce the acceptability of the products. As reported by Muhimbula *et al.* (2011) aroma is an integral part of taste and general acceptance of the food before it is put in the mouth. The contour plot in figure below indicates that as the proportion of okara flour increase the undesirable smell of cookies increase. The regression model for Aroma was presented below equations from 32 Where A= Red teff, B=Wheat, C=okara.

Aroma=32.2A+0.40B-45.3C-156.8AB+ 46.5AC+175.7BC...... (Equation 32)

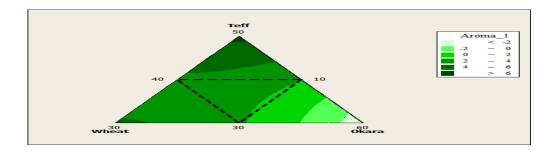


Figure 23. Blending effect of mixed flours on cookies Aroma indicated by contour plot

## 4.1.6.2. Taste acceptability

Taste attributes consist of saltiness, sweetness, bitterness and acidity which is detected by the taste buds at the tip, sides, and back of the tongue is one of the important sensory quality parameters of a food product was also studied and it was found that highly significantly influenced by both of the interaction of red teff with okara observation proportion (p< 0.05). Significance difference was also obtained from quadratic models while non-significance difference was observed for linear model and other interaction. The mean value of taste was found in the rage of 2.5 to 4.2 (Table15). The highest mean score of taste was obtained for cookies from 40% red teff, 20% wheat and 40% okara and the lowest value was obtained for cookies from 30% red teff, 20% wheat and 50% okara blended. The acceptability of the taste was decreased as the level of okara proportion increase because okara was beany flavor and taste for eating. The significance decreasing trend of taste may be due to the own taste of soy product which dominated when used in high amount (Drobot and stabikon1976; Husain 1993). The contour plot in figure below indicates that as the proportion of red teff and wheat flour increase the taste of cookies increase. The regression model for taste was presented below equations from 33(where A=Redteff, B=Wheat, C=okar)

*Taste=39.10A+14.55B47.3C+12.77AB+183.345AC+67.38BC......* (*Equation 33*)

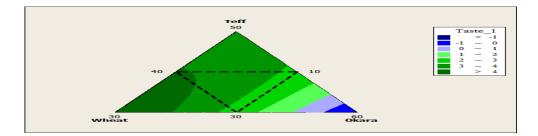


Figure 24. Blending effect of mixed flours on cookies Taste indicated by contour plot

Teff	Wheat	Okara	Colour	Aroma	Crispness	Taste	Over.A
35	15	50	3.46	1.67	4.80	2.65	4.00
40	20	40	3.00	3.89	3.25	4.00	2.68
30	20	50	4.06	2.00	4.35	2.50	3.68
30	20	50	2.40	2.40	4.45	2.70	4.55
37	16	47	4.00	2.42	3.60	3.53	4.35
40	10	50	2.06	2.70	4.60	2.78	3.50
34	20	46	2.32	3.10	3.50	3.60	4.00
35	18	47	4.00	3.40	4.00	3.45	3.50
40	17	43	3.70	3.76	3.20	3.78	4.50
38	18	44	2.00	3.20	3.00	3.86	3.32
40	15	45	3.90	3.28	4.30	3.50	2.50
40	10	50	4.50	1.53	4.50	2.63	3.73
34	20	46	3.03	2.50	3.40	3.55	2.50
35	15	50	3.20	1.65	4.40	2.72	3.55
40	20	40	3.60	3.69	2.80	4.20	3.00
33	18	49	2.50	2.30	4.20	2.54	4.25
0	100	0	4.5	5	5	5	4.5

Table 15. Sensory acceptability mean scores for cookies samples

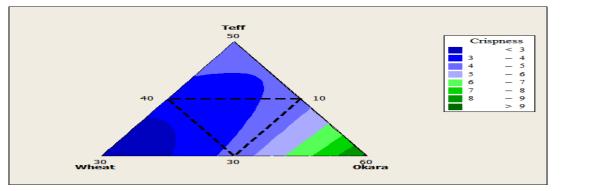
Table 16. Analysis of variance p-value of sensory acceptability evaluation

		-			
Regression	Color	Aroma	Taste	Crispness	O.A
Linear	0.592	0.0142	0.141	0.473	0.643
Quadratic	0.789	0.409	0.024	0.143	0.816
Teff*Wheat	0.854	0.257	0.801	0.993	0.707
Teff*Okara	0.497	0.734	0.005	0.039	0.635
Wheat*Okara	0.494	0.247	0.241	0.306	0.485
Lack of fit	0.657	0.622	0.104	0.069	0.269

O.A= Overall acceptability

### 4.1.6.3. Crispness

Crispiness is one of the textural properties of a food product in which attributes of a food material resulting from combination of physical and chemical properties, perceived largely by the sense of touch, sight and hearing is also one of the most important quality parameters of a food product was also studied. As it can be seen from the result that the crispiness of the cookies was found significantly differences (P  $\leq 0.05$ ) was observed in red teff with okara while nonsignificance difference was observed for other interaction Both of quadratic and linear models were observed non-significance as judged by untrained panelist among all tested samples in terms of crispness. The result of cookies crispness in the present study was varied from 2.8-4.8. The highest value was obtained from 35% red teff, 15% wheat and 50% okara blend while the lowest result was obtained from 40% of red teff, 20% of wheat and 40% okara. Even though the blend proportion did not significantly affect the crispiness of the cookies, the cookie which was made from hundred percent wheat score the highest and a decreased in crispiness was observed as the proportion of quality protein okara and red teff flour increases. The contour plot in figure below indicates that as the proportion of okara flour increase the crispness of cookies increase. The regression model for crispness over all acceptability was presented below equations from 34 (where A= Red teff, B=Wheat, C=okara).



*Crispness*= 61.5A-3.2B+71.0C+0.9AB-246.1AC-120.288BC...... (Equation34).

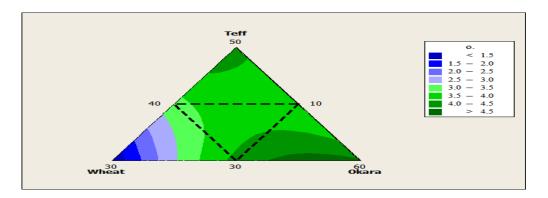
Figure 25. Contour plot of displaying crispness of cookies.

### 4.1.6.4 Overall acceptability

The final sensory analysis conducted by the panelist was the overall acceptability of the cookies. The overall acceptability of cookies showed non significant difference (P<0.05) in the blended flour of red teff, wheat and okara and both of linear and quadratic models. All of the result

implies that the evaluated sensory properties of developed cookies are not much different from the 100% wheat (control) cookies. All of the cookies made from both the 100% wheat and the mixed flours were generally well accepted.

The regression model for over all acceptability was presented below equations from35 where A= Red teff, B=Wheat, C=okara.



*O*.*A* =30.00*A*-87.83*B*+15.96*C*+65.95*AB*-85.26*AC*+135.54*BC*..... (*Equation 35*)

Figure 26. Contour plot of displaying Overall acceptance of cookies.

## 4.2. Region of optimum formulation

## I. Optimization based on proximate composition

The white area of Figure 26 indicates the optimum point of formulation to develop composite Cookies with good chemical composition which can serve the expected purpose. Thus the sweet point which includes all the optimum points of fat %, fiber %, protein %, carbohydrate %, gross energy Kcal/100gm, Iron mg/100gm, calcium mg/100gm, and zinc mg/100gm. The optimum point was found in the cookies samples prepared within the range of red teff 33-38%, Wheat 18-20% and 45-47 Okara%. From the optimal value it can be seen that the amount of Okara can be used from the lowest value to the maximum without affecting the nutritional content whereas the optimum nutritional content was found at the maximum amount of okara. Superimposition of contour plot regions of interestconsist of protein (15.4-18.56%), fat(14-18.2%), Fiber( 7.4-8.9%), Carbohydrate (45.11-57.45%), Energy (406.8-416.2kcal/100gm), iron ( 6.2-7.3mg/100g), Zinc (2.66-2.88 mg/100g,) Calcium(16.6-18 mg/100g).

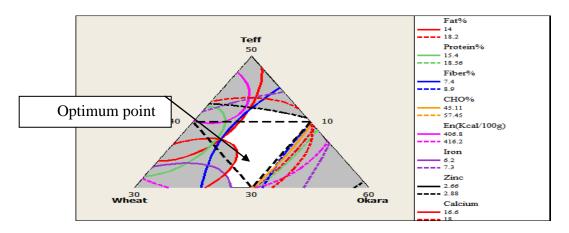


Figure 27. Overlaid contour plot of optimum proximate composition

## **II.** Optimization based on sensory evaluation

The optimum formation should maximize consumer acceptance, it is impossible to develop a product with all five sensory qualities that would satisfy consumers in most applications (Moskowitz, 1994). Since, acceptability of the product by consumers is an essential parameter. To obtain the optimum region the ingredient formulation that would obtain optimum color, aroma, taste, Crispness and overall acceptability for Cookies. The optimum point for all sensory attributes was graphically presented. The white region of Figure 28 shows the optimum sensory acceptability of the formulation. The Point prediction shows that 37-40% red teff, 16-20% wheat and 47-50 % okara. The optimal point for color, aroma, taste, crispness and overall acceptability were found to be ranged from 3-4.5, 3.28-3.89, 3.25-4.8, 3.5-4.2 and the 3.32-4.55 respectively.

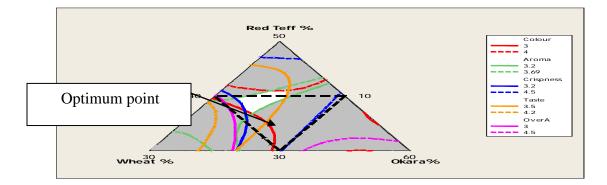


Figure 28. Overlaid contour plot of optimum sensory quality

## III. Optimization based on physical properties of cookies

The white region in these figures 29 indicates that any point with in this region represents an optimum combination of red teff, wheat, and okara, which results in desirable physical attributes. The optimum point prediction shows that 35-40% red teff; 18-20% wheat and 40-50% okara. The optimum point for texture, Diameter, Thickness, and spread ratio was varied between 66.6-71%, 6.4-6.9%, 3.2-3.7., 18.57-20.67 respectively with respect to physical properties of cookies samples.

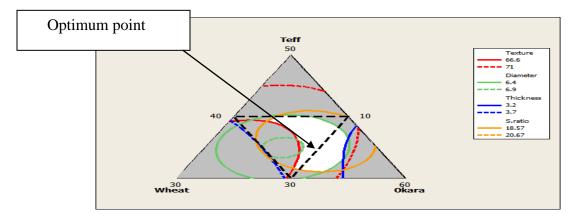


Figure 29.0verlaid contour plot of Texture, Diameter, Thickness and Spread ratio of cookies

## IV. Optimization based on Functional properties of cookies

The sweet point which includes both the optimum points of funcitional properties (bulk density from 0.66-0.75 and water absorbtion capacity from 133-140) is indicated in Figure 19. The sweet point was found in the cookies samples prepared within the range of red teff from 30- 40, wheat 17-20%, 43-50 % (figure 30). From the optimal value it can be seen that the amount of wheat can be used from the lowest value to the maximum without affecting the functional properties where as the optimum nutritional content was found at the maximum amount of okara.

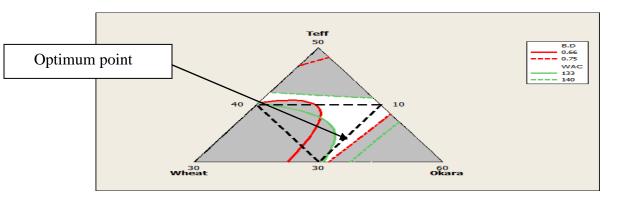
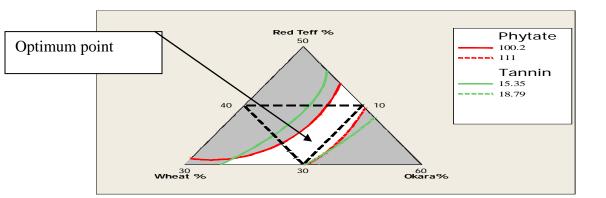
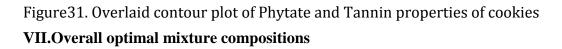


Figure30. Overlaid contour plot of Bulk density and Water Absorvable capacity of cookies **VI.Optimum point based on anti nutritional properties of cookies** 

The sweet point which includes both the optimum points of Anti-nutritional factors (Phytate from 100.2-111and Tannin from15.35-18.79) is indicated in Figure 30. The sweet point was found in the cookies samples prepared within the range of red teff from the lowet result was present on 30% red teff, wheat 20%, and okara 45% the highest result was found on red teff 40% 10% wheat and 50 % okara. From the optimal value it can be seen that the amount of wheat can be used from the lowest value to the maximum without affecting the anti-nutritional factors where as the optimum nutritional content was found at the maximum amount of okara.





The study focused on determining the optimal formulation ratio of individual food source that are suited to produce cookies with desirable nutrient compositions. In order to determine the optimum formulation, the regions of acceptability in the contour plot for protein, fat, gross

energy, iron, calcium, zinc ,Water absorption capacity,breaking strength,phytate andoverall acceptability of sensory attribute were super imposed.

Super imposition of contour plot regions of interest over all (protein 15.4-18.56%, fat14.8-18.2%, energy 409.88-416.2 kcal/100gm, iron6.66-7.3mg/100g,calcium16.6-18mg/100gm, zinc 2.66-2.88 and phytate 100.2-111, breaking strength 66.6-71,wac 133-140,overall acceptance received hedonic ratings 3.32-4.55 resulted in optimum regions for cookies (Figure 32). The white region in these figures 32 indicates that any point with in this region represents an optimum combination overall optimum values were found in a range of red teff 35-40%, Wheat15-20% and 45-50% Okara.

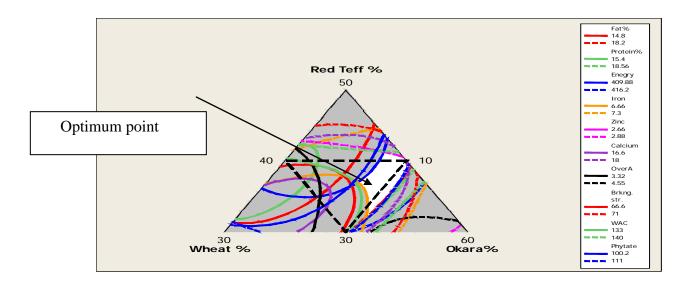


Figure 32. Overlaid contour plot of fat, protein, energy, iron, calcium, zinc, and over all acceptability, breaking strength, water absorvable capacity, and phytate of cookies

The results also indicated that there may be a possibility to make a product which contains optimal levels of iron sufficient for combating iron deficiency problem and enriched protein product for malnutrition problem. Iron content was associated with increased proportion of red teff flour in the formulation and also increased protein and energy by increase okara on the formulation. Hence, accessing of cookies enriched with red teff and okara provides iron and protein can play a key role in developing countries for sustainable long term food-based strategy to control iron deficiency and protein malnutrition patient.

## **5. SUMMARY AND CONCLUSION**

The current study was conducted to evaluate the possibility of using okara to produce cookies as a means of value addition to this by prodcuts and minimize its impact as a waste to the environment.

The study showed that blending, of okara powder had significant effect on nutritional, antinutritional, mineral, physical, functional and sensory property of the cookies. Thus, new aspects concerning the use of this by-product for further exploitation to utilize for human food with high nutritional is good alternative potential and resulted nutritionally attractive and having good sensory acceptability. This is very important, especially in developing countries where many people struggle to get high protein contained foods because of their expensive costs.

The result revealed that, the protein, fat, fiber and mineral contents were significantly improved with increasing the proportion of okara (40-50%) flour in the formulation. The phytate and tannin contents in the cookies were increased as the levels of okara and red teff flour increases from 30-40 and 40- 50% respectively. The sensory acceptability score of the developed cookies were got lower values compared with wheat cookies in terms of color, aroma, and taste, but it was better interms of crispness and overall acceptability than control. Optimization was done with the objectives of getting maximum nutritional contents and minimum anti-nutritional values. According the optimum values were in a range of red teff 35-40%, Wheat15-20% and 45-50% Okara. Thus, enriching cookies with okara using this optimum range can inrich nutritional contents of the products instead of being wasted hence enriched cookies with okara substitutions were found to be nutritionally superior than the whole-wheat cookies.

# **6. RECOMMENDATION**

- Okara utilization of based products with increasing extensive development prospects need further research and summarization
- > More investigation should be done on other quality parameters of value added cookies
- To improve the color and beany flavor of cookies made from okara different coloring and flavoring agents should be added during production of enriched cookies
- Further research work should be focused on the shelf life stability, microbial and optimization of the enriched cookies by considering the daily allowance cookies nutrient content for different ages like childrens, and adults.

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**8. APPENDICES** 

Terms	Mo		protein		Fat		Fiber		Ash		СНО		G. ENE	RGY
	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE
Teff	4.1	14.177	100.7	31.48	73.30	41.52	16.7	52.93	32.5	0.2900	-127.4	80.49	552.1	178.5
Wheat	45.4 27.9	23.234	74.4	51.59	124.0	68.04	20.5	91.45	11.1	0.5011	-172.2	131.91	804.7	292.6
Okara		9.691	126.1	21.52	134.0	28.38	58.4	37.28	25.6	0.2043	-272.8	55.05	607.0	122.0
T*W	-36.0	40.785	-112.7	90.56	-146.1	119.44	45.1	156.54	-67.8	0.8578	310.5	231.55	-664.5	513.6
T*O	-29.3	41.714	-374.4	92.62	-333.8	122.16	-117.2	156.41	-101.8	0.8571	958.7	236.82	-628.5	525.3
W*O	-101.0	44.745	-268.7	99.35	-369.7	131.04	-148.6	178.36	-30.9	0.9773	917.4	254.03	-799.9	563.5
$\mathbb{R}^2$	96.28		96.19		97.17		90.13		95.64		98.27		83	.82

Appendix Table 1. Estimated regression coefficients of proximate compositions of mixed product.

RC= Regression coefficient

SE= Standard error  $R^2$ = Determination coefficient

	Phytate		Tannin		Bulk Density		Water absorption	
Terms	RC	SE	RC	SE	RC	SE	RC	SE
Teff	172	213	81.9	44.36	0.049	0.891	987	302
wheat	663	350	42.5	72.7	1.474	1.46	-578	495
Okara	439	146	110	30.32	2.664	0.609	629	206
Teff*wheat	-812	614	-119	127.6	4.488	2.562	-95	868
Teff*Okara	-722	628	-309	130.5	-2.291	2.62	-2639	888
wheat*Okara	-1369	673	-160	140	-7.011	2.811	386	953
R 2	96.41		96.35		93.18		78.42	

**AppendixTable 2** Estimated regression coefficient of anti –nutritional factors and functional properties

RC= Regression coefficient SE= Standard error

 $R^2$ = Determination coefficient

AppendixTable 3. Estimated coefficient of physical properties

Terms	B.S		Diam.		Tickn.		S.ra	S.ra	
	RC	SE	RC	SE	RC	SE	RC	SE	
Teff	297.8	116.90	-81.82	29.75	0.78	4.321	-226.5	73.16	
Wheat	180.0	191.57	-77.69	48.75	30.96	7.081	-326.2	119.90	
Okara	257.9	79.9	-41.35	20.34	-3.10	2.954	-101.2	50.01	
Teff*Wheat	-300.6	336.29	221.93	85.59	-43.97	12.430	785.6	210.48	
Teff*okara	-791.6	343.95	250.94	87.53	19.6	12.713	670.9	215.27	
Wheat*Okara-	-506.3	368.94	162.51	93.90	-15.33	13.637	535.0	230.92	
$R^2$	75.73		70.94		95.91		79.50		

B.S=Breaking strength RC= Regression coefficient SE= Standard error

 $R^2$ = Determination coefficient

Terms	Aroma		Colour		Taste		Crispness		O.A	
	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE
Teff	32.2	45.29	81.1	99.43	-39.10	17.11	61.5	35.32	30.00	59.23
Wheat	0.40	74.22	-145.7	162.94	14.55	28.04	-3.2	57.88	-87.83	97.07
Okara	-45.3	30.96	26.7	67.96	-47.3	11.70	71.0	24.14	15.96	40.49
Teff*Wheat	-156.8	130.28	54.0	286.03	12.77	49.23	0.9	101.61	65.95	170.41
Teff*Okara	46.5	133.25	-206.1	292.54	183.345	50.35	-246.1	103.92	-85.26	174.29
Wheat*Okara	175.7	142.93	223.0	313.80	67.38	54.01	-120.288	111.47	135.54	186.95
$R^2$	81.58		13.41		95.31		77.77		26.97	

Appendix Table4 Estimated regression coefficients of sensory evaluation

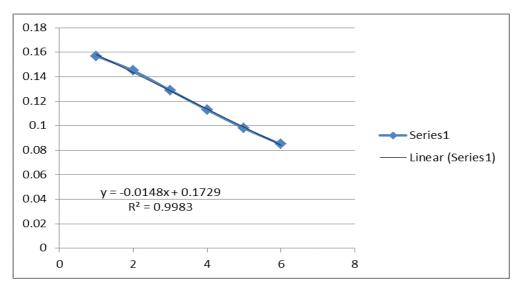
RC= Regression coefficient SE= Standard error

 $R^2$ = Determination coefficient

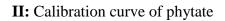
Appendix Table5 Estimated regression coefficients of mineral evaluation

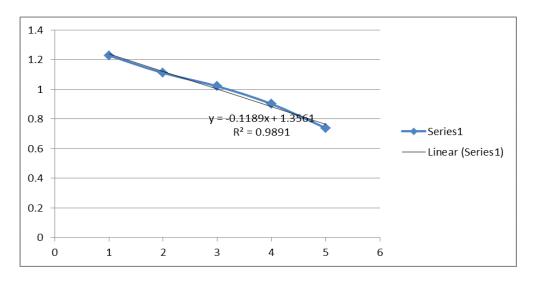
Terms	Iron		Zinc		Calcium		
	RE	SE	RE	SE	RE	SE	
Teff	50.4	25.66	15.70	3.665	118.6	29.29	
Wheat	8.9	42.06	10.01	6.006	60.4	48.00	
Okara	45.1	17.54	7.35	2.505	95.4	20.02	
Teff*Wheat	-2.3	73.83	-34.15	10.543	-139.6	84.27	
Teff*Okara	-160.1	75.51	-31.44	10.783	-342.6	86.27	
Wheat*Okara	-88.6	81.00	-8.73	11.567	-181.3	86.18	
R2	68.29		81.62		80.93		

## I: Calibration curve of tannin



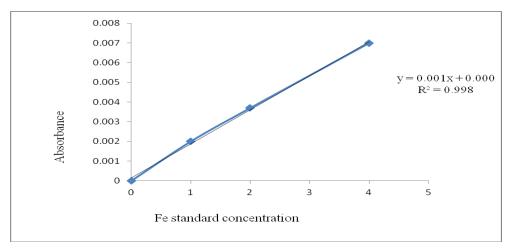
Y= Concentration for tannin ( $\mu g/g$ )





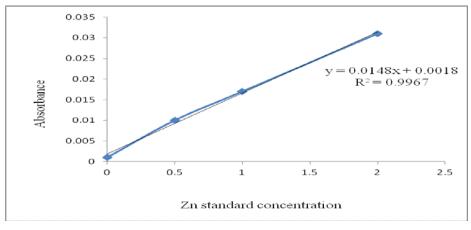
Y= Concentration for phytate ( $\mu g/g$ )

# **III** Iron calibration curve



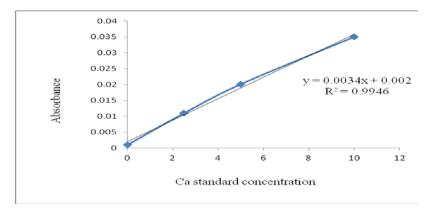
Y=Concentration for iron (mg/100gm)

#### IV Zinccalibration curve



Y= Concentration for Zinc (mg/100gm)

## V Calcium calibration curve



Y=Concentration for calcium (mg/100gm)

## VI Sensory evaluation form

Please look at and taste each sample of cookies in order from left to right 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. Dislike Very Much
- 2. Dislike
- 3. Neither Like or Dislike
- 4. Like
- 5. Like Very Much

Sample code	Colour	Aroma	Taste	Crispness	O.Acceptability