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ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL SCIENCE
CENTER FOR FOOD SCIENCE AND NUTRITION

Effect of Composite Flour Blend of Teff, Sorghum and Maize on Macro Nutritional Quality, Sensory Acceptability and pH Fermentation Kinetics in Preparation of *Injera*

By

Solomon Legesse

A thesis submitted to center for Food Science and Nutrition Program in partial fulfillment of the requirement for the degree of masters of Science in Food Science and Nutrition

Advisors:

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June, 2015

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Dedication

This research work is dedicated to:

My wife Hiwot Shenkoru

And

My grandmother Askale Kidane (Akalye), My father Legesse W/gyorgis (Legessye) and My sister Bezuayehu Legesse (Buzeye)..... You were the best family in the world before you go to your father (Almighty God).

TABLE OF CONTENTS

Content	Page
Acknowledgement	i
Dedication	ii
Table of contents	iii
List of tables	vi
List of figures	vii
Abbreviations	viii
Abstract	ix
1. Introduction	1
1.1. Back ground of the study	1
1.2. Statement of the problem	3
1.3. General objective	4
1.4. Specific objectives	4
2. Literature review	5
2.1. Fermentation and fermentation kinetics	5
2.2. Cereals commonly used in fermented food preparation	7
2.2.1. Teff	7
2.2.2. Sorghum	8
2.2.3. Maize	9
2.3. Foods based on fermented cereals	10
2.4. Food formulation based on cereals blend	12
2.5. The use of design expert in food formulation optimization	13
3. Materials and method	15
3.1. Chemicals used	15

3.2. Apparatuses and instruments used	15
3.3. Sample collection	15
3.4. Study site	15
3.5. Conceptual frame work of the study	16
3.6. Sample preparation	20
3.7. Preparation of raw flour	21
3.8. Estimation of the dry weight of <i>Injera</i>	21
3.9. Preparation of flour blend	21
3.10. Preparation of fermentation starter (<i>Ersho</i>)	21
3.11. Dough preparation	22
3.12. <i>Injera</i> baking process	23
3.13. Sun drying and grinding of dried <i>Injera</i>	23
3.14. An overall diagrammatic display of <i>Injera</i> preparation	24
3.15. Analytical experimental procedures	25
3.15.1. Determination of crude protein	25
3.15.2. Determination of crude fat	26
3.15.3. Determination of total ash	26
3.15.4. Determination of moisture	27
3.15.5. Determination of total carbohydrate content	27
3.15.6. Determination of energy	27
3.15.7. Determination of crude fiber	28
3.16. Data entry and analysis	29
4. Results and Discussion	30
4.1. Raw material proximate composition	30
4.2. Determination of dry weight of <i>Injera</i>	32
4.3. Proximate composition of the 14 experimental trials	32
4.4. Sensory acceptability of the experimental trials	35
4.5. Formulations optimized	39
4.5.1. Proximate composition of the optimized formulations	42
4.5.2. Sensory acceptability of the optimized formulations	44
4.5.3. Number of eyes of <i>Injera</i> 's of the optimized formulations including control	49

4.5.4. Fermentation pH kinetics of the optimized formulations	49
5. Conclusion and Recommendation	52
5.1. Conclusion	52
5.2. Recommendation	53
6. References	54
7. Appendix	62
7.1. Sensory evaluation score sheet and questionnaire	62
7.2. Panelists photograph while conducting sensory analysis	63
7.3. Picture of <i>Injera</i> prepared based on optimized formulation F1	64
7.4. Picture of <i>Injera</i> prepared based on optimized formulation F2	65
7.5. Picture of <i>Injera</i> prepared based on optimized formulation F3	66
7.6. Picture of <i>Injera</i> prepared based on optimized formulation F4	67
7.7. Picture of teff grain with Varsity of Kuncho (DZ-Cr-387)	68
7.8. Picture of sorghum grain with Varsity of Gambella (1107)	69
7.9. Picture of maize grain with Varsity of (M-6Q)	70
7.10. Declaration	71

List of tables

Table		Page
Table 1	Metabolites of lactic acid bacteria which may be inhibitory to other pathogenic and food spoilage organisms	6
Table 2	Macro, amino acid and fatty acid composition of teff grain compared to maize, sorghum, wheat and rice.....	12
Table 3	Simplex lattice coded design for <i>Injera</i> preparation	16
Table 4	Design constraints	17
Table 5	Proximate composition of raw ingredients result	30
Table 6	Dry <i>Injera</i> weight	32
Table 7	Proximate composition of the 14 experimental trials	33
Table 8	Sensory acceptability of the experimental trials	36
Table 9	Mean number of eyes in 1x1 inch square of <i>Injera</i> 's of the 14 experimental trials	38
Table 10	Proximate composition of the optimized formulations	42
Table 11	Mean sensory acceptability of the optimized formulations due to variation of formulation	45
Table 12	Mean sensory acceptability of the optimized formulations due to variation of days	45
Table 13	Mean sensory acceptability of the optimized formulations due to the interaction of the main effects	46
Table 14	Mean number of eyes in 1x1 inch square of <i>Injera</i> 's of the optimized formulations.....	49
Table 15	The mean maximum rate of change of pH per hour of the optimized formulations and the control fermenting dough	51

List of figures

Figure		Page
Figure 1	The diffusion of a weak organic acid into a microbial cell, and its dissociation yielding protons (H ⁺) and potentially toxic anions (A ⁻) (Adams, 1990).....	6
Figure 2	Conceptual frame work of raw material preparation, proximate and sensory acceptability determination	17
Figure 3	Photograph of <i>Injera</i> taken at a distance and used for determination of number of eyes of <i>Injera</i> samples	20
Figure 4	Picture indicating the process of dough preparation	22
Figure 5	Picture indicating the process of <i>Injera</i> baking preparation	23
Figure 6	Picture indicating the process of sun drying and grinding of <i>Injera</i> for further experimental analysis.....	23
Figure 7	Diagrammatic description of the process of <i>Injera</i> baking followed	24
Figure 8	Contour plot for formulation two (F2)	40
Figure 9	Contour plot for formulation three (F3)	41
Figure 10	Contour plot for formulation four (F4)	42
Figure 11	Graphical display of change of pH of fermenting dough over time	50

List of abbreviations

AOAC	Association of official Analytical chemists
ANOVA	Analysis of variance
BSTID NRC	Board on Science and Technology for International Development-National Research Council
CHO	Carbohydrate
CSA	Central Statistics Agency
DZARI	Debrezeit Agricultural Research Institute
EPHI	Ethiopian public health institute
FAO	Food and Agriculture Organization
ICRISAT	International Crops Research Institute for the Semi Arid Tropics
SD	Standard deviation
SPSS	Statistical product and Service solutions
USDA	United States Department of Agriculture
USDA/FAS	United States Department of Agriculture and Foreign Agricultural Service
CHO	Carbohydrate

Abstract

The study attempted to investigate the effect of composite teff flour blend with sorghum and maize through optimization of formulation based on simplex lattice mixture design expert soft ware, on macro-nutritional composition, sensory acceptability and pH fermentation kinetics while looking for opportunities of preparing Injera with an affordable cost from such composites. The study was based on 14 experimental trials including four replication points on the contour triangle plot, three vertex points ([100% teff], [50% teff: 50% sorghum] & [50% teff: 50% maize]) and one point on the center edge (75% teff: 25% maize) which were generated from the soft ware with primarily defined constraint. Proximate and sensory acceptability determinations were carried out on the experimental trials resulting in content of protein (11.27-14.73)%, fat (1.79-3.59)%, crude fiber (5.65-9.57), total carbohydrate (6.97-19.82)%, total energy value (110.36-157.60)Kcal/100g and sensory acceptability scores from liked to extremely liked (color[4-5], taste[3-4], texture[3-4], odor[3-4], appearance[3-4] and over all acceptability[3-4]) based on 5-point hedonic scale which these response results were used for formulation optimization to finally came up with three optimized formulations; (formulation one [F1-control; 100% teff], formulation two [F2; 55.40% teff: 37.20% sorghum: 7.30% maize], formulation three [F3; 50% teff: 30.70% sorghum:19.30% maize], and formulation four [F4; 66.6% teff:19.80% sorghum:13.60% maize]) based on objective criterion. Further determination of proximate, sensory acceptability and pH fermentation kinetics were performed on the three optimized formulations and the control to result in content of (protein [11.34-16]%, fat [1.79-3.01]%, crude fiber [6.00-8.66]%, total carbohydrate [10.33-18.02]% & total energy value [132.39-162.37]Kcal/100g), sensory acceptability scoring from liked to very liked (color[3-4], taste[3-4], texture[3-4], odor[3-4], appearance[3-4] & over all acceptability[3-4]) within three days of preparation of Injera for all the optimized formulations plus the control, and maximum rate of change of pH per hour of (-0.011639 up to -0.040486) within 72 hours of fermentation. Composite teff flour blend with sorghum & maize had brought effect in different manner with regard to macro nutritional composition and sensory acceptability. With regard to pH fermentation kinetics, the fermentation rate of the three optimized formulations (F2,F3 & F4) was faster as compared to the control (F1) that the pH of the optimized formulations reached at a pH value of (3.901-3.906) within (22-26)hrs of fermentation while the control F1 reached at a pH value of 3.903 after 72 hours of fermentation; thus, by limiting the fermentation time of the optimized formulations from 72 hours to one & half days it could be possible to score comparable sensory acceptability responses with that of the control. Therefore, it was found out that F2 is preferable and healthy formulation especially for people whose life style is sedentary and not energy demanding while F3 and F4 are the preferred formulations for people who demand energy in any case.

Key words: *Formulation optimization, design expert, pH fermentation kinetics, composite flour blend, Injera.*

3. Introduction

3.1. Back ground of the study

Most developing countries rely largely on cereals as staple. Major cereals grown in Ethiopia include teff, sorghum, maize, wheat, and barley, but the consumption and preference of these cereals vary throughout the country among rural and urban societies due to different factors (Bart *et al.*, 2012). Cereal based foods are eaten either in fermented or unfermented form depending on the habit or tradition of a given community. In Ethiopia, the most widely consumed fermented food by young children and adults alike is *Injera*, which is a thin, flat, traditional fermented pancake. However, depending on the agro-ecology of the area concerned (highlands versus lowlands), different cereal blends are used to make *Injera*, (Baye *et al.*, 2012). Cereal grains constitute a major source of dietary nutrients all over the world; although, cereals are deficient in some basic components (e.g. essential amino-acids), fermentation may be the most simple and economical way of improving their nutritional value, sensory properties, and functional qualities (Blandino *et al.*, 2002).

Teff, *Eragrostis teff*, is believed to have originated in Ethiopia (Vavilov, 1951). Currently, teff is cultivated as a forage crop in countries like Australia, India, Kenya and the Republic of South Africa (Costanza *et al.*, 1979) with limited human consumption in the USA. The crop is the second most widely produced and consumed cereal in Ethiopia next to maize (CSA, 2010/11). Teff has remained an important crop to Ethiopian farmers for several reasons, namely: the price for its grain and straw are higher than other major cereals; the crop performs better than other cereals under moisture stress and waterlogged conditions; its grain can be stored for a long period of time without being attacked by weevils; there is no disease epidemic that has threatened its performance (Ketema, 1989).

Teff flour is used to prepare *Injera*, while the straw provides a nutritious feed for cattle or can be used as a house plastering material. According to CSA (2010/11) data, over the past few years cultivation of teff ranked first in terms of area coverage (accounting for 28% of the area) and is

second to maize in terms of volume of production among cereals, accounting for about 20% of the total produce in the category.

A report by Bekabil *et al.*, (2011) shows that 16 out of the 20 released improved varieties of teff were developed by Debre Zeit Agricultural Research Center (DZARC). According to the same source, previously released varieties have not been widely accepted by farmers because of their color, despite higher yield levels. However, because of its acceptable color and yield, the recently released Quncho (DZ-Cr-387) variety has become popular particularly around the Ada'a area, which is one of the known teff growing localities in the country.

Teff is the one of the high-priced cereals grown in Ethiopia and following the prevalence of high food price inflation in the country in 2008, the price of teff has also experienced a huge increase in recent years (Bekabil *et al.*, 2011). White teff, for instance, on average has increased by 200% from 2005 to 2010. The price of teff took off, starting in May and June of 2008 when wheat and maize prices also peaked. It has increased from Birr 593 per quintal in April to Birr 810 per quintal in May and then to Birr 931 per quintal in June of the same year. Since then, it has become a grain that fetches a high price per quintal, albeit less per hectare (on account of its inferior yields) than wheat or chickpeas. Currently the price of one quintal of teff is estimated between birr xxx and yyy depending on the variety of the grain and harvest season of the year (CSA, 2010/11). Similar price increases were observed for mixed and red teff as well (Bekabil *et al.*, 2011).

In Ethiopia *Injera* is made from teff flour whose preparation consists of two stages of natural fermentation that lasts for about 1 to 3 days depending on fermentation temperatures. The only required ingredients to prepare *Injera* are teff flour and water (Zewdu, 2012). The method of processing of *Injera* from its raw materials to the final product involves preparing and mixing the ingredients to dough, which is fermented and subsequently thinned to a batter. The batter is then baked by pouring onto a hot griddle in a thin layer to develop a characteristic color, flavor and texture.

3.2. Statement of the problem

Teff grain does not contain gluten and is an increasingly important dietary component for individuals who suffer from gluten intolerance (Spaenij-Dekking *et al.*, 2005). Despite its nutritional and health benefit teff has relatively high concentration of phytic acid, an anti nutritional factor, which can compromise the bioavailability of vitamins and minerals. Furthermore, due to the high and increasing cost of teff *injera* made from teff alone is limited to few segment of the society in the country. Therefore, teff is often blended with cheaper cereals to make *Injera*. Such blends have organoleptic and nutritional implications which is not an easy task to achieve though. For instance, Yetneberk (2004) reported that by compositing sorghum flour with teff flour, it was possible to improve the sensory acceptability of *Injera* made from sorghum-teff blend. Baye *et al.*, (2012) have also showed that different flour blends affect the fermentation kinetics and nutritional quality of *Injera*. More recently,, teff-wheat blend *Injera* enabled higher phytate degradation than teff alone (Maren *et al.*, 2014).

Even if teff has commonly been used by most Ethiopians to prepare *Injera*, other cereals such as sorghum, millet, barley and maize are also utilized for such purpose especially in the rural communities. In terms of sensory acceptability, nutritional composition, and health benefits *Injera* made from teff alone is the most preferred. But due to the high price of teff that most Ethiopians can't afford, nowadays most households and small *Injera* shops blend teff with other cereals like sorghum and maize to make *Injera*. However, it was not scientifically well studied and the impact of such blending on the macro-nutritional quality, sensory acceptability, and fermentation kinetics is poorly understood. Moreover, studies involving multiple cereal blends for making *Injera* are scarce. Therefore the present study was conducted to look for opportunities of preparing high quality *Injera* by compositing teff flour with sorghum and maize flours through optimization of formulation based on 'mixture design expert' method. The design was constrained as the ratio of teff should be at a lower limit of 50% and upper limit of 100% purposely, in-order not to compromise the nutritional and health benefit of teff significantly while the design constraint for those of sorghum and maize was at a lower limit of 0.00% and upper limit of 50%.

3.3. General objective

To evaluate the effect of composite teff flour blended with sorghum and maize flours on fermentation pH kinetics, sensory acceptability, and macro-nutritional quality of *Injera* from the blend.

3.4. Specific objectives

- To investigate the recommended composite flour recipe in terms of sensory acceptability.
- To investigate the recommended composite flour recipe in terms of macro-nutrient composition.
- To investigate the recommended composite flour recipe in terms of macro-nutrient composition and sensory acceptability.
- To investigate the effect of flour blend on fermentation pH kinetics of the optimized recipes for the preparation of *Injera*.

4. Literature review

7.4. Fermentation and fermentation kinetics

According to WHO food safety unit, fermentation is considered as a technique for preparation/storage of food. One main reason for this is that in developing countries, one tenth of the children under five years of age dies due to dehydration (Peter, 1999). The dehydration is mainly caused by incidences of diarrhea. The main cause for getting diarrhea is the ingestion of food not having the appropriate standard regarding the hygienic condition. The hygienic standard of a food is based on the processing and handling of the food, as well as on the conditions of the raw materials. A food item prepared from water contaminated with pathogenic microorganisms will successively be contaminated, and a health risk. Lactic acid fermentation of food has been found to reduce the risk of having pathogenic microorganisms grow in the food.

Fermentation due to micro-organisms such as lactic acid bacteria has a significant effect on the growth of pathogenic micro-organisms; Adams (1990) suggested that lactic acid bacteria are inhibitory to many other microorganisms when they are cultured together and this is the basis of the extended shelf life and improved microbiological safety of lactic-fermented foods. The microorganisms involved in fermentation of the Ethiopian pan cake, *Injera*, are mainly yeasts, some fungi species including *Pullaria*, *Aspergillus*, *Penicillium*, *Rhodotorula*, *Hormodendrum*, *Candida* and a number of unidentified bacteria (Ashenafi, 1993 and Steinkraus, 1983). In addition to this lactobacillus species can produce a variety of metabolites, such as lactic and acetic acids which lower the pH of the growth environment, that are inhibitory to competing bacteria including psychrotrophic pathogens (Breidt and Fleming, 1997). This effect could be due to a combination of many factors as shown below in Table 1.

Table 1: Metabolites of lactic acid bacteria which may be inhibitory to other pathogenic and food spoilage organisms

Product	Main target organisms
Organic acid	
Lactic acid	Putrefactive and gram negative bacteria, some fungi
Acetic acid	Putrefactive bacteria, clostridia, some yeasts and some fungi
Hydrogen peroxide	
	Pathogens and spoilage organisms, especially in protein rich foods
Enzymes	
Lactoperoxidase system with hydrogen peroxide	Pathogens and spoilage bacteria (milk and dairy products)
Lysozyme (by recombinant DNA)	Undesired gram-positive bacteria
Low molecular weight metabolites	
Reuterin	Wide spectrum of bacteria, yeasts and molds
Diacetyl	Gram negative bacteria
Fatty acids	Different bacteria
Bacteriocins	
Nisin	Some LAB and gram-positive bacteria, notably endo-spore formers
Other	Gram positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

Source: (Breidt and Fleming., 1997).

The inhibition by organic acids has been attributed to the protonated form of these acids, which are uncharged and may therefore cross biological membranes (Figure 1). The resulting inhibition of growth may be due to acidification of the cytoplasm and/or accumulation of anions inside the cell (Adams, 1990; Russel, 1992; Breidt and Fleming, 1997).

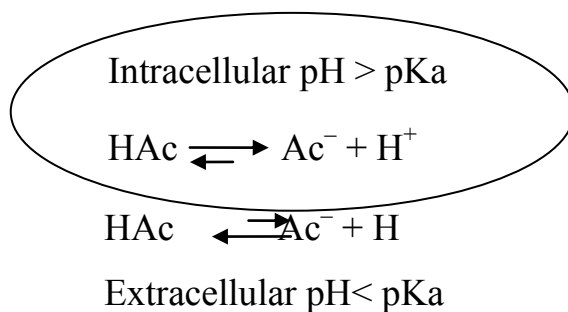


Figure1: The diffusion of a weak organic acid into a microbial cell, and its dissociation yielding protons (H+) and potentially toxic anions (A-) (Adams, 1990).

Fermentation can activate several endogenous enzymes including phytases and may thus result in products with reduced anti-nutritional factors (Greiner *et al.*, 2006). The extent to which enzymes like phytases are activated depends on the fermentation kinetics, which in turn, depends on the raw materials used (Hammes *et al.*, 2005). Thus fermentation has beneficial effect in improving the nutritional and sanitary quality of food (Nout, 2009 and Svanberg *et al.*, 1997).

The effect of fermentation on the protein and amino acids levels is a topic of controversy. For example, during the fermentation of corn meal the concentrations of available lysine, methionine, and tryptophan increase (Nanson *et al.*, 1984). In the same way, fermentation significantly improves the protein quality as well as the level of lysine in maize, millet, sorghum, and other cereals (Hamad *et al.*, 1979). On the contrary, investigations of the nutritive value of sorghum kiswa bread showed no increase in the lysine content, although tyrosine and methionine levels did increase (McKay *et al.*, 1990). In the same line, it has been reported that the tryptophan content increases during Uji manufacture while a significant drop in lysine content was measured (McKay *et al.*, 1990). It appears that the effect of fermentation on the nutritive value of foods is variable, although the evidence for improvements is substantial. With regard to carbohydrate, natural fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non digestible poly and oligosaccharides (Blandino *et al.*, 2002).

7.5. Cereals commonly used in fermented food preparation

7.5.1. Teff

Teff is currently the most expensive grain to purchase in Ethiopia as it requires labor-intensive harvesting and processing techniques, and produces especially low yields, thus its availability is limited by high cost (Doris *et al.*, 2010). Although teff covers the greatest space of arable land in Ethiopia, it has the lowest yield per hectare of an average of 910kg.. During the 1996-1997 farming season, teff covered 31% of the total landmass, as compared to 17% and 13% for corn and wheat respectively. The total yield for the teff grown in that year was only 26-28% (Doris *et al.*, 2010).

The chemical composition of teff is not far from those of other cereals, nonetheless the micro- and macronutrients level of grain teff is apparently higher than that of barley, wheat and sorghum (Bultosa *et al.*, 2002) and therefore the nutrient composition of grain teff indicates that it has good potential to be used in foods and beverages worldwide. The amino acid composition of grain teff is reported to be comparable to that of egg protein, except for its lower lysine content (Bultosa, 2007). Teff flour has high water absorption capacity which is related to the higher degree of swelling of the teff starches, that have a small and uniform granule size, hence, providing larger surface area and thus higher water absorption (Bultosa *et al.*, 2002 and Bultosa, 2007). Teff starch has slow retrogradation tendency (Bultosa *et al.*, 2002), hence, it could have a potentially positive impact on shelf life of baked products. The crude fat of grain teff is around 2%, lower than that of grain maize which is around 4.45%. The typical composition of Protein, fat, ash and carbohydrate contents of teff grain are given as 9.6%, 2.0%, 2.9% and 73.0%, respectively (Patricia and Lisette, 2008).

Teff is a very small grain in which the flour consists of the bran and the germ making teff flour high in nutrient value, as the bran and germ are the most nutritious parts of any grain (Patricia and Lisette, 2008). Teff has very high calcium content, and also contains high levels of phosphorous, iron, copper, aluminum, barium, and thiamin (Patricia and Lisette, 2008). It is considered that teff has an excellent amino acid composition, with lysine levels higher than wheat or barley and slightly less than rice or oats (Patricia and Lisette, 2008). Teff is high in protein, carbohydrates, and fiber. The protein composition offers an excellent balance among the essential amino acids (Yu, 2006). It contains no gluten so it is appropriate for people with gluten intolerance (Patricia and Lisette, 2008).

7.5.2. Sorghum

It is believed that sorghum originated in Africa, more precisely in Ethiopia, between 5000 and 7000 years ago (ICRISAT, 2005). From there, it was distributed along the trade and shipping routes around the African continent, and through the Middle East to India at least 3000 years ago. It then journeyed along the Silk Route into China. Sorghum was first taken to North America in the 1700-1800's through the slave trade from West Africa. It was re-introduced in

Africa in the late 19th century for commercial cultivation and spread to South America and Australia. Sorghum is now widely found in the dry areas of Africa, Asia (India and China), the Americas and Australia (ICRISAT, 2009).

According to FAO STAT (2008) among the major staple foods based on cassava, maize, yam, plantains, rice, wheat, millet, sweet potato, and bananas; sorghum occupies a unique position due to its hardiness as a crop. Sorghum is drought-tolerant and resistant to water-logging (Doggett, 1988), and grows in various soil conditions (Dillon *et al.*, 2007) and, also grows in temperate & arid climate (Lloyd *et al.*, 2010). These characteristics contribute toward it being the staple crop of Africa's most food-insecure people, who live in the desert-margin, semiarid tropics of about 300 million people (Doggett, 1988). Like maize, sorghum does not have a true hull or husk (Taylor, 2003). Because of its similarity to maize (hard and floury endosperm that are large fat-rich germ), sorghum can be processed using technologies of dry and wet milling applied to maize (Taylor, 2003). The recent elucidation of the genome sequence will enhance future production and nutritional quality of sorghum (ICRISAT, 2009).

Starch is the main component of sorghum grain, followed by proteins, non-starch polysaccharides (NSP) and fat. The average energetic value of whole sorghum grain flour is 356 kcal/100g (BSTID NRC, 1996). Sorghum has a macromolecular composition similar to that of maize and wheat (BSTID-NRC, 1996). However, sorghum contains resistant starch, which impairs its digestibility, notably for infants (FAO, 1995). This resistance is desired in other applications to fight human obesity and to feed diabetic people. Foods prepared from high tannin sorghum varieties have a longer passage in the stomach (Awika and Rooney, 2004). Edible products incorporating slowly digestible starch are known to exhibit a low glycemic index and increase satiety (Shin *et al.*, 2004).

7.5.3. **Maize**

Corn (*Zea mays*), also known as maize, is one of the world's leading cereal grains along with rice and wheat (Emily *et al.*, 2010). In 2008 over 750 million metric tons were produced, with the United States, the European Union, China, Brazil, Mexico, and India being the world's

leading producers (USDA/FAS, 2008). Its popularity as a crop is largely due to its diverse functionality as a food source for both humans and animals (Emily *et al.*, 2010). Kernels can be consumed off the cob, parched, boiled, fried, roasted, ground, and fermented for use in breads, porridges, gruel, cakes, and alcoholic beverages. Further processing leads to its use as food thickeners, sweeteners, oils, and non-consumables (Alexander, 1987; Gardner and Inglett, 1971; and Whistler *et al.*, 1970). Maize, providing an estimated 15% of the world's protein and 20% of the world's calories (Brown *et al.*, 1985), is a dietary staple for more than 200 million people. This number can be expected to grow as the world's population approaches 8 billion in 2025 (Lutz *et al.*, 2001 and USDA, 2009a), indicating maize's status as a paramount important crop in the context of global nutrition.

More than 80% of the world starch market originates from corn (Jobling, 2004). Corn starch is a valuable ingredient to the food industry, being widely used as a thickener, gelling agent, bulking agent and water retention agent (Singh *et al.*, 2003). Maize contains about 72% starch, 10% protein, and 4% lipid (Gardner and Inglett, 1971), supplying an energy density of about 365 kcal/100 g (USDA, 2009b). In common maize varieties, the percentage of insoluble fiber is generally 12%, while soluble fiber is less than 2%. Taken as a dietary supplement, maize fiber (89% dietary fiber, <0.5kcal/g), which improved serum lipid levels in individuals with hypercholesterolemia, is a low-calorie alternative to other high fiber lipid-lowering products like wheat bran (49% dietary fiber, 2 kcal/g) (Earll *et al.*, 1988).

7.6. Foods based on fermented cereals

A number of cereal based fermented foods exist in Africa including Kenkey in Ghana, togwa in Tanzania, mawe in Benin and ben-saalga in Burkina Faso (Baye, 2014; Guyot, 2010, and Nout, 2009). In Ethiopia, *Injera* is the most common and popular fermented food from cereals such as teff, sorghum, maize, millet, and barley. *Injera* (Enjera) is the undisputed national food of Ethiopians (Chavan and Kadam, 1989 and Oda *et al.*, 1983). Out of these cereals, teff (*Eragrostis teff*) is the major cereal ingredient in Ethiopian *Injera* (Blandino *et al.*, 2002). Kebede *et al.*, (1984) reported that sorghum ranks second to teff in preference for making *Injera*. This could be due to the relative brittleness and dryness of sorghum *Injera* after storage (Zegeye,

1997). However due to the high cost of fertilizer, engagement of intense labor for cultivation, weeding and harvesting, and the losses during harvesting, the price of teff is generally high (Bekabil *et al.*, 2011) as compared to sorghum and maize so that blending and compositing on the base teff is common in making *Injera* in spite of the fact that teff has an excellent nutritional value (Table 2).

According to Baye (2014) the nutritional composition of the above mentioned cereals is high which suggests that foods prepared from such cereals can also be nutritious particularly in terms of protein, energy, fat and amino acid content. When teff is compared with other cereals, it has the highest content of lysine and other essential amino acids. In addition teff is also rich in starch but in terms of energy, it is slightly lower which is advantageous to diabetic type II patients. It has also high fiber content. In terms of micronutrient content teff is rich in Iron, Calcium and Zinc (Abebe *et al.*, 2007). Considering phytate, polyphenol and tannin content (Abebe *et al.*, 2007), these cereals are rich in it with content variation among them. These phytochemicals are important as an antioxidant. At the same time they are also inhibitory to the micronutrients obtained from those cereals. But fermentation and cooking can improve the inhibitory effect of the phytochemicals (Peter, 1999).

Table 2: Macro, amino acid and fatty acid composition of teff grain compared to maize, sorghum, wheat and rice.

Nutrient	Teff	Maize	Sorghum	Wheat	Rice
Energy (Kcal)	357	375	370	359	357
Starch (%)	73	72	63	71	64
Crude protein(%)	11	8-11	8.3	11.7	7.3
Amino acid (g/16gN)					
Lysine	3.7	0.3	2.1	3.7	3.7
Isoleucine	4.1	0.7	3.7	4.5	4.1
Leucine	8.5	2.1	7.0	8.2	8.5
Valine	5.5	0.8	4.1	6.0	5.5
Phenyl alanine	5.7	0.9	4.9	5.5	5.7
Tyrosine	3.8	0.7	2.3	5.2	3.8
Tryptophan	1.3	0.2	1.1	1.2	1.3
Threonine	4.3		0.5	2.7	3.7
Histidine	3.2		0.4	2.1	2.3
Arginine	5.2		0.6	3.5	8.5
Methionine	5.2		0.6	3.5	8.5
Cystine	2.5		0.3	2.4	1.8
Asparagines	6.4			5.1	9.0
Serine	4.1		0.8	5.0	5.0
Glutamine + glutamic acid	21.8			29.5	17.0
Proline	8.2		1.3	10.2	5.0
Glycine	3.1		0.5	4.0	4.5
Alanine	10.1		1.6	3.6	5.5
Crude fat (%)	2.5	4.9	3.9	2	2.2
Total unsaturated fatty acid	1.1	1.8	1.4	0.5	0.8
Linoleic acid (LA)	0.9	1.7	1.3	0.5	0.78
α -linoleic acid (ALA)	0.14	0.05	0.07	0.03	0.03
LA:ALA ratio	7:1	34:1	20:1	17:1	26:1
Crude fiber (%)	3.0	-	0.6	2.0	0.6-1.0
Total dietary fiber	4.5	2.6			-
Soluble dietary fiber	0.9	0.6			-
Ash (%)	2.8	1.4	1.6	1.6	1.4

Adapted from Agren *et al.*, (1968) in “Food composition table for use in Ethiopia.

7.7. Food formulation based on cereals blend

Low-cost, high-protein food product development for infants is a constant challenge. This is particularly important in developing countries where malnutrition problems are still common, particularly during weaning (Schmidt, 1983). Complementary foods in most developing countries are based on staple cereal or root crops and, commercial foods of high quality are occasionally available, they are often expensive and therefore unaffordable by low-income rural

households; thus, different approaches are needed to offer families the opportunity to feed their infants on improved formulations using low cost and locally available staples (Happiness *et al.*, 2011). In a same research conducted to improve the protein and energy intake of infants in Iringa region, Tanzania, nine complementary foods were formulated (F1-F9) based on maize, sorghum and finger millet as staples and common beans, cowpeas and green peas as protein supplements that the samples were germinated and spatially roasted for the purpose of improving the nutritive value and sensory attribute of formulated recipes where all the formulations were evaluated for their sensory acceptability by both semi- and untrained panelists using a five point hedonic scale. Complementary feeding improvement should be of highest priority for nutrition of infant and young children because of its crucial role in preventing mortality and enhancing children development (Lutter *et al.*, 2003). Over 70% of dietary protein in developing countries is supplied by cereals that are relatively poor sources of protein (Glover, 1976). Formulation and development of nutritious weaning foods from local and readily available raw materials has received a lot of attention in many developing countries (Plahar *et al.*, 1994). For instance, Del Valle *et al.*, (1981) formulated "Soyaven," a highly nutritive infant formula already in commercial production; Bressani *et al.*, (1963) reported the development of cereal-based foods supplemented with amino acids; Del Angel *et al.*, (1982) and Yepiz *et al.* (1983) also reported the use of cereal and legume combinations to improve the nutritive value of food proteins for human consumption.

7.8. The use of design expert in food formulation optimization

A number of researches involving food formulation optimization from different ingredients of cereals and legumes have been conducted with the methodology of design expert. Dibyakanta *et al.*, (2012), for instance, used a response surface methodology based on a five-level-three-factor central composite rotatable design (CCRD) for optimization of formulation for the production of a soy-fortified millet-based extruded snack by using soy, sorghum, millet and rice blend. The objective of their work was to investigate the effect of amount of ingredients such as ragi (40–50%), sorghum (10–20%) and soy (5–15%) on the physical properties like bulk density, expansion ratio, water absorption index and water solubility index. In another research conducted by Ashley (2004) entitled “consumer sensory characteristics of butter cake made from wheat and

rice flours” the experimental design of three component constrained simplex lattice mixture design was followed in a mixture design consisting of wheat flour (X1), rice flour (X2), and pre-gelatinized rice flour (X3). Also in a similar research done by Prinyawiwatkul *et al.*, (1997) entitled “Optimizing Acceptability of Chicken Nuggets Containing Fermented Cowpea and Peanut Flours” a mixture design was used to optimize the sensory acceptability (appearance, color, flavor, texture, and overall liking) of chicken nuggets containing flours processed from fermented cowpeas (FCF) and fermented partially defatted peanuts (FPDPF), a three-component constrained simplex lattice mixture design consisting of mixture components of chicken (X), flour from fermented cowpeas (FCF, X), and flour from fermented partially defatted peanuts (FPDPF, X), made up 84% of the total formulation. All formulations contained 1% salt, 0.6% garlic powder, 0.4% black pepper, and 14% added water.

According to Douglas (2001), simplex lattice design can be used for 2 up to 24 components (ingredients). A simplex -lattice mixture design of degree m consists of $m+1$ points of equally spaced values between 0 and 1 for each component. If $m = 2$ then possible fractions are 0, $1/2$, 1. For $m = 3$ the possible values are 0, $1/3$, $2/3$, 1. The points include the pure components and enough points between them to estimate an equation of degree m . This design differs from a simplex-centroid design by having enough points to estimate a full cubic model. Since the number of points may be equal to just the number needed to estimate the model, these designs should be augmented to allow for detection of lack of fit. It should be noted that the software does this by default. Simplex-lattice augmentation adds the overall centroid plus all check blends. Check blends are 50-50 combinations of the center point and each vertex. To test the lack of fit, an estimate of pure error is used. This estimate of pure error comes from replication of several design points. By default, design expert software adds a number of replications equal to the number of components plus one, up to a maximum of 5.

8. Materials and method

8.1. Chemicals used

Ethanol, diethyl ether, boric acid, sodium hydroxide, hydrochloric acid, sulphuric acid, 30% hydrogen peroxide, potassium sulphate, selenium metal as a catalyst, distilled water, potassium chloride, and buffer solutions of pH 7.01, 4.01 and 9.21.

8.2. Apparatuses and instruments used

PH-meter, volumetric flask (1000ml, 25, 50ml), tecator tube, digester, soxhlet apparatus, desicator, extraction thimble, extraction chamber, fat-free cotton, flask holder, porcelain crucible, muffle furnace, drying oven, pipette, beaker (600ml), analytical balance, spatula, cereal grain disintegrator (flour grinder), wash bottle and filter paper (Whatman).

8.3. Sample collection

The samples, Sorghum (Gambella 1107) & Maize (M-6Q), were obtained from Melkasa Agricultural Research Center (located 112km south east of Addis Ababa) and teff (Kuncho DZ-Cr-387) from Debrezeit Agricultural Research Institute (located around 40kms south east of Addis Ababa). These sampling sites were chosen purposely (purposeful sampling), as it was possible to get widely consumed varieties among our society. All preventive and precautionary measures were taken while collecting the grain samples to avoid adventitious contamination including wearing gloves, using clean plastic poly ethylene bags, etc.

8.4. Study site

Experiments were conducted at the research laboratory of the Center for Food Science and Nutrition, College of Natural Science, Addis Ababa University; at the food chemistry laboratory of the Ethiopian Public Health Institute and at the laboratory of Addis Pharmaceutical Factory PLC located in Akaki on the way to Debrezeit, where all are located in Addis Ababa.

8.5. Conceptual frame work of the study

Simplex lattice mixture design was followed for the generation of experimental test trials, analysis of the results, model building and formulation optimization with the use of a statistical software package (*Design- Expert* ®, version 7.0, Stat-Ease, SaMeep104 Inc., 2021 East Hennepin Ave., Suite 480 Minneapolis, MN 55413, 2005).

There were 14 experimental test trials (Table 3) generated based on predefined constraint (Table 4) up on which the experiment was conducted. Four out of the 14 experimental trials ([100% teff], [50% teff: 50% sorghum], [50% teff: 50% maize] and [75% teff: 25% sorghum]) were replicated, points with relatively maximum standard error.

A total of 15 kilogram teff, 5 kilogram of sorghum and 4 kilogram of maize were consumed for the preparation of 14 different *Injera* types and for the 4 optimized formulations.

Table 3: Simplex lattice coded design for *Injera* preparation

Trial	Type	Component 1 A: Teff	Component 2 B: Sorghum	Component 3 C: Maize	Color	Odor	Taste	Appearance	Overall acceptability	Crude Protein	Crude fat	Crude fiber	Total carbohydrate	Total Energy
1	Vertex	1.000	0.00	0.00										
2	Axial CB	0.833	0.083	0.083										
3	Cent Edge	0.75	0.25	0.000										
4	Cent Edge	0.75	0.25	0.000										
5	Cent Edge	0.50	0.25	0.25										
6	Vertex	0.50	0.50	0.00										
7	Axial CB	0.583	0.083	0.333										
8	Cent Edge	0.75	0.00	0.25										
9	Vertex	1.00	0.00	0.00										
10	Center	0.667	0.167	0.167										
11	Vertex	0.50	0.00	0.50										
12	Vertex	0.50	0.50	0.00										
13	Vertex	0.50	0.00	0.50										
14	Axial CB	0.583	0.333	0.083										

Table 4: Design constraints

Low	\leq Constraints	\leq High
0.5	\leq A:Teff	\leq 1
0	\leq B:Sorghum	\leq 0.5
0	\leq C:Maize	\leq 0.5

B+C=0.5
A+B+C=1

Proximate determination was conducted on the raw materials (teff, sorghum and maize), on the *Injera* samples of the experimental trials and on the optimized formulations while sensory acceptability of six parameters was conducted for the experimental trials only once but for the optimized formulations, each sample was examined after first day, second day and third day of the *Injera* prepared (Figure 2).

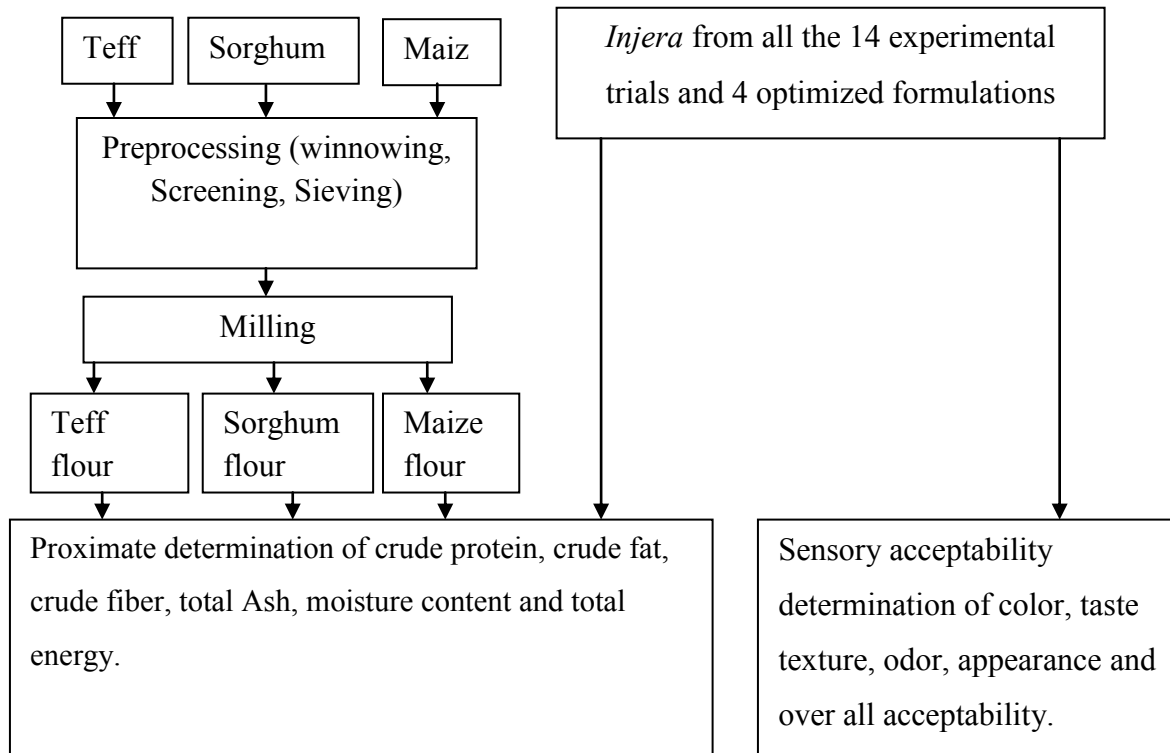


Figure 2: Conceptual frame work of raw material preparation, proximate and sensory acceptability determination.

Fermentation kinetics of pH determination was conducted on the dough samples of the optimized formulations after every two hours of fermentation starting from the initial dough preparation up to the final time of fermentation for three days, that was [initial 0 hour, after 2 hours, 4 hours, 6 hours, 8 hours,, after 72 hours] (Baye *et al.*, 2012). During fermentation, the pH of the batter dough was recorded using a calibrated pH meter. The rate of change in pH (dpH/dt) was calculated for each of the four optimized batter dough samples observations by using the following equation:

$$\text{dpH/dt} = [\text{pH}(t+2) - \text{pH}(t)] / [(t+2) - t],$$

Where

- t: fermentation time in (hours),
- pH(t): pH of the dough after “t” hours of fermentation, and
- pH(t+2): pH of the dough after (t+2) hours of fermentation.

The maximal value of (dpH/dt) for each observations was then be averaged to give the maximal rate of change in pH ((dpH/dt)_{max}), (Baye *et al.*, 2012). The maximum rate of change in pH is explained us how fast the pH of the dough changed every hour during the course of fermentation.

For the sensory acceptability study, 20 informed panelists were used. When we say informed panelist, all the panelists took sensory analysis course (Msc & Phd students at the Center for Food Science & Nutrition program and lecturers of the centre, College of Natural Science, AAU), were in a good health conditions and were frequent *Injera* consumers aged from 28 up to 40 years old both male and female sexes, and this was checked with four questionnaire questions included with the score sheet. At the beginning of the experiment, panelists were instructed about the objective of the study, the required discipline and how they could fill the score card and questionnaire (Appendix 7.1) based on their evaluation. Then panelists were asked to evaluate the different types of *Injera* samples on the basis of color, taste, texture, odor, appearance and overall acceptability with the use of 5 point hedonic scale (1= the characteristics is not liked, 2= the characteristics is least liked, 3= the characteristics is liked, 4= the characteristics is very liked, 5= the characteristics is liked extremely). Appropriate time (time when the panelists were

not full and not hungry, which was from 2:00pm up to 4:00pm) was chosen to conduct the experiment. Five days were spent to complete sensory analysis of the 14 samples, a maximum of only 3 samples were served and tasted every day so as not to bore and bias the panelists that may eventually affect the responses. All the 14 experimental *Injera* samples were tasted after 12 hours of preparation, thus *Injera* for a maximum of 3 independent samples were prepared every day, here there was no time (day) variation therefore time was constant and hence the only factor studied was variation of trial formulations . Whereas the four *Injera* samples resulted from optimization were tested for three days while four of the *Injera* samples were prepared the same day, in which the test was conducted after the first day, after the second day and after the third day of its preparation, here variation of days was considered as one factor in addition to formulation variation thus we had two main factors. The seating pattern of the panelists was made sure that they seat far enough separately while samples were provided step wise one at a time. A bottle of natural spring water (Aqua Addis®) was provided to each panelist for rinsing their mouth at the beginning and after scoring each sample. Newly purchased clean disposable tray and cup materials were used to serve samples and to provide water for the panelists. Important notes during the experiment were properly taken and written down on a note book.

Photographs of all the 14 experimental *Injera* samples and those of the 4 optimized formulations were taken with the use of digital camera from the same distance, and the number of eyes in 1x1inch square at 0.16 inch separation was approximately determined to objectively describe a given *Injera*. The numbering of eyes was conducted by taking 9 observations from 9 1x1inch squares at 9 points of equal distance in 5x5 inch square of *Injera* samples (Figure 3). For *Injera* eyes which were not fully included in the 1x1 inch square, it was counted in the square where more than half of the eyes were included.

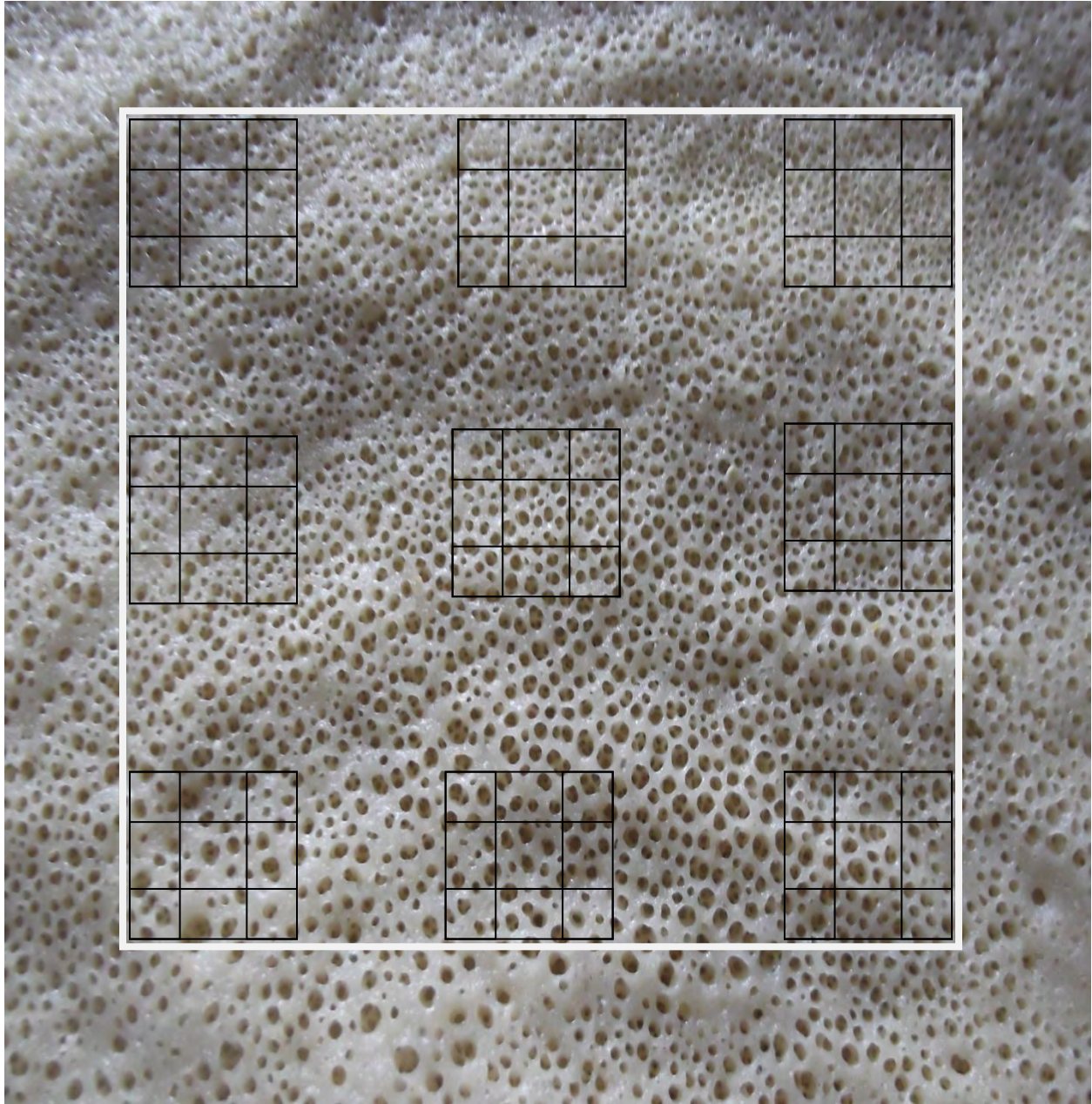


Figure 3: Photograph of *Injera* taken at a distance and used for determination of number of eyes of *Injera* samples.

8.6. Sample preparation

All samples of teff, sorghum and maize were taken separately to be winnowed, cleaned, and then stored in an air tight closed polyethylene bags so that it was used for the entire study. The samples were divided in to two portions in which one portion was used for the preparation of 14 different experimental *Injeras* so that proximate nutritional composition, and sensory

acceptability determinations were conducted, and the second portion was used to prepare 4 different optimized batter formulations for the determination of pH kinetics and to prepare 4 different optimized *Injera* formulations.

8.7. Preparation of raw flour

The samples of teff, sorghum and maize were ground separately by using a small grinding mill (High Speed Universal Disintegrator FW 100) available at the Center for Food Science and Nutrition, College of Natural Science, AAU. The grounded flour was finally collected in to an air tight dry clean poly ethylene bags and was labeled properly (Figure 7).

8.8. Estimation of the dry weight of *Injera*

The dry weight of one *Injera* was estimated to be 117g. This estimation was done by randomly collecting 3 *Injera* each from 3 different shops located around piazza area and another 3 injera from home, therefore a total of 12 *Injera* samples were collected. These *Injera* samples were each weighed initially and after careful sun drying, and then the dry weight of one *Injera* was estimated by disregarding the weight of water content of fresh *Injera*.

8.9. Preparation of flour blend

Enough quantities of flour blends for all the 14 trials were prepared by estimating 117g as the dry weight of one *Injera* (generated with the use of design expert STAT EASY version 7 software). The weight flours was measured by means of analytical balance (Adventure Ohasu and Shimadzu) and preparation of the blends was conducted by thorough mixing (Figure 7).

8.10. Preparation of fermentation starter (*Ersho*)

50g of flour from each of the 14 blended samples, 30ml of home *Ersho* (about 2%, estimated by taking 100ml of home Ersho, weighing initially, after evaporation to dryness and calculating the percentage) and 50 ml of clean water was used to prepare each of the fermentation starter of all

the 14 experimental trials that also includes the 4 formulations resulted from optimization. The fermentation starter was then become ready for use after 10 hours of fermentation and was used for the preparation of the main dough (Figure 7).

8.11. Dough preparation

After 10 hours of preparation, the whole *Ersho* was mixed with 351g of flour followed by the addition of 200ml of clean water for further mixing. The prepared thick dough was kept covered for 72 hours at room temperature to allow fermentation (primary fermentation) and then about 70 ml of supernatant was discarded followed by dough thinning through the addition of 200ml clean water which this was then left covered for 15 minutes to allow further fermentation. The so called *Abscit*, which serves as a binder, was prepared with a ratio of 1:3 (thin dough: water) by boiling on a hot oven (about 120g) and then cooled to a temperature of about 43⁰C. It was then added fully onto the thinned batter, mixed carefully and left for 1 hour up until it rises (secondary fermentation) that became ready for baking to prepare *Injera* (Figures 4 and 7).



Figure 4: Picture indicating the process of dough preparation.

8.12. *Injera* baking process

With the use of *Mesti*, a small container, about 450ml of the final batter was poured on to hot clay griddle in a circular motion working toward the centre from outside. After 2 to 3 minutes of cooking on a traditional baking equipment (*Metad*), the *Injera* was removed and stored inside a short cylindrical bowl called *Mesob* on and over clean poly bag after cooling. The baking of all *Injera* samples was done by the same baker throughout the study (Figures 5 and 7).



Figure 5: Picture indicating the process of *Injera* baking preparation

8.13. Sun drying and grinding of dried *Injera*

The prepared *Injera* of all the trials was properly sundried by spreading onto a flat clean plastic sheet, was then ground into a dry fine sample with the use of grinder mill previously used and finally collected and stored in to a clean poly bag for further laboratory analysis (Figure 6 and 7).



Figure 6: Picture indicating the process of sun drying and grinding of *Injera* for further experimental analysis.

8.14. An overall diagrammatic display of *Injera* preparation followed

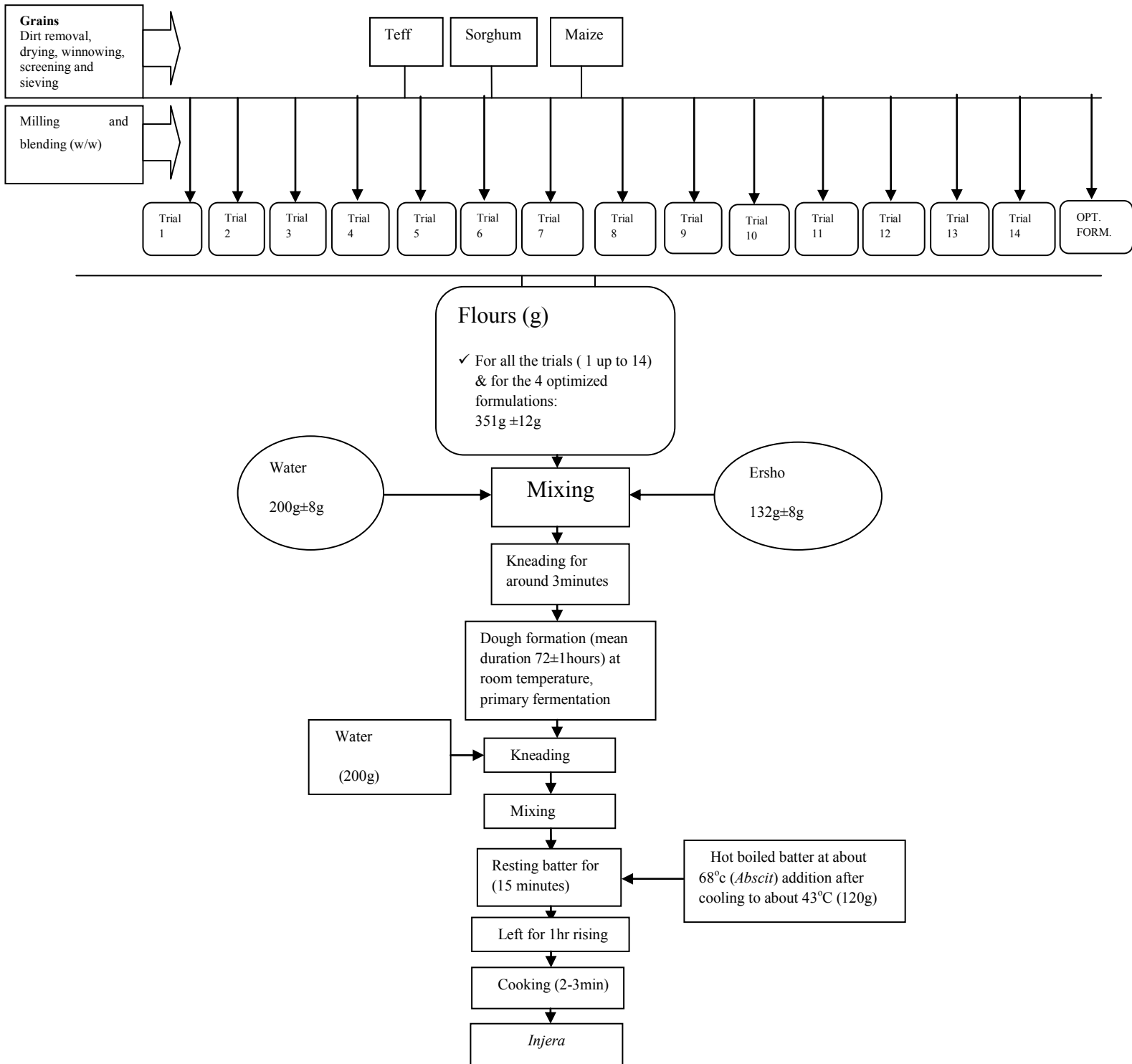


Figure 7: Diagrammatic description of the process of *Injera* baking followed.

*OPT. FORM: Optimized Formulations.

8.15. Analytical experimental procedures

8.15.1. Determination of crude protein

Protein content was determined according to AOAC (2000) using the official method 979.09.

Digestion

Fresh samples (in duplicate) of each 0.5g were added in to Tecator tubes and 6ml of acid mixture (5parts of concentrated ortho-phosphoric acid and 95 parts of concentrated sulfuric acid) was added in to each tubes, mixed, left over night to allow enough reaction facilitating the digestion process and then 3.5ml of 30% hydrogen peroxide was added step by step, resulting in a violent reaction. Following the cessation of violet reaction, the tubes were shaken and placed back to the rack. Catalyst mixture (ground 0.5g of selenium metal with 100g of potassium sulfate) of 3g was added in to each tube and allowed to stand for about 10 minutes before digestion. Following the attainment of digester temperature at 370°C, the tubes were lowered in to the digester. The digestion had been continued until a clear solution was obtained after 4 hours. The tubes in the rack were cooled in a fume hood. About 25 ml of de-ionized water was added and shaken to avoid precipitation of sulfate in the solution.

Distillation and titration

The digested and diluted sample solution was distilled using 2% boric acid and 40% sodium hydroxide, then the distillate was subjected in to titration using 0.1N hydrochloric acid until reddish color was appeared.

Computing of crude protein values

$$\text{Nitrogen \%} = \frac{V_{\text{HCl}} * [\text{HCl}]}{W_0} * 14 * 100 \dots \dots \dots (1)$$

$$\text{Protein \%} = 6.25 * \% \text{ Nitrogen} \dots \dots \dots (2)$$

Where

- V: volume of 0.1N HCl consumed in litere,
- N: normality of HCl (0.1N),
- W₀: sample weight on dry mass basis and
- 14: molecular weight of nitrogen.

8.15.2. Determination of crude fat

The fat content of *Injera* samples had been evaluated by using semi-continuous solvent extraction method (Sohxlet method) following the procedures of AOAC (2000) official methods 960.39. The solvent had been built up in the extraction chamber for 5-10 minutes and completely surrounded the sample and then siphoned back to the boiling flask. The fat content was measured by weight loss of the sample or by weight of the fat removed. The materials used were the Soxhlet machine, the extraction thimble, the extraction chamber and the desiccators.

First the extraction thimble was cleaned, dried and weighed (W_1). The extraction thimble covered primarily with fat free cotton containing the primarily weighed 2gm sample (W) was covered over again with a layer of fat free cotton. Then the thimble was put in to the extraction chamber. The cleaned and dried extraction cylinder which was previously kept inside a desiccator was then taken out from the desiccators, was put in the flask holder; 50ml ether was added in the extraction cylinder and was moved in to the heating plank. The extraction had been continued for 4hours at 55 °C. Then after the extraction cylinder was disconnected and had been kept in the drying oven at 70°C for 30 minutes and was again put in to the desiccators to let it cool for 30 minutes. Finally the extraction cylinder had been taken at once out of the desiccators and was weighed (W_2). The crude fat content percent weight by weight was computed by the formula below:

$$F = \left(\frac{W_2 - W_1}{W} \right) * 100\% \dots\dots\dots (3)$$

Where,

F= Crude fat content in percent weight by weight

W_1 = Weight of the extraction flask in gram (g)

W_2 = Weight of the extraction flask and the dried crude fat in gram (g)

W = Weight of sample in gram (g)

8.15.3. Determination of total ash

The ash content was determined based on AOAC (2000) using the official method 923.03. Previously washed (by using distilled water) and dried porcelain crucibles were placed inside a

muffle furnace at 550°C for 30 minutes. After withdrawing from the furnace the crucibles were cooled by placing in desiccators for 30 minutes at room temperature. Following this step, the crucible was weighed to the nearest milligram (M_1). Using the dried, cooled and weighed crucible, fresh 2.5g of sample was weighed together (M_2). Then the sample was thoroughly charred in a fume hood by placing it on a hot plate, increasing the temperature slowly until smoking ceases. After the completion of charring, the sample was placed in muffle furnace at 550°C for 5 hours. The ignition was continued by cooling for 1 hour and weighing until getting a clean and white ash to the nearest milligram (M_3).

$$\text{Total ash (in \% weight by weight)} = \left(\frac{M_3 - M_1}{M_2 - M_1} \right) * 100 \dots \dots \dots (4)$$

Where: ($M_2 - M_1$) is the weight of sample in gram (g) on dry basis and ($M_3 - M_1$) is the weight of ash in gram (g).

8.15.4. **Determination of moisture**

The moisture content of the samples were determined by drying 10g of sample in an air oven at 105°C to constant weight (16 -18 h),(AOAC, 2000, 17th edition, Vol.2).

8.15.5. **Determination of total carbohydrate content**

The formula indicated below, equation (5), was used to determine the carbohydrate content of the samples.

$$\% \text{Carbohydrate} = 100\% - (\% \text{moisture} + \% \text{protein} + \% \text{fat} + \% \text{ash}) \dots (5)$$

8.15.6. **Determination of energy**

The formula indicated below, equation (6), was used to determine the total energy value of the samples.

$$\text{Total energy in Kcal/100g} = [9x\% \text{cont. Fat} + 4x(\% \text{cont. protein} + \% \text{cont. CHO})] \dots (6)$$

8.15.7. Determination of crude fiber

Crude fiber content was determined by using the official method of AOAC (2000).

Digestion

Fresh sample of 1.7000g (W_1) was placed in to a 600ml beaker; 200 ml of 1.25% sulphuric acid was added and boiled gently for 30 minutes while watch glass was placed over the mouth of the beaker. The level of the sample solution was kept constant by using hot distilled water during boiling. After exactly 30 minutes heating, 20 ml of 20% KOH was added and boiled gently for further 30 minutes with occasional stirring.

Filtration

The bottom of a sintered glass crucible was covered with 10 mm sand layer and wet with distilled water. The solution was then poured in to a sintered glass crucible, and filtered with the aid of vacuum pump. The wall of the beaker was rinsed with hot distilled water several times. The washing was transferred in to the crucible and filtered.

Washing

The residue in the crucible was washed with hot distilled water and filtered twice. Again the residue was washed with 1% H_2SO_4 , filtered, and again washed with hot distilled water, filtered and finally washed with 1% KOH, and filtered. At this level also, the residue was washed with hot distilled water, filtered and again washed with 1% H_2SO_4 , and filtered. Finally the residue was washed with water - free acetone.

Drying and combustion

The crucible with the content was dried in a drying oven for 2 hours at $130^\circ C$, cooled for 30 minutes in a desiccators and then weighed (W_2). The crucible was then transferred in to a muffle furnace, and heating was continued for 30 minutes at $550^\circ C$. The crucible was cooled in a desiccator and then the crucible was weighed with the content (W_3). The crude fiber was determined by the formula below:

$$\text{Crude fiber in (\%)} = \frac{[(W_2 - W_3) * 100]}{W_1} \dots\dots\dots (7)$$

Where:

W_1 = weight of the fresh sample,

W_2 = weight of crucible with the sample after oven draying and

W_3 = weight of the crucible with the sample after ashing.

8.16. Data entry and analysis

All analytical determinations were conducted in duplicate with the exception of total ash and moisture content, done in triplicate, and the values were presented as mean \pm standard error. Design expert soft ware package (*Design- Expert* ®, version 7.0, Stat-Ease, SaMeep104 Inc., 2021 East Hennepin Ave., Suite 480 Minneapolis, MN 55413) of mixture design (simplex lattice design) was used to generate experimental test trials, to check for homogeneity of variance of response parameters result, to analyze the response data's and for modeling & optimization of experimental test trials. SPSS (Statistical Product and Service Solution) version 20 soft ware package had also been used to analyze experimental results of the experimental trials and of the optimized formulations through the use of one way ANOVA in comparing means of the 14 trials, and two-way ANOVA in comparing sensory acceptability means of the four optimized formulations for three different days. All the data analysis was conducted at a significance level of $p < 0.05$ and "Duncan's multiple comparison" was followed for separation of significantly different means.

9. Results and Discussion

9.1. Raw material proximate composition

The main purpose of conducting proximate experimental analysis on the raw materials was that unless we know the nutritional quality of the initial raw materials, it is difficult to get a clear understanding about the variations and changes on the nutritional quality of the finished blend product, thus it help us to know and reason out the real nutritional contribution of each raw materials on the finished product, *Injera* during discussion.

Table 5: Proximate composition of raw ingredients

Ingredients	Crude Protein (%)	Crude Fat (%)	Crude fiber (%)	Total Ash (%)	Moisture content (%)	Total CHO (%)	Total Energy (Kcal/100g)
Teff	12.24±0.06 ^a	2.69±0.03 ^a	8.57±0.12 ^a	2.93±0.06 ^a	9.69±0.04 ^a	72.37±0.07 ^a	362.65±0.23 ^a
Sorghum	15.06±0.10 ^b	3.69±0.08 ^b	12.70±0.01 ^b	1.69±0.05 ^b	9.50±0.24 ^a	69.85±0.18 ^b	372.83±0.38 ^b
Maize	9.92±0.09 ^c	5.18±0.10 ^c	8.87±0.02 ^a	1.45±0.05 ^c	8.75±0.01 ^b	74.74±0.07 ^c	385.38±0.66 ^c

*The data is expressed as mean ± standard error on a dray weight basis. *Any two means in the same column not followed by the same letters are significantly different at (p<0.05).

The mean crude protein content of teff, sorghum and maize was significantly different (p<0.05). When these results were compared with the results in Table 2 (teff=11%, sorghum=8.3% & maize=8-11%), there was a difference from minimum of 4% up to 7%. The mean crude protein composition of teff was 9.60% (Patricia *et al.*, 2008) which was about 2% less than the result obtained but the protein composition of maize was 10% (Gardner and Inglett, 1971) which was almost in agreement with the result obtained in the current study (9.92%). The reason might be due to difference in the varieties of teff and similarity of varieties of maize. The difference in mean crude protein composition of teff, maize and sorghum might obviously arise due to their specious difference and the mean crude protein content of sorghum was the highest which might be due to specious difference from the other two cereals and the uniqueness of the Gambela variety and it was expected that sorghum contributes higher crude protein composition to the composite blend products, *Injera*.

The crude fat composition of teff and maize was 2.0% and 4.45% respectively (Bultosa et al., 2003) which was nearly in agreement with the current study result (teff=2.69% & maize=5.18%). The mean crude fat content of teff, sorghum and maize was significantly different at significance level of $p<0.05$. The difference might obviously arise due to their species difference and the mean crude fat content of maize was the highest and it was expected that maize contributes higher fat composition to the composite blend product, *Injera*.

The mean crude fiber content of teff and maize was similar while that of sorghum was significantly different at a significance level of $p<0.05$ from both of them. The highest crude fiber content of sorghum under the current study is not in agreement with a literature by (Kaleab, 2014) and the crude fiber content of maize was about 14% (Earll et al, 1988) which was 5% higher than the result from the current study (8.98%) which such difference might be due to difference in variety.

The mean total ash composition of teff, sorghum and maize was significantly different at significance level of $p<0.05$. The total ash composition of teff was 2.90% (Patricia *et al.*, 2008) and the result from the current study is 2.93% which is in agreement. But the results among the three cereals were significantly different which obviously might arise due to their species difference. The mean total ash content of teff was the highest which might be associated with the higher mineral content of teff as compared to sorghum and maize.

The mean moisture content of teff and sorghum was similar while that of maize was significantly different at significant level of $p<0.05$. The water absorption capacity of teff was high (Bultosa *et al.*, 2002; Bultosa 2007) and thus in agreement with the study under the title, moisture content of teff was the highest. The reason might be due to the similarity of the water holding capacity of teff and sorghum, and difference of that of maize plus the moisture content of maize was relatively the least which might also be associated with its highest fat content.

The mean total carbohydrate content of teff, sorghum and maize was significantly different at significance level of $p<0.05$. The total carbohydrate composition of teff was 73% (Bultosa *et al.*, 2002) which was almost in agreement with the studied result (72.37%). The difference in the

total carbohydrate among teff, sorghum and maize might arise from difference in their protein, fat, moisture and ash content. The carbohydrate content of maize was the highest which was due to its highest fat content and due to its least protein, moisture and ash content.

The mean total energy value of teff, sorghum and maize was significantly different at a significance level of $p < 0.05$ in which the value for that of maize was the highest because of its highest fat and carbohydrate composition.

9.2. Determination of dry weight of *Injera*

The *Injera* dry weight determination was needed to make appropriate blend of ingredients per *sample* so that the variation factor on the sensory and nutritional quality of the *Injera* from lack of proper weight estimation was eliminated or minimized.

Table 6: Dry *Injera* weight

<i>Injera</i> type	Mean <i>Injera</i> weight	Grand mean weight of one <i>Injera</i>
Home <i>Injera</i> (control)	105.44±0.30 ^a	
Shope1 <i>Injera</i>	124.09 ±12.22 ^a	116.76±3.98≈117±4
Shope2 <i>Injera</i>	118.11±2.66 ^a	
Shope3 <i>Injera</i>	119.40±2.27 ^a	

*The data is expressed as mean± standard error on a dry weight basis. *Any two means in the same column not followed by the same letters are significantly different at ($p < 0.05$). *The weights are all given in grams.

The mean weights of *Injeras* from all the three shops were similar with the home *Injera* at significance level of $p < 0.05$, thus the average dry one *Injera* weight of is estimated to be 117g.

9.3. Proximate composition of the 14 experimental trials

The experimental trials were generated from the mixture simplex lattice design expert soft ware as per the defined constraints. The soft ware had primarily resulted in enough experimental test trials with replication of the 3 vertex points and one center edge on the contour plot augmenting that most of the possible recipe points on the lattice space were included thus finding out and

getting appropriate optimal blend composite formulations in terms of macro-nutritional quality, sensory acceptability and with an affordable cost was likely for *Injera* preparation. The proximate and sensory acceptability determination mean results for 13 response parameters had been fed to the soft ware as per the earlier defined recipes, and the design expert soft ware had analyzed the data to generate appropriate model to all the response parameters.

Table 7: Proximate composition of the 14 experimental trials

Trials	Recipe			Crude Protein (%)	Crude fat (%)	Crude fiber (%)	Total ash (%)	Moisture content (%)	CHO (%)	Total energy (Kcal/100g)
	Teff	Sorghum	Maize							
control (Trial1)	1.000	0.00	0.00	11.34±0.00 ^{ab}	1.79±0.03 ^a	6.00±0.03 ^b	2.98±0.03 ^g	65.94±0.06 ^d	17.98±0.02 ⁱ	133.39±0.35 ^{dc}
Trial 2	0.833	0.083	0.083	12.02±0.10 ^c	3.26±0.21 ^{fg}	9.29±0.01 ⁱ	2.59±0.02 ^{ef}	66.86±0.10 ^f	15.18±0.07 ^g	137.98±1.37 ^f
Trial 3	0.75	0.25	0.000	14.22±0.10 ^{ef}	2.94±0.16 ^{ef}	7.17±0.03 ^c	2.74±0.01 ^{fg}	67.35±0.03 ^g	12.74±0.21 ^{cd}	134.26±1.00 ^c
Trial 4	0.75	0.25	0.000	14.50±0.10 ^f	2.57±0.06 ^{cde}	7.96±0.11 ^f	2.78±0.04 ^{fg}	66.31±0.02 ^e	13.88±0.04 ^{ef}	136.59±0.24 ^{ef}
Trial 5	0.50	0.25	0.25	13.14±0.34 ^d	2.82±0.06 ^{de}	7.71±0.05 ^e	2.34±0.02 ^{cde}	69.98±0.06 ^j	11.74±0.23 ^b	124.86±0.10 ^b
Trial 6	0.50	0.50	0.00	14.73±0.10 ^f	2.54±0.08 ^{cde}	9.52±0.04 ^j	2.51±0.01 ^{def}	73.25±0.16 ^k	7.14±0.05 ^a	110.36±0.54 ^a
Trial 7	0.583	0.083	0.333	11.31±0.29 ^{ab}	3.53±0.06 ^g	7.54±0.02 ^d	2.19±0.07 ^{bc}	69.37±0.16 ⁱ	13.39±0.36 ^{de}	130.51±0.24 ^{cd}
Trial 8	0.75	0.00	0.25	11.69±0.24 ^{abc}	2.82±0.11 ^{de}	5.65±0.08 ^a	2.58±0.03 ^{ef}	65.37±0.14 ^c	17.45±0.56 ⁱ	141.92±0.27 ^g
Trial 9	1.00	0.00	0.00	11.27±0.17 ^a	2.06±0.10 ^{ab}	7.95±0.11 ^f	2.94±0.03 ^g	66.25±0.23 ^{de}	16.28±0.49 ^h	128.60±3.40 ^c
Trial 10	0.667	0.167	0.167	13.92±0.11 ^e	2.14±0.20 ^{ab}	8.15±0.04 ^g	1.99±0.18 ^b	69.59±0.04 ⁱ	12.51±0.33 ^{bc}	124.90±0.04 ^b
Trial 11	0.50	0.00	0.50	11.90±0.10 ^c	3.42±0.04 ^g	8.38±0.04 ^h	2.05±0.03 ^{bc}	62.81±0.04 ^a	19.81±0.08 ^j	157.60±0.42 ^h
Trial 12	0.50	0.50	0.00	14.59±0.15 ^f	2.44±0.14 ^{bcd}	7.44±0.02 ^d	2.25±0.10 ^{bcd}	73.08±0.19 ^k	7.76±0.20 ^a	110.98±0.48 ^a
Trial 13	0.50	0.00	0.50	11.83±0.10 ^{bc}	3.59±0.14 ^g	7.47±0.02 ^d	1.65±0.21 ^a	63.67±0.03 ^b	19.45±0.07 ^j	157.41±1.40 ^b
Trial 14	0.583	0.333	0.083	12.69±0.15 ^d	2.37±0.14 ^{bc}	9.57±0.01 ^j	2.28±0.18 ^{bcd}	68.49±0.03 ^h	14.27±0.25 ^f	129.17±0.34 ^c

*The data is expressed as mean± standard error on a dry weight basis. *Any two means in the same column not followed by the same letters are significantly different at (p<0.05) and any one means in the same column followed by the same letters are similar.

The mean crude protein content of the experimental trials varies from each other depending on the type and amount of ingredients incorporated in each of the recipe of the trials. Most of the experimental trials containing sorghum had shown the highest protein content while the experimental trials containing maize had shown the least protein content as compared to the control experimental trial (trial1). The first reason for the highest and least protein content was associated with the type and quantity of the ingredients. That was the protein content of sorghum was the highest while that of maize was the least out of the three. Thus experimental trials containing sorghum (trial 6) had relatively shown the highest protein content while those containing maize (trial 7) had shown the least protein content. The other reason might be

associated with the blending that flour compositing affects fermentation kinetics (Baye *et al.*, 2012) and fermentation affects protein availability (Hamad *et al.*, 1979).

The mean fat content of the experimental trials vary from each other depending on the type and amount of ingredients incorporated in each of the recipe of the trials. All of the experimental trials containing sorghum and maize had shown the highest fat content as compared to the control (trial1). Those experimental trials containing maize had shown even higher result as compared to those containing sorghum. The reason is associated with the highest fat content (5.18%) & least moisture content (8.75%) as compared to the least fat composition (2.69%) and the highest moisture content (9.69%) of the control teff.

The mean crude fiber content of the experimental trials varies from each other depending on the type and amount of ingredients incorporated in each of the recipe of the trials. The crude fiber content of the experimental trials containing sorghum had shown the highest fiber content as compared to the control and those containing maize. The first reason is associated with the highest fiber content of sorghum (12.70%) as compared to teff (8.57%) and maize (8.87%).

The mean total ash content of some of the experimental trials varies depending on the type and amount of ingredients incorporated in each of the recipe of the trials. Experimental trials containing significant amount of teff had the highest and similar total ash content as that of the control (trial 1) than the other experimental trials. The reason is due to the highest total ash content of teff (2.93%) as compared to sorghum (1.69%) and maize (1.45%).

Throughout the mean moisture content of the 14 experimental trials, those experimental trials containing significant amount of sorghum in the recipe had shown the highest moisture content as compared to the moisture content of the control (trial1) which might be due to enhanced water absorption capacity of the recipes when sorghum was incorporated in relatively higher amount. The moisture content of those experimental trials whose teff proportion was the highest had shown the least moisture content as compared to the control. But in experimental trial 13 the moisture content (63.67%) was even the least as compared to the control (trial1). In the recipe of trial 13 the content of maize (50%) and teff (50%) were equal. Such least moisture content may

be associated with the highest proportion of maize and the minimal proportion of teff in the recipe since the highest fat composition of maize might significantly affect the water absorption capacity of trial 13. The reason for the other situation might be associated with a better water holding capacity of the experimental trials containing significant amount of sorghum in the recipe, also the blending and processing effect might also have impact on the moisture content.

The mean total carbohydrate content of the experimental trials varies from each other depending on the type and amount of ingredients incorporated in each of the recipe of the trials, and depending on their moisture, total ash, protein & fat content. Those experimental trials whose moisture content was the least trial 13 (19.45%) and trial 11 (19.81%) had shown the highest carbohydrate content as compared to the control trial 1 (17.98%). With regard to all other experimental trials their carbohydrate content was higher than the control trial 1 which was in a similar manner associated with their highest moisture content as compared to the control trial 1.

The mean total energy of those experimental trials containing large amount of maize in the recipe had shown the highest total energy value, trial 11 and trial 13 had 50% maize in the recipe, and their total energy was 157.6 Kcal/100g and 157.41 Kcal/100g which was the highest as compared to the control trial 1 (133.39Kcal/100g). The reason was due to the highest fat content of maize (5.18%) as compared to sorghum (3.69%) and teff (2.69%).

9.4. Sensory acceptability of the experimental trials

The mean sensory acceptability results of the 14 experimental trials for color, taste, texture, appearance, odor and over all acceptability obtained from the experiment was as in (Table 8) and had been used for formulation optimization.

Table 8: Sensory acceptability of the experimental trials

Trials	Recipe			Color	Taste	Texture	Odor	Appearance	Overall acceptability
	Teff	Sorghum	Maize						
Trial1 (control)	1.000	0.00	0.00	4.00±0.23 ^{ab}	3.53±0.22 ^{bcd}	2.79±0.26 ^{abc}	3.68±1.06	2.74±0.30 ^{ab}	3.37±0.19 ^{bcd}
Trial 2	0.833	0.083	0.083	4.68±0.14 ^c	3.79±0.18 ^{cd}	4.16±0.18 ^f	3.63±0.83	4.05±0.18 ^d	4.16±0.14 ^c
Trial 3	0.75	0.25	0.000	3.84±0.23 ^a	3.16±0.22 ^{abc}	3.05±0.20 ^{abcd}	3.32±1.11	3.05±0.27 ^{ab}	3.16±0.21 ^{bc}
Trial 4	0.75	0.25	0.000	3.86±0.22 ^a	3.67±0.20 ^{cd}	3.67±0.23 ^{def}	3.43±0.98	3.38±0.22 ^{abcd}	3.52±0.20 ^{bcd}
Trial 5	0.50	0.25	0.25	3.90±0.21 ^a	2.86±0.19 ^{ab}	2.57±0.24 ^a	2.71±1.19	2.71±0.23 ^a	2.43±0.20 ^a
Trial 6	0.50	0.50	0.00	4.24±0.15 ^{abc}	3.86±0.19 ^d	3.71±0.23 ^{def}	3.38±1.07	3.52±0.24 ^{bcd}	3.71±0.23 ^{cde}
Trial 7	0.583	0.083	0.333	4.19±0.19 ^{abc}	3.19±0.23 ^{abcd}	2.63±0.32 ^{ab}	3.38±0.96	3.13±0.24 ^{abc}	3.00±0.23 ^{ab}
Trial 8	0.75	0.00	0.25	4.25±0.17 ^{abc}	3.31±0.24 ^{abcd}	3.44±0.24 ^{cdef}	3.38±1.03	3.44±0.20 ^{abcd}	3.25±0.17 ^{bcd}
Trial 9	1.00	0.00	0.00	3.69±0.22 ^a	3.88±0.22 ^d	3.81±0.25 ^{ef}	3.69±0.87	3.44±0.30 ^{abcd}	3.69±0.20 ^{cde}
Trial 10	0.667	0.167	0.167	4.12±0.21 ^{abc}	3.12±0.17 ^{abc}	3.35±0.21 ^{bcd}	3.53±0.87	3.47±0.23 ^{abcd}	3.41±0.17 ^{bcd}
Trial 11	0.50	0.00	0.50	4.06±0.20 ^{abc}	2.76±0.20 ^a	2.82±0.21 ^{abc}	3.24±0.90	3.12±0.23 ^{abc}	2.88±0.19 ^{ab}
Trial 12	0.50	0.50	0.00	4.00±0.23 ^{ab}	3.24±0.25 ^{abcd}	3.06±0.23 ^{abcd}	3.47±0.87	3.41±0.21 ^{abcd}	3.29±0.21 ^{bcd}
Trial 13	0.50	0.00	0.50	4.60±0.16 ^{bc}	3.67±0.19 ^{cd}	3.73±0.21 ^{def}	3.60±0.74	3.87±0.24 ^{cd}	3.82±0.20 ^{de}
Trial 14	0.583	0.333	0.083	3.93±0.15 ^a	3.87±0.22 ^d	4.13±0.19 ^f	3.93±0.80	4.00±0.14 ^d	4.15±0.15 ^c

*The data is expressed as mean± standard error. *Any two means in the same column not followed by the same letters are significantly different at (p<0.05) and any one means in the same column followed by same letters are similar.

The mean color response of the 14 experimental trials varies from 3.68 to 4.68 (from very liked to extremely liked). Even though the preference of the panelists varies significantly at significance level of p<0.05 in a group of three among the 14 experimental trials, all the formulations were liked more than average by the panelists, similar to that of the control trial 1(4.00). Those formulations having maize in the recipe had shown a relatively maximum color response (Trial13=4.6 and Trial 2=4.68) suggesting that the ingredient maize had good coloring effect.

The mean taste response of the 14 experimental trials varies from 2.76 to 3.87 (from liked to very liked). Even though the preference of the panelists varies significantly at significance level of p<0.05 in a group of four among the 14 experimental trials, all the formulations were liked by the panelists. Those experimental trials that relatively scored the least value (Trial11=2.76) might

be associated with processing effect thus through improvement of the process it might be possible to improve the scores.

The mean texture response of the 14 experimental trials varies from 2.57 to 4.16 (from liked to very liked). Even though the preference of the panelists varies significantly at significance level of $p < 0.05$ in a group of six among the 14 experimental trials, all the formulations were liked by the panelists. Those experimental trials that relatively scored the least value (Trial5=2.57) might be associated with processing effect thus through improvement of the process it might be possible to improve the scores.

The mean odor response of the 14 experimental trials varies from 2.71 to 3.93 (from liked to very liked). The preference of the panelists for 14 experimental trials was similar that was there was no variation among all the trials. In the same manner as discussed above best maximum score can be achieved through improvement of the processing in *Injera* preparation.

The mean appearance response of the 14 experimental trials varies from 2.71 to 4.05 (from liked to very liked). Even though the preference of the panelists varies significantly at significance level of $p < 0.05$ in a group of four among the 14 experimental trials, all the formulations were liked by the panelists. In the same manner as discussed above best maximum score can be achieved through improvement of the processing in *Injera* preparation.

The mean overall acceptability response of the 14 experimental trials varies from 2.88 to 4.16 (from liked to very liked). Even though the preference of the panelists varies significantly at significance level of $p < 0.05$ in a group of five among the 14 experimental trials, all the formulations were liked by the panelists. Exception was trial 5 (2.43) which was least liked and this was associated with processing problem that it was observed during slurry dough preparation the binder which we call it *Absit* was extra added un intentionally which affected the result, thus still improving the process can improve the overall sensory acceptability of all the formulations.

Above 70% of the panelists response described on the questionnaire, that their preference of *Injera* in terms of ingredient proportion was 100% teff but on the actual sense the panelists gave

a mean score of more than 3.50 (very liked) for over all acceptability, trial 13 (3.87), 50% maize and 50% teff blended *Injera* which was more than the control trial1 (3.37=liked), 100% teff *Injera*. Thus it may be possible to infer that the panelists thought the actually 50:50 teff and maize *Injera* was 100% teff *Injera* but actually not thus the *Injera* from even up to 50% maize/sorghum blended teff can be eaten being liked.

Nine equally spaced points on each of the 5x5 inch *Injera* samples were selected, 1x1 inch square was drawn, and again inside the 1x1 inch square 0.4x0.4 & 0.3x0.3 inch squares were joined to get nine small four sided quadrilaterals in which four of them were 0.3x0.3 inch squares at the corner, one was 0.4x0.4 inch square at the center, two 0.4x0.3 inch rectangles and another two 0.3x0.4 inch rectangles were on the side edge of the 1x1 inch squares. The counted number of eyes were at 0.16 inch separation inter distance, those *Injera* eyes which were not fully included in the 1x1 inch square were counted in the square where it was included more than half and the total number of eyes from the nine 1x1 inch square were averaged and compared for similarity/difference at significance level of $p < 0.05$.

Table 9: Mean number of eyes in 1x1 inch square of *Injera*'s of the 14 experimental trials

Experimental trials	Recipe			Mean Number of eyes in 1x1inch square
	Teff	Sorghum	Maize	
Trial 1 (control)	1.000	0.00	0.00	43.00±1.05 ^{ab}
Trial2	0.833	0.083	0.083	53.00±1.90 ^{ef}
Trial3	0.75	0.25	0.000	49.78±2.91 ^{cde}
Trial4	0.75	0.25	0.000	44.78±1.22 ^b
Trial5	0.50	0.25	0.25	39.78±1.16 ^a
Trial6	0.50	0.50	0.00	51.89±1.20 ^{ef}
Trial7	0.583	0.083	0.333	46.22±0.74 ^{bcd}
Trial8	0.75	0.00	0.25	54.78±2.08 ^f
Trial9	1.00	0.00	0.00	46.33±1.00 ^{bcd}
Trial10	0.667	0.167	0.167	45.44±1.44 ^{bc}
Trial11	0.50	0.00	0.50	52.11±1.35 ^{ef}
Trial12	0.50	0.50	0.00	50.67±1.29 ^{def}
Trial13	0.50	0.00	0.50	59.89±1.42 ^g
Trial14	0.583	0.333	0.083	55.56±0.65 ^{fg}

*The data is expressed as mean± standard error. *Any two means in the same column not followed by the same letters are significantly different at ($p < 0.05$) and any one means in the same column followed by the same letters are similar means.

The mean number of eyes of *Injera*'s of the 14 experimental trials in 1x1 inch square ranges from 40 (Trial 5) up to 60 (Trial 13). When this number of eyes were correlated with the overall acceptability scores, trial 5 had score of 2.43 (least liked), trial 13 had score of 3.82 (very liked), trial 1(control) had a score of 3.37 (liked) and with its number of eyes was 43. The number of eyes score for most of the experimental trials were within 40 and around 50 which were quite far from the score for trial 13 (60); thus, liked number of eyes limit was established as the mean value of the control ($43 \pm$ its standard deviation that was 43 ± 3) and the preference of the panelists for the number of eyes of *Injera* was 43. Based on this limit 8 of the trials were out of the limit while 6 of them were within the limit which means compositing teff with sorghum and maize had affected the number of eyes of *Injera* made from the blend.

9.5. Formulations optimized

Formulation optimization was performed based on the three specific objectives. The first was formulation optimization based on macro-nutritional criterion (crude protein, crude fat, total carbohydrate, total energy and crude fiber where their model gave significant response, used to navigate the design) of the experimental trials, in terms of ingredient the ratio of teff was constrained to be minimum considering the associated cost, and sorghum & maize were constrained to be maximum. The mean crude protein composition (11.34-14.73) %, mean crude fat composition (1.79-3.59) % & mean total carbohydrate composition (7.14 -19.8) % were in the range, the mean crude fiber composition was maximized and the mean total energy was minimized. The second formulation optimization was based on sensory acceptability criterion of the experimental trials, and in terms of ingredient the same as the first one. The mean color response was in range (3.69-4.65, from very liked to extremely liked) since all the color of the experimental trials were liked by the panelists more than average, and the mean taste, texture, odor, appearance & over all acceptability responses were maximized. The third optimization was based on the combination of the above two objectives. Note that best optimization formula was obtained with the first objective that was based on the macro-nutritional parameters since the

model responses were sound and significant provided the remaining were also important at the above defined criteria. Thus three optimized formulations and one control were resulted which were:

- A. The first formulation was labeled to be formulation one (F1) which was the control 100% teff containing formulation.
- B. The second formulation was labeled to be formulation two (F2) which was in the ratio of 0.554 (teff), 0.373 (sorghum) and 0.073 (maize), the formulation optimized based on both macro-nutritional & sensory parameters with a desirability of 0.538 (Figure 8).
- C. The third formulation was labeled to be formulation three (F3) which was in the ratio of 0.500 (teff), 0.307 (sorghum) and 0.193 (maize), the formulation optimized based on macro- nutritional parameter, the best formulation with maximum desirability of 0.684 (Figure 9).
- D. The fourth formulation was labeled to be formulation four (F4) which was in the ratio of 0.666 (teff), 0.198(sorghum) and 0.136 (maize), the formulation optimized based on only sensory acceptability parameters with a better desirability of 0.548 and better chosen than F2 (Figure 10).

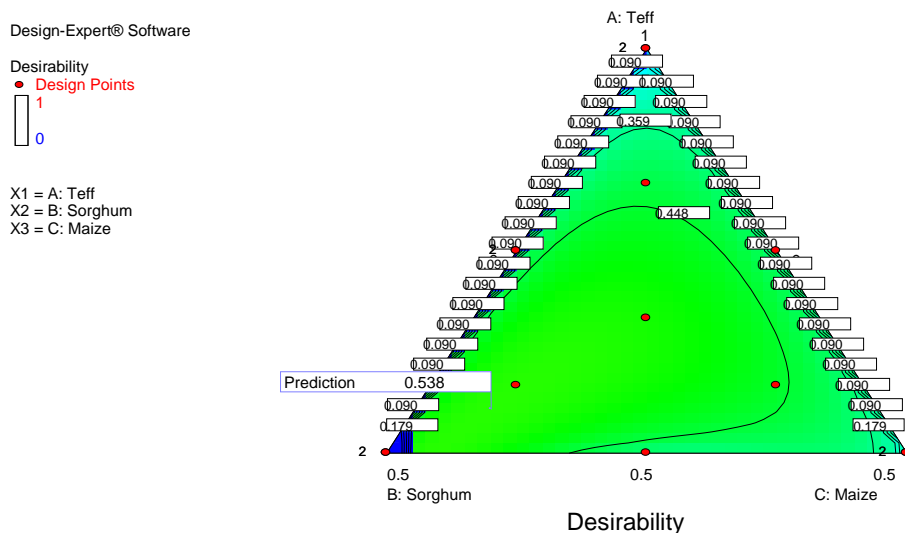


Figure 8: Contour plot for formulation two (F2)

In the contour plot (Figure 7) the desirability multiple response function was predicted to be 0.538 which means the desirability reflected the desirable response range for each of the response parameters at the point indicated on the contour plot. As per the criteria defined for optimization, the region indicated on the contour plot is the most desired region where we got needed response with a desirability value of 0.538.

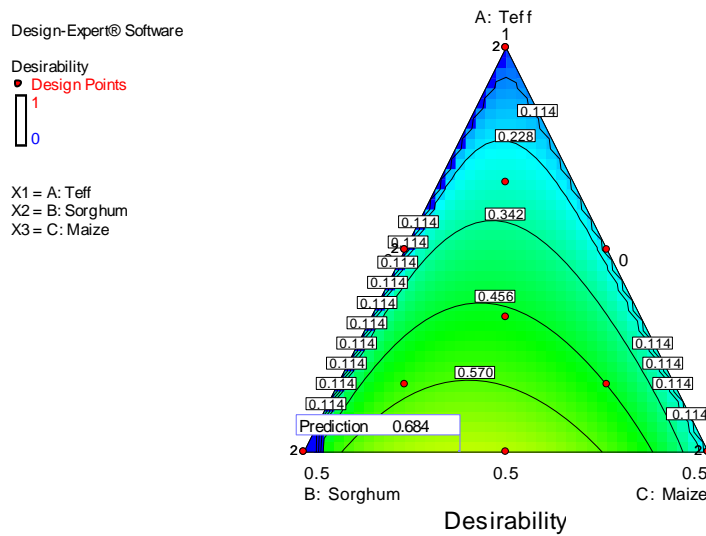


Figure 9: Contour plot for formulation three (F3)

In the contour plot (Figure 9) the desirability multiple response function was predicted to be 0.684 which means the desirability reflected the desirable response range for each of the response parameters at the point indicated on the contour plot. As per the criteria defined for optimization, the region indicated on the contour plot is the most desired region where we got needed response with a desirability value of 0.684.

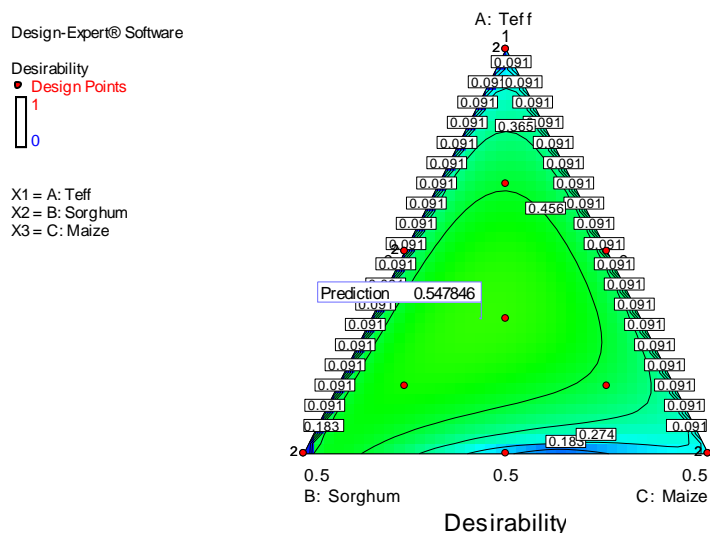


Figure 10: Contour plot for formulation four (F4)

In the contour plot (Figure 10) the desirability multiple response function was predicted to be 0.547846 which means the desirability reflected the desirable response range for each of the response parameters at the point indicated on the contour plot. As per the criteria defined for optimization, the region indicated on the contour plot is the most desired region where we got needed response with a desirability value of 0.547846.

9.5.1. Proximate composition of the optimized formulations

The proximate composition results of the optimized formulations were presented in table 10 below.

Table 10: Proximate composition of the optimized formulations

Formulation	Recipe			Crude Protein (%)	Crude Fat(%)	Crude Fiber(%)	Total ash (%)	Moisture content (%)	Total CHO (%)	Total Energy (Kcal/100g)
	Teff	Sorghum	Maize							
F1-control	1.000	0.000	0.000	11.34±0.00 ^a	1.79±0.03 ^a	6.00±0.03 ^a	2.98±0.03 ^c	65.94±0.06 ^c	17.98±0.02 ^c	133.39±0.35 ^a
F2	0.554	0.372	0.073	16.00±0.10 ^c	3.01±0.27 ^b	8.47±0.04 ^{bc}	2.43±0.03 ^b	68.37±0.20 ^d	10.33±0.15 ^a	132.39±2.21 ^a
F3	0.500	0.307	0.193	15.81±0.15 ^c	3.01±0.27 ^b	8.66±0.09 ^c	2.22±0.01 ^a	60.98±0.11 ^a	18.02±0.06 ^c	162.37±2.07 ^c
F4	0.666	0.198	0.136	15.02±0.05 ^b	2.74±0.00 ^b	8.38±0.03 ^b	2.41±0.02 ^b	64.77±0.08 ^b	15.03±0.20 ^b	144.82±0.60 ^b

*The data is expressed as mean± standard error on a dry weight basis. *Any two means in the same column not followed by the same letters are significantly different at (p<0.05) and any one means in the same column followed by the same letters are means.

The mean crude protein composition of the optimized formulations including the control ranges from 11.34%-16.00% and the values for optimized formulations had shown to have maximum crude protein composition (F2=16%, F3=15.81% & F4=15.02%) as compared to the control F1 (11.34%). When these values were compared with the predicted value of 14.06%, the values of optimized formulations were close to the predicted crude protein composition. When we compare these results in relation to their recipe, those recipes having large amount of sorghum (F2 & F3) were found to have improved maximal protein composition, even formulation F4 had shown to have high crude protein composition as compared to the control F1 owing to its sorghum proportion.

The mean crude fat composition of the optimized formulations including the control ranges from 1.79%-3.01% and the values for the optimized formulations had shown to have maximum crude fat composition (F2=3.01%, F3=3.01% & F4=2.74%) as compared to the control F1 (1.79%). When these values were compared with the predicted value of 2.64%, the values of optimized formulations were close to the predicted crude fat composition. When we compare these results in relation to their recipe, all the optimized formulations had shown maximal crude fat composition as compared to the control due to the presence of maize in their recipe. In addition all the mean crude fat composition of the optimized formulations were similar and all were significantly different from the control at the significance level of $p < 0.05$.

The mean crude fiber composition of the optimized formulations including the control ranges from 6.00% -8.66%. When the crude fiber composition of the three optimized formulations were compared with control, maximal crude fiber content had been shown owing to the blending especially incorporation of sorghum in their recipe and as compared to the predicted value (8.42%) the fiber content of the formulations was closer and improved. Thus in relation to our objective criteria to maximize fiber composition, the optimized formulations had met the target.

The total ash composition of the optimized formulations including the control ranges from 2.22%-2.98%. The total ash composition of F3(2.22%) which was the least and those of F2 (2.43%) and F4(2.41%) higher than F3 but lower than F1(2.98%). Those formulations containing larger amount of teff had shown relatively higher total ash content.

The mean moisture content of the optimized formulations including the control ranges from 60.98%-68.37%. The moisture content of F2 (68.37%) was the maximum which might be due to its high sorghum proportion in its recipe and F3 (60.98%) was the least which might be due to its high maize proportion in its recipe.

The mean total carbohydrate composition of the optimized formulations including the control ranges from 10.33%-18.02%. The carbohydrate composition of F3 (18.02%) was the maximum which might be due to its high maize proportion in its recipe and F2(10.33%) was the least which might be due to its high sorghum proportion in its recipe.

The mean total energy value of the optimized formulations including the control ranges from (132.39-162.37)Kcal/100g. The total energy value of F3 (162.37Kcal/100g) was the maximum which was due to its high fat and carbohydrate content and F2(132.39Kcal/100g) was the least which was due to its least carbohydrate content. All changes were directly associated with their recipe proportion.

9.5.2. Sensory acceptability of the optimized formulations

The sensory acceptability results of the optimized formulations in (Table 11) were mean results of color, taste, texture, appearance, odor and over all acceptability of main effect formulation variation keeping day variation constant (Table 11), main effect day variation keeping formulation variation constant (Table 12) and effect from the interaction of the two main effects (Table 13) based on two way variance analysis.

Table 11: Mean sensory acceptability of the optimized formulations due to variation of formulation.

Effect of variation of the different optimized formulations on the sensory acceptability of <i>Injera</i> (first main effect)						
Formulation	Color	Taste	Texture	Odor	Appearance	Overall acceptability
F1	3.68±0.12 ^a	3.47±0.14 ^a	3.70±0.13 ^b	3.17±0.14 ^a	3.50±0.13 ^a	3.52±0.12 ^a
F2	3.92±0.12 ^a	3.20±0.14 ^a	3.28±0.13 ^a	3.18±0.14 ^a	3.53±0.13 ^a	3.33±0.12 ^a
F3	3.68±0.12 ^a	3.18±0.14 ^a	3.08±0.13 ^a	3.32±0.14 ^a	3.32±0.13 ^a	3.15±0.12 ^a
F4	3.87±0.12 ^a	3.22±0.14 ^a	3.00±0.13 ^a	3.18±0.14 ^a	3.22±0.13 ^a	3.33±0.12 ^a

*The data is expressed as mean± standard error. *Any two means in the same column not followed by the same letters are significantly different and any two means in the same column followed by the same letters are similar means at (p<0.05).

When the mean color, taste, odor, appearance and over all acceptability of the three optimized formulations (F2, F3, & F4) were compared with the control formulation (F1), there was no significant difference due to the first main effect (formulation) variation, thus the optimized formulations were liked by the panelists with a similar liking degree for all the sensory parameters with the exception of texture at a p<0.05 (Table 11).

Table 12: Mean sensory acceptability of the optimized formulations due to variation of days.

Effect of variation of days on the sensory acceptability of <i>Injera</i> (second main effect)						
Day	Color	Taste	Texture	Odor	Appearance	Overall acceptability
Day1	4.01±0.10 ^b	3.56±0.12 ^b	3.59±0.11 ^b	3.4±0.12 ^b	3.73±0.11 ^b	3.59±0.10 ^b
Day2	3.73±0.10 ^a	3.20±0.12 ^a	3.14±0.11 ^a	3.36±0.12 ^b	3.30±0.11 ^a	3.30±0.10 ^a
Day3	3.63±0.10 ^a	3.04±0.12 ^a	3.08±0.11 ^a	2.83±0.12 ^a	3.15±0.11 ^a	3.11±0.10 ^a

*The data is expressed as mean± standard error. *Any two means in the same column not followed by the same letters are significantly different and any two means in the same column followed by the same letters are similar means at (p<0.05).

The mean color, taste, texture, odor, appearance and over all acceptability of the three optimized formulations were similar with the control formulation (F1) in the first day that was all of them for all of the six parameters had a very liked score while the scores for all the sensory parameters

with the exception of odor were significantly different after the first day (day 2) and after the second day (day 3) for all the formulations including the control at a significance level of $p < 0.05$ but the formulations were still liked by the panelists even after three days storage of the *Injera* samples for all the six sensory parameters (Table 12), thus the *Injera* prepared from the optimized formulations can be eaten for three days being liked in a comparable manner with 100% teff *Injera*.

Table 13: Mean sensory acceptability of the optimized formulations due to the interaction of the main effects.

Effect on sensory acceptability of <i>Injera</i> due to the interaction of change of formulation and days (factor from interaction)							
Day	Form.	Color	Taste	Texture	Odor	Appearance	Overall acceptability
Day1	F1	4.30±0.21 ^a	3.85±0.24 ^a	4.10±0.22 ^a	3.45±0.24 ^a	4.00±0.22 ^a	3.90±0.21 ^a
	F2	4.20±0.21 ^a	3.35±0.24 ^a	3.60±0.22 ^a	3.40±0.24 ^a	4.10±0.22 ^a	3.75±0.21 ^a
	F3	3.60±0.21 ^a	3.45±0.24 ^a	3.30±0.22 ^a	3.55±0.24 ^a	3.40±0.22 ^a	3.20±0.21 ^a
	F4	3.95±0.21 ^a	3.60±0.24 ^a	3.35±0.22 ^a	3.40±0.24 ^a	3.40±0.22 ^a	3.50±0.21 ^a
Day2	F1	3.50±0.21 ^a	3.35±0.24 ^a	3.50±0.22 ^a	3.25±0.24 ^a	3.40±0.22 ^a	3.50±0.21 ^a
	F2	3.90±0.21 ^a	3.25±0.24 ^a	3.20±0.22 ^a	3.50±0.24 ^a	3.50±0.22 ^a	3.25±0.21 ^a
	F3	3.60±0.21 ^a	3.10±0.24 ^a	3.20±0.22 ^a	3.35±0.24 ^a	3.25±0.22 ^a	3.15±0.21 ^a
	F4	3.90±0.21 ^a	3.10±0.24 ^a	2.65±0.22 ^a	3.35±0.24 ^a	3.05±0.22 ^a	3.30±0.21 ^a
Day3	F1	3.25±0.21 ^a	3.20±0.24 ^a	3.50±0.22 ^a	2.80±0.24 ^a	3.10±0.22 ^a	3.15±0.21 ^a
	F2	3.65±0.21 ^a	3.00±0.24 ^a	3.05±0.22 ^a	2.65±0.24 ^a	3.00±0.22 ^a	3.00±0.21 ^a
	F3	3.85±0.21 ^a	3.00±0.24 ^a	2.75±0.22 ^a	3.05±0.24 ^a	3.30±0.22 ^a	3.10±0.21 ^a
	F4	3.75±0.21 ^a	2.95±0.24 ^a	3.00±0.22 ^a	2.80±0.24 ^a	3.20±0.22 ^a	3.20±0.21 ^a

*The data is expressed as mean± standard error. *Any two means in the same column not followed by the same letters are significantly different and any two means in the same column followed by the same letters are similar means at ($p < 0.05$).

The mean sensory acceptability response of all the six parameters were not affected significantly at a $p < 0.05$ due to the effect from the interaction variation of both factors (Table 13), thus the interaction of the main effects had no significant effect on the sensory parameters.

The mean color response of the *Injera*'s from all the formulations was shown to range from 3.63 to 4.01 after the first day of its preparation to the third day which was all liked (Table 12). The mean color response of the *Injera*'s during all the three days was shown to range from 3.68 to 3.92 all very liked across the four formulations (Table 11) and were all similar at significance level of $p < 0.05$. The mean color response due to the interaction of the two factors (Table 13, different days and different formulations) was shown to range from 3.25 to 4.30 (from liked to very liked). When the grand mean (3.79=very liked) was compared with the control F1 (4.3=very liked) of all the three days, the color response was maximal and was similar with the control without being affected due to storage of *Injera* for three days, due to compositing or blending of teff (formulation variation) and due to variations from the interaction of the two factors.

The mean taste response of the *Injera*'s from all the formulations was shown to range from 3.04 to 3.56 after the first day of its preparation to the third day which was from liked to very liked (Table 12). The mean taste response of the *Injera*'s during all the three days was shown to range from 3.18 to 3.47 (from liked to very liked) across the four formulations (Table 11) and were all similar at significance level of $p < 0.05$. The mean taste response due to the interaction of the two factors (Table 13, different days and different formulations) was shown to range from 2.95 to 3.85 (from liked to very liked). When the grand mean (3.27= liked) was compared with the control F1 (3.85=very liked) of all the three days, the taste response was still liked even after three days storage of *Injera* samples, after compositing or blending of teff (formulation variation) and after interaction of the two factors.

The mean texture response of the *Injera*'s from all the formulations was shown to range from 3.08 to 3.59 after the first day of its preparation to the third day which was from liked to very liked (Table 12). The mean texture response of the *Injera*'s during all the three days was shown to range from 3.00 to 3.70 (from liked to very liked) across the four formulations (Table 11). The mean texture response due to the interaction of the two factors (Table 13, different days and different formulations) was shown to range from 2.65 to 4.10 (from liked to very liked). When the grand mean (3.27= liked) was compared with the control F1 (4.10=very liked) of all the three days, the texture response was still liked even after three days storage of *Injera* samples, after compositing or blending of teff (formulation variation) and after interaction of the two factors.

The mean odor response of the *Injera*'s from all the formulations was shown to range from 2.83 to 3.40 after the first day of its preparation to the third day which was all liked (Table 12). The mean odor response of the *Injera*'s during all the three days was shown to range from 3.18 to 3.32 all liked across the four formulations (Table 11). The mean odor response due to the interaction of the two factors (Table 13, different days and different formulations) was shown to range from 2.65 to 3.55 (from liked to very liked). When the grand mean (3.21= liked) was compared with the control F1 (3.45=very liked) of all the three days, the odor response was still liked even after three days storage of *Injera* samples, after compositing or blending of teff (formulation variation) and after interaction of the two factors.

The mean appearance response of the *Injera*'s from all the formulations was shown to range from 3.15 to 3.73 after the first day of its preparation to the third day which was from liked to very liked (Table 12). The mean appearance response of the *Injera*'s during all the three days was shown to range from 3.22 to 3.53 from liked to very liked across the four formulations (Table 11). The mean appearance response due to the interaction of the two factors (Table 13, different days and different formulations) was shown to range from 3.00 to 4.10 (from liked to very liked). When the grand mean (3.39= liked) was compared with the control F1 (4.00=very liked) of all the three days, the appearance response was still liked even after three days storage of *Injera* samples, after compositing or blending of teff (formulation variation) and after interaction of the two factors.

The mean over all acceptability response of the *Injera*'s from all the formulations was shown to range from 3.1 to 3.59 after the first day of its preparation to the third day which was from liked to very liked (Table 12). The mean over all acceptability response of the *Injera*'s during all the three days was shown to range from 3.15 to 3.52 from liked to very liked across the four formulations (Table 11). The mean over all acceptability response due to the interaction of the two factors (Table 13,different days and different formulations) was shown to range from 3.00 to 3.90 (from liked to very liked). When the grand mean (3.33= liked) was compared with the control F1 (3.90=very liked) of all the three days, the overall acceptability response was still

liked even after three days storage of *Injera* samples, after compositing or blending of teff (formulation variation) and after interaction of the two factors.

9.5.3. Number of eyes of *Injera*'s of the optimized formulations including the control

Result for the counted number of eyes of the optimized formulations as in (Table 14) below:

Table 14: Mean number of eyes in 1x1 inch square of *Injera*'s of the optimized formulations

Optimized formulations	Recipe			Mean Number of eyes in 1x1 inch square
	Teff	Sorghum	Maize	
F1 (control)	1.000	0.00	0.00	44.11±2.20 ^a
F2	0.554	0.372	0.073	43.44±2.59 ^a
F3	0.500	0.307	0.193	43.89±2.72 ^a
F4	0.666	0.198	0.136	43.33±2.46 ^a

*The data is expressed as mean± standard error. *Any two means in the same column not followed by the same letters are significantly different and any two means in the same column followed by the same letters are similar means at (p<0.05).

The mean number of eyes of *Injera*'s of the optimized formulations in 1x1 inch square ranges from 43.33 (F4) up to 44.11 (F1, control). When this number of eyes were correlated with the grand mean overall acceptability score (3.33=liked), and the control f1 (3.90=very liked). Thus the number of eyes range of the *Injera* from (43-44) was the liked preference of the panelists and it was in the range of the established limit based on the experimental trials (40-46).

9.5.4. Fermentation pH kinetics of the optimized formulations

The fermentation pH kinetics of the optimized formulations including the control was conducted through the measurement of the pH of the dough every 2 hours for 72 hours then plotting pH versus time (Figure11), calculating and averaging the rate of change of pH over time (pH kinetics, Table 15).

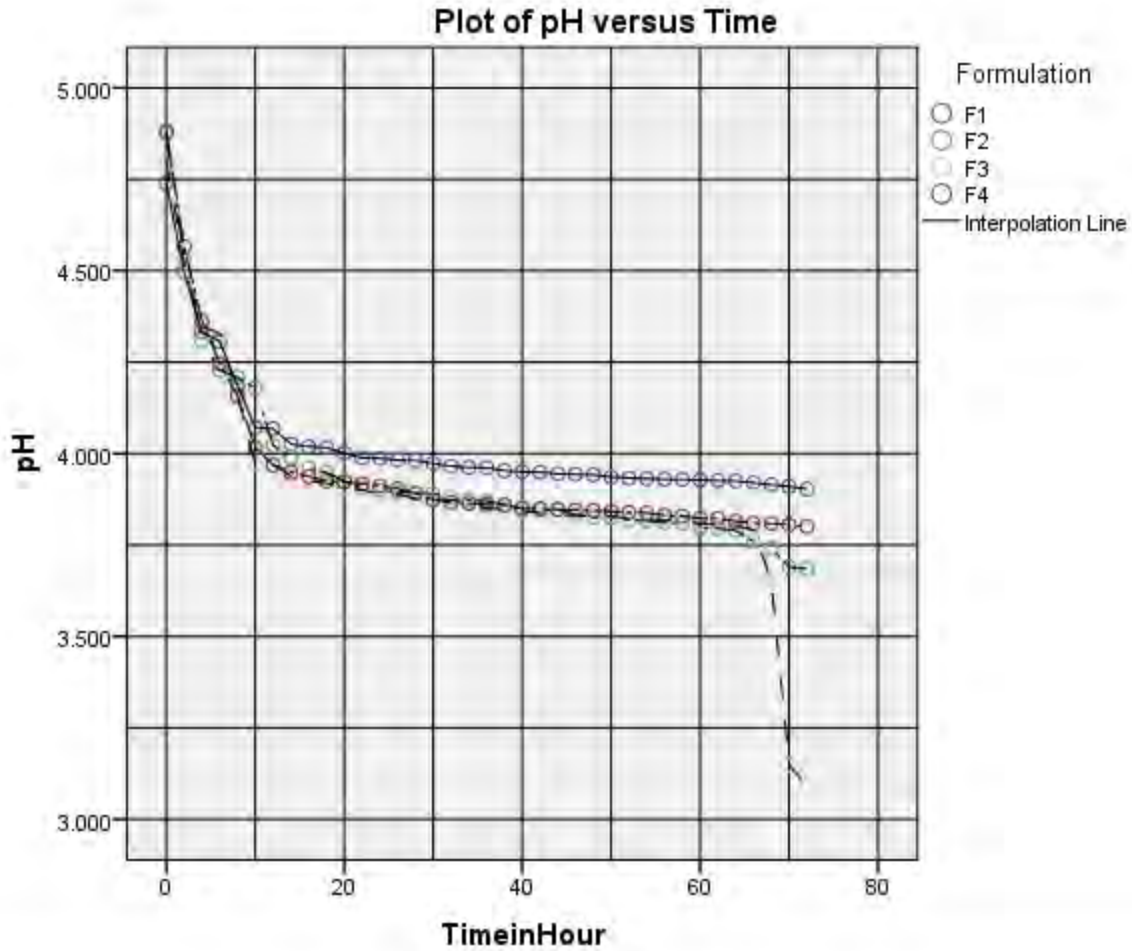


Figure 11: Graphical display of change of pH of fermenting dough over time

* F1 (100%) is formulation one (control) and F2 (teff=0.554, sorghum=0.372 and maize=0.073), F3(teff=0.50, sorghum=0.307 and maize=0.193)&F4(teff=0.666, sorghum=0.198 and maize=0.136) are the three optimized formulations.

As the graph on (Figure 11) indicate, the change of pH over time of the three optimized formulations and the control decreased in a similar manner up to 10hours of fermentation but the decrease in pH over time of the optimized formulations continued then after with relatively higher rate of change of pH per hour than the control while the control formulation pH continued decreasing in a relatively slow rate up to the end of the 72 hours. The rate of decrease in pH of the three formulations continued in a similar manner up to the 60hours then after the pH decrease rate of F2 & F3 became faster up to around 70 hours and again the pH decrease rate of F3 increased significantly while that of the other formulations including the control remained decreasing in an almost constant rate (Table 15 below). The difference in pH change rate was

obviously affected due to composite flour blending of teff with sorghum & maize in an almost same or similar pH kinetics in 72 hours fermentation time and pH change rate can be best predictor of fermentation rate but further microbiological & chemical studies need to be conducted to best predict the rate of fermentation and investigate the contributing factors of fermenting dough for *Injera* preparation. In addition the pH of F1 reached at 3.903 in 72 hours, F2 reached at 3.900 after 24 hours, F3 reached at 3.901 after 22 hours and F4 reached at the pH of 3.904 after 26 hours of fermentation. Therefore decreasing the fermentation period of the three optimized formulations from 72 hours to 24hours up to 30 hours might improve the sensory acceptability as compared to the control F1 especially taste since the relatively lower taste response of the optimized formulations might be due to sourness as fermentation continued for extended period.

Table 15: The mean maximum rate of change of pH per hour of the optimized formulations and the control fermenting dough

Formulation	Mean pH kinetics in pH per unit time (hr.)
F1(control)	-.011639±0.004401 ^a
F2	-.017431±0.005834 ^a
F3	-.040486±0.020394 ^a
F4	-.016181±0.005558 ^a

* The maximum values of rate of change of pH over time is expressed as mean ±standard error.

The maximum rate of change of the pH of the optimized formulations as compared to the control was similar at a significance level of $p < 0.05$. Even if effect of blending had not brought significant impact on the pH kinetics as the mean rate of change of pH indicates in (Table15), the mean rate of change of pH of F2, F3 &F4 was higher than F1(control). The reason why of this was probably associated with the proportion of the ingredients in their formulation that as the amount of teff increases the pH change rate decreases while the rate increases when the teff proportion decreases, thus in this respect the compositing of teff with sorghum and maize slightly affected or had impact on pH kinetics of the dough for *Injera* preparation, especially the increase of maize proportion while teff proportion was decreasing, which also might be due to improvement of the starch digestibility rate for fermenting organisms.

10. Conclusion and Recommendation

10.1. Conclusion

Injera prepared from the composite flour blend of teff with sorghum and maize had brought effect in different ways as compared to the *Injera* prepared from 100% teff as follows:

With regard to sensory acceptability, the acceptability of *Injera* from 100% teff was superior in preference over *Injeras* from the three optimized formulations for its taste, texture, appearance and over all acceptability; however, similarity was found out for color & odor responses. But all the three optimized formulations were liked while the control was very liked for taste, texture, appearance & odor responses within three days.

With regard to the macro-nutritional quality, the protein, fat and crude fiber content of the *Injeras* from the three optimized formulations were increased by up to 5%, 1% and 2.66%, respectively as compared to the *Injera* prepared from 100% teff (control). The carbohydrate content of the *Injera* prepared from the optimized formulations was decreased from 3% (F4=15.03%) up to 8% lower (F2=10.33%) as compared to the 100% teff *Injera* (F1=17.98%) with exception of *Injera* prepared from (F3=18.02%) which had similar or un-affected carbohydrate content as compared to the control *Injera*. Concerning total energy value, the energy value for formulation F3 & F4 was increased by 11.43Kcal/100g & 28.98Kcal/100g respectively while that of F2 (132.39Kcal/100g) remains un-affected significantly due to flour composite blending as compared to the control F1(133.39Kcal/100g).

With regard to pH kinetics and change of pH over time, teff flour composite blending with sorghum and maize flour in the preparation of *Injera* had accelerated the change of pH over time in an almost constant rate or constant pH kinetics. Thus the fermentation period of the optimized formulations should be limited to one day up to one and half day to get comparable sensory acceptability response with that of the control.

Formulation two, F2 (teff=0.554, sorghum=0.372 and maize=0.073) is the recommended recipe in terms of decreased energy value (from F1=133.39Kcal/100g to F2=132.39Kcal), increased fiber content (from F1=6.00% to F2=8.47%), in terms of sensory acceptability even if F1 was

very liked F2 was also liked and this sensory acceptability value can be improved by shortening fermentation period from three days up to one and half day. Formulation F2 is preferable and healthy especially for people whose life style is sedentary and not energy demanding in any case.

Formulation F3(teff=0.500, sorghum=0.307, maize=0.193) is the most desired & best formulation with maximum desirability of 0.684 in terms of cost and quality. F3 is the best since its model gives sound response at a significant level of $p < 0.05$.

Formulation F4(teff=0.666, sorghum=0.198 and maize=0.136) is relatively costly formulation as compared to F2&F3 as its teff proportion is almost 67% but it is still the preferred formulation with desirability of 0.548 in terms of quality and also for people who demand energy in any case. Thus when people make use of the optimized formulations properly, the cost burden from teff can be reduced significantly with comparable quality as that of teff.

10.2. Recommendation

The following recommendations are made based on the study from the present research:

The study from this research has resulted in an optimized formulations for *Injera* preparation thus there is a possibility to make use of the formulations so that the public can be benefited cost wise plus nutritionally in a comparable manner with that of *Injera* prepared from 100% teff, entrepreneurs and so industries can make business by making use of these formulations.

Based on the information from this research, further studies as starch digestibility especially to definitely verify how suitable the optimized formulations for people who are affected by diabetes, protein digestibility and further microbiological and chemical studies on fermentation kinetics are recommended to be conducted.

With the use of design expert software more researches need to be done for food formulation and optimization especially on Ethiopian foods based on cereals.

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12. Appendix

12.1. Sensory evaluation score sheet and questionnaire

Occupation:	Health status: ok <input type="checkbox"/> not ok <input type="checkbox"/>	Age:	Sex: Male <input type="checkbox"/> Female <input type="checkbox"/>			
Name:		Product:				
Panelist No.:		Date:				
Instruction Taste the given samples, and then place “√” mark on the scale point which best describes your feeling. Thank you in advance.						
Score	Sample code:					
	Color	Taste	Texture	Odor	Appearance	Over all acceptability
(5) The characteristics is extremely liked						
(4) The characteristics is very liked						
(3) The characteristics is liked						
(2) The characteristics is least liked						
(1) The characteristics is not liked						

Name:		Product:				
Panelist No.:		Date:				
Instruction Taste the given samples, and then place “√” mark on the scale point which best describes your feeling. Thank you in advance.						
Score	Sample code:					
	Color	Taste	Texture	Odor	Appearance	Over all acceptability
(5) The characteristics is extremely liked						
(4) The characteristics is very liked						
(3) The characteristics is liked						
(2) The characteristics is least liked						
(1) The characteristics is not liked						

Name:		Product:				
Panelist No.:		Date:				
Instruction Taste the given samples, and then place “√” mark on the scale point which best describes your feeling. Thank you in advance.						
Score	Sample code:					
	Color	Taste	Texture	Odor	Appearance	Over all acceptability
(5) The characteristics is extremely liked						
(4) The characteristics is very liked						
(3) The characteristics is liked						
(2) The characteristics is least liked						
(1) The characteristics is not liked						

Questionnaire: please fill the following few inquiries after you finish filling the score card, thank you in advance

1. Please specify your work place: _____, and living place: _____
2. From your perspective, how do you describe a good *Injera* in terms of color: _____, thickness: _____ and eye size: _____?
3. Please specify the type of *Injera* you usually eat in terms of ingredient: _____, and if you know the proportion please mention it: _____
4. From the alternatives indicated which type of *Injera* is your preference?
 - a) Fresh and hot just after baking
 - b) Cold (place “√” mark on your preference): 1st day after baking 2nd day after baking 3rd day after baking

12.2. Panelists photograph while conducting sensory analysis



12.3. **Picture of *Injera* prepared based on optimized formulation F1**



7.5 **Picture of *Injera* prepared based on optimized formulation F2**



7.11. **Picture of *Injera* prepared based on optimized formulation F3**



7.12. **Picture of *Injera* prepared based on optimized formulation F4**



7.13. **Picture of teff grain with Varsity of Kuncho (DZ-Cr-387)**



7.14. **Picture of sorghum grain with Varsity of Gambella (1107)**



7.15. **Picture of maize grain with Varsity of (M-6Q)**



7.16. **Declaration**

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

Name: Solomon Legesse

Signature: _____

Date: _____

The thesis has been submitted with my approval as a supervisor:

Name : Kaleab Baye (Ph.D)

Signature: _____

Date: _____

Tilahun Bekele (Assistant professor)

Signature: _____

Date: _____