OPTIMIZATION of *TEFF* (*Eragrostis Teff*) *INJERA* MAKING PROCESS CONDITIONS for BETTER PHYSICOCHEMICAL and SENSORY QUALITY

M.Sc. THESIS

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MARCH 2020

JIMMA, ETHIOPIA

Optimization of *Teff* (*Eragrostis Teff*) *Injera* Making Process Conditions for Better Physicochemical and Sensory Quality

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M.Sc Thesis

*Submitted to the School of Graduate Studies, Jimma University, College of Agriculture and Veterinary Medicine, in Partial Fulfillment of the Requirements for the Degree of Master of Science (*M.Sc*) in Food Science and Technology*

March 2020

Jimma, Ethiopia

DEDICATION

This thesis manuscript is dedicated to my beloved father, who passed away before I have started my M.Sc. study and to my beloved mother who passed away while I was following my M.Sc. program. The dedication also extends to my beloved families and close relatives for all the sacrifices, wishes and laudable to my success in all my activities.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

Gizachew Merga was born in September 1989 in North Shoa, Ware Jarso Woreda to his mother Mrs. Wide Mola and Father Mr. Merga Bikila. He completed his Elementary Education in July 2004 at Jarso Tuti Elementary School and attended his junior and Secondary Schools in Gohatsion Senior and Secondary School from September 2004 to July 2010 in Gohatsion town.

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ACKNOWLEDGEMENTS

First, I would like to express my thanks to almighty God who gave me strength, endurance and enabling me to overcome all the challenges during my study and for successful completion of my research work within time.

Second, I would like to express my sincere gratitude to Dr. Yetenayet Bekele and Mr. Chala Gowe for their encouragement, supervision, scientific advice, and useful suggestions throughout this research work. Their moral support and continuous guidance enabled me to complete my work successfully.

I am grateful to the Debrezeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR) for providing research samples. I would also like to say a big thank to my wife Muliye Gemeda, families as well as friends like Gemeda Worku, Tolcha Techane, Abeya Tarefe, and others. Their support was much appreciated.

Finally, I would like to thank the Department of Post-Harvest Management, Jimma University College of Agriculture and Veterinary Medicine for their technical support and laboratory facilities during my work and special thanks goes to Mrs. Yirgalem Mokonin and Miss. Meskerem Haile for their support during baking *injera* and providing me stationery materials for the work.

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LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
AOAC	Association of Analytical Chemists
ANOVA	Analysis of Variance
BC	Before Christs
CCD	Central Composite Design
CRD	Completely Randomized Design
DZARC	Debrezeit Agricultural Research Center
EIAR	Ethiopian Institute of Agricultural Research
EPS	Exopolysaccharides
ESA	Ethiopian Standard Agency
Hcl	Hydrochloric Acid
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
KJ	Kilo Jule
LAB	Lactic Acid Bacteria
M.Sc	Master of Science
pH	Power of Hydrogen
PHM	Postharvest Management
RCBD	Completely Randomized Block Design
RSM	Response Surface Methodology
TA	Titratable Acidity; Texture Analyzer
TG	Transition Temperature
WAI	Water Absorption Index

ABSTRACT

Injera is a fermented and naturally leavened flatbread indigenous to Ethiopia. However, variation in processing steps results in the difference in the quality of injera, which might even baked from the same variety of teff. To minimize such variability it is necessary to study and develop pre-baking and baking standard procedures that fit for industrial and export purposes. In line with this, the research initiated to optimize pre-baking processing and baking conditions for the better quality of teff injera. Four consecutive experiments were conducted: The first and second phases of the study were conducted using response surface methodology with a central composite design to optimize the effect of primary fermentation condition (time and temperature), and absit mixing ratio, cooking time and secondary fermentation time respectively. Optimization was made by taking primary fermentation temperature and time in the range of 25–38℃ and 24–96hrs, absit mixing ratio of 5-15%, the cooking time of 5-15min and secondary fermentation time of 2-6hrs. Standard methods were used to conduct physicochemical quality analysis of injera. The result showed that primary fermentation condition and absit mixing ratio, absit cooking time and secondary fermentation time were influenced most of physicochemical quality parameters. Numerical optimization for these parameters were carried out by setting criteria (minium, maximum and in the range) for dependent variables. Accordingly, the best response variables were obtained at the optimal condition of 25℃ primary fermentation temperature for 64 hrs fermentation time, 8% mixing ratio of absit with 10 min of cooking and 4 hrs of secondary fermentation time. The third phase of the study was conducted using randomized complete block design with the factorial arrangement in 3 replications to evaluate the effect of baking temperature (195±5, 215±5, 235±5 and 255±5℃) and time (1, 2 and 3 minutes) on physicochemical and sensory quality of injera. The result indicated that the interaction effect of baking temperature and time differ significantly ($P < 0.05$) and 255±5℃ for 2 min or 235±5℃ for 3 min out weight other treatments in terms of all physicochemical and sensory quality evaluated. Finally, validation study was performed to evaluate the robustness of optimized pre-baking processing steps and baking condition for five different varieties of teff using a completely randomized block design in three replications. Results indicated that there were insignificant differences ($P > 0.05$) among four teff varieties in terms of physicochemical and sensory quality except for red teff. Therefore, the optimized pre-baking processing steps and baking condition could be used to produce better quality of teff injera at large commercial scale capacity to reach both local and export markets.

Keywords: *Baking Condition; Injera; Optimization; Prebaking Process*

1. INTRODUCTION

1.1. Background and Justification

Teff (Eragrostis *teff*) is one of the cereal crops widely cultivated in different agro-ecology zones of Ethiopia (Assefa *et al.,* 2015). It is believed to have originated in Ethiopia between 4000 and 1000 BC and primarily cultivated cereal crop in Ethiopia with high market price and scio-economic values (Dijkstra *et al.*, 2008). It is cultivated on over one million hectares of land each year comprising about 28.4% of the total cereal crops cultivated area (Jemal *et al.*, 2018) and gets popularity as an especial nutritious and healthy grain in recent time throughout the world. The crop is considered as one of African's traditional "Orphan crop" or "Lost crop" in developed nations, even though it is widely cultivated and consumed as a staple food by millions of Ethiopians (Minten *et al.*, 2013).

Teff grain has recently been receiving global awareness, because of its nutritional importance and does not contain gluten and is an increasingly important dietary component for individuals who suffer from gluten intolerance (Bemihiretu *et al.,* 2013; Satheesh and Fanta, 2018). The dietary fiber content of *teff* is several folds higher than that of maize, sorghum, wheat, and rice (Hager *et al.,* 2014). Moreover, it is rich in Ca, Cu, Fe, Mn, Zn, thiamin, vitamin K, and low in Na, bad fat and cholesterol as compared to other cereals (Bemihiretu *et al.,* 2013). It is sharing the crown of quinoa in the world market due to its nutritional importance, rich in digestible proteins and essential amino acid (Capriles and Areas, 2014). It is also recommended as a functional food and supply more nutrients as consumed as whole-grain in fermented form like *injera* (Bergamo *et al.,* 2011).

In Ethiopia, *teff* is traditionally used to make various baked foods and a significant volume of *teff* production is mainly used to make *injera* (Minten *et al.*, 2013). *Injera* is common and widely consumed throughout the country and abroad. Almost in all parts of Ethiopia, $2/3^{rd}$ of the major meal part served is *injera* with different sauces (Stallknecht *et al.,* 1993). This traditional fermented food could be made of either from whole *teff* grains or *teff* mixed with other cereal grains. Some people tend to blend *teff* with other cereals for either its nutritional purpose or high prices of *teff* grains compared with others (Menure, 2017). *Teff* is preferably selected for its unique quality compared to any other cereals in making *injera* with all the required qualities attributes (Bultosa *et al.* 2008).

1.2. Statement of the Problem

Starting in the past years, *injera* is commonly made in most of the Ethiopian kitchen through different traditional processing steps inherited from parents. Different people due to their specific experience, use different pre-baking procedures and baking conditions to make *injera*. Variation in prebaking processing steps and baking condition result *in* variations in physicochemicals and sensory quality which might even be baked from the same variety of *teff* (Yetneberk *et al.,* 2005). These cause differences in terms of different *injera* quality attributes, which is very often observed in restaurants, shops or private houses. *Teff injera* is a major food for the majority of Ethiopians, its preparation takes several days due to lack of well standardized *injera* making process conditions (Mulaw and Tesfaye, 2017). The variations are mainly associated with an absence of standardized prebaking and baking conditions that can fit to a wide range of *teff* varieties.

So far, different attempts have been made to characterize the nutritional, physical and health benefits of *teff* grain for human consumption (Bultosa and Taylor, 2004; Matos and Rosell, 2015). Investigations also conducted on the potential of *teff* for developing value-added food products with nutritional and health benefits (Forsido and Ramaswamy, 2011; Girma *et al*., 2013; Teshome *et al.*, 2017). Significant studies were also done on *teff* processing and *injera* shelf life extension aspects (Assefa *et al.,* 2018; Attuquayefio, 2014; Yoseph, 2019; Geta, 2019; Ashagire and Abate, 2012; Bemihiretu *et al*., 2013).

However, available data to show effects of prebaking steps like fermentation conditions, preparation, and use of "*absit*" (thin gelatinized cooked fermented dough) and mix ratio of "*absit*" with a batter of *teff* dough on quality of *injera* are scarce. Baking condition of *injera* (temperature and time) varies in the literature, all reports are in the range, and no one reported the single optimum baking temperature and time for good quality *injera*. Bultosa and Taylor (2004) stated that *injera* was baked on a *mitad* or griddle for about 2-3 minutes at a temperature of 180 to 220°C. Tesfaye *et al.* (2014) also tested for different ranges of baking temperature and found that the quality of *injera* in the range of 135-220°C remains the same when baked for 2.5 min baking time.

These gaps need to be scientifically studied and optimized to produce consistent quality *injera* to support large scale commercial production and export of the product to other countries. The developed standard methods could improve existing conventional practices and promote local knowledge at the global level for *teff injera* to be exported to other countries. With existing traditional processing and the absence of well-written and documented standard processing methods, the wide use and consumption of *teff injera* are restrained in international markets. However, developing the optimum processing method will enhance the wide commercialization and use of *teff injera* all over the world. This, in turn, creates better market opportunities for *teff* grower farmers, and those associated with its value chain. In view of this, this work was proposed to achieve the following objectives.

1.3. Objectives

1.3.1. General objective

To optimize conventional pre-baking processing steps and baking condition to produce ~~more~~ consistent and better quality of *teff injera* (old)

1.3.2. Specific Objectives

a. To optimize the primary fermentation time and temperature of *teff* dough for consistent and better quality of *teff injera*

b. To optimize *absit* mixing ratio, *absit* cooking time and secondary fermentation time of *teff* dough for consistent and better quality of *teff injera*

c. To determine the optimum baking time and temperature for consistent and better quality of *teff injera*

d. To validate robustness of optimized pre-baking process steps and baking condition for five *teff* varieties

1.4. Expected outputs

The following were expected outputs this findings and recommended for further personal or commercial use to produce consistent and better quality of *teff injera*.

- Optimized pre-baking conditions determined.
- Optimum baking temperature and time ranges recommended.

2. LITERATURE REVIEW

2.1. *Teff* (Eragrostis *teff*) grains Production and Utilization

Teff (Eragrostis *teff*) grain is cultivated as a major cereal in Ethiopia and is a staple food for the majority of Ethiopians (Cheng *et al.,* 2017). In Ethiopia, the main *teff* producing areas have been concentrated in the Amhara and Oromia regional states (the northwestern and central highlands of Ethiopia) and about 47.8% and 37.6% of the land is covered in these regional states respectively (Abewa *et al.,* 2019). *Teff* grain production ranged from 0.84 to 1.75 tons per hectare annually in Ethiopia depending on the location of cultivation and agronomic practices (Abewa *et al.,* 2019). Almost all *teff* grain produced in Ethiopia is mainly used for local consumption in the form of "*injera*" a thin, malleable, with many eyes, fermented bubbly spongy type Ethiopian bread (Stallknecht *et al.,* 1993).

There are four major categories of *teff* grain colors specified by the Ethiopian Standard Agency (ESA) and the grains are classified as very white (Magna), white (nech), mixed (sergegna) and brown/red (key) *teff* (Abewa *et al.,* 2019). The grain color is a measure of quality that also determines the market demand and value. The very white and white *teff* provide the highest price and is consumed by the wealthiest individuals whereas the brown *teff* is sold at a lower price to low-income communities (Hassen *et al.,* 2018). According to Minten *et al.* (2013), the premiums for the white and magna *teff* partially come from the social preference for the white color. Besides the social preference for the white color *teff*, the popularity of the white comes from the introduction of the improved variety Kuncho (Minten *et al.,* 2016). Kuncho is the top in the local language and the major achievement of the *teff* breeding program in Ethiopia (Cheng *et al.,* 2017). Globally, the demand for *teff* grain has increased over the last decade due to many health benefits of this gluten-free product. Because of the demand, the Ethiopian government has prohibited the export of *teff* grain to ensure food security and protection of local markets in Ethiopia. However, the government of Ethiopia did not place a ban on value-added *teff* based products due to this; *teff* bread (*injera*) is exported to the Middle East, Europe, and the United States of America. Ethiopia is the only country where *teff* is used for human consumption before the 19th century (Stallknecht *et al.,* 1993). Nowadays, countries such as the Netherlands, some parts of the USA and South Africa are also using *teff* for human consumption.

2.2. Nutritional importance of *teff* grain

The nutritional value of *teff* is similar or even higher than that of wheat (Spaenij-Dekking *et al.*, 2005). *Teff* grains are milled into whole-grain flour (germ, bran, and endosperm included) due to its small size (Alaunyte, 2013). This condition makes the grain has higher fiber contents, high nutritional importance, high levels of essential amino acids (lysine and methionine) and other nutrients such as minerals, vitamins and bioactive phenolic compounds than most other cereals (Gebremariam *et al.*,2014; Zhang *et al.*, 2016).

Research on celiac disease patients who are using *teff* products reported a significant reduction in symptoms of diabetes because it is particularly important in dealing with in assisting with blood sugar control (Gebremariam *et al.*, 2014). This is possibly related to a reduction in gluten intake or to increase in fiber, intake of *teff injera*, can be a valuable addition to the gluten-free diet of celiac disease patients. In recent years, cereals and their products are accepted as functional foods due to play a role beyond its provision of energy and body forming, which contributes as an extra role of imparting health benefits to the consumer (Kalui *et al.*, 2010). It can be used to prevent and reduce risk factors for several diseases and enhancing certain physiological functions, beyond adequate nutritional effects (Fardet, 2010).

2.3. *Teff* grain utilization

In the past, a major determinant of *teff* grain consumption was its production area (Berhane *et al.*, 2011). However, with the improvement of market linkages, this conditon is gradually changing. For instance, Oromia region is known as the second highest *teff* producing area next to Amhara region in Ethiopia but its consumption expenditure of *teff* is only 8 percent (Berhane *et al.* 2011). In contrast, the Afar region is little known by *teff* producing area but *the* consumption expenditure of *teff* grain in this region is about 10 percent (Berhane *et al.*, 2011). Now a days, in Ethiopia consumption of cereal grains could be based on several factors such as agro-ecology of the country, livelihoods and income of of the consumers (Baye *et al.*, 2013; Berhane *et al.*, 2011). From the analyses of national representative household consumption and income survey conducted in Ethiopia in 2004/05 indicated that energy (calorie) intake is obtained from *teff* is only 11 %, Maize 17 %, sorghum 14 % and wheat 13 % as indicated in figure 1 (Berhane *et al.*, 2011).

Teff is the most expensive cereal in Ethiopia, especially in urban and semi-urban areas where incomes are relatively higher than rural peoples are there (Berhane *et al*., 2011). Globally, the demand for *teff* grain has increased over the last decade due to many health benefits of this gluten-free product. Because of the demand, the Ethiopian government has prohibited the export of *teff* grain to ensure food security and protection of local markets in Ethiopia. However, the government of Ethiopia did not place a ban on value-added *teff* based products due to this; *teff* bread (*injera*) is exported to the Middle East, Europe, and the United States of America (Lee, 2018).

2.4. *Teff injera* proessing steps

2.4.1. *Teff* flour production and dough making process

To produce the flour *teff* grain is grind in between two stone-discs in the case of disc mill and the hammer mill and crush the grain repeatedly until it passes through the sieve that is fitted inside the mill and the blade mill having rotary blades to grind the grain (Assefa *et al.*, 2018). The *teff* flour, water and *irsho* are usually mixed in different proportions to make the dough. Some of the flour to water ratio proportion reported by different researchers are; 1:1.6 by Girma *et al.* (2013), and 1:2 by Ashagrie and Abate (2012) and Girma *et al.* (2013). Proper dough making is important for the quality of baked goods including *injera* (Alaunyte *et al.*, 2012).

2.4.2. Primary fermentation process

The fermentation stages of *teff* flour for *injera* making have two stages, which are independently processed. The first fermentation stage is where the dough left to ferment for about 24 to 72hrs or 48–72hrs depending on fermentation temperatures (Steinkraus, 2002; Mezemir, 2015). The primary fermentation starts after adding the back slope or a clear yellow liquid that accumulates on the surface of the dough towards the final stage of a previous fermentation. The initial 18 hrs of fermentation are characterized by a vigorous evolution of gas and maximum dough-rising (Mezemir, 2015). This is followed by the appearance of an acidic yellowish liquid on the surface of the dough at about 30-33 hrs of fermentation (Ashenafi, 2006). Gas evolution decreases after the pH has fallen below 5.8 (31 hrs) (Firstenberg-Eden and Zindulis, 1984).

Stewart and Getachew (1962) were the first to investigate the fermentation process associated with *injera* and concluded that both the yeast and bacteria have important roles in fermenting *teff* for *injera* production. Gashe (1985) stated that the progression of microbial flora during the wild fermentation of *teff* (no starter culture) determining that lactic acid bacteria were responsible for the acidic flavor and pH development. Members of *Enterobacteriaciae* decrease the pH from 6.6 to 5.8 over the first 18 hrs after which they are succeeded by *Streptococcus faecalis* and *Leuconostoc mesenteroides*. *Pedicoccus cerevisiae*, *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus plantarum* appear in significant numbers (10^6 CFU/g) at 30 hrs (pH 4.7) and dominate the fermentation after 42 hrs. Yeasts become the most widespread flora at 60 hrs, after which there is little change in pH, but high counts of lactic acid bacteria remain until the termination of fermentation at 72 hrs. Megersa *et al*. (2017) identified that *Lactobacillus pontis* is the dominant species in *teff* sourdoughs that were spontaneously initiated and stabilized by back slopping.

2.4.3. *Absit* making process and secondary fermentation

After primary fermentation, the yellowish liquid layer is discarded and about 10% of the fermenting dough is mixed with three parts of water and boiled for 15 minutes to form the *absit* (Ashenafi, 2006). The hot cooked dough (*absit*) is cooled to about 45^0C before it is mixed into the main part of the dough and sufficient potable water is added to make a batter (Dessalegn, 2018). The second fermentation time start after *absit* is added on the first fermented dough and lasts for 2-4hrs (Ashagrie and Abate, 2012; Attuquayefio, 2014). However, the secondary fermentation time is affected by the altitude of the area, the amount of the *irsho*, temperature of the environment and the container used (Mezemir, 2015).

Different literature stated that, the amount of *absit* added to the batter for secondary fermentation does varies. Ten percent (10%) (Ashenafi, 2006; Girma *et al.*, 2013; Umeta and Faulks, 1988) of the weight of the fermented batter is commonly used to make *absit*. However other amounts such as 5%, 15% and 20% (Zannini *et al.*, 2012) of the fermented batter are sometimes used. Moreover, 10-20% was used by Dessalegn (2018). Parker *et al.* (1989) stated in their study that the major contributor to the *injera* matrix is gelatinized starch. Although, there were many trials conducted so far on secondary fermentation conditions, no optimized primary fermentation condition (temperature and time), *absit* mixing ratio, cooking time and secondary fermentation time reported obtained because, in different literature data the process condition were found in the range.

2.4.4. Baking of *injera*

Baking of *injera* is the final process of the fermented *teff* batter and it is the process where heat is transferred from the hot pan to the surface of the food baked on it and as a result, moisture continuously removed out (Desalegn, 2019). After the batter is poured in a circular manner on the hot pan, the pan will be covered and left for 2 to 3 minutes to be steam cooked (Ashagrie and Abate, 2012). A lid is used to cover the *injera* during baking process after eyes forming started on the surface. This is to allow steam to cook the upper surface of the *injera* and prevent it from drying out.

Tesfaye *et al* (2014) tested the electric *injera* stove for different ranges of baking temperature, and the quality of *injera* in the range of 135-220°C remains the same and a slightly baking time difference was observed. Similarly, Pyle (2005) reported a typical temperature range for baking crumpets is 200-230°C, and observed that baking temperature increased the elasticity of the crumpets. Bultosa and Taylor (2004) also stated *injera* baked on a *mitad* or griddle for about 2-3 min at a temperature of 180 to 220°C got the highest quality than other temperatures and time tested. Although, different studies were conducted on *injera* baking condition no one optimized the temperature and time at which good quality *injera* could be baked. Baking condition of *injera* (temperature and time) vary from literature to literature and no single optimum time and temperature is reported. Therefore, it is necessary to obtain an optimum temperature and time at which *injera* should be baked in order to obtain proper physicochemical and sensory quality.

2.5. Effect of *injera* processing steps on *injera* quality

2.5.1. Fermentation and *absit*

Fermentation refers to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage (Karovicova and Kohajdova, 2007). According to Steinkraus (2002), the traditional fermentation of foods is used for the enhancement of diet through the development of flavor, aroma, and texture in food substrates, preservation and shelf-life extension through lactic acid, alcohol, acetic acid and alkaline fermentation. Similarly, improvements in the protein digestibility of fermented products are mainly associated with enhanced proteolytic activity of the fermenting microflora. Fermentation has been shown to improve the nutritional value of grains such as wheat, maize, *teff* and rice, by increasing the content of the essential amino acids lysine, methionine and tryptophan (Zannini *et al.*, 2012).

During fermentation time, Lactic Acid Bacteria (LAB) and yeasts are responsible micro floras in starch/carbohydrates degradation and production of different exopolysaccharides (EPS) and CO_2 gases (Assefa, 2017; Stewart and Getachew 1962). During fermentation process, microorganisms and the enzymes found in *teff* flours involved in a biochemical modifications of the flours prior to process or bake for human consumption. This condition leads to changes in texture, taste, aroma, nutritional value and digestibility of the food products. During fermentation, yeast and mainly lactic acid bacteria play an important role because LAB produces lactic acid that lowers the pH to 3.5-4. The lactic acid bacteria perform second proteolysis and this activity makes an increase of amino acids that is associated with an improvement in protein digestibility (Ganzle *et al*., 2008).

Absit added to the fermented batter can be used as hydrocolloids in products which provide the batter with a better gas-holding capacity by increasing its viscosity (Zannini *et al.*, 2012). It is a dough enhancer (improves the texture of the dough) and a dough binder (Ashenafi, 2006; Girma *et al*., 2013). *Absit* also has other possible functions in activation of yeasts responsible for CO_2 production and the development of eyes during baking of *injera* (Dessalegn, 2018). According to Assefa *et al.* (2018), *injera* made from batter lacking *absit* has a powdery look and lacks the air spaces or the so-called eyes of the *injera, which* give it an “attractive look”. *Injera* baked at 24 hrs or less is called *aflegna* and has sweet taste and such type of *injera* is recommended for people suffering from gastritis who do not tolerate acidic foods (Mezemir, 2015; Sahlin and Nair, 2012). The objective of starch gelatinization is primarily to bring about the cohesiveness of the batter and secondly to provide easily fermentable carbohydrates to leave the *injera* (Yetneberk *et al.,* 2004). The researchers also reported that by cooking part of the fermented batter, the carbon dioxide produced by the fermentation is trapped and leavens the *injera* during baking. Therefore, it can be concluded from the literature that the main role of *absit* is to enhance the quality of *injera* as an enhancer of fermentation microorganism and improving texture and binding ability of the dough.

2.5.2. Fermentation temperature and time

Fermentation temperature has an effect on the physicochemical quality of spontaneous *teff* fermentations and the quality of *injera*. According to Chavan (2011), temperature control is important in sourdough production as changes in fermentation temperature may cause variation in the microflora of sourdough and affects the final bread quality and flavor. Ashagrie and Abate (2012) stated that temperature in the highlands of Ethiopia is generally between 17 and 25°C, hence, *injera* made at these temperatures should still have the desired quality characteristics.

Fermentation is a time-dependent and known to cause changes in the physicochemical and sensory quality of the food products. During the fermentation process in order to observe whether there are any significant changes in pH, titratable acidity, viscosity, elasticity, eye formation and moisture of *injera*, it was necessary to monitor fermentation at different temperature and time. According to Attuquayefio (2014), *teff* flour was fermented for different fermentation time starting from 0 and 72 hrs for the primary fermentation process at ambient temperature (22-25°C). The researcher observed that different primary fermentation time (0, 24, 48 and 72 hrs) made difference on *injera* quality indicators such as viscosity of batter, moisture content, elasticity, eye numbers, pH and titratable acidity. It was concluded that fermentation time would have a significant effect on the physicochemical and sensory quality of *injera*.

2.5.3. Baking temperature and time

During the baking process of *injera*, heat is transferred from the hot pan to the surface of the food material, while moisture is transferred from the interior to the surface of the product and then evaporates. As a result, changes in temperature and moisture conditions develop as cooking proceeds and bring about the desirable characteristics (color, texture, and flavor) of the food (Getenet, 2011). During baking of *teff injera*, starch is completely gelatinized and forms a steam-leavened spongy matrix in which bubbles of gas, microorganisms, and fragments of bran and the outer layer of endosperm are surrounded (Alaunyte, 2013). *Teff* starch is the major contributor to the texture and keeping other properties of Ethiopian bread *injera* quality (Alaunyte, 2014). The water absorption index (WAI) of *teff* starches has been reported to be considerably higher when compared to other starchy cereals (Bultosa *et al.,* 2002). This can be related to the small *teff* starch granule size and narrower granule diameter ranges and *teff* starch granule is - 2-6µm; maize- 5-30µm; wheat- 2-55µm; barley- 0.9-44.9µm (Bultosa *et al.,* 2002). The smaller the granule size and the larger surface area will result in the higher the water absorption capacity of the grains.

2.6. Quality parameters of *injera*

2.6.1. Physicochemical quality parameters

2.6.1.1. Carbon dioxide concentration

Carbon dioxide produced during the primary fermentation stage significantly affects the number of eyes formed on the surface of *injera.* According to Pyle (2005, the small bubbles of CO_2 resulting from fermentation play an important role in nuclei or pore development and without these nuclei a porous structure in the final product could not be formed. These nuclei are the source of dominant pore structure in the final product, which results from the initial explosive release of water vapor from the batter together with the desorption of CO_2. The increase in gas bubbles at around 24 and 48 hrs during primary fermentation could have caused an increase in the viscosity of the batter which results in the high number of eyes and elasticity of *injera*.

2.6.1.2. Number of 'eyes' per square centimeter on injera

The number of eyes on the surface of *injera* has always been taken as a good indicator of *injera* quality. In the work of Attuquayefio (2014), it was indicated that the gas bubbles formed during fermentation create nuclei like hole due to the developing of CO_2. These gas bubbles in the batters will try to find out a way to escape by the temperature of baking which in turn leaves a hole like structure called the honeycomb-like eye (Stewart and Getachew, 1962). *Injera* with large unevenly spaced eyes or those with tiny eyes is both considered poor quality (Attuquayefio, 2014). While the former signifies insufficient fermentation, the latter indicates too much *absit* in the dough. Different factors affect the quality, size and number of eyes on *injera*. According to different studies on *injera,* these factors are mentioned as fermentation process, *absit*-making process, the viscosity of batter, fermentation temperature and time, baking temperature and time (Stewart and Getachew, 1962; Attuquayefio, 2014). Therefore, these factors should be optimized in order to get good quality *injera*.

2.6.1.3. Rollability of injera

The *injera* should be soft enough to cut piece easily with the fingers but resilient enough so that it does not crack or crumble when folded or used to wrap around wot portions during eating. The spongy and soft structure of *teff injera* is essential for its keeping freshness, flavor and better keeping qualities (Desalegn, 2019). *Injera* is a medium thickness (4 - 6 mm) and is soft, spongy, and resilient, and its texture should not be gluey or stick to the fingers when handled. It becomes drier and more brittle as the number of days of storage increases. *Teff injera* is preferred to other cereals since it can be stored for 3 days without losing its pliability (Desalegn, 2018). Pliability is related to the ability of *injera* to roll or wrap around a 1-5 cm wooden dowel without tearing or cracking and this is a mark of good quality *injera*.

2.6.1.4. Tensile strength/ extensibility of injera

The texture is the overall experience of how a substance feels in hand and mouth. It contributes to the overall eating experience. The malleability of *injera* makes it good for folding or rolling and its eyes also good for picking up wot. A non-powdery, soft appearance is also characteristic of good quality *injera*. Lack of textural quality and the interaction of flavor and texture are more likely to cause rejection of *teff injera* (Yegrem, 2019). Free sugars in fermented *teff* may also affect glass transition temperature (TG) of *injera* (Abbas *et al.*, 2010). The flexibility of *injera* is based on its glass transition temperature and an important attribute as it relates to its elasticity. Instrumental texture measurement techniques are mainly based on evaluation of mechanical properties. The amount of force required to produce a given amount of deformation is used for quantitative evaluation of texture.

2.6.1.5. pH

pH is defined as the logarithm of the reciprocal of the hydrogen ion concentration. In modern food analysis, pH is usually determined instrumentally with a pH meter, but chemical pH indicators also exist. Cereal mashes with a pH of 5-6.2, which are rich in fermentable carbohydrates, that has been preferentially fermented by LAB, at least to a pH below four, and below this point, acid-tolerant yeasts dominate the fermentation (Stolz, 2003). Good quality *injera* is a slightly sour taste due to acidic or low PH nature. If the batter is over fermented, the acidity of *injera* will increase due to the secretion of acid by bacteria during fermentation time (Xiang *et al.,* 2019). Even though the more acidic *injera* is less exposed to microbial growth, the more acidic it is less acceptable in its taste by consumers. Different kinds of literature data were reported the pH values in *teff injera* and recorded different ranges. Ashagrie and Abate (2012) determined the pH of *teff injera* 3.4 while Attuquayefio (2014) recorded the pH values of *teff injera* samples were between 3.65 and 4.02. However, the optimum amount of pH was not determined and it should be determined in this study.

2.6.1.6. Titratable Acidity

Titratable Acidity (TA) refers to the total concentration of free protons and undissociated acids in a solution that can react with a strong base and could be neutralized. It is dealing with a measurement of the total acid concentration contained within a portion of food and called total acidity (Sadler and Murphy, 2010). It is a better predictor of acid impact on flavor than pH. Foods establish complex buffering systems that dictate how hydrogen ions (H^+), the fundamental unit of acidity, are expressed. Even in the absence of buffering, less than 3% of any food acid is ionized into H^+ and its anionic parent species (it is conjugate base). The ability of microorganisms to grow in a specific food is an important example of a process that is more dependent on hydronium ion concentration than on titratable acidity (Rahman, 2007). The amount of organic acids in food directly affects the food flavor, color, microbial stability (via inherent pH-sensitive characteristics of organisms), growth of microorganisms or germination of spores and its final quality (Andres-Bello *et al.*, 2013). Organic acids may be present in the food, naturally, during fermentation or they may be added as part of a specific food formulation.

2.6.1.7. Moisture content

One of the most important ingredients in any gluten-free formulation is water. Moisture in foods is known to have an effect on quality both positively and negatively. The moisture content in the product might affect the water activity level that is responsible for the microbial growth and affects the shelf life of the product (Ashagrie and Abate, 2012). The glass transition is strongly dependent on water content, which often causes large differences in reported glass transition temperatures (Roos, 2010). Gelatinization of starch is strongly affected by water content (Pyle, 2005). Therefore, the moisture content of *injera* has an effect on its texture. In some literatures the moisture content are reported to be 65.23% for *injera* baked at 72hrs on the other literature it was found in the range 62-65% (Attuquayefio, 2014). In the specification for Ethiopian *injera* the moisture content that the *injera* should have indicated as 58% to 63% (ESA, 2013).

2.6.2. Sensory quality parameters

The first and most important parameter of food is sensory characteristics. Sensory evaluation is a science that measures, analyzes, and interprets the reactions of people to products as perceived by the senses (Stone, 2018). It is a means of determining whether product differences are perceived, the basis for the differences, and whether one product is liked more than others. Zegeye (1997) evaluated *injera* acceptability made from *teff*, maize, sorghum and barley for sensory panel responses with and without stewed chicken (Doro-wot) by preference and difference tests, respectively. He found no significant difference between fresh sorghum and maize *injera* in flavor. However, *teff injera* preferred over other *injera* types. *Injera* from *teff* substituted with two flaxseed forms at 3%, 6% and 9% studied by Girma *et al.* (2013) results indicated that with an increase in the flaxseed substitution, most sensory acceptance increased, whereas *injera* eyes and color decreased and appeared superior for control (100% *teff injera*). Desselagn (2019) evaluated the sensory acceptability (color, taste, texture, eye distribution, overall acceptability and others) of *injera* baked from kuncho *teff* and observed no significant difference. The researcher stated that since the *injera* is baked from the same variety *teff* no variation was observed for all treatments.

2.7. Summary

As compared to other common cereals, *teff* grain is better in its nutrient composition as it contains slowly digestible starch, a favorable amino acid composition and high in minerals, especially iron and calcium. Different researchers characterized the nutritional, physical and health benefits of *teff* grain for human consumption (Bultosa and Taylor, 2004; Jonnalagadda *et al.*, 2011) and obtained an excellent results. It is the main staple in Ethiopia mostly used to make *injera*. Nowadays, *teff injera* is becoming popular in developed world because of its being a whole-grain product, gluten free nature and other quality parameters. The potential of *teff* for developing value-added food products with good nutritional and health benefits was studied by different authors (Forsido and Ramaswamy, 2011; Girma *et al*., 2013, Teshome *et al.*, 2017) and promising results were obtained.

However, *injera* from different shops/markets and private houses lack uniformity in physicochemical and sensory quality. This might be due to different factors related with processing factors during *injera* making. Among many processing factors, prebaking processing steps (primary fermentation time and temperature, *absit* mixing ratio, cooking time and secondary fermentation time) and baking condition (baking temperature and time) are the most important factors identified in literature for quality of *teff injera*.

So far, different studies have been attempted on *teff injera* processing like the influence of milling type on quality (Assefa *et al.*, 2018), processing parameters on eye size and elasticity (Attuquayefio, 2014). Moreover, Ashagire and Abate (2012) addressed a Shelf-life extension aspect and Bemihiretu *et al*. (2013) addressed antioxidant properties of traditional Ethiopian bread (*injera*) as affected by processing techniques and *teff* grain varieties. However, research on optimization of pre-baking processing steps like primary fermentation condition, preparation and use of "*absit*" (thin gelatinized cooked dough) and mix ratio of "*absit*", secondary fermentation time on the quality of *injera* are scarce.

In conventional *injera* baking, no one knows optimum baking temperature and time for good quality *injera*. Commonly a trial and error method based upon the experience of a baker is employed to estimate sufficiency of baking condition. Baking condition of *injera* (temperature and time) varies in the literatures, all reports are in the range, and no one reported the single optimum baking temperature and time for good quality *injera*. Different studies were conducted on *injera* baking condition Tesfaye *et al* (2014) tested the electric *injera* stove is tested for different ranges of baking temperature; the quality of *injera* in the range of 135-220^{o}C remains the same, however, a slightly baking time difference was observed. They studied that, one *injera* needs an average of 0.1kWh of heat power and 2.5 min to be well cooked.

Pyle (2005) stated that a typical temperature range for baking crumpets is 200-230°C, and observed that baking temperature increased the elasticity of the crumpets. According to Feleke Fanta and Oquino (2019) good quality *injera* is baked at a temperature range of 150-200°C for 2minutes. Bultosa and Taylor (2004) stated that *injera* is baked on a *mitad* or griddle for about 2-3 minutes at a temperature of 180 to 220°C. Variation of the temperature in the middle of the *injera* during the baking process might be due to the frequent polishing of *injera* stoves with oil or oilseeds to avoid sticking between baking intervals to produce. This practice takes considerable time and power. Tesfaye *et al.* (2014) also observed polishing drops stove temperature from 165 to 100^{o}C and 158 to 120^{o}C. Therefore, scientific investigations towards the optimization of prebaking processing steps and baking condition for the better quality of *teff injera* are important.

3. MATERIALS AND METHODS

3.1. Description of sample site

The experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) at Post-Harvest Management laboratory between 2018 and 2019.

3.2. Sample collection

Kuncho (DZ-Cr-387) and red (DZ-Cr-2124) *teff* varieties were collected from Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). However, three different *teff* varieties (white (T-BT), white (T-GK) and sergegna (T-E) were randomly purchased from the local market and the spontaneous culture for back slopping for all phases was collected from different houses and stored to be used at different stages of the experiments.

3.3. Sample preparation

Upon arrival at the Postharvest Department laboratory of JUCAVM, the *teff* grain samples were cleaned manually by sifting and winnowing before milling to remove stones, dust, light materials and other extraneous materials. It was milled and ground into fine flour using small- scale commercial hammer mill. Following grinding, the flour sifted to pass through 0.5 mm sieve (Abebe *et al.*, 2015), sealed in polyethylene plastic bags, and stored at room temperature until further used.

3.4. Experimental setup

The experiments were carried out in four consecutive phases to optimize prebaking and baking process conditions for better quality of *teff injera*. The first phase of the study was to optimize primary fermentation time and temperature to make the dough, the second phase was focused on optimizing *absit*-mixing ratio, cooking time and secondary fermentation time, the third phase was to optimize baking condition (baking temperature and time) using temperature regulated baking plate. Finally, a validation study was conducted to verify the robustness of optimized pre-baking process steps and baking condition for better quality of *injera* from flour of different *teff* varieties purchased from the local market.

3.5. Experimental design and treatment combinations

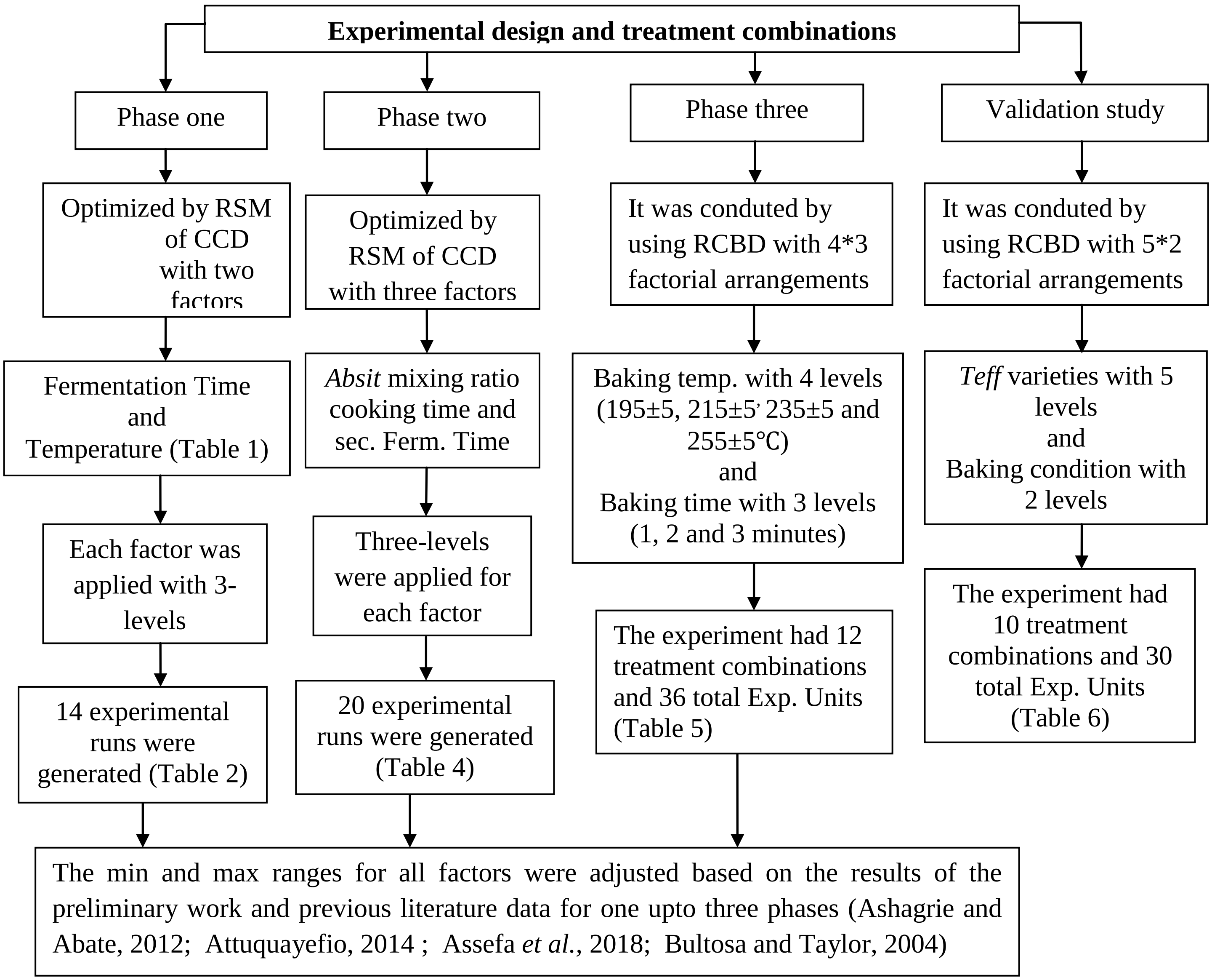

Figure 1: General experimental frameworks for objective 1, 2, 3 and 4

The first and second phases of studies were laid out with the aid of the design expert software (design expert ® version 6.02, Minneapolis, USA) using the response surface methodology experimental design. To optimize the first phase of the study, the central composite design

(CCD) with two factors (fermentation time and temperature) applied, which generated 14 experimental runs. The minimum and maximum primary fermentation time and temperature ranges were adjusted based on the results of the preliminary work and described in Table 1. The actual independent variables and their levels were described in Table 1 while Table 2 shows the 14 experimental runs generated using response surface design for primary fermentation condition.

Table 1 Factors and their levels for the central composite response surface design

Primary fermentation Conditions	Minimum	Average	Maximum
Time (hrs)	24	60	96
Temperature (℃)	25	31.5	38

Table 2 Experimental run generated using CCD for primary fermentation condition

Std Order	Run Order	PtType	Blocks	Time (hrs)	Temperature (℃)
2	1	0	B-1	60	32
7	2	-1	B-1	60	22
5	3	0	B-1	60	32
3	4	-1	B-1	111	32
6	5	0	B-1	60	32
1	6	-1	B-1	9	32
4	7	-1	B-1	60	41
13	8	1	B-2	96	25
10	9	0	B-2	60	32
12	10	0	B-2	60	32
9	11	1	B-2	24	38
14	12	0	B-2	60	32
8	13	1	B-2	24	25
11	14	1	B-2	96	38

To optimize the 2nd phase of the study, a response surface methodology of CCD with three factors (*absit* mixing ratio, cooking time and secondary fermentation time) was applied, which generated 20 experimental runs (Table 4). The minimum and maximum ranges for the three factors were adjusted based on the previous literature data (Ashagrie and Abate, 2012; Attuquayefio, 2014 and Assefa *et al.*, 2018) and taken as described in Table 3.

Table 3 Factors and their levels for the central composite response surface design

Pre-baking processing steps	Minimum	Average	Maximum
Absit mixing ratio (%)	5	10	15
Absit cooking time (min)	5	10	15
Secondary fermentation time (hr)	2	4	6

Table 4 Experimental run generated using response surface methodology of ccd for absit mixing ratio, cooking time and secondary fermentation time

Std	RO	Blocks	*Absit* mixing ratio (%)	*Absit* cooking time (min)	Secondary fermentation time (hrs)
3	1	Block1	15	5	2
8	2	Block1	15	15	6
5	3	Block1	5	15	2
11	4	Block1	10	10	4
12	5	Block1	10	10	4
7	6	Block1	15	15	2
1	7	Block1	5	5	2
6	8	Block1	5	15	6
2	9	Block1	5	5	6
4	10	Block1	15	5	6
9	11	Block1	10	10	4
10	12	Block1	10	10	4
17	13	Block2	10	2	4
19	14	Block2	10	10	4
20	15	Block2	10	10	4
18	16	Block2	10	18	4
14	17	Block2	10	10	7
16	18	Block2	18	10	4
15	19	Block2	2	10	4
13	20	Block2	10	10	1

Optimization of baking temperature and time was laid out in a completely randomized block design (RCBD) with 4*3 factorial arrangements in three replications in third phase of the study. The factors consisted of baking temperature (195±5, 215±5, 235±5 and 255±5℃) and time (1, 2 and 3 minutes). Therefore, the experiment had 12 treatment combinations and 36 total experimental units. The minimum and maximum baking temperature and time ranges were adjusted based on the previous literature data by different researchers (Bultosa and Taylor (2004); Pyle (2005); Tesfaye *et al.*, 2014). Data collection days were used as an experimental block since it is not possible to complete within one day.

Table 5 Factors and their levels combinations for baking temperature and time

Run	Blocks	Baking temperature (℃)	Baking time (min)
1	1	190-200	1
2	1	190-200	2
3	1	190-200	3
4	1	210-220	3
5	1	210-220	1
6	1	230-240	1
7	1	250-260	3
8	1	210-220	2
9	1	250-260	2
10	1	230-240	3
11	1	250-260	1
12	1	230-240	2
13	2	210-220	2
14	2	250-260	2
15	2	250-260	1
16	2	190-200	3
17	2	190-200	1
18	2	210-220	1
19	2	250-260	3
20	2	230-240	2
21	2	190-200	2
22	2	210-220	3
23	2	230-240	1
24	2	230-240	3
25	3	230-240	1
26	3	190-200	3
27	3	250-260	2
28	3	230-240	2
29	3	190-200	1
30	3	250-260	1
31	3	230-240	3
32	3	190-200	2
33	3	210-220	3
34	3	250-260	3
35	3	210-220	2
36	3	210-220	1

Finally, to check the robustness of optimized pre-baking processing steps and baking condition for the production of better quality *teff injera* the validation study was conducted. For this phase, a completely randomized block design (RCBD) with 5*2 factorial arrangements in three replications was used. The factors consisted of: *teff* varieties with five levels (DZ-Cr-387 (A), DZ-Cr-2124 (B), white (T-BT) (C), white (T-GK) (D), and sergegna *teff* (T-E) (E), and optimum baking condition obtained on the 3rd objective with two levels 255±5°C for 2 min and 235±5°C for 3 min. Therefore, the experiment had 10 treatment combinations and 30 total experimental units as indicated in table 6.

Table 6 Factors and their levels for teff varieties and optimum baking condition for validation work

Run Order	Blocks	*Teff* varieties	Optimum baking conditions
1	1	DZ-Cr-2124	255±5°C for 2min
2	1	White (T-GK)	255±5°C for 2min
3	1	White (T-BT)	255±5°C for 2min
4	1	Sergegna *Teff*	235±5°C for 3min
5	1	White (T-BT)	235±5°C for 3min
6	1	White (T-GK)	235±5°C for 3min
7	1	Sergegna *Teff*	255±5°C for 2min
8	1	DZ-Cr-387	255±5°C for 2min
9	1	DZ-Cr-2124	235±5°C for 3min
10	1	DZ-Cr-387	235±5°C for 3min
11	2	Sergegna *Teff*	255±5°C for 2min
12	2	DZ-Cr-2124	255±5°C for 2min
13	2	White (T-GK)	235±5°C for 3min
14	2	Sergegna *Teff*	235±5°C for 3min
15	2	DZ-Cr-2124	235±5°C for 3min
16	2	White (T-BT)	255±5°C for 2min
17	2	DZ-Cr-387	255±5°C for 2min
18	2	White (T-BT)	235±5°C for 3min
19	2	White (T-GK)	255±5°C for 2min
20	2	DZ-Cr-387	235±5°C for 3min
21	3	DZ-Cr-387	255±5°C for 2min
22	3	White (T-GK)	255±5°C for 2min
23	3	White (T-GK)	235±5°C for 3min
24	3	White (T-BT)	255±5°C for 2min
25	3	DZ-Cr-387	235±5°C for 3min
26	3	Sergegna *Teff*	255±5°C for 2min
27	3	DZ-Cr-2124	235±5°C for 3min
28	3	Sergegna *Teff*	235±5°C for 3min
29	3	DZ-Cr-2124	255±5°C for 2min
30	3	White (T-BT)	235±5°C for 3min

3.6. *Injera* making process

3.6.1. Dough making and primary fermentation process

Dough making for primary fermentation undertaken by taking 1 Kg of *teff* flour, 2L tap water or 1:2 (w/w) as stated in Ashagrie and Abate (2012). About 60 mL (6% of culture from previous spontaneous fermentation of dough by the weight of the dough) was added (Yoseph *et al.,* 2019) and it was kneaded in a bowl by hands with continuous addition of a measured amount of water for each treatment. The dough was allowed to ferment in an oven at a specified temperature and time as per treatment combination. After the specified time and temperature scheduled completed, the supernatant (the slightly yellowish liquid) floated on the surface of the fermented batter decanted before used at the next steps.

3.6.2. *Absit* preparation and secondary fermentation process

After the yellowish liquid settled on the surface of the fermented batter poured off, (2, 5, 10, 15 and 18%) of the weight of fermented batter was mixed with boiling water by 1:3 (v/v) (Aseffa *et al.,* 2018) and cooked for (2, 5, 10, 15 and 18 min) at 100℃. The gelatinized batter (*absit*) was cooled to approximately 45°C and added back to the fermented dough and sufficient equal amount of tap water added for all treatments until to attain desired viscosity with continuous stirring. Then, the fermented dough was covered and left for secondary fermentation time (1, 2, 4, 6 and 7 hrs). The *absit* making process (*absit* mixing ratio, cooking time and secondary fermentation time) were separately prepared for each treatment.

3.6.3. Baking of *injera*

After specified secondary fermentation time, about half (500 ml) of the plastic beaker of batter was taken from fermented dough and filled in a container and poured out onto the hot WASS Electronics (Mitad 16″ Grill made in USA) baking plate, and baked at the indicated baking temperature and time in Table 5. After a specified temperature and time, *injera* was removed from the hot plate and cooled to room temperature before measuring response variables.

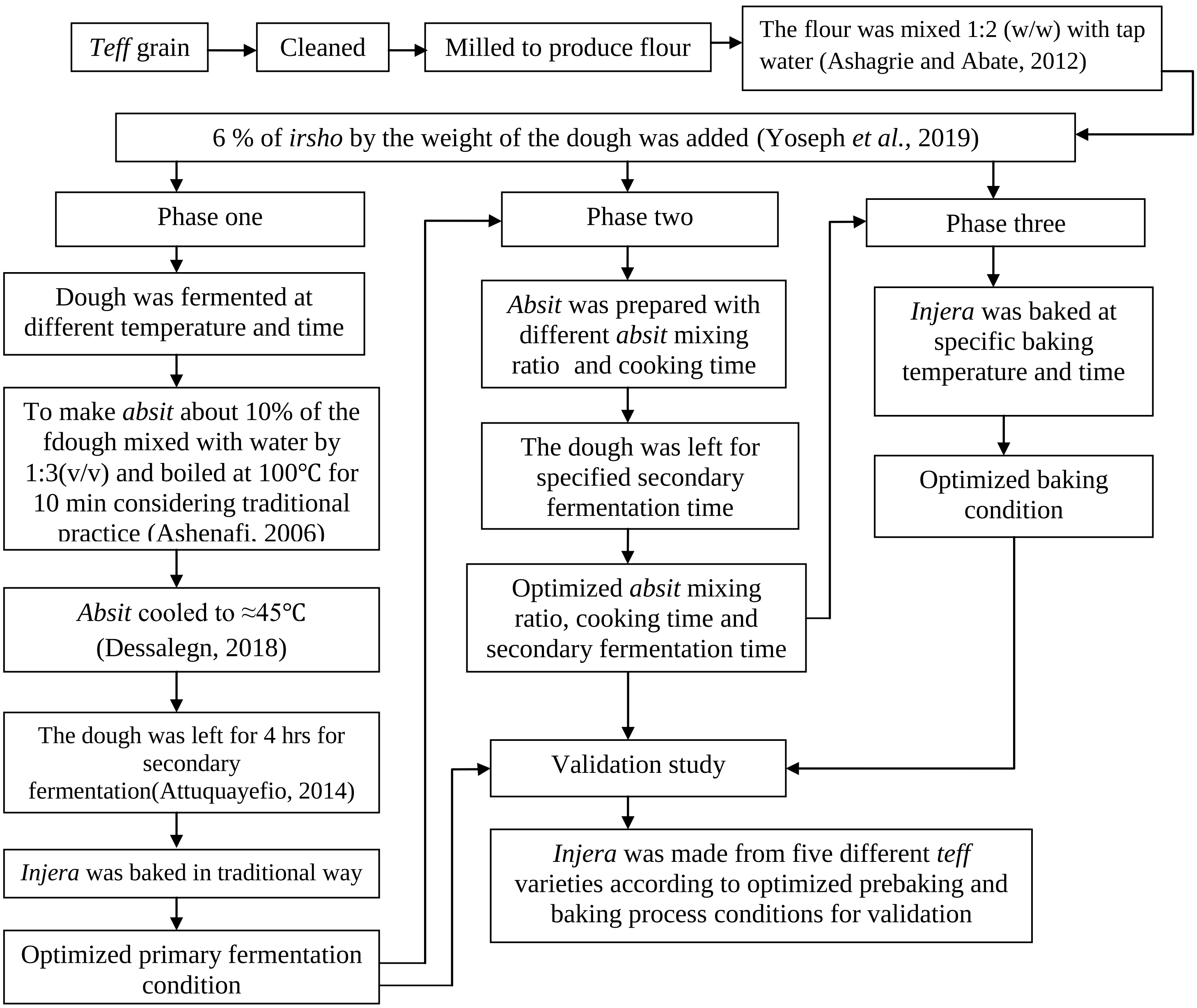

Figure 2 *Injera* making process flow diagram for all phases of the study

3.7. Quality paraeters of *injera*

3.7.1.Physico-chemical quality parameters

3.7.1.1. Percent of carbon dioxide formed during primary fermentation

Effect of fermentation temperature and time on CO_2 concentration during the primary fermentation process was determined by using CO_2 meter (Oxybaby M+i O_2/CO_2, E7, made in Germany) at the end of fermentation. The dough was properly sealed in air tight condition with gas proof materials to keep the gases produced in a material during fermentation time. The dough samples were kept in an oven (Leicester, LE675FT, England) at specified temperatures and time.

3.7.1.2. Determination of eye numbers in per unit area of injera

The number of eyes on the surface of *injera* was determined by counting the number of eyes on a portion of the sample 3cm x 3cm and dividing the total number of *injera* eyes counted from four different portions of *injera* each having surface area of 9 cm^2 (3cm x 3cm). Therefore, the number of eyes was reported as the number of eye/cm^2 using the formula mentioned below (Cherinet, 1993).

$$Number\ of\ eyes = \frac{total\ number\ of\ eyes}{3cmx3cm} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq\,(1)$$

3.7.1.3. Determination of rollability

Injera sample of 12 cm long with a 2cm width was cut from full *injera* and wrapped or rolled around on 2 cm diameter of wooden dowel to determine its rollability. The rollability score was rated from 1-5 scales (one= breaks immediately after one roll /cannot be rolled, two= breaks in two rolls, 3= breaks in three rolls, 4= breaks in four rolls and five = no cracks; very flexible) (Girma *et al.,* 2013). *Injera* was considered unacceptable when the rollability score is below three.

Figure 3 Rollability determination manually (by athour)

3.7.1.4. Determination of extensibility

The extensibility of *injera* was determined by using a texture analyzer (TA Micro Stable System, UK). The machine was adjusted before starting the evaluation by taking 1mm/s test speed, 1.5mm/s pre-test speed, 10mm/s post speed, 60mm of bottom distance and target distance bottom. The length of the *injera* sample was 10 cm and measured by holding and pulling the two ends of *injera* on the machine, as indicated in figure 4.

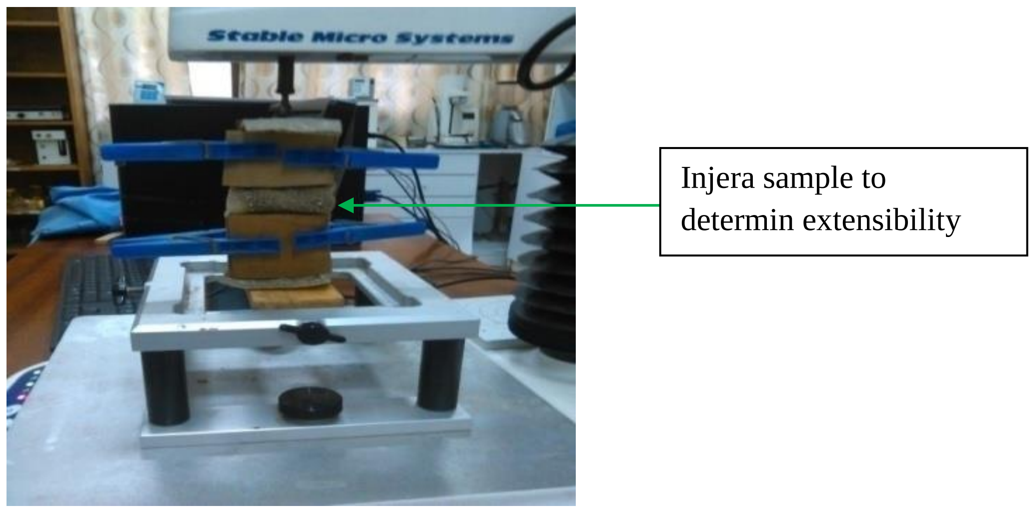

Figure 4: The extensibility (N) of *injera* recorded by using texture analyzer (by Athour)

3.7.1.5. Determination of pH

The pH of the experimental samples was determined according to AOAC (1995). The fresh *injera* samples (10gm) were weighed and mixed with 100 mL of distilled water prepared at the PHM laboratory and the dispersion of samples was homogenized using a shaker to measure the pH of each sample. The supernatant solution was decanted into a 250 mL beaker and pH was measured using a scientific electronic bench top pH meter of nine series (model pH-016, made in China).

3.7.1.6. Determination of titratable acidity

The titratable acidity (TA) of the experimental samples was determined according to AOAC (1995). Ten (10) gm of *injera* sample was mixed with 100 mL of distilled water and titrated with fresh 0.1 N NaOH solution, using four drops of 0.1% phenolphthalein endpoint indicator. The volume of NaOH used for each titration was recorded and titratable acidity was expressed as percentage lactic acid using the formula:

$$Titratable\ acidity\ (\%) = \frac{0.1Nb \times Vb \times 0.09}{Ws} \times 100\% \text{...} Eq\ (3)$$

Where,

Nb = normality of base,
0.09 = acid equivalent factor for lactic acid,
Vb =volume of NaOH solution used in ml,
Ws = weight of the sample

3.7.1.7. Determination of moisture content

The moisture contents of the experimental samples were determined, according to AOAC (2000) method 925.09**.** The empty crucible was cleaned and dried in an oven at 105 ℃ for 130 min and placed in a desiccator to cool and weighed the mass of the crucible after cooling (W1). About 5gm of the sample was weighed (W2) and transferred to the crucible and then it was placed inside the oven for 2 hrs (Leicester, LE675FT, England) at 105℃ to dry the samples to a constant weight and cooled in desiccators and re-weighed (W3). Then, the moisture content was estimated by the formula.

$$Moisture\ content\ (\%) = \frac{W_2 - W_3}{W_2 - W_1} * 100\% \text{...} Eq\ (4)$$

Where,

W1= weight of the crucible

W2= Weight of crucible and fresh sample

W3= Weight of the crucible and dry sample

3.7.2. Sensory evaluation

The panelists were chosen from staff and graduating class students of post harvest management department, Jimma University. *Injera* prepared from the different process conditions were evaluated for its sensory acceptability and preference by using 50 consumer participants. All the panelists were frequent consumers of *Injera* and free from any drug addiction (epecially alcohol and narcotic drugs). The age ranges of the participants were 22-40 years old, so that they could fill the scorecard properly. According to Mekuria and Admassu (2011), the orientation was given to panelists before sensory evaluation. Ten grams each sample was cut and placed on a plate with a random coded number within 3-4 hrs after baking (Kobue *et al.*, 2012). The sensory quality was evaluated by five point hedonic scale system (from 1 to 5) where, 5= Like extremely, 4=Like moderately, 3= neither like nor dislike, 2=Dislike moderately and 1=Dislike extremely. During evaluation panelists were informed about parameters and requested to use water for palate cleaners in between each samples of sensory analysis. Each sample was placed some distance far from each other to reduce discussion of the consumers with each other about the sample evaluation.

3.8. Statistical Analysis

The data were analyzed and modeled using Design expert ® version 6.0.2, Minneapolis, USA to generate second-degree polynomial models with response surface effects for the first and second phases of the study. The significant terms in the models were identified by analysis of variance (ANOVA) for each response and accepted at 0.05 level of probability ($p<0.05$). The model adequacy was checked by the regression coefficient (adjusted R^2). The response surface plots were generated to visualize the combined effects of two of the factors on the response, and contour plots were generated for two of the factors while keeping the other third factor at its middle value for the second phase. Numerical optimization of pre-baking process steps and baking condition were conducted by setting target values response variables (minimum, maximum and within the range). It was carried out to find optimum levels of independent variables that would give optimum levels of response. For the third and fourth phases of the study, the results were subjected to analysis of variance (ANOVA) by using Minitab statistical computer software program version 16. Differences were determined by the Tukey test when p-values are significant at 5% probability level.

4. RESULTS AND DISCUSSION

Physicochemical and sensory quality data were collected for pre-baking processing steps (primary fermentation time and temperature, *absit* mixing ratio, cooking time, and secondary fermentation time), and baking condition (time and temperature) were analyzed and detail results and discussion are presented below in tables and figures.

4.1. Fitting the models

To check the adequacy of the models, a lack of fit test was analyzed as a measure of the efficiency of a model to represent data in the experimental domain, at which points were not included in the regression (Montgomery, 1984). ANOVA results showed that R^2 value of all dependent variables was greater than 0.83 indicating that the data explained a high proportion of variability. Additionally, lack of fit was not significant for most of the parameters. Therefore, the results showed that the experimental model was adequate due to no significant lack of fit and satisfactory levels of R^2. Moreover, for all analyzed data diagnostic tools like a normal plot of residuals were tested and indicated that the residuals of all parameters normally distributed.

PHASE I

4.2. Optimization of primary fermentation time and temperature of *teff* dough

The effects of primary fermentation condition (time and temperature) on selected physicochemical quality parameters were analyzed and the results showed significant differences ($P < 0.05$) for all quality parameters. Table 7 indicates the mean values of physicochemical properties of *injera* prepared from varying fermentation time and temperature while Table 8 shows responses are influenced by linear, quadratic and interaction terms of factors.

The results showed that CO_2 production and accumulation was affected by linear and quadratic term of fermentation time, and by linear term of fermentation temperature. Moreover, both number of eyes and rollability of *injera* were affected by linear and quadratic term of fermentation time. Extensibility was affected both by linear and quadratic terms of fermentation time and temperature respectively (Table 8). Both pH and titratable acidity were affected by linear and quadratic terms of fermentation time, and the pH was also affected by linear interaction terms of fermentation time and temperature while TA was affected by quadratic term of fermentation temperature (Table 8). However, moisture content was affected by linear term of fermentation temperature and quadratic terms of fermentation time.

Table 7 Mean values of some physico-chemical quality parameters of *teff injera*

RO	Block	A	B	CO_2 (%)	No of E/cm^2	Roll./2 cm	FWt.L	Ext.(N)	pH	TA (%)	MC (%)
1	1	60	32	93.00	16.04	4.40	12.45	1.48	3.38	0.36	59.80
2	1	96	38	81.00	15.16	4.12	12.86	1.46	3.39	0.59	63.56
3	1	24	25	94.50	8.91	2.85	11.75	1.43	3.85	0.19	61.75
4	1	96	25	83.00	15.73	4.30	10.05	1.36	3.31	0.48	62.59
5	1	60	32	94.00	16.50	4.55	10.25	1.48	3.43	0.49	62.60
6	1	24	38	91.50	13.60	3.45	13.06	1.30	3.67	0.21	64.00
7	1	60	32	94.20	14.85	4.65	9.50	1.47	3.37	0.34	62.82
8	2	60	32	93.90	16.04	4.68	10.50	1.48	3.42	0.35	61.75
9	2	60	22	96.70	16.15	4.80	8.05	1.42	3.45	0.52	58.90
10	2	9	32	78.00	4.73	2.65	13.55	1.29	4.12	0.12	65.35
11	2	111	32	79.30	14.73	4.05	10.55	1.39	3.37	0.53	63.25
12	2	60	32	94.00	16.44	4.70	9.63	1.47	3.40	0.34	63.80
13	2	60	41	88.50	16.23	4.25	11.65	1.39	3.39	0.57	63.00
14	2	60	32	95.25	16.53	4.45	12.35	1.48	3.38	0.36	61.00

RO = *run order, A= Fermentation Time (hrs); B= Fermentation Temperature; No* of *E = Number of eyes/cm^2; Roll= Roll ability (2cm of the wood roller diameter), FWL=fresh weight loss (%) Ext= Extensibility (N); TA= Titaratable acidity (%) and MC= Moisture content (%)*

The results in Table 7 indicate that the primary fermentation condition has significant role in influencing both physical and chemical quality of *injera* and developing optimum condition is necessary in order to get better *injera* with desired physicochemical quality.

4.2.1. Percent of carbon dioxide formation

There were significant differences ($P<0.05$) between treatments in terms of volume of CO_2 produced at the end of primary fermentation time (Table 8). The CO_2 concentration of dough was ranged from 78% to 96.70% (Table 7), the highest value (96.7%) was from dough fermented at 22℃ for 60 hrs, and the lowest for a sample fermented at 32℃ for 9 hrs. The lower CO_2 production was observed at a relatively higher temperature and extended fermentation time, which might be due to less favorable temperature and the conversion of produced CO_2 to different compounds with extended fermentation time. During the fermentation process, the main fermenting microorganisms are lactic acid bacteria (LAB) (Lactobacillus species) (Gashe, 1985) and yeast (Saccharomyces species) (Girma *et al.,* 1989). A similar observation was reported by Michel *et al.* (2016) indicated that a decrease in microflora (yeast/LAB) level with extended fermentation time which could finally influence the production of CO_2. The microorganisms are responsible for the fall of pH to 4.0, gas production and dough rising and are responsible for desired final product flavor and acidity (Umeta and Faulks, 1988). However, temperature control is an important during the fermentation process as changes in fermentation temperature may cause variation in the microflora of the dough and final product quality and flavor (Ganzle, 2014).

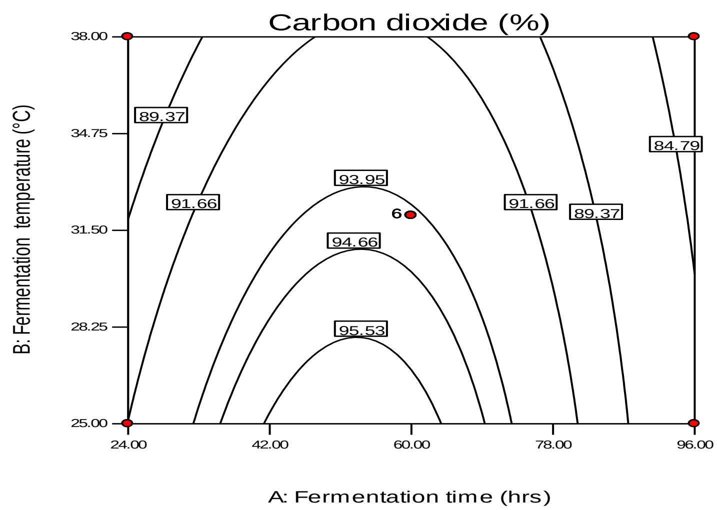

Figure 5 the effect of fermentation time and temperature on carbon dioxide formed during primary fermentation condition

Figure 5 indicates that higher CO_2 production was in between 50-55 hrs of fermentation but with optimum temperature level between 25-28^{o}C. The observed trend in the production of CO_2 could be related to the activity of microflora responsible for fermentation (whether they are on active or dormancy stage) which might be associated with these optimum conditions. During extended fermentation process, the food energy used for the growth of microorganisms is diminished after a certain duration of time and this condition creates a dormancy stage for fermenting microorganisms and inhibits the fermentation process and gas production. A similar observation reported by Heitmann *et al.* (2018). The observations of Yetneberk *et al*. (2004) also support these findings of the study which stated that when the activities of microorganisms and enzymes are limited, the fermentation process could be inhibited.

4.2.2. Number of eyes

There were significant differences ($P<0.05$) between treatments in terms of eye formation on the surface of *injera* (Table 8). The total eye numbers per unit area ranged from 4.73 to 16.53/cm^2 (Table 7). A result reported by Cherinet (1993) (11-15/cm^2) is in the range with results of the present work. As indicated in Table 7, for most of the cases the higher values (16.04-16.23 eyes/cm^2) recorded from samples fermented at 32℃ for 60 hrs while the sample fermented at 32°C for 9 hrs revealed the lowest number of eyes (4.43 /cm^2). This indicates that, fermentation time had an important factor in determining the number of eyes formed per unit area. As indicated in Table 7, there was an association between the number of eyes and CO_2 produced during fermentation.

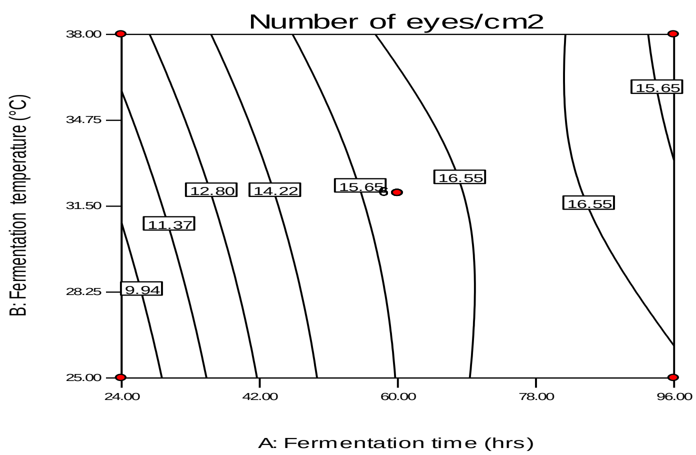

Figure 6: The effect of fermentation time and temperature on the number of eyes

Figure 6 shows that the number of eyes increased with increasing fermentation time irrespective of temperature and decreased for extended fermentation time, approximately 85 hrs. The more accumulation of the gas during fermentation time than the temperature would result in the more number of eyes which corresponds with the better quality *injera*. This observation is supported by the work of Pyle (2005) who stated that CO_2 resulting from the fermentation stage plays a significant role in pore development, and without these nuclei, the final product would lack a porous structure. In this study, it was observed that the higher CO_2 percentage the better the number of eyes formed on the surface of *injera* which mainly influenced by fermentation time than temperature.

Table 8 Regression coefficients of predicted quadratic polynomial and percentage of precision (R^2) for some physical and chemical quality parameters of teff *injera*

Source	CO_2 (%)	No of E /cm^2	Roll/2cm	FWt.L	Ext(N)	pH	TA (%)	MC (%)
Model	93.81**	1.53**	-1.52**	22.12	0.66**	5.12**	1.32**	59.10**
Block	0.39	0.21	-0.09	0.95	0.00	-0.01	-0.01	0.09
A	0.41*	0.41**	0.10**	0.11*	0.00*	-0.03**	0.00**	-0.12
B	-0.20*	0.00	0.17	0.03**	0.05*	-0.03	-0.08	0.19*
A^2	-0.01**	0.00**	0.00**	0.08	0.00**	0.00**	0.00*	0.00**
B^2	-0.01	0.00	0.00	0.71**	0.00*	0.00	0.00**	0.00
AB	0.01	0.00	0.00	0.58	0.00	0.00*	0.00	0.00
Lack of fit	0.00	0.50	0.108	0.8289	0.10	0.11	0.06	0.61
R-Sq	89.32%	89.32%	95.36%	90.52%	87.82%	98.67%	98.52%	82.55%
R-Sq(adj)	80.82%	80.17%	91.39%	79.50%	77.38%	97.54%	97.26%	67.60%

***Significant at 1%, *Significant at 5%, A=fermentation Time (hr), B= Fermentation Temperature (℃), No of E /cm^2 = Number of eyes /cm^2, Roll/2cm = Rollability/2cm, FWL= fresh weight loss (%), Ext (N) = Extensibility (N), TA= Titrable acidity (%) and MC= moisture content (%)*

4.2.3. Rollability of *injera*

The results showed that this rollability was significantly influenced ($P<0.05$) by treatment factors (Table -8) and ranged from 2.65 to 4.80/2cm diameter of the roller wood (Table 7). The highest value for rollability corresponded to the sample fermented at 22℃ for 60 hrs, while the sample fermented at 32℃ for 9hrs revealed the lowest rollability values.

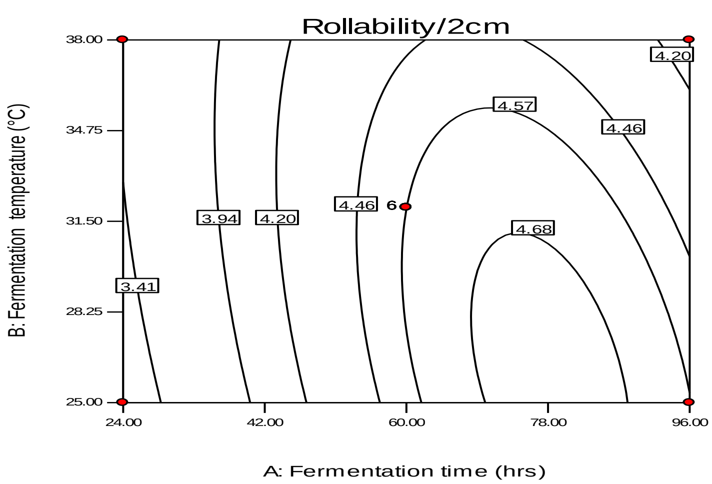

Figure 7: The effect of fermentation time and temperature on rollability of *injera*

It was observed that both short fermentation time and higher fermentation temperature hurt rollability. The present finding is in line with the work of Mihretie and Bultosa (2017), who reported that *injera* baked from dough fermented for shorter fermentation time becomes more brittle. As fermentation time extended, the components of *teff* flour could be degraded by microorganisms and contribute to elasticity. As indicated in figure 7, the optimum condition were at temperature range of 25-29^{o}C with a fermentation time of 70-80 hrs. The lower value for shorter fermentation time might be less chance of modification properties of the starch by microflora which results in cracking or trearing of *injera* during rolling. Rollability is one of the desired properties of *injera* to have the desired texture before consumption. Good quality *injera* is being soft and able to be rolled without cracking and this is the mark of good quality (Yetneberk *et al.,* 2004). So far, there is no standard range for the rollability of *injera*, but there is a common understanding that the higher the value is the better.

4.2.4. *Fresh weight loss of injera*

There were significant differences (P<0.05) among treatments in terms of fresh weight loss of *injera* (Table -8). The fresh weight loss of *injera* samples was ranged from 8.05- 13.55%. The highest value for fresh weight loss corresponded to the sample fermented at 32°C for 9 hrs while the sample fermented at 22°C for 60 hrs revealed the lowest weight loss (Table 7). This is probably due to the lack of moisture content of *injera* at this fermentation temperature and time among different treatments.

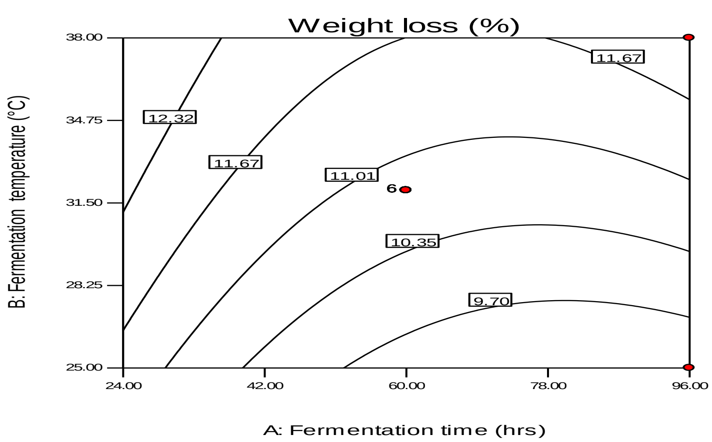

Figure 8 Response surface plots showing the effect of fermentation time and temperature on fresh weight loss

Figure 8 indicated that fresh weight loss of *injera* during primary fermentation increased directly with increment in fermentation temperature and fermentation time upto certain point.

The ability to water holding capacity of products could be rated with its biological molecules like fermentable sugars (Fuentes-Zaragoza *et al.,* 2010). There are high fermentable sugars at lower fermentation time and their ability to hold moisture in the final product (*injera*) could be high at this condition. The more moisture in the food leads higher moisture/fresh weight loss from the products. Another possible reason for the variations in the *injera* fresh weight loss could be related with fermentation temperatures. Pinnavaia and Pizzirani (1998) reported that there is a good correlation between the water holding capacity and the degree of gelatinization, for certain starchy products. As fermentation temperature increases it attribute to increament in starch gelatinization of batters and result in higher water holding capacity. Since, the more moisture in the food leads higher moisture/fresh weight loss from the products and correlated with the result of the present finding. Gelatinization progress along the granule is determined by the physicochemical properties of the starch, the availability of water and process parameters applied (temperature and time) (Schirmer *et al.,* 2015).

4.2.5. Extensibility of *injera*

There were significant differences (P<0.05) between treatments in terms of extensibility of *injera* (Table -8). The extensibility values of *injera* were ranged from 1.29 to 1.48N. The highest extensibility value of *injera* was measured from the sample fermented at 32°C for 60 hrs while the sample fermented at 32°C for 9hrs revealed the lowest extensibility value (Table 7). Likewise other properties, extensibility shows similar trends which highly influenced by fermentation time than temperature. The dough should ferment for sufficient time to degrade the structural components of *teff* flour-like proteins, starch and hemicelluloses and produce higher exopolysaccharides that are responsible for the elasticity of *injera* (Attuquayefio 2014). The author stated that *injera* fermented for primary fermentation time of 72 hrs had the highest modulus and this may be due to more bacterial exopolysaccharides produced after primary fermentation time. Ruhmkorf *et al.* (2012) also stated that Exopolyssachides produced can act as hydrocolloids and hence may have contributed for the elastic texture of *injera*.

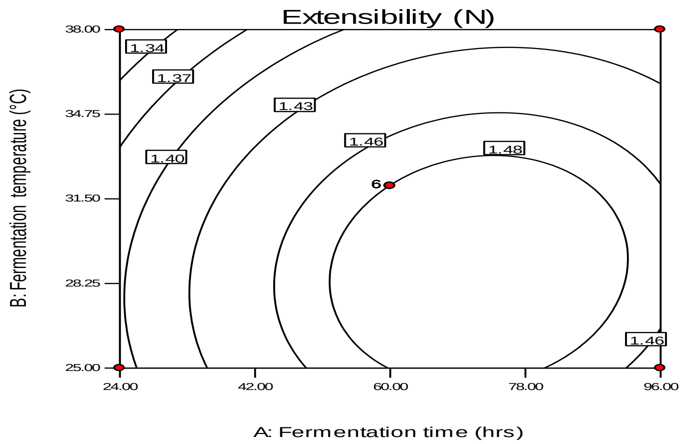

Figure 9: The effect of fermentation time and temperature on extensibility

As indicated in figure 9, the extensibility of *injera* attained the higher value for fermentation time between 60-80 hrs and fermentation temperature of 25-32^{o}C. Lazaridou *et al.* (2007) stated that the protein matrix is a major determinant of the important rheological characteristics of dough during primary fermentation, like elasticity, extensibility, resistance to stretch and gas-holding ability which might critically be attained at these ranges. This protein matrix could be affected at a higher temperature and decrease the matrix contribution in the elasticity of the *injera*. Therefore, applying optimum fermentation temperature and time is very important to increase the extensibility of *injera*.

4.2.6. pH of *injera*

The mean values of pH showed significant differences (P<0.05) among treatments (Table -8). Analysis of different treatment of *teff injera* showed that the pH of fresh *injera* samples ranged from 3.31 to 4.12 (Table-7). The values recorded are slightly related to what was reported in different literature (Ashagrie and Abate, 2012; Attuquayefio, 2014) for *teff injera*. The highest (4.12) pH value as compared to other *injera* measured from the sample fermented at a temperature of 32°C for 9 hrs while the sample fermented at 25°C for 96 hrs revealed the lowest (3.31) pH value (Table-7) (figure 10). The lower the pH from the food safety point of view, always the better which inhibits the growth of bacteria. However, it might alter the taste and flavor of *injera*. It observed that as primary fermentation time and temperature increased the pH values decreased due to the act of LAB on *teff* batter (Table 7). According to Urga *et al.* (1997), the pH readings of *injera* was reported with different figures due to variation in fermentation time and temperature. The pH value was dependent on the lactic acid content in the fermented batter on the day of baking which indicated that as fermentation time and temperature increased the pH value of *injera* decreased (Attuquayefio, 2014; Urga *et al.*, 1997). Moisture, amylose and starch contents of the *teff* are significant in affecting pH values depending on the primary fermentation time and temperature (Sahlin, 1999).

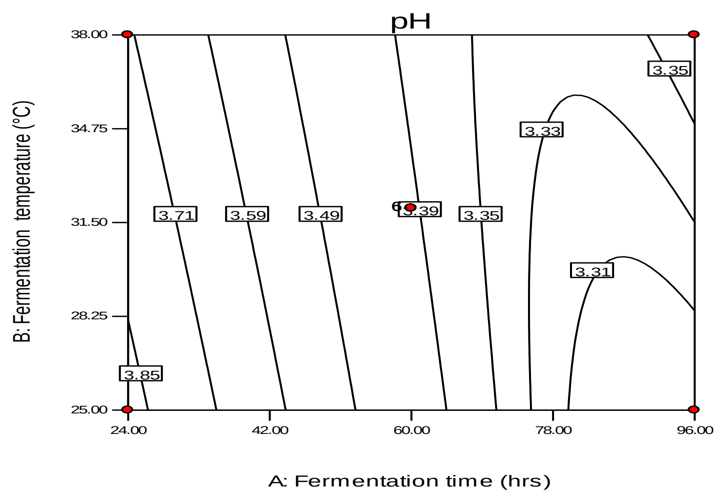

Figure 10: The effect of fermentation time and temperature on pH

At the initial primary fermentation process, the number of fermentable sugars is very high and these conditions varied pH values after baking of *injera*. The sourness taste of traditionally fermented Ethiopian *injera* is caused by its pH that indicates the sensory quality of the products due to change in Lactic acid concentration during fermentation (Yigzaw *et al.*, 2004; Urga and Narasimha, 1997).

4.2.7. Titratable acidity of *injera*

There were significant differences (P<0.05) among treatments in terms of mean values of titratable acidity of *injera* (Table -8). The titratable acidity (TA) values in this study were ranged from 0.12 to 0.59% (Table-7). This result is related to the observation by Megersa *et al*. (2017) who reported TA value of 0.279 to 0.586 on optimization of processing condition for dough to manufacture *injera* and varies with the work of Attuquayefio (2014) who recorded 2.33% on *teff* *injera*. At around 32^{o}C of primary fermentation temperature, the acidity of *injera* increased with the increase of fermentation time (Figure 11). When fermentation time and temperature increased, *teff* batter becomes more acidic, and this condition leads to an increment of an acidic value for the final product (*injera*).

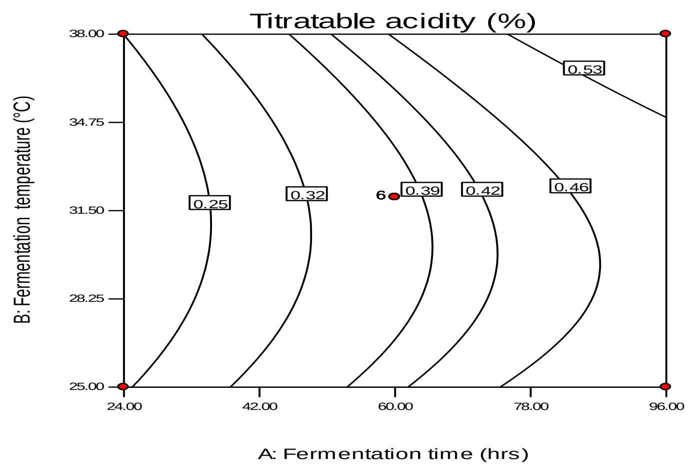

Figure 11: The effect of fermentation time and temperature on titratable acidity (TA) concentration

The highest (0.59%) value of titratable acidity as compared to other treatments was recorded from the sample fermented at 38°C for 96 hrs while the sample fermented at 32°C for 9hrs revealed the lowest (0.12%) titratable acidity value (Table-7). This variation is probably due to the extent of primary fermentation time which make a high amount of acid could be produced in the highest fermentation time which corresponds with lower pH value.

4.2.8. Moisture content of *injera*

There were significant differences (P<0.05) among treatments in terms of mean values of moisture content of *injera* (Table -8). In this study, the moisture content of *injera* for each run of the experiment was measured and ranged between 58.90 to 65.35% (Table-7). The measured values in this study is in line with the specification of moisture content value set by standard Ethiopian agency (58% to 63%) (ESA, 2013) except for treatment conducted at 32°C for 9 and 24hrs. It was also in line with other literature results conducted on *teff injera* 65.23% reported by Attuquayefio (2014), in the range of 62-65% reported by (Ashagrie and Abate, 2012) and 61.04 % - 62.32% reported by (Dessalegn, 2018). The highest (65.23%) value of the moisture content of fresh *injera* was obtained at 32°C for 9hrs as compared to other treatments, while the sample fermented at 22°C for 60 hrs revealed the lowest (58.90 %) moisture content value (Table 7).

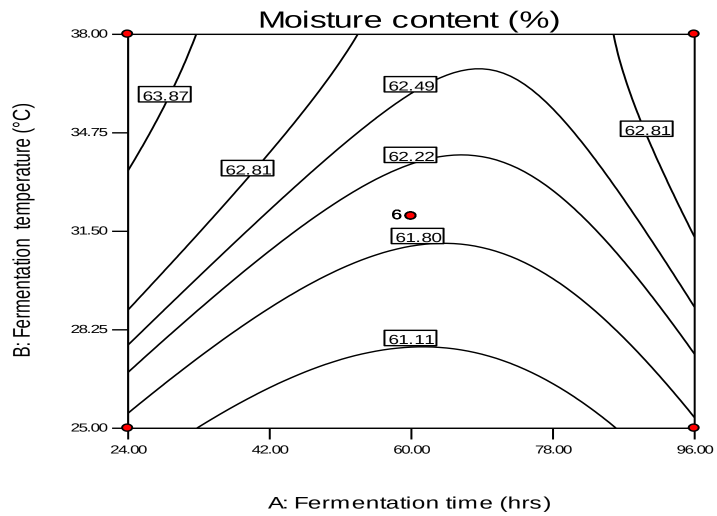

Figure 12: The effect of fermentation time and temperature on moisture content

Figure 12 indicated that the moisture content of *injera* is increased directly with the increase of fermentation temperature at constant time. Fermentation temperature mainly influenced the moisture content in comparison with fermentation time. The possible reason for increment in moisture content with fermentation temperature might be the gelatinization of starch at high fermentation temperature. The higher starch gelatinization formed due to higher fermentation temperature attributes to higher water holding capacity of batters. This, in turn, contributed to higher moisture content, as observed in the present finding. A similar observation was reported by Pinnavaia and Pizzirani (1998) who stated that there is a good correlation between the water holding capacity and the degree of gelatinization for certain starchy foods.

4.2.9. Optimization of primary fermentation condition for response variables

A numerical optimization procedure was used for identifying the best condition by incorporating appropriate constraints to establish the independent and dependent variables on the response of processing conditions. It was carried out by setting criteria (minimum, maximum and in the range) for each response to find the optimum level of independent variables (time and temperature) that could produce the best quality *injera*. Accordingly, 64 hrs of primary fermentation time and 25°C of primary fermentation temperature were numerically optimized as optimum values at 0.86 desirability value. The optimum values and optimization criteria for selected dependent variables are indicated in table 9.

Table 9 Response optimization for values of primary fermentation process parameters

Response	Goals	Optimized values
Carbon dioxide concentartion	Maximum	95.56%
Number of eyes	In range	$16.06/cm^2$
Rollability	Maximum	4.60/2cm
Fresh Weigh loss	minimum	9.30%
Extensibility elasticity	Maximum	1.45N
pH	In range	3.40
Titratable acidity	In range	0.43%
Moisture content	In range	60.44%

In general, temperature and time of primary fermentation condition significantly influenced both the physical and chemical quality of *injera*. However, the results of this study indicated that the impact of fermentation time is more significant than fermentation temperature.

PHASE II

4.3. Optimization of *absit* mixing ratio, cooking time and secondary fermentation time

This phase of the work was conducted to optimize *absit* mixing ratio, *absit* cooking time and secondary fermentation time for better *injera* quality and consistency. Optimized primary fermentation time and temperature from objective one (64 hrs and 25°C) were used as an input to optimize the intended objective in this phase. Selected physicochemical quality parameters (number of eyes, rollability, fresh weght loss, extensibility, pH, titratable acidity and moisture content) were measured and analyzed, and the ANOVA results showed significant differences ($P < 0.05$) for the parameters. Table 10 shows the mean values of physico-chemical property of *injera* prepared from varying *absit* mixing ratio, cooking time and secondary fermentation time.

Results indicated that the number of eyes and rollability of *injera* were affected both by linear and quadratic terms of *absit* mixing ratio, while rollability was specifically affected by linear terms of *absit* cooking time and by quadratic terms of secondary fermentation time (Table 11). Moreover, both extensibility and pH were affected by linear terms of *absit* mixing ratio, while extensibility was specifically affected by both quadratic term of *absit* mixing ratio and cooking time. However, pH was affected by linear terms of *absit* cooking time and linear interaction terms of *absit* mixing ratio and cooking time. Both titratable acidity and moisture content were affected by linear terms of *absit* mixing ratio, and TA was specifically affected by linear terms of secondary fermentation time (Table 11).

Table 10 Mean values of some physico-chemical quality parameters for absit mixing making process of *teff injera*

RO	Blocks	A	B	C	No of E/cm^2	Roll./2 cm	Ext.(N)	pH	TA (%)	MC (%)
1	Block 1	4	10	10	16.55	4.72	1.53	3.40	0.52	61.55
2	Block 1	4	10	10	16.58	4.55	1.48	3.43	0.46	61.85
3	Block 1	6	5	5	15.66	4.38	1.52	3.49	0.32	63.00
4	Block 1	4	10	10	16.59	4.73	1.48	3.40	0.42	61.00
5	Block 1	2	15	5	10.60	4.15	1.36	3.50	0.38	63.25
6	Block 1	6	15	15	7.50	2.89	1.32	3.41	0.55	63.56
7	Block 1	6	5	15	14.55	4.45	1.40	3.48	0.39	63.87
8	Block 1	2	5	15	13.43	3.96	1.28	3.45	0.45	63.95
9	Block 1	2	15	15	8.56	2.96	1.24	3.47	0.48	62.50
10	Block 1	4	10	10	16.54	4.75	1.51	3.43	0.41	58.85
11	Block 1	2	5	5	11.48	3.25	1.41	3.54	0.24	64.00
12	Block 1	6	15	5	10.35	3.95	1.45	3.41	0.47	62.30
13	Block 2	4	10	10	16.60	4.74	1.50	3.40	0.43	59.90
14	Block 2	4	18	10	6.23	2.25	1.38	3.52	0.55	65.23
15	Block 2	4	10	18	14.87	4.25	1.24	3.42	0.49	63.00
16	Block 2	4	10	2	14.31	4.73	1.38	3.46	0.44	62.45
17	Block 2	1	10	10	9.87	4.25	1.35	3.41	0.42	60.52
18	Block 2	7	10	10	15.54	4.25	1.52	3.43	0.53	64.95
19	Block 2	4	2	10	6.50	2.95	1.22	3.48	0.46	62.45
20	Block 2	4	10	10	16.60	4.75	1.57	3.41	0.42	61.00

RO= Run order, A= Secondary fermentation time (hr), B= absit mixing ratio (%), C= absit cooking time (min), Roll= Rollability/2cm wooden roller of diameter, Ext. = extensibility (N), TA= Titaratable acidity (%), and MC= Moisturecontent(%)

The results in Table 10 indicated that the *absit* mixing ratio, *absit* cooking time and secondary fermentation time have a significant role in influencing both physical and chemical quality of *injera* and developing the optimum condition is necessary in order to get better *injera* with desired physicochemical quality.

4.3.1. Number of eyes

There were significant differences ($P < 0.05$) among treatments in terms of eye numbers of *injera* (Table 11). The mean values of eye numbers ranged from 6.23 to 16.6/cm^2 (Table-10). The values were a little bit higher than the finding of Dessalegn (2018), who reported the number of eyes between 11.32 and 13.65 eyes/cm^2 for *teff injera*. The higher (16.6 eyes /cm^2) values were from *injera* made with 10% *absit* mixing ratio, 10 min of cooking time and 4 hrs of secondary fermentation time. Both the higher and lower mixing ratio values result in the lower number of eyes per unit area. For instance, the sample baked from 18% *absit* mixing ratio, cooked for 10 min and 4 hrs of secondary fermentation time resulted in the lowest (6.23 eyes/cm^2) number of eyes (Table-10). Similarly lower *absit* mixed ratio value of 2% with 10 min cooking time and 4 hrs secondary fermentation time resulted in similar eye numbers (6.5 eyes/ cm^2).

Optimum *absit* mixing ratio with batter after gelatinization of starch for 10 min cooking results in the hydrocolloids and contributes more to enhance the gas holding capacity of the batter through modifying the viscosity. Moreover, *absit* could have a role in the activation of yeasts responsible for CO_2 production in secondary fermentation time for the occurrence of eyes during baking of *injera.* Cooking could break down starch components to form more sugars for yeast activity for more fermentation and CO_2 production. Different authors reported that *absit* mixing ratio contributes significantly to eye numbers (Assefa *et al.,* 2018; Dessalegn 2018).

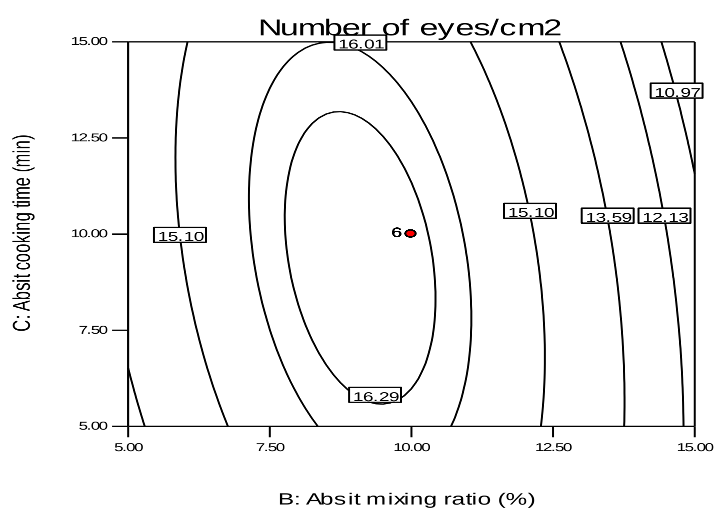

Figure 13: The effect of *absit* mixing ratio and cooking time on number of eyes of *injera*

Figure 13 shows that *absit* mixing ratio between 8 to 10% with the cooking time of 5 to 11 minutes identified as the optimum range for *absit* preparation to get a high number of eyes per unit area after 4 hrs secondary fermentation. Particularly with an increase in mixing ratio above 10% decreases the number of eyes formed per unit area. Ashenafi (2006) reported that *injera* baked with less *absit* or overdose amount of *absit* ratio has a lesser amount of eyes.

4.3.2. Rollability

There were significant differences (P < 0.05) among treatments in terms of rollability of *injera* (Table 11). The mean values of rollability for different treatments were ranged from 2.25 to 4.75/2cm (Table 10). The highest mean value (4.75/2cm) for rollability corresponded to the sample baked with 10% of *absit* mixing ratio, 10 min of cooking time and 4hrs of secondary fermentation time at ambient temperature. However, the samples baked with a high percentage of *absit* ratio (18%), cooked for 10 min and fermented for 4 hrs secondary fermentation revealed the lowest rollability value. These differences were probably due to the variation in *absit* mixing ratio which made it to brake easily during rolling time.

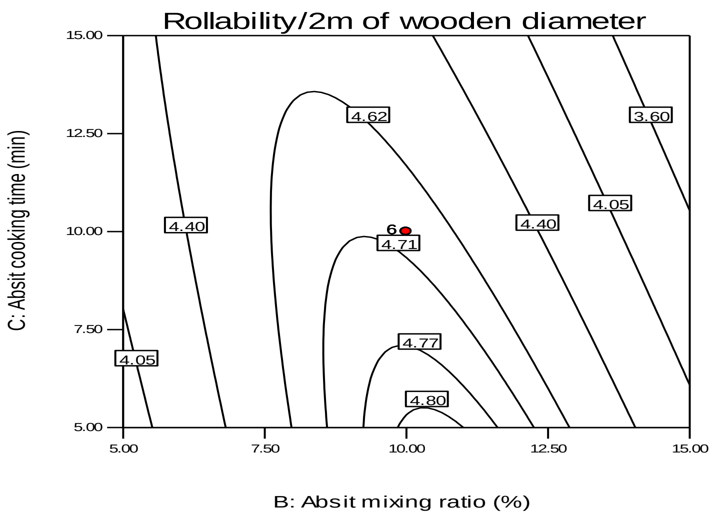

Figure 14: The effect of *absit* mixing ratio and cooking time on rollability of *injera*

As indicated in figure 14, rollability of *injera* decreased with increasing mainly *absit* mixing ratio and cooking time. In this observation, the result indicated that both the lowest (2%) and the highest (18%) levels of *absit* ratio cause significant change on rollability of *injera* and the optimum being close to 10% *absit* mixing ratio. A similar finding also reported in Houben *et al.*

(2012); the proper amount of gelatinized starch added to the original fermented dough is used to increase the viscosity of the batter and responsible for the elasticity of baked *injera*. Ashenafi (2006) also indicated that *injera* baked without *absit* or with less *absit* is easily breakable.

4.3.3. Extensibility

There were significant differences ($p<0.05$) among different treatments in terms of extensibility of *injera* (Table 11). The extensibility of *injera* samples was ranged from 1.22- 1.57N (Table 10). The highest (1.57N) value measured from the sample baked with 10% of *absit* mixing ratio, cooked for 10 min and 4 hrs of secondary fermentation time. However, the samples baked with the highest (2%) *absit* mixing ratio, cooked for 10min and 4 hrs of secondary fermentation time revealed the lowest extensibility value (Table 10). Attuquayefio (2014) stated that the elastic modulus of *injera* samples increased gradually with increasing viscosity of batters by adding some amounts of *absit* to the original dough and this condition could be used to increase the elasticity/extensibility of baked *injera* reported in similar finding.

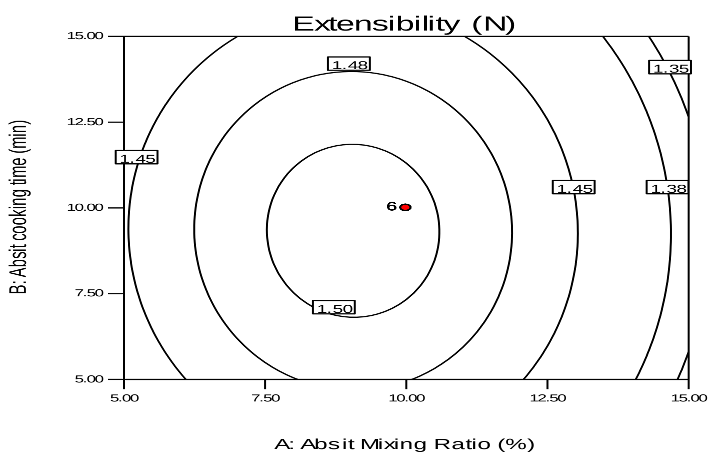

Figure 15: The effect of *absit* mixing ratio and cooking time on the extensibility of *injera*

The viscosity, texture and rheological properties of many cooked starchy foods are affected by the interaction of starch with protein or change in the structure due to bacterial or enzymatic activities or other processes (Bultosa and Taylor, 2004; Wang *et al.*, 2015). The result of this study shows that extensibility moderately decreased with an increased mixing ratio of the *absit* from the fermented dough, as indicated in figure 15.

Table 11 Regression coefficients of predicted quadratic polynomial and percentage of precision (R^2) for some physicochemical quality parameters of *teff injera*

Source	N of E/cm^2	Roll/2cm	Ext. (N)	pH	TA (%)	MC (%)
Model	-1.31**	1.27**	0.91**	3.85**	0.17**	72.49**
Block	0.41	0.17	0.01	0.01	-0.02	-0.04
A	2.88**	0.39**	0.06**	-0.02**	0.00**	-0.72**
B	0.32	0.16*	0.03	-0.03*	0.02	-0.51
C	2.20	0.65	0.08	-0.03	0.02**	-0.76
A^2	-0.15**	-0.02**	0.00**	0.00	0.00	0.00
B^2	-0.02	-0.01	0.00**	0.00	0.00	0.01
C^2	-0.27	-0.07*	-0.01	0.00	0.00	0.01
AB	-0.01	-0.01	0.00	0.00*	0.00	0.02
AC	-0.03	0.00	0.00	0.00	0.00	0.04
BC	0.04	0.00	0.00	0.00	0.00	0.02
Lack of fit	0.00	0.051	0.066	0.068	0.24	0.32
R-Sq	86.48%	88.04%	90.22%	88.36%	88.91%	93.02%
R-Sq(adj)	71.45%	74.75%	79.36%	75.43%	76.58%	85.26%

***Significant at 1%, *Significant at 5% A= Secondary fermentation time (hrs), B= Mixing ratio of absit (%), C= Cooking time of absit (min), Roll= Rollability/2cm wooden roller of diameter, Ext. = extensibility (N), TA= Titaratable acidity (%) and MC= Moisturecontent (%)*

4.3.4. pH

There were significant differences (P<0.05) among different treatments in terms of *injera* pH (Table 11). In this study, the pH of *injera* samples was ranged in between 3.4 and 3.54 (Table-10). The values recorded for pH in the present study were related with the result obtained by Attuquayefio (2014) who recorded 3.73 of pH on *teff injera*. The highest (3.54) value of pH corresponded to the sample baked with 5% of *absit* mixing ratio from fermented dough, 5 min of cooking time and 2 hrs of secondary fermentation time while the sample baked with 10% of *absit* mixing ratio, 10 min of cooking time and 4 hrs of secondary fermentation time revealed lowest (3.4) pH. The variation was probably due to the higher fermentation time and *absit* mixing ratio that causes the dough becomes more acidic leading to a decrease in pH after baking. In a different kind of literatures the pH readings of *injera* is variable due to the variation of fermentation time (Urga *et al.,* 1997; Yigzaw *et al.,* 2004). Figure 16, indicates that the pH values decreased with the increase of *absit* mixing ratio.

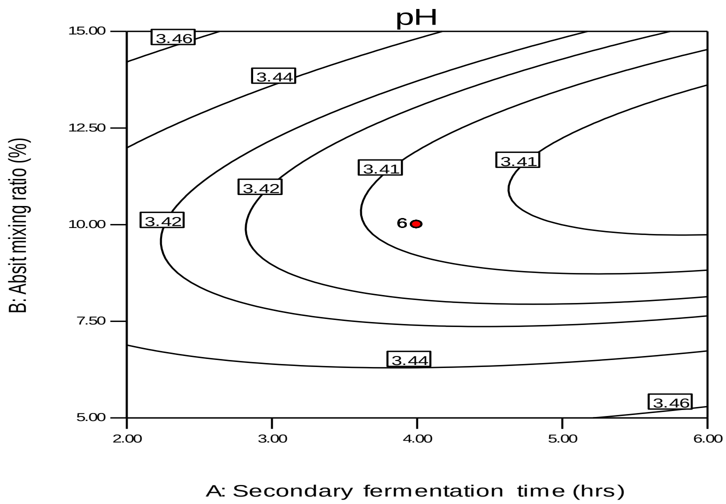

Figure 16: The effect of *absit* mixing ratio and secondary fermentation time on pH of *injera*

4.3.5. Titratable acidity

There were significant differences (P<0.05) among different treatments in terms of titratable acidity of *injera* (Table -11). The TA of *teff injera* was ranged from 0.24 to 0.55% (Table-10). The results related to the observation of Megersa *et al.* (2017) who recorded in the range of 0.279 to 0.586% on *teff* dough for *injera* production. The highest (0.55%) value of TA recorded from sample baked with (15 and 18%) of *absit* mixing ratio, (10 and 15 minutes) of cooking time and (4 and 6 hrs) of secondary fermentation time, while the sample baked with 5% *absit* mixing ratio, 5min cooking time and 2 hrs of secondary fermentation time revealed the lowest titratable acidity contents (Table-10). This variation is probably due to the high amount of *absit* added to the original dough, cooking time and secondary fermentation time that made a difference among treatments. The content of lactic acid at a certain pH is very much dependent on fermentation time and *absit* added to the original dough (Sahlin, 1999). According to Attuquayefio (2014), environmental conditions such as pH and moisture content of batter may increase the activity of the flour amylases as well as starch hydrolyzing bacteria during fermentation. This, in turn, increases the amounts of fermentable sugars and acid production. The result of this study shows that titratable acidity moderately increased with the increasing of mixing ratio of the *absit* from the fermented dough and cooking time as indicated in the fig-17.

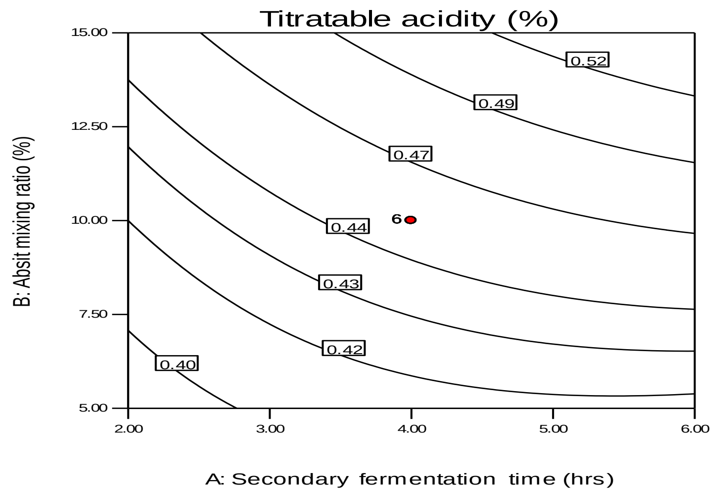

Figure 17: The effect of *absit* mixing ratio and secondary fermentation time on titratable acidity of *injera*

4.3.6. Moisture content

There were significant differences (P<0.05) among different treatments in terms of moisture content of *injera* (Table -11). In this study, the moisture content of fresh *injera* samples (as soon as it was baked) were ranged from 58.850 to 65.23% (Table-10). The result obtained by Dessalegn (2018) was similar to this study which reported in the range of 61.04% to 62.32% of moisture content for *teff injera*. The highest (65.23%) moisture content was obtained from *injera* made with *absit* mixing ratio of 18%, cooked for 10 min and left for 4hrs of secondary fermentation time, while the sample with 10% of *absit* mixing ratio, 10 min of cooking time and 4hrs of secondary fermentation time revealed the lowest of moisture content value. This variation was probably due to the low and high amount of *absit* added to the original dough.

Moisture in food is known to affect quality. The more water content in the batter due to high amount of *absit* added will result in the more moisture content in the final product. Gelatinization of starch is strongly affected by the water content of food products (Pyle, 2005). Therefore, the moisture content of *injera* has an effect on its texture and shelf life. Ashagrie and Abate (2012) stated that the moisture content of the *injera* samples were affected the shelf life of *injera*. As indicated in figure 18, the moisture content of *injera* increased with the increasing of *absit* mixing ratio in comparison with secondary fermentation time.

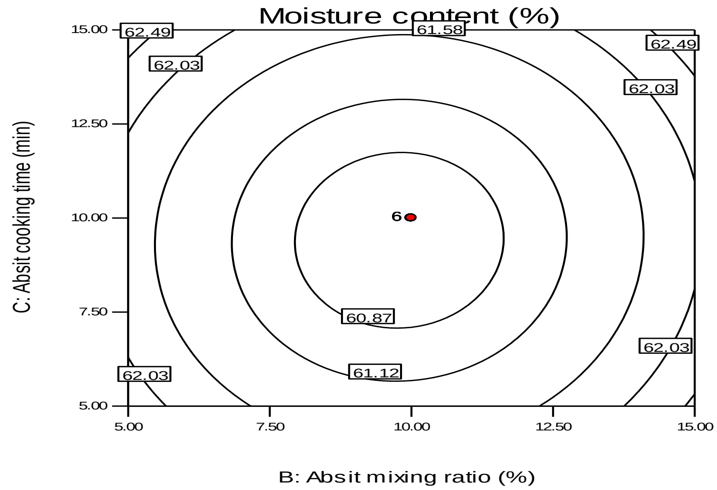

Figure 18: The effect of *absit* mixing ratio and cooking time on the moisture content of *injera*

4.3.7. Optimization of *absit* mixing ratio, cooking time and secondary fermentation time for response variables

A numerical optimization procedure applied for identifying the best condition by incorporating appropriate constraints to establish the independent and dependent variables on responses of processing conditions. It was carried out by setting criteria (maximum and in the range) for each response to find optimum levels of independent variables (*absit* mixing ratio, cooking time and secondary fermentation time) that could produce the best quality *injera*. Accordingly, 8% of *absit* mixing ratio, 10 min of cooking time and 4 hrs of secondary fermentation time were obtained as optimum values for independent variables at 0.89 overall desirability value. The optimum values and optimization criteria for selected dependent variables are indicated in Table 12 and used as an input together with optimized values of objective one to accomplish objective three.

Table 12 Response optimization of dependent variables for the second phase of study

Response	Goals	Optimized values
Number of eyes	In range	16.46/cm^2
Rollability	Maximum	4.68/2cm
Extensibility	Maximum	1.5N
pH	In range	3.42
Titratable acidity	In range	0.44%
Moisture content	In range	60.82%

PHASE III

4.4. Optimization of baking time and temperature of *teff injera*

This phase of the work was conducted to optimize baking condition for better *injera* quality and consistency. Optimized primary fermentation time and temperature from objective one (64 hrs and 25°C) and optimized *absit* mixing ratio (8%), *absit* cooking time (10 min) and secondary fermentation time (4 hrs) from objective two were used as an input to optimize the intended objective in phase three. Different analysis were conducted on *injera* prepared from kuncho *teff* treated with baking temperatures of 195±5, 215±5, 235±5 and 255±5°C for 1, 2 and 3 minutes. The analysis results revealed that the interaction effect of baking temperature and time were significantly differed ($P < 0.05$) on all physicochemical and sensory quality parameters.

4.4.1. Physico-chemical quality parameters of *teff injera* affected by baking condition

Table 13 shows the range values for number of eyes, extensibility, pH, TA and moisture contents of *injera* as affected by baking temperature and time. The ranges of values of these parameters were eye numbers (10.50–16.9/cm^2), extensibility (1.26–1.55N), pH (3.42–3.75), titratable acidity (0.40–0.62%, and moisture content (59–66.11%).

Table 13 Mean values of selected physicochemical quality parameters of *injera* based temperature and time

A(min)	B(^{o}C)	No of E/cm^2	Ext. (N)	pH	TA (%)	MC (%)
	195±5	10.50±0.19^{g}	1.26±0.04^{fg}	3.42±0.04^{g}	0.62±0.06^{a}	66.11±0.65^{a}
	215±5	11.30±0.15^{fg}	1.30±0.11^{f}	3.43±0.04^{g}	0.60±0.06^{ab}	65.60±0.62^{ab}
1	235±5	13.10±0.82^{cde}	1.36±0.14^{ef}	3.50±0.08^{ef}	0.50±0.06^{bc}	64.90±0.53^{abc}
	255±5	13.40±0.30^{def}	1.39±0.09^{cd}	3.54±0.06^{de}	0.52±0.06^{cd}	63.30±0.47^{bcd}
	195±5	12.30±0.74^{ef}	1.40±0.06^{bc}	3.46±0.07^{fg}	0.50±0.04^{bc}	65.30±0.54^{ab}
	215±5	14.80±0.12^{cde}	1.45±0.07^{bcd}	3.50±0.04^{ef}	0.51±0.06^{cd}	64.80±0.67^{abc}
2	235±5	15.80±0.17^{abc}	1.52±0.08^{ab}	3.55±0.06^{d}	0.54±0.07^{cde}	64.30±0.51^{abcd}
	255±5	16.80±0.43^{a}	1.55±0.10^{a}	3.73±0.06^{ab}	0.42±0.06^{g}	60.50±0.52^{de}
	195±5	16.30±0.11^{ab}	1.45±0.04^{bcd}	3.53±0.06^{de}	0.53±0.08^{cd}	64.20±0.42^{abc}
	215±5	15.50±0.48^{bcd}	1.53±0.06^{ab}	3.64±0.06^{bc}	0.46±0.06^{f}	61.70±0.58^{cd}
3	235±5	16.90±0.47^{a}	1.55±0.08^{a}	3.74±0.06^{a}	0.45±0.07^{f}	60.50±0.41^{de}
	255±5	15.80±0.38^{abc}	1.52±0.08^{ab}	3.75±0.06^{a}	0.40±0.06^{g}	59.00±0.43^{e}
	CV	6.54	4.64	2.55	3.34	3.73

A= Baking time (min), B= Baking temperature (^{o}C), No of E = *Number of eyes/cm^2, Ext. =Extensibility (N), TA= Titaratable acidity (%) and MC= Moisture content (%)*

4.4.1.1. Number of eyes

Injera baked at 195±5°C for 3 min, 235±5°C for 2 and 3 minutes, and 255±5°C for 2 and 3 minutes had the highest number of eyes (15.80–16.9/cm^2) as compared to other treatment combinations while the lowest eyes number (10.50–11.30/cm^2) were from *injrea* baked at 195±5°C and 215±5°C for 1 min (Table -13). These results were related to the work of Cherinet (1993), who determined an appropriate number of eyes on the surface of *injera* and recorded the value in range of 11-15 eyes/cm^2. There was some variation in *injera* eyes observed from different treatments in this study. During baking time, the gas bubbles created nuclei and result in eye formation on the surface of *injera*. The nuclei to be created, it might need sufficient baking temperature and time. Therefore, lower temperature and shorter time produced less gas bubbles and less number of eyes as a result. Similar finding was recorded by Pyle (2005) who stated that the dissolved CO_2 released from batter during cooking of *injera* could contribute to pore development and overall expansion of the baked *injera*. In general baking temperature of 235 or 255^{o}C with 3 to 2 min baking time was found better baking combinations for large number of eyes per unit area.

4.4.1.2. Extensibility/ Elasticity of injera

The highest mean value of extensibility (1.5–1.55N) was recorded from *injera* made at a temperature of 215±5°C for 3 min, 235±5 and 255±5°C for 2 and 3 minutes respectively (Table -13). The lowest (1.26 –1.36N) mean values of extensibility was recorded from at 195±5, 215±5 and 235±5°C for 1 min. Likewise, eye number, extensibility also associated with relatively higher baking temperature for 2 to 3 min as compared to the lower temperature and time. The variation observed in terms of extensibility could be caused by a lack of sufficient starch gelatinization due to lower temperature and shorter time during baking. Similar finding was reported by Parker *et al*. (1989) who stated that, during baking of *injera*, the starch found in the batter gelatinize to form a steam-leavened and spongy starch matrix and contributes for elastic property of *injera*.

4.4.1.3. pH of injera

Analysis of different treatments of *teff injera* indicated that the highest pH of fresh *injera* (3.73–3.75) was measured from *injrea* baked at 255±5°C for 2 min and 235±5 and 255±5°C for 3 min respectively (Table 13). The lowest pH (3.42–3.46) value was recorded at 195±5 and 215±5°C for 1 min and 195±5°C for 2 min respectively. The values recorded are slightly related to the work of Ashagrie and Abate (2012) on *teff injera*. The variations observed among treatments are probably due to the decrease in the moisture content during baking that causes a change in pH. In this study, the pH and moisture content have an inverse relationship, as pH increase the moisture content decrease. This might be related with higher baking temperature, lower moisture content and volatile loss of hydrogen ions could be formed and result in lower acidity (high pH). A similar finding was reported by Attuquayefio (2014) who reported that the inverse relationship between the pH and moisture content for commercially available *injera* which was recorded the highest moisture (60.40%) content for pH of 3.65 and lowest moisture content (44.46%) for pH of 4.02. Moreover, the Ethiopian Standard Agency (ESA) (2013) has set the pH of *teff injera* has to be in the range of 3.45 to 4.0 and the value of pH obtained in this work was not significantly different from the set values of ESA since it was in between 3.46 to 3.75 except for *injera* baked at 195±5 and 215±5°C for 1 min (Table 13).

4.4.1.4. Titratable acidity of injera

The highest mean value of titratable acidity (0.6 – 0.62%) of fresh *injera* was recorded from *injera* baked at 195±5 and 215±5°C for 1 min while the lowest mean value (0.40–0.42%) was registered from *injera* baked at 255±5°C for 2 and 3 minutes respectively (Table -13). This result was related to what was reported by Megersa *et al.* (2017) on the optimization of processing condition for *injera* manufacturing while varied with the experiment conducted by Attuquayefio (2014). The variation in this study was probably due to the high amount of temperature and the lowest baking time, which made a difference among different treatments. As baking time and temperature increased the amount of TA production decreased since it has inverse relationship with the pH value (Table -13).

4.4.1.5. Moisture content of injera

The highest mean value of moisture content (64.20 – 66.11%) of fresh *injera* samples was obtained from *injera* baked at 195±5, 215±5 and 235±5°C for 1min, 195±5, 215±5 and 235±5°C for 2min and 195±5°C for 3 min respectively (Table 13). However, the lowest mean value (59–60.5%) was measured from sample baked at 255±5°C for 2 min and 235±5 and 255±5°C for

3min respectively. As baking temperature and time increased, the moisture content of *injera* is decreased (Table -13). Most often, the moisture content is changed with the movement of water both within and out of the product. Some changes occur due to inherent properties that have been built into the product during the baking process itself and others. These changes could affect their physicochemical quality of *injera* either individually or collectively. A similar finding was reported by Assefa (2018), who stated that moisture content of baked *injera* is dependent on the amount of moisture during baking, baking temperature and time.

4.4.2. Sensory property of *injera*

Selected sensory property of *injera* prepared from optimized primary fermentation condition, *absit* mixing ratio, *absit* cooking time and secondary fermentation time, and baking temperature and time range were analyzed. The analysis results revealed that the interaction effect of baking temperature and time were significantly differed (P< 0.05) on all sensory quality parameters except color. Table 14 shows the range of values for sensory property of *injera* as affected by baking temperature and time.

Table 14 Mean values of selected sensory quality parameters for *injera* quality attributes

A	B	Color	Taste	Texture	Eye distribution	Overall acceptability
	195±5	3.9±0.12^{ab}	2.9±0.04^{g}	2.8±0.03^{g}	3.0±0.05^{f}	2.8±0.15^{g}
	215±5	3.9±0.02^{ab}	3.0±0.11^{fg}	2.95±0.04^{fg}	3.0±0.05^{f}	3.1±0.62^{fg}
1	235±5	4.2±0.82^{a}	3.3±0.14^{de}	3.4±0.04^{cde}	3.3±0.06^{ef}	3.3±0.53^{def}
	255±5	4.2±0.30^{a}	3.5±0.09^{cd}	3.4±0.06^{cde}	3.5±0.06^{d}	3.8±0.47^{bcd}
	195±5	4.2±0.74^{a}	3.9±0.06^{bcd}	3.2±0.07^{ef}	3.8±0.04^{cd}	4.3 ±0.24^{abc}
	215±5	3.9±0.14^{ab}	4.2±0.07^{bc}	4.0±0.04^{bc}	3.9±0.06^{c}	4.4 ±0.67^{ab}
2	235±5	4.2±0.24^{a}	4.3±0.08^{ab}	4.4±0.06^{a}	4.2±0.07^{abc}	4.4±0.51^{ab}
	255±5	4.2±0.43^{a}	4.5±0.10^{a}	4.3±0.06^{ab}	4.3±0.06^{a}	4.5±0.12^{a}
	195±5	4.2±0.10^{a}	4.5±0.04^{a}	4.0±0.06^{bc}	4.2±0.08^{ab}	4.4±0.42^{ab}
	215±5	4.2±0.28^{a}	4.5±0.06^{a}	4.1±0.06^{abc}	4.3±0.06^{a}	4.5±0.58^{a}
3	235±5	4.2±0.47^{a}	4.6±0.08^{a}	4.3±0.06^{ab}	4.4±0.07^{a}	4.6±0.42^{a}
	255±5	4.20±0.08^{a}	4.5±0.08^{a}	4.0±0.06^{bc}	4.3±0.06^{a}	4.3±0.43^{abc}
	CV	9.25	5.34	2.55	6.21	4.75

A= Baking time (min) and B= Baking temperature (^{o}C)

Sensory qualities are the main criteria that make the product to be liked or disliked by consumers (Garber *et al,* 2003). During baking process, heat is transferred from the hot pan to the surface of the food material, while moisture is transferred from the interior to the surface of the product and then evaporates. As a result, changes in temperature and moisture conditions develop as cooking proceeds, and bring about the change in sensory characteristics of the food.

4.4.2.1. Color of injera

Visual appearance or the color of a food is the first quality parameter that the consumer perceives and uses as a tool either to accept or reject it. The best color of *injera* could generally be whitish, cream, reddish-brown, or brown depending on the color of the flour used. Downham and Collins, (2000) stated that color is one of the physical properties often used by food customers and manufacturers to assess the quality of food materials. The average color score values of panelists were insignificant ($P > 0.05$) varied among treatments.

4.4.2.2. Taste of injera

Good *injera* must be slightly sour to have the desired taste combination with the spicy wot. The average score value of taste of baked *injera* was ranged from 2.9 to 4.6. The highest taste (4.3 to 4.6) of fresh *injera* samples were obtained from *injera* baked at 235±5°C and 255±5°C for 2min, and 195±5, 215±5, 235±5°C and 255±5°C for 3min (Table 14). However, the lowest (2.9 to 3) was recorded from sample baked at 195±5 and 215±5°C for 1 min. The variation observed among treatments is intems of taste could be due to change in pH during baking. Therefore, the higher taste score for sample baked at higher temperature and time could be related with volatile loss of hydrogen ions at higher baking temperature which result in moderate acidity and increase panalistis taste acceptability.

4.4.2.3. Texture of injera

The texture value for the baked *injera* scored by the panelists was found to be in the range of 2.8 to 4.4. Panelists' texture response varies among the experimental runs and the maximum value (4.1 to 4.4) was obtained from *injera* baked at 235±5°C and 255±5°C for 2min, and 215±5, 235±5°C for 3min. However, the minimum value (2.8 to 2.95) was obtained from *injera* baked at 195±5 and 215±5°C for 1 min. The possible reasons for lower texture score for sample baked at lower temperature for shorter time as compared to the higher temperature and time is associated with lack of sufficient starch gelatinization due to short baking temperature and time. The relative softness of *teff injera* could be related to starch gelatinization and better starch gelatination might occur at optimum baking temperature and time.

4.4.2.4. Eye distribution

The sensory response for eye distribution of baked *injera* was ranged from 3.0 to 4.4. The highest eye distribution value (4.2 to 4.4) was recorded from *injera* baked at 235±5°C and 255±5°C for 2min, and 195±5, 215±5, 235±5 and 255±5°C for 3min (Table 14). However, the lowest (3.3 to 3.3) was recorded from sample at baked 195±5, 215±5 and 235±5°C for 1 min. There were variations on eye distribution among treatments. The sensory score for eye distribution followed similar trends with the results obtained for eye number in this work.

4.4.2.5. Overall Acceptability

The overall acceptability of baked *injera* was found to be in the ranges of 2.8 to 4.6 for all treatments. *Injera* made at 195±5, 215±5, 235±5 and 255±5°C for 1min was indicated lower acceptability value and most of the treatment combinations of experiments were found in the range of moderately liked by panelists (Table 14). Therefore, the best baking temperature and time combinations for better quality *injera* was found to be 3min and 2min of baking time at either 235±5 or 255±5°C for most of them.

Table 15 Summary of optimized values for prebaking and baking conditions for consistent and better quality of *teff injera*

No.	Processing conditions	Optimum values obtained
1	Primary fermentation temperature	25 ^{o}C
2	Primary fermentation time	64hrs
3	*Absit* mixing ratio	8%
4	*Absit* cooking time	10min
5	Secondary fermentation time	4hrs
6	Baking temperature and time	235+5^{o}C for 3min or 255+5^{o}C for 2min

Generally by considering all evaluated physicochemical and sensory property of the product, baking temperature of 235±5^{o}C for 3 min or 255±5^{o}C for 2min is the best treatment combination to produce better and consistent quality *injera*. The optimized prebaking and baking conditions achieved from objective one to objective three and to be used in validation to test their robustness are indicated in Table 15.

PHASE IV

4.5. Validation of pre-baking processing steps and baking condition for consistent and better quality of *teff injera*

To validate the robustness of optimized pre-baking processing steps and baking condition, randomly collected five different *teff* varieties were used to produce *injera*. *Injera* from all varieties were produced according to optimized pre-baking and baking conditions of objective one to three. Tables 16 and 17 indicate the mean values of the selected physicochemical and sensory quality of *injera* prepared from five different *teff* varieties. The results indicated that there were insignificant differences (P > 0.05) among four *teff* varieties in terms of eye numbers, extensibility, moisture content, color, taste, texture, eye distribution and overall acceptability exept *injera* baked from red *teff*.

4.5.1. Physicochemical property of *teff injera*

Table 16 Mean values of selected physico-chemical quality parameters for validation study

Teff varieties	Optimum baking conditions	No of E/cm^2	Ext. (N)	pH	TA (%)	MC (%)
DZ-Cr-387	255±5°C for 2min	16.59±0.15^a	1.55±0.04^a	3.47±0.04^a	0.32±0.05^a	65.19±0.17^a
	235±5°C for 3min	16.07±0.20^a	1.51±0.04^a	3.46±0.04^a	0.33±0.04^a	64.98±0.08^a
DZ-Cr-2124	255±5°C for 2min	13.88±0.17^b	1.44±0.04^b	3.45±0.04^a	0.34±0.04^a	64.23±0.31^{ab}
	235±5°C for 3min	13.13±0.24^c	1.44±0.04^b	3.43±0.13^a	0.34±0.07^a	63.39±0.23^b
White *teff* T-BT	255±5°C for 2min	16.50±0.12^a	1.54±0.07^a	3.47±0.06^a	0.34±0.06^a	65.02±0.21^a
	235±5°C for 3min	16.25±0.27^a	1.52±0.08^a	3.44±0.08^a	0.33±0.07^a	64.78±0.40^a
White teff T-GK	255±5°C for 2min	16.43±0.14^a	1.53±0.08^a	3.43±0.10^a	0.32±0.04^a	65.01±0.24^a
	235±5°C for 3min	16.12±0.28^a	1.53±0.08^a	3.44±0.08^a	0.34±0.05^a	64.69±0.46^a
Sergna teff	255±5°C for 2min	16.32±0.17^a	1.52±0.08^a	3.44±0.06^a	0.33±0.05^a	64.90±0.24^a
	235±5°C for 3min	16.22±0.29^a	1.53±0.06^a	3.44±0.08^a	0.33±0.06^a	64.32±0.36^a
CV		7.52	2.64	0.73	3.93	0.92

DZ-Cr-387 = Quncho teff, DZ-Cr-2124 = red teff, optimum baking conditions (255±5°C *for 2min and 235±5*°C *for 3min),* No of E/cm^2= *Number of eyes/cm^2*, Ext. (N) =*Extensibility (N), TA= Titaratable acidity (%) and MC= Moisture content (%)*

There were insignificant variations (P > 0.05) in terms of some physicochemical property (eye numbers, extensibility and moisture content) on *injera* prepared from five commonly used *teff* varieties and optimum prebaking processing steps and two optimum baking conditions (255±5°C for 2min or 235±5°C for 3min) except for *injera* made from red *teff* variety (DZ-Cr-2124). However, insignificant variations (P > 0.05) were observed in terms of pH and titratable acidity among all treatments.

Injera made from red *teff* (DZ-Cr-2124) had few eye numbers, less extensibility and low moisture content as compared to *injera* from other *teff* varieties. The variation observed on red *teff* variety (DZ-Cr-2124) could be due to the inherent property of this variety as compared with others (which is different in color and chemical composition of flour from other). However, the evaluated physicochemical property on *injera* had slightly close to each other numerically and observed insignificant differences among four *teff* varieties (Table-16).

4.5.2. Sensory property of *teff injera*

The mean values of sensory quality property (taste, texture, eye distribution, and overall acceptability) were ranged from 3.11 to 4.41, 3.20 to 4.40, 3.55 to 4.46 and 3.23 to 4.76 respectively (Table-17).

Table 17 Mean values of sensory quality analysis affected by *teff* varieties and baking teperature and time

Teff varieties	Optimum baking conditions	Color	Taste	Texture	Eye distribution	Overall acceptability
DZ-Cr-387	255±5°C for 2min	4.41±0.06[a]	4.01±0.06[a]	4.40±0.00[a]	4.46±0.09[a]	4.76±0.17[a]
	235±5°C for 3min	4.35±0.09[a]	4.2±0.09[a]	4.34±0.12[a]	4.30±0.16[a]	4.70±0.05[a]
DZ-Cr-2124	255±5°C for 2min	3.43±0.18[b]	4.0±0.18[b]	3.20±0.18[b]	3.71±0.17[b]	3.23±0.13[b]
	235±5°C for 3min	3.11±0.30[b]	4.01±0.32[b]	3.23±0.24[b]	3.55±0.12[b]	3.24±0.14[b]
White teff	255±5°C for 2min	4.43±0.12[a]	3.6 ±0.12[ab]	4.35±0.12[a]	4.40±0.23[a]	4.67±0.14[a]
T-BT	235±5°C for 3min	4.18±0.09[a]	4.0±0.09[a]	4.21±0.24[a]	4.31±0.17[a]	4.64±0.35[a]
White teff	255±5°C for 2min	4.19±0.14[a]	3.8±0.14[ab]	4.38±0.21[a]	4.23±0.26[a]	4.70±0.30[a]
T-GK	235±5°C for 3min	4.28±0.09[a]	3.9±0.09[a]	4.36±0.20[a]	4.31±0.17[a]	4.69±0.14[a]
Sergna teff	255±5°C for 2min	4.36±0.14[a]	3.9±0.14[a]	4.20±0.31[a]	4.44±0.17[a]	4.65±0.24[a]
	235±5°C for 3min	4.16±0.22[a]	3.9±0.22[a]	4.31±0.16[a]	4.17±0.21[a]	4.71±0.18[a]
CV		11.06	8.88	11.63	7.62	3.50

DZ-Cr-387 = Quncho teff, DZ-Cr-2124 = red teff and optimum baking conditions (255±5 for 2min and 235±5 for 3min)

The results showed that there were insignificant differences (P > 0.05) in terms of all selected sensory property for *injera* prepared from four *teff* varieties and optimized conditions (objective one to three) except for *injera* made from red *teff* variety. *Injera* made from red *teff* had less values of color, taste, texture, eye distribution (eye uniformity) and overall acceptability as compared to other treatments. The variation observed on red *teff* variety (DZ-Cr-2124) could be due to the inherent property of this variety that affects the final quality of products as compared with others. Moreover, the evaluated sensory quality on *injera* had nearly close to each other numerically and non-significant differences among four *teff* varieties were observed (Table-17).

It is indicated that the optimum prebaking processing steps and baking condition developed are robust for these four varieties and other optimum prebaking processing steps and baking condition development is needed for red *teff*.

5. CONCLUSION AND RECOMMENDATION

Teff injera produced in most Ethiopian houses and served in most of restaurants lacks consistency in desired product properties and unique quality parameters. This mainly because of lack of standardized pre-baking process consitions and optimized baking time and temperature. According to optimized works, better quality *injera* interms of determined physicochemical and sensory quality parameters obtained, at primary fermentation temperature of 25^{o}C for 64 hrs, and fermented dough mixed with *absit* at mixing ratio of 8%, cooked for 10 min, after holding the secondary fermentation time for 4 hrs at ambient temperature and with baking temperature of 235±5^{o}C for 3 min or 255±5^{o}C for 2min. It was observed that primary fermentation time, *absit* mixing ratio and baking temperature are the most significant factors to determine the quality of *injera* as compared with other studied factors.

6. FUTURE LINE OF WORKS

Based on the limitations of time and resources, the following points have been put forward for any research in the future.

i. This study was based on optimization of prebaking procssing steps and baking conditions in terms of some physicochemical and sensory properties of *teff injera*. It is recommended to study the proximate composition and minerals on *injera* prepared at these optimum conditions on future studies.

ii. As indicated in the validation part, optimized conditions showed no variations for measured physicochemical and sensory properties except for red *teff* variety. Further studies are required on the effect of other *teff* varieties and process conditions for consistent and better quality of *teff injera*.

iii. Further studies are required on the shelf life of teff injera at optimum process conditions obtained in these findings for consistent and better quality of *teff injera*. It is also advised to link starch gelatinization with some *injera* quality parameters on future studies.

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8. APPENDICES

Appendix 1 Design summary for optimization of primary fermentation time and temperature

Study Type			Response Surface			Runs 14	
Initial Design		Central composite				Blocks	2
Design Model		Quadratic					
Factor Name	Units	Type	Low actual	High actual	Low coded	High coded	Mean
A	Hrs	Numeric	24	96	-1	1	60
B	℃	Numeric	25	38	-1	1	31.50
Response Name	Units	Observations	Analyses	Minimum	Maximum	Mean	Trans formation
Y1 CO_2	%	14	Polynomial	78.00	96.70	87.35	None
Y2 Number of eyes	$Cm/^2$	14	Polynomial	4.47	16.5	10.49	None
Y3 Rollability	cm	14	Polynomial	2.65	4.80	3.73	None
Y4 Extensibility	N	14	Polynomial	1.29	1.48	1.39	None
Y5 pH	-	14	Polynomial	3.31	4.35	3.83	None
Y6 TA	%	14	Polynomial	0.12	0.59	0.36	None
Y7 MC	%	14	Polynomial	58.9	65.35	62.13	None

Primary fermentation time =A, fermentation temperature =B, titratable acidity = TA and moisture content =MC

Appendix 2 Design summary for optimization of *absit* mixing ratio, cooking time and secondary fermentation time of *teff* dough for consistent and better quality of *teff injera*

Study Type			Response Surface			Runs 20	
Initial Design		Central composite				Blocks	2
Design Model		Quadratic					
Factor Name	Units	Type	Low actual	High actual	Low coded	High coded	Mean
A	%	Numeric	5	15	-1	1	10
B	min	Numeric	5	15	-1	1	10
C	hrs	Numeric	2	6	-1	1	4
Response Name	Units	Observations	Analyses	Minimum	Maximum	Mean	Trans formation
Y1 Number of eyes	$Cm/^2$	20	Polynomial	6.23	16.60	11.42	None
Y2 Rollability	cm	20	Polynomial	2.25	4.75	3.50	None
Y3 Extensibility	N	20	Polynomial	1.22	1.53	1.38	None
Y4 pH	-	20	Polynomial	3.36	3.85	3.61	None
Y5 TA	%	20	Polynomial	0.24	0.55	0.40	None
Y6 MC	%	20	Polynomial	58.50	67.25	62.88	None

A= absit mixing rati (%), B= Cooking time of absit (min), C= Secondary fermentation time (hrs), T&BS = Top and bottom surface and OAA = Over all acceptability

Appendix 3 The p-value of physicochemical properties for optimization of primary fermentation time and temperature of *teff* dough for consistent and better quality of *teff injera*

Source	CO_2 (%)	Number of eyes /cm^2	Rollability/ 2cm	Extensibility (N)	pH	TA (%)	MC (%)
Blocks	0.635	0.597	0.126	0.896	0.377	0.045	0.740
Regression	0.002	0.003	0.000	0.004	0.000	0.000	0.014
Linear	0.018	0.005	0.001	0.007	0.000	0.000	0.035
A	0.020	0.002	0.000	0.010	0.000	0.000	0.408
B	0.045	0.769	0.533	0.020	0.116	0.074	0.014
Square	0.001	0.002	0.000	0.003	0.000	0.001	0.007
A*A	0.000	0.001	0.000	0.002	0.000	0.030	0.002
B*B	0.743	0.844	0.266	0.015	0.451	0.000	0.896
Interaction	0.396	0.515	0.091	0.595	0.011	0.068	0.500
A*B	0.396	0.515	0.091	0.595	0.011	0.068	0.500

Primary fermentation time =A and fermentation temperature =B, TA= titratable acidity and MC = moisture content

Appendix 4 The p-value of physicochemical properties for optimization of *absit* mixing ratio, cooking time and secondary fermentation time of *teff* dough for consistent and better quality of *teff injera*

Source	Number of eyes /cm^2	Rollability/2cm	Extensibility (N)	pH	TA (%)	MC (%)
Blocks	0.418	0.094	0.288	0.068	0.026	0.821
Regression	0.006	0.004	0.001	0.004	0.003	0.000
Linear	0.031	0.003	0.004	0.000	0.000	0.000
A	0.006	0.001	0.001	0.000	0.000	0.000
B	0.315	0.044	0.100	0.037	0.074	0.057
C	0.552	0.225	0.276	0.074	0.005	0.364
Square	0.001	0.003	0.000	0.522	0.279	0.803
A^2	0.000	0.001	0.000	0.668	0.065	0.603
B^2	0.345	0.098	0.005	0.197	0.970	0.441
C^2	0.143	0.043	0.079	0.670	0.725	0.923
Interaction	0.889	0.804	0.496	0.063	0.133	0.163
A*B	0.689	0.353	0.879	0.036	0.075	0.092
A*C	0.706	1.000	0.151	0.571	0.318	0.152
B*C	0.599	0.865	0.761	0.077	0.184	0.508

A= absit mixing ratio (%), B = absit cooking time (min) and C= Secondary fermentation time (hrs), TA= titratable acidity and MC = moisture content

Appendix 5 Numerical optimizations and desirability values of primary fermentation condition

Response variables	Goal	Lower	Target	Upper	Predicted values	Over all Desirability
CO_2 (%)	Maximum	78.00	96.70	87.35	0.00%	0.95
Number of eyes (cm^2)	Maximum	4.47	16.5	10.49	0.00%	0.95
Rollability/2cm	Maximum	2.65	4.80	3.73	0.00%	0.95
Extensibility (N)	Maximum	1.29	1.48	1.39	96.41%	0.95
pH	In range	3.31	4.35	3.83	82.24%	0.95
TA (%)	In range	0.12	0.59	0.36	92.71%	0.95
MC (%)	Maximum	58.9	65.35	62.13	0.00%	0.95

Titratable acidity = TA and moisture content =MC

Appendix 6 Numerical optimizations and desirability values of *absit* mixing ratio, cooking time and secondary fermentation time of *teff* dough

Response variables	Goal	Lower	Target	Upper	Predicted values	Over all Desirability
Number of eyes (cm^2)	In range	6.23	16.60	11.42	16.87%	0.96
Rollability/2cm	Maximum	2.25	4.75	3.50	22.49%	0.96
Extensibility (N)	Maximum	1.22	1.53	1.38	19.12%	0.96
pH	In range	3.36	3.85	3.61	0.00%	0.96
TA (%)	In range	0.24	0.55	0.40	0.00%	0.96
MC (%)	Maximum	58.50	67.25	62.88	0.00%	0.96

TA= titratable acidity and MC = moisture conten

Appendix 7 p value of ANOVA for physicochemical and sensory quality of baking temperature and time

Source of variation	Df	Number of e/cm^2	Ext. (n)	Ph	Ta (%)	Mc (%)	Color	Taste	Texture	Ed	Oaa
Block	2	0.043	0.240	0.585	7.78	0.696	0.198	0.724	0.256	0.448	0.724
Treatments	11										
A	3	0.000	0.007	0.000	10.52	0.000	0.816	0.000	0.000	0.000	0.000
B	2	0.000	0.000	0.000	25.99	0.046	0.717	0.000	0.000	0.000	0.000
A*B	6	0.000	0.000	0.000	5.55	0.170	0.803	0.000	0.000	0.000	0.000
Error	22										

A= baking temperature and B=baking time

Appendix 8 p value of ANOVA for physico-chemical and sensory qualities for validation study

Source of variation	DF	Number of E/cm^2	Ext. (N)	pH	TA (%)	MC (%)	Color	Taste	Texture	ED	OAA
Block	2	0.078	0.009	0.009	0.026	0.185	0.025	0.401	0.538	0.515	0.631
Treatments	9										
A	4	0.000	0.000	0.111	0.198	0.000	0.000	0.695	0.000	0.000	0.000
B	1	0.000	0.087	0.260	0.251	0.010	0.000	0.351	0.849	0.029	0.825
A*B	4	0.005	0.043	0.467	0.179	0.567	0.000	0.884	0.791	0.298	0.318
Error	18										

Teff varieties =A, two optimum baking conditions = B, Extensibility = Ext., titratable acidity = TA, moisture content = MC, Eye distribution = ED and Overall acceptibility = OAA