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Optimization of Absit process factors to improve the physicochemical and sensory quality of Teff Injera

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Declaration

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

Signature: - ________________ Date: - _____________

Dedication

This work is dedicated to my family.

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List of abbreviations

a*	CIE red(+)/green(−) color attribute
b*	CIE yellow(+)/blue(−) color attribute
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
CHO	Carbohydrate
CIE	Commission Internationale d"Eclairage
DCC	Digital Color Camera
DM	Dry matter
DZARI	Debrezeit Agricultural Research Institute
EPHI	Ethiopian Public Health Institute
FAO	Food and Agriculture Organization
JPEG	Joint Photographic Experts Group
L*	CIE lightness coordinate
LAB	Lactic Acid Bacteria
RSM	Response Surface Methodology
SD	Standard deviation
SPSS	Statistical Product and Service Solutions
WHO	World Health Organization

Abstract

Teff Injera is the staple Ethiopian fermented bread processed from fermented tef [Eragrostis tef (Zucc.) Trotter] grain flour. ***Absit*** *is a portion of the fermented dough diluted to paste with water and then cooked and gelatinizes the starch which enhances proper fermentation of the batter. The proper fermentation process of dough can be enabled and attained by appropriate absit cooking parameters (dough level %, agitation speed rpm and adding temperature* ^{O}*C) to make good injera. There is no research made about absit process factors before to improve the physicochemical and sensory quality of teff injera. This study aimed to optimize the above absit cooking process factors to improve the physicochemical and sensory quality of teff injera using Box-Behnken response surface methodology (RSM). Absit process factors ((dough level(10-20 %), agitation speed(200-300 rpm) and adding temperature (45-65* ^{O}*C)) were considered and made 15 samples of absit including 3 replicates using RSM. The investigated responses were viscosity , sensory attributes (color, taste, texture, number of eyes, eye size, eye distribution, top & bottom surfaces and overall acceptability) and lightness (L* value) of the injera. RSM with Box-Behnken Design was used to develop and optimize absit process factors in response with the baked injera. Moreover, Both numerical and graphical optimizations were carried out to determine the optimum values of injera in terms of taste, texture, number of eyes, eye size, eye distribution, top & bottom surfaces and overall acceptability. The sensory scores for color, taste, texture, number of eyes, eye size, eye distribution, top & bottom surface and overall acceptability were 5.63 to 7.72, 5.22 to 6.21, 56.63 to 72.58 , 47.47 to 65.05, 57.11 to 74.88,53.09 to 77.62, 41.26 to 66.26, 46.76 to 71.71, 51.34 to 77.80 and 50.03 to 68.38, respectively, while the L* value(lightness) ranged from 60 – 75.78%. The optimum process factors of absit while considering taste, texture, number of eyes, eye size, eye distribution, top & bottom surface and overall acceptability were dough level of 11.06 %,agitation speed, 200rpm and temperature, 45 °C with a desirability of 0.91. The sensory scores for color, taste, texture, number of eyes, eye size, eye distribution, top & bottom surface and overall acceptability were selected as 64.84, 74.88, 74.61, 62.79, 68.16, 70.41, 68.27 % respectively. The study showed that all the absit process factors were important parameters influencing the physicochemical and sensory attributes of injera except the visual color, L*values and viscosity of injera.*

Key words : Absit, Box-Behnken Design Teff Injera, Optimization, Response Surface Methodology

CHAPTER ONE

1. Introduction

1.1. Background

Teff Injera is the staple Ethiopian fermented bread processed from tef [Eragrostis tef (Zucc.) Trotter] grain flour. Grain tef bears about 11% protein, 73% carbohydrate (virtually all starch), 3% crude fiber (CF), 2.5% fat and 2.8% ash (Bultosa & Taylor, 2004). The very small size grain tef starch granules (2–6µm) are implicated to have a fat mimetic, flavor and aroma carrier functionalities as that of small starch granules (Bultosa & Taylor, 2004). During fermentation of teff flour for injera making, about 9% of starch is known to be utilized by fermenting microorganisms (Parker et al., 1989). Tef grain micronutrient is regarded high particularly in iron partly because of agronomic practices used in tef productions, and tef is usually consumed as injera in which mineral inhibitors like phytates are reduced on fermentation (Abebe et al., 2007). Grain tef is rich with digestible-type proteins, and the essential amino acid profile is considered as well well-adjusted when compared to FAO reference pattern (FAO/WHO, 1973) excluding lysine (Bultosa &Taylor, 2004). Grain tef is recommended as functional food for celiac patients (Bergamo et al., 2011), because it is gluten-free and offers in many respects more nutrient supply as it is consumed as whole grain often fermented as injera.

Absit is a gruel or function as a dough binder in the course of second fermentation of dough. It can be cooked by taking about 10% from the fermenting dough, mixing with water (1:3 ratio) and boiling (2–5 min) and agitating for starch gelatinization. After cooling to about 46°C, absit will be mixed into fermenting vat for the second phase of fermentation that had lasted for about 2 h. Adding absit develops gas formation and causes the paste to rise (Gashe, 1985). Adding absit is also critical to develop the desired texture and consistency, as injera made without absit tends to be powdery and have fewer of the "eyes" which are so prized by Ethiopian consumers. It is important to note that tef, millet, and corn are the only grains that require absit during the process of making injera (Steinkraus, 1996). Since absit has a significant influence on the physicochemical and sensory quality of making injera, therefore identifying and optimizing the process factors of absit preparation and finally reaching the optimum conditions has a very good scientific input to make teff injera.

1.2. Problem Statement

Quality and standardized food products are the central aspects during the whole chain from producers to the ultimate consumers. Now a day's injera is becoming popular in Ethiopia as well as in the developed world because of its being a whole grain product and gluten free nature, the cause for celiac disease. It is high in protein, carbohydrates, and fiber.

Teff injera found in home and market do not have a proper and consistent physicochemical and sensory quality which is found in Ethiopia. This might be due to different factors like type of teff, and processing factors of absit cooking. Among the many processing factors of absit cooking: dough levels, agitation speed, and final temperature of absit were found very important for quality teff injera during preliminary study.

Therefore, scientific investigations towards optimization process factors of absit for quality teff injera based on traditional injera baking are limited, virtually absent in the country.

In order to alleviate such kind of problems in injera production in home and in industrial scale for a better health and economically benefited. This study were done for optimization of absit processing factors that are majorly affected the physicochemical and sensory quality of tef injera and finally reached the optimum possible and controlled factors using the a Box-Behnken design with response surface methodology(RSM).

1.3. Objectives of the Study

- **General objective**

The general objective of this study was to optimize of process factors of absit cooking to improve the physicochemical and sensory quality of Teff Injera by using response surface methodology.

- **Specific objectives**
  - ✓ Identification of process factors for absit making
  - ✓ Selecting the major absit processing factors that influence the physicochemical and sensory quality of tef injera
  - ✓ Determination of the lower and higher values of processing factors by repeated experimentation and from literatures
  - ✓ Evaluating the physicochemical and sensory quality of injera by using RSM
  - ✓ Determination of the viscosity of prepared absit as a response variable

1.4. Significance of the study

- ✓ The study will provide significant information about the processing parameters of absit for baking good Injera.
- ✓ The findings will help the injera bakers and traders to save their resources and to supply the consistent injera quality.
- ✓ contribute good information about the basic factors that influence the quality of Teff based injera and help us to control and produce injera in homemade and in industrial scale so that we can add our economy and health benefits.
- ✓ To produce good quality injera, a standardized method of injera production will have to be put into place.

CHAPTER TWO

2. Literature Review

2.1. Teff grain and injera

The cereal grain teff (Eragrostis tef [Zucc.] Trotter) is one of the major cereal crops of Ethiopia, where it is believed to have originated (Uraga K and HV Narasimha, 1997). It is the most popular cereal grain for making injera, which forms the traditional basic diet in Ethiopia, although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used (Bultosa G. 2004). Teff has the largest share of area (23.42%, 2.6 million hectares) under cereal cultivation and third (after maize and wheat) in terms of grain production (18.57%, 29.9 million quintals) in Ethiopia (Central Statistical Agency of Ethiopia, 2008).

The principal use of teff grain for human food is the Ethiopian injera, a soft porous thin pancake with a sour taste (Yigzaw Y, et al .2001). Injera is made from teff flour, water and starter irsho (Ashenafi M,2006). Irsho is a fluid saved from previously fermented dough. Teff provides over two-thirds of the human nutrition in Ethiopia, with grain protein content (10-12%) similar to other cereals. Teff proteins have non-gluten nature and owing to prevailing portion of protamine belong to easily digestible ones, which make it a suitable alternative to wheat in the case of celiac disease and gluten-free diet. Besides providing protein and calories, it has high nutritional content, including better amino acid composition, especially lysine, more mineral content (mainly iron, calcium, phosphorus and copper) than other cereal grains, contain B1vitamin and is rich in fibre.

Fermentation is one of the oldest and most economical methods of producing and preserving food. It is found to destroy undesirable components, to enhance the nutritive value, flavour and taste of the food, and to make the product safe from pathogenic microorganisms. In indigenous fermented foods, the microorganisms responsible for the fermentation are usually the microflora naturally present on the raw substrate. Back slopping, that is, inoculation of the raw substrate with a small quantity of a previously performed successful fermentation is used to optimize spontaneous fermentation. This kind of a starter, which is a previously fermented product, is used not only to initiate the fermentation but also to accelerate the initial phase of fermentation and keep a uniform quality from batch to another. Foods that, in addition to their basic nutrients, contain biologically active components that can have a positive impact on the health of the consumer are defined as functional foods (Nyanzi R *et al*,2012) associated with fermentation are useful microorganisms, referred to as probiotics, most of which belong to the genera *Lactobacillus* and *Bifidobacterium.* These health enhancing microorganisms bring about fermentation resulting in production of lactic acid and hence commonly referred to as lactic acid bacteria (LAB). The term LAB is used to describe a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, usually non-motile, that utilize carbohydrates fermentative and form lactic acid as the sole or major end product. Prebiotics, on the other hand, are non-digestible food ingredients that affect the host by selectively targeting the growth and/or activity of one or a limited number of beneficial bacteria in the colon, and thus have the potential to improve health (Desai AR, *et al*, 2004) Cereals are good substrates for the growth of probiotic strains and due to the presence of non-digestible components of the cereal matrix may also serve as prebiotics (Charalampopoulos D, *et al.*, 2002).

The principal use of teff *[Eragrostis tef (Zucc) Trotter]* is in *injera* production that constitutes the 70% of Ethiopians diet (Gamboa PA and LV Ekris. 2008). Teff injera is the most common and the main staple food in much of the central, western and northern highlands of Ethiopia as well as among the urban community.

2.1.1. Fermentation Process of Injera

The preparation of teff injera consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures (Steinkraus KH, 1983). Temperature in the highlands of Ethiopia is generally between 17 and 25°C.The only required ingredients are the teff flour and water. An appropriate amount of flour is mixed with twice its weight of water (Gamboa PA and LV Ekris. 2008). This is kneaded thoroughly to produce a thick paste. Inoculation is accomplished by consistently using partially cleaned fermentation container (back slopping) and by adding some irsho, a clear, yellow liquid that accumulates on the surface of the dough towards the final stage of a previous fermentation (Desiye A and K Abegaz. 2013). About 480 g irsho is added to 3 kg teff flour and 6 L of water (Gamboa PA and LV Ekris. 2008). The initial 18 hours of fermentation are characterized by vigorous evolution of gas and maximum dough-rising (Gashe BA.1983). This is followed by the appearance of an acidic yellowish liquid on the surface of the dough at about 30-33 hours of fermentation. Gas evolution decreases after the pH has fallen below 5.8 (31 hours). The liquid layer is discarded at the end of the first stage of fermentation. As soon as the liquid layer is poured off, about 10% of the fermenting dough is mixed with three parts of water and boiled for 2 to 5 minutes (Gamboa PA and LV Ekris. 2008). This is called absit, a dough enhancer, and it is mixed with the rest in the fermentation vat. Absit ensures that injera will have the proper texture and consistency and the dough-rising and gas formation processes are enhanced so they occur in a short time. This process signals the initiation of the second stage of fermentation.

Injera baked without absit or with less absit than the required will have fewer amounts of eyes (pits) on the upper surface. A higher number of larger eyes are a very desirable attribute of an attractive injera. Injera baked at 24 hours or less is called aflegna injera and has sweet taste. It is recommended for people suffering from gastritis and those who do not tolerate acidic foods.

Maximum dough-rising, which normally takes 30 minutes to 2 hours, signals the termination of fermentation. At this stage the fermenting dough is thin enough to pour onto the hot flat pan, locally known as *mitad* for steam-baking into *injera*. The total baking time for one *injera* is 2 ½ - 3 ½ minutes (Ashenafi M. 2006).

2.1.2. Nutritional Importance of Fermentation

Teff grain contains less than 1% (528-842mg/100g) phytic acid and other inositol phosphates, which are strong inhibitors of Fe and Zn absorption (Umeta M, West CE and H Fufa. 2005; 18: 803-817). Fermentation gives injera its sensorial characteristics, as flavour, aroma and colour. But the more important effect of teff fermentation is the increase in the nutritional content, because of the decreasing relationships of iron with phytates and of iron and tannins. One study on content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia reported that amount of phytates in injera is considerably reduced to 35-76 mg/100 g (91-93% destruction) due to fermentation and the acidity nature of injera. Microorganisms are able to produce various metabolites during the fermentation through their enzymatic action on the substrate and anti-nutritional factors are also reduced. Indeed, the lactic acid and volatile fatty acids (C2 - C6) were reported as the major organic acids produced during fermentation and contributing to good aroma and sour taste of injera (Umeta M and RM Faulks, 1988).

Different authors have demonstrated that during the teff paste fermentation, the phytate: iron molar ratio decreases. That is why, Ethiopian food scientists and nutritionist agree about the need to improve the practice to ferment teff before use in injera production. Given the high iron content and the relatively favorable phytate: iron molar ratio, teff injera was the best source of bioavailable iron of all foods analyzed by researchers, between the Ethiopian foods.

2.2. Physical Characteristics and Quality Attributes of Injera

Injera is a fermented and naturally-leavened flatbread indigenous to Ethiopia, ~50 cm in diameter with a honeycomb-like texture, rather like a giant crumpet (Belton and Taylor, 2004).

Other grains such as sorghum, millet, maize, wheat and mixtures thereof have also been used in making injera. Sorghum is the second most preferred flour for injera preparation in Ethiopia; however, teff injera is the most preferred because it can be stored for 3 days without losing its pliability (Steinkraus, 1996).

Quality characteristics of injera are directly related to its appearance, texture and taste. According to Gebrekidan and GebreHiwot (1982), a normal and typical injera is round, soft, spongy and resilient, about 6 mm thick and ~60 cm in diameter with uniformly spaced honeycomb-like ‘‘eyes’’ on the top. Injera is unique in that despite the fact that it is not made from gluten-containing wheat, it is leavened.

Injera with large unevenly spaced eyes or those with tiny eyes are both considered poor quality. While the former signifies insufficient fermentation, the latter signifies too much absit in the dough. The backside of injera is normally smooth and devoid of eyes. A good injera is also spongy and can be folded without cracking. A non-powdery, soft appearance is also characteristic of a good quality injera. Poor quality injera is brittle, crumbles easily when handled, and looks powdery and dry, or sticky and rusty brown on the back.

The color of injera could generally be whitish, cream, reddish brown, or brown depending on the color of the Teff flour used. The most preferred kind of injera is whitish or cream in color, has a soft and pliable texture for as long as 3 days after its preparation, is relatively thin, and has uniformly spaced medium sized eyes. The backside of injera must be smooth, not sticky and should not look rusty or brown with burns. Good quality injera does not fluff off and stick to the fingers when it is handled.

In taste, good injera must be slightly sour to have the desired taste combination with the spicy wot. Injera made from dough that has not been sufficiently fermented has sweetish taste and is not considered good for eating with wot. This type of injera is called aflegna. When there is an urgent need for injera and the housewife cannot wait until sufficient fermentation has taken place, she would normally make Aflegna injera. Injera made from over fermented dough is too sour and is undesirable for food.

The texture of injera should be spongy as well. Texture is the overall experience of how a substance feels in the hand and mouth. It contributes to the overall eating experience and can impact flavor release of a food product. *Injera* is used as an eating utensil and this makes its texture an important quality attributes.

The quality of injera is influenced to a large extent by the fermentation process and the length of time for fermentation. In teff, the primary agent of fermentation of the injera dough has been identified as the yeast Candida guilliermondii (Cast) Langeron and Guerra (Stewart and Getachew 1962).

2.2.1. Number of eyes

The number of eyes and the distribution on the surface of injera has always been taken as a good indicator of injera quality. Ideally, eyes should neither be too few nor too numerous, they must be rather deep, interlocked with thin cross walls between them and be evenly distributed. Eyes need not be of uniform size, but variation in dimension should not be large (Cherinet, 1988).

Carbon dioxide produced during fermentation is known to play a fundamental role in the formation of cellular structure of leavened breads. Thus the absence of eyes or limited number of eyes in the prepared injera is indicative of very little carbon dioxide being produced during fermentation. So if the activities of microorganisms and enzymes are limited, the fermentation process will be inhibited (Yetneberk *et al*., 2004).

2.2.2. Color

Visual appearance or the color of a food is the first quality parameter that the consumer perceives and uses as a tool to either accept or reject it. Color is one of the physical properties often used by food customers and manufacturers to qualitatively assess the quality of feed and food materials (Leon *et al*., 2006). Color is a perceptual phenomenon that depends on the observer and the conditions in which the color is observed. It is a characteristic of light, which is measurable in terms of intensity and wavelength (Pathare *et al*., 2013).Color is one of the most important physical attributes in the assessment of flour quality in injera preparation. According to Gebrekidan and

Gebrehiwot, (1982), the color of injera could generally be whitish, cream, reddish-brown, or brown depending on the color of the flour used. The most preferred kind of injera is whitish or cream in color, has a soft and pliable texture for as long as 3 days after its preparation, is relatively thin, and has uniformly spaced medium sized eyes.

The color of food products can be evaluated using sensory panelists or instrumental analysis. Instrumental color analysis can be specified by three co-ordinates in the color space which can be obtained directly with a tristimulus colorimeter. The L *a*b* system is the more frequently used scale to measure objectively the color of food products (Pathare *et al*., 2013).

Instrumental evaluation of color must relate to sensory assessment of appearance. Visual scoring by a trained panel is the preferred method for subjective color assessment, and although it may be difficult to perform and control, it sets the benchmark for instrumental measurement comparisons (Stevenson *et al*., 2015)

In his study "A comparison of two different instruments for measuring venison CIELAB values and color assessment by a trained panel" on meat products, Stevenson et al. (2015) identified that there is close relationship between perceived color and CIE (1976) L *a*b* values and suggests that both instruments can be used as a satisfactory substitute for a trained panel, provided they are calibrated appropriately. Literature is scarce with respect to the effect of blending ratios on color quality (L* values) of injera.

CIELab color space

L*a*b* color space (Figure 2.1) is an international standard for color measurements, adopted by the Commission Internationale d‟Eclairage (CIE) in 1976 (Leon *et al.*,2006; Pathare *et al.*, 2013).L* is the luminance or lightness component, which ranges from 0 to 100, and parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components, which range from -120 to 120(Leon *et al.*, 2006).

The Lab color is device independent, providing consistent color regardless of the input or output device such as digital camera, scanner, monitor, and printer. The L*a*b* values are most frequently used in food research studies to measure the color of food products (Yam & Papadakis, 2004).

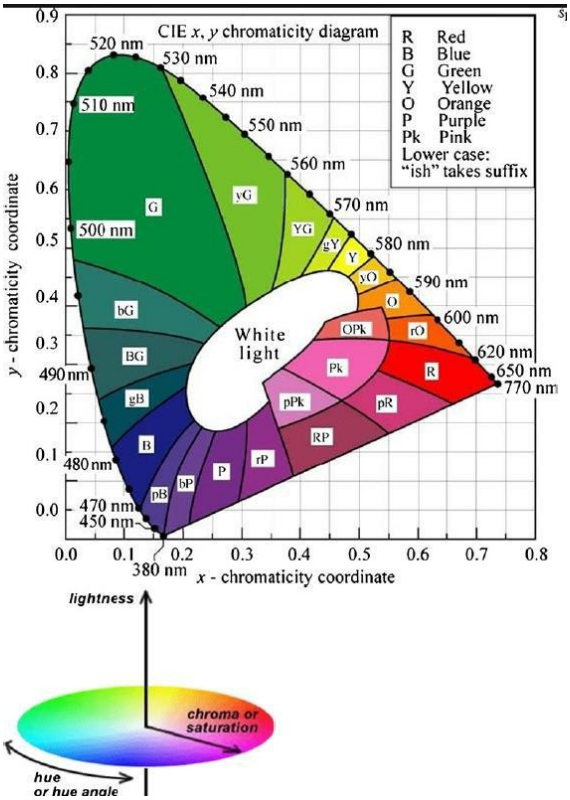


Figure 2.1: Illustration of the CIE 1931 color space.

Unlike the RGB and CMYK color models which are mostly used for screen display and printing respectively (Yam & Papadakis, 2004), L*a*b* color is designed to approximate human vision.

It aspires to perceptual uniformity, and it is L* component closely matches human perception of lightness. It can thus be used to make accurate color balance corrections by modifying output curves in a* and b* components, or to adjust the lightness contrast using the L*component (Vyawahare *et al.*, 2013).

Adobe Photo ShopTM

Photoshop^{TM} is standard software used primarily by graphics producers and photographers for photo re-touching and image editing (Adobe Photoshop, 2002). However the software also has several features that maybe adopted for analyzing color of food samples. Its richness in image editing features and its color analysis capability comparable to the more expensive color analysis software make the Adobe Photoshop^{TM} more preferable(Yam, 2004).The software provides more sophisticated capability for man-aging color and producing consistent color than other graphics software. The software is also available in many laboratories, and it is strongly supported by the manufacturer and user (Yam and Papadakis, 2004).

Image Analysis of Injera

The digital imaging method allows measurements and analyses of the color of food surfaces that are adequate for food engineering research. Photoshop™ is shown to be able to analyze the color for food samples, although the software was not originally designed for this purpose. However, it is already one of the most powerful software for color analysis, and the manufacturer and users are regularly making enhancements (Yam and Papadakis, 2004).

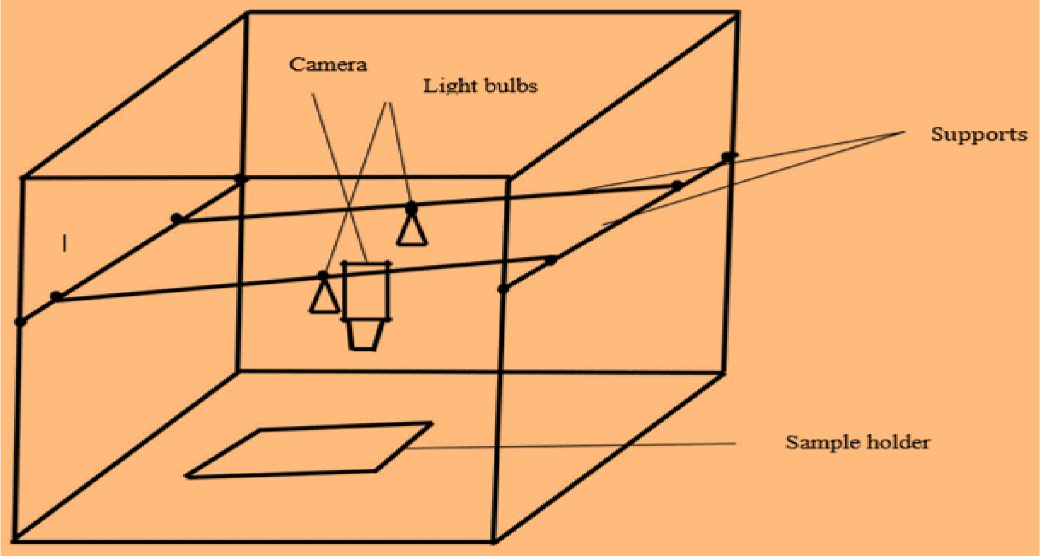


Figure 2.2: Image processing acquisition systems.

2.2.3. Taste

A good injera must be slightly sour to have the desired taste combination with the spicy wot. Injera made from dough that has not been sufficiently fermented has sweetish taste and is not considered good for eating with wot. This type of injera is called aflegna. Injera made from over fermented dough is too sour and is undesirable for food (Mezemir, 2015; Boka *et al*., 2013).The quality of injera is influenced to a large extent by the fermentation process and the length of time for fermentation.

2.2.4. Appearance

The shape is properly round and the top surface has uniformly distributed eyes and closely located to one another. There should be no blind spots on the surface. The general appearance should be rich and not powdery. The backside should be smooth and not browned. Rough circumference, powdery and cracked top surface, too large or tiny and irregularly placed eyes and if the backside is browned are considered as poor quality injera (Gebrekidan and Gebrehiwot, 1982; Yetneberk *et al*., 2004)

2.2.5. Texture

The injera should be soft enough to cut a piece easily with the fingers but resistant enough so that it does not crack or crumble when folded or used to wrap around wot portions when eating. The texture should not be gluey or stick to the fingers when handled. It should not be too thin, dry, brittle, or leathery (Gebrekidan and Gebrehiwot, 1982).

2.3. Eye formation on injera

2.3.1. Characteristics of eyes on injera

Naturally leavened breads have a variable porous structure produced by a fermentation process that evolves carbon dioxide as a gas. According to Niranjan and Silva (2008), the presence of bubbles in a number of food products, such as bread, champagne, ice cream and beer, has dominated our perception of product quality. A characteristic trait of *injera* is the eyes or pores on its surface and numerous eyes on *injera* are an indication of good quality. A study by Cherinet (1988) on composite flour development for *injera* determined the appropriate number of eyes on the surface to be 11-15eyes/ cm^2

2.3.2. Effect of processing on eye formation

The size and shape of the resultant gas cells in wheat bread is determined by many factors such as mixing rate, fermentation rate, starch gelatinization, and protein denaturation (Autio and Laurikainen, 1997). According to Taylor and Emmambux (2008), as the temperature of the teff batter rises during baking, the carbon dioxide in the batter comes out of solution.

At the same time, the starch in the batter gelatinizes increasing the viscosity of the batter. This creates gas bubbles in the batter that turn into cells as the gas escapes and the batter sets. Niranjan and Silva (2008) stated that in crumpet production, the batter expands due to the formation of CO_2 during fermentation; the larger bubbles then escape leaving behind a population of small nuclei.

The authors further stated that the water in the batter evaporates into the nuclei during hot plate baking at 200–230°C to form a series of vertical cones. Hence, the rise of bubbles during baking is due to the gravity acting on bubbles of lower density than the tef batter. This phenomenon has been described by Stokes' law, which relates the rise velocity of particles or bubbles in a liquid to the density difference between the bubble and the liquid phase, the diameter of the bubble, and the viscosity of the liquid phase.

The viscosity of tef batter may have a significant role to play in the formation of 'eyes' on the surface of *injera* during baking. According to Niranjan and Silva (2008), even though a continuous phase may be purely viscous, bubble incorporation tends to make the dispersion viscoelastic. Pyle (2005) stated that dissolved carbon dioxide released from batter during heating contributed to pore development and overall expansion of the baked crumpet, and that the main role of the fermentation process is in the production of carbon dioxide. This was the conclusion drawn from an experiment that compared vacuum degassed batter to batter that was not degassed. From this experiment, Pyle (2005) observed that pore formation was absent in the degassed white wheat flour batter when baked on a hot plate. The baked product from the degassed batter was also similar to the baked product obtained from a batter which did not have any yeast or leavening agents. In this study, it is hypothesized that carbon dioxide produced during tef fermentation significantly affects eye formation on the surface of *injera*.

2.3.3. Measurement of 'eyes' on injera

In a study conducted by Cherinet (1993), a 3 x 3 cm frame was used to count the eyes of injera at four randomly selected sites. They found the number of eyes to be in a range of 11-15 eyes/cm^2. However, according to Gebrekidan and GebreHiwot (1982), about 4 eyes are contained per cm2 of surface. There is no standard on how many eyes should be on the surface of an injera, but according to Cherinet (1993), eyes should neither be too few nor too numerous, they must be rather deep, interlocked with thin cross-walls between them and evenly distributed Stanley and Baker

(2002) stated that the characteristic honeycomb structure of bread can be studied using the micro-structural imaging capabilities of an ordinary flatbed scanner.

Computer measurements can also provide accurate numerical values provided that good measurement algorithms are used, which is not always the case. There is still debate about which of the many size measurements to use in any particular situation (Russ, 2005). The area covered by a feature can be determined by counting the number of pixels, and applying the appropriate calibration factor to convert to square centimeters (Russ, 2005). Hence, the size of each eye on the surface of injera can be measured using a scanner and imaging software.

The equivalent diameter calculated from the area of an eye on the surface of injera gives an indication of the size of the eye. The average of all the equivalent diameters is determined and the mean equivalent diameter of the eyes on the injera scanned is obtained. The percentage of the area covered by all the eyes on the surface of the injera is obtained by dividing the total area of all the eyes by the surface area of the injera scanned and multiplying by 100.

2.4. Elasticity of injera

Gluten-free breads lack the elasticity provided by gluten, the protein found in wheat flour. According to Keiffer (2006), elasticity, which is closely connected to the cohesive structures, seems to be nothing but an indicator of a persisting good continuous gluten network.

Even though *injera* lacks gluten, it has an elastic texture. It is therefore not clear what is responsible for this property. There is lack of research on the mechanism of *injera* elasticity and hence the importance of delving into this area.

‘Elasticity’ in bakery science is a sensory perception that is felt when the dough is rapidly stretched, then released, and is “good” when the dough contracts rapidly to approximately its original shape (Keiffer, 2006). Protein degradation during sourdough fermentation in wheat flour affects the overall quality of sourdough bread by modifying its viscoelastic properties. Structural components in teff such as hemicellulose, proteins and starch (amylose and amylopectin) undergo changes during fermentation and these changes affect the rheology of the final product. It is essential to determine how the changes occurring during fermentation affect the texture of *injera*. Studies of proteolytic events occurring during sourdough fermentation and their effects on gluten-free bread quality are still limited. There are different procedures used to measure the texture of food materials. According to Dobraszczyk and Morgenstern (2003), the most common types of fundamental rheological tests used in cereal testing are: (i) small deformation dynamic shear oscillation; (ii) small and large deformation shear creep and stress relaxation; (iii) large deformation extensional measurements; and (iv) flow viscometry.

Dobraszczyk and Morgenstern (2003) stated that the dynamic oscillation measurements are the most popular and widely used fundamental rheological techniques for measuring cereal dough and batters and these tests measure rheological properties (such as elastic and viscous moduli) by the application of sinusoidally oscillating stress or strain with time and measuring the resulting response.

Dynamic mechanical analysis is used to determine the mechanical behavior of food materials. Studies on the dynamic mechanical properties of *injera* are lacking. Most studies using dynamic mechanical analyses have been conducted on wheat breads. In a study conducted on bread by Wang and Sun (2002), it was observed that the bread crumb behaved similarly to a soft rubberlike solid in the frequency sweep. They also observed that typical viscoelastic behaviors of bread crumb involved a transition from rubberlike to glasslike consistency with increasing frequency. According to (Duncan, 2007), it is desirable to check whether a material is within its linear viscoelastic range before commencing a series of measurements. He stated that this can be done by performing a strain scan on the material over the range 0.01–5% (or the maximum possible strain) and if a constant modulus is obtained, then it is within the linear range. If the modulus starts to drop (or increase) at a certain level then this is the onset of non-linear behavior, and unless there is a specific reason to study this effect, strains should be kept below this level (Duncan, 2007)**.**

2.5. Structural components of tef and fermented tef batter

Among the components of tef flour, proteins, starch (amylose and amylopectin), and hemicelluloses could possibly contribute to the elastic texture of injera, as could bacterial exopolysaccharides formed during fermentation. However, studies on characteristics of these structural components and how they affect the textural quality of injera are limited. The elasticity and pore or eye formation of injera is major quality attributes, however it is not clear what component(s) of tef flour is (or are) responsible for elasticity and the formation of eyes.

2.5.1. Starch

Starch is made up of amylose and amylopectin. The inherent molar masses and fine structures of amylose (AM) and amylopectin (AP) are the primary determinants of starch properties and functionalities (Bultosa *et al*., 2008). A study by Parker et al. (1989) on injera showed that during cooking, the starch within the injera is totally gelatinized to form a steam-leavened, spongy starch matrix, in which fragments of bran and embryo, micro-organisms and organelles are embedded. Petrofsky and Hoseney (1995) indicated that increased starch-gluten interaction increased the viscoelasticity of gluten dough. Petrofsky and Hoseney (1995) further stated that starch had an active role in determining dough rheological characteristics. The role of starch in the texture of *injera* is further substantiated by Taylor and Emmambux (2008), who stated that good *injera* making quality seems to be related to the starch found in tef and finger millet and that it is probably of great significance that both of these cereals have compound, and not simple type, starch granules. Hamada *et al.* (2013) also reported that the rheological properties of rice batter facilitated adequate gas retention during yeast fermentation, which was caused by protein-starch interaction that resulted from the partial degradation of storage proteins surrounding the starch granules. Furthermore, Edwards *et al.* (2002) concluded that starch measurably contributes to durum dough rheological properties and also that increased proportions of smaller granules increased dough elastic character**.** Umeta and Faulks (1988**)** stated that tef starch has a smaller granule size of 2-6 µm compared to sorghum starch (approximately 20 µm) and according to Yetneberk *et al.* (2004), the relative softness of tef *injera* compared to sorghum *injera* could be related to starch granule size. They further stated that the cell walls and aleurone components of tef could affect the texture of *injera* positively**.** It is proposed that tef starch forms networks which might have a significant role to play in the elasticity of *injera*. The pasting properties of tef starch have an effect on the texture of *injera*. According to Ekris and Gamboa (2008), this property is important and helps to predict the behavior of the flour in baking. Ekris and Gamboa (2008) compared the pasting properties of tef and maize and observed that the breakdown viscosity of tef starch paste was considerably lower than that of maize starch paste. The breakdown viscosity is the decrease in viscosity of the paste as a result of the rupturing of starch granules at high temperatures (Sciarini *et al.*, 2008). This gives an indication of the shear-thinning behavior of the pastes. Yetneberk *et al.* (2004) stated that the difference in pasting properties of sorghum and tef flours could also be related to inherent morphological differences in their starches. More syneresis is likely to be seen in a viscous fluid with a high setback viscosity. The study on tef starch by Bultosa *et al.* (2002) reported a low setback viscosity and slow syneresis. Yetneberk *et al.* (2004) hence stated that this finding by Bultosa *et al.* (2002) is probably related to the softer texture of tef *injera* compared with sorghum *injera*. According to Bultosa (2007), tef starch and its flour pasting are shear tolerant and thus have a potential for use in foods processed under high shear conditions. The *absit* (pre-gelatinized starch) added to the tef batter after primary fermentation is known to enhance the texture of *injera*. According to Taylor and Emmambux (2008), the increased viscosity of the tef batter resulting from cooking the absit seems to enable it to better hold the carbon dioxide produced during fermentation. Zannini *et al.* (2012) stated in their paper that starch gelatinization could play an important role in gluten-free formulation because of the ability of starch pastes to trap air bubbles that aid the gas-holding capacity of batter. According to Abdel-Aal (2009), modified starches such as partially cross-linked and pre-gelatinized starches could play an important role in gluten-free bakery formulations due to their ability to form highly viscous slurries and pastes. Native and modified starches are added to batter formulations in order to soften crumb texture, improve batter consistency and control starch gelatinization during baking (Abdel-Aal, 2009). The absit therefore plays an important role in the formulation of good quality injera.

2.6. The effect of other components in fermented tef on injera quality

2.6.1. Fermentable sugars

The level of sugar in tef flour is known to decrease during fermentation as lactic acid bacteria acts on it to produce lactate. Sugar has been shown to have an effect on *injera* quality as most Ethiopians prefer the characteristic sour taste of injera. The *aflegna injera* (baked at 24 hours) is sweet and is preferred by some Ethiopians, but is not as pliable as fully fermented *injera*. During fermentation, amylase breaks down starch into dextrins, which increases levels of fermentable sugars in the wheat dough (Goesaert *et al.*, 2006) and hence, increases bread volume. Umeta and Faulks (1988) studied two varieties of tef and observed that both varieties contained free sugars that were predominantly sucrose (95%) with fructose being the principal free sugar in the fermenting batter and cooked product. Baye *et al*. (2013) showed glucose to be the main fermentable sugar in a tef-white sorghum composite *injera.* Free sugars may also have an effect on the texture of *injera* as Rühmkorf *et al.* (2012) showed in their study that the higher the sucrose concentration at the beginning of fermentation, the higher the amount of exopolysaccharides produced. The free sugars in fermented tef may also have an effect on the glass transition temperature (Tg) of *injera*. Tg defines a transition from brittle, metastable amorphous solid to a rubbery, unstable, amorphous liquid (Kaletunc and Breslauer, 1993). According to Lasekan and Khalil (2010), amorphous sugar particles are highly hygroscopic and will absorb water at higher humidity resulting in plasticization that lowers the Tg of the particles significantly. There is limited study on the Tg of tef, however, a study by Adebowale *et al.* (2011) reported that tef prolamin has a relatively low thermal stability compared to kafirin and this may be related to the good bread making functionality of tef flour. Lawton (1992) in a study on zein dough observed that it exhibited good viscoelastic properties above its Tg. According to Welti-Chanes *et al.* (2008), a polymer is brittle (a glassy solid state) below its Tg but above Tg, it is flexible and malleable. The flexibility of injera is an important attribute as it relates to its elasticity. As mentioned in previous paragraphs, the pliability of *injera* enables it to be used as a utensil to scoop up *wot***.** It is however unknown whether Tg of the prolamins, starches or sugars in tef have a significant effect on the texture of *injera*.

2.7. Moisture

A study by Ashagrie and Abate (2012), the moisture content of their *injera* samples ranged between 63 to 65%. Moisture in foods is known to have an effect on quality both positively and negatively. Parker *et al.* (1989) likened cooking of *injera* to wafer production and stated that for rapid gelatinization of starch and entrapment of gas bubbles, the batter-like dough should have a high water content, that a steamy atmosphere should be maintained throughout the cooking period, and that heat should be efficiently transferred from the cooking surface. 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). Pyle (2005) observed that the structure of baked crumpets depended on a number of factors including water content of the batter. The moisture content of injera will therefore have an effect on its texture.

2.8. Tef fermentation and the contribution of '*absit*' to *injera* quality

The fermentation of *injera* begins with adding water to tef flour and mixing or kneading it with a starter (back-slopped culture) called *irsho*. This process commences the 'primary fermentation'. According to Dobraszczyk and Morgenstern (2003), even though it is obvious that mixing in the development of rheology and texture in wheat dough is important, there is very little information in the literature on these changes during the different stages in the mixing process. There is little information on mixing and its effect on the texture of *injera*. In the traditional preparation of *injera*, the tef flour, water and *irsho* are kneaded into a thick paste or dough (Zegeye, 1997; Girma *et al.*, 2013; Abraha *et al.*, 2013; Ashagrie and Abate, 2012). Kneading in bread making is known to aerate the dough and according to Maloney and Foy (2003), gas retention depends on the development of the proper dough structure which requires adequate dough mixing. According to Keiffer (2006), during kneading, the wheat dough will wind up the hook when the kneading optimum approaches. He described this as the 'so-called Weissenberg effect' and stated that it is a sign of elasticity. It is not known whether the Weissenberg effect (rod-climbing phenomenon) occurs in gluten-free dough or whether kneading enhances this phenomenon and hence has a significant effect on the quality of the final baked *injera*. Some studies conducted on *injera* reported varying amounts of tef flour to water ratio in tef fermentation. The tef flour, water and *irsho* are usually mixed in different proportions. The flour to water ratio varies in literature from 1:1 to 2:3. A flour: water ratio of 1:1 was used by Abraha et al. (2013), 1:1.6 was used by Girma et al. (1989), 1:2 was used by Ashagrie and Abate (2012), Girma et al. (2013) and Abiyu et al., (2013), while a ratio of 2:3 was used by Zegeye (1997) and (Parker et al., 1989). According to Stewart and Getachew (1962), the time of fermentation depends on the altitude of the area, the concentration of the irsho, and the container used. Stewart and Getachew (1962) stated that the time for optimum fermentation, *i.e.* when gas production ceases and the dough and liquid phase separate, varies depending on how the fermentation is initiated, the numbers and type of organisms present in the *irsho* or flour, ambient temperature, and the type and bacterial cleanliness of the container used. After about 48 to 72 hours of primary fermentation, part of the fermented batter is gelatinized by cooking to form the *absit* which is then added back to the fermented batter. This step initiates the 'secondary fermentation'.

Figure 2.3: Fermented teff batter ready to bake during the work

The role of the *absit* in *injera* making is not clear. Zannini *et al.* (2012) stated that the functionality of *absit* in the *injera* flatbread can be described as that of hydrocolloids in gluten-free breads, providing the batter with a better gas-holding capacity because of increased viscosity. Ashenafi (2006) also reports that the *absit* is a dough enhancer (improves the texture of the dough) and Girma et al. (2013) also mentioned that the absit is a dough binder, but did not define these terms or suggest a mechanism for the effect. It is believed that the main function of a dough enhancer and dough binder is to enhance the viscosity of batters. Other possible functions of the *absit* are that it activates yeasts responsible for CO_2 production (Abiyu *et al.*, 2013) and the development of eyes during baking of *injera*. Ashenafi (2006) mentioned that *injera* baked without *absit* or with less *absit* than required will have a lesser amount of eyes on the upper surface. Also according to Stewart and Getachew (1962), *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 “inviting look”. Yetneberk *et al.* (2004) stated that the objective of gelatinization is primarily to bring about cohesiveness of the batter and secondly to provide easily fermentable carbohydrate to leaven the *injera*.

Yetneberk (2004) reported that by cooking part of the fermented batter to gelatinize the starch, the carbon dioxide produced by the fermentation is trapped and leavens the *injera* on baking. Umeta and Parker (1996) stated that an objective of cooking part of the batter is to increase the amount of gluey material between the batter particles to form more cohesive starch matrix in the injera. It is still not known whether the *absit* has all these functions or whether other processes during fermentation are responsible for eye formation and elasticity of *injera* during baking. However, from all the different functions of the *absit* it is very clear that it helps to improve the quality of *injera*. From studies on *injera*, the amount of *absit* to use for secondary fermentation varies. Ten percent (10%) (Ashenafi, 2006; Girma et al., 2013; Umeta and Faulks, 1988; Zegeye, 1997) 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. There are no studies on *injera* elasticity or any research to show whether *absit* contributes to this elasticity. Parker *et al.* (1989) stated in their study that the major contributor to the *injera* matrix is gelatinized starch.

2.9. Viscosity of teff batter

The overall effect of batter viscosity on the quality of baked *injera* has not been studied. Zannini *et al.* (2012) stated that the *absit* added back to the fermented batter increases the viscosity of the batter and provides the batter with a better gas-holding capacity. According to Shelke *et al.* (1992), the minimum viscosity maintained by a wheat flour batter during heating is considered to be important because it reflects the ability of the batter to retain gas bubbles and to resist settling of starch. A study conducted by Schober *et al.* (2007) on sorghum flour, showed that during fermentation the sourdough becomes thinner. They observed that the extrusion force between a fresh (2 h) and ripe (24 h) sourdough differed significantly by 48% (4.6 N for fresh vs 2.4 N for ripe sourdough), and described the drop in consistency of the batters as being due to degradation of mechanically damaged starch by amylases from sorghum and the degradation of proteins. Degradation of starch and proteins of tef flour occurs during fermentation. It is however unknown whether a similar effect observed in the sorghum flour is likely to be seen in tef flour as both cereals are gluten-free. Hamada *et al.* (2013) related kneading of rice flour dough to the viscosity of batter. They observed that if rice flour is kneaded with water, the dough has greater fluidity than wheat dough and its viscosity resembles that of cake batter. According to Admassu (2006), *injera* generally requires a batter mixture that is viscous enough (200- 1500 centipoise) to retain leavening gasses while cooking, but the batter must also be thin enough so as to result in a finished *injera* which is one centimeter or less in thickness. Gebrekidan and Gebrehiwot (1982) in their study on sorghum *injera* reported that normal *injera* should be thin, about 6 mm, the same thickness reported by KamalEldin and Chiwona-Karltun (2008).

Figure 2.4: viscosity measurement of teff batter ready to bake during the work

The viscosity ranges of 200-1500 cP appears to be a wide range as tef batter used in preparing *injera* usually has a thin pancake-like consistency. According to Sahi (1999), the increase in batter consistency, as observed by increase in elastic and viscous moduli would be expected to slow down the migration of air bubbles in the batter. Sahi (1999) further stated that this would slow down the rate of disproportionation of the bubbles and improve batter stability. Shear thinning behavior is observed with structured foods, where viscosity decreases with applied shear (Cullen and Connelly, 2009). Tef batter is expected to exhibit shear thinning or non-Newtonian behavior at increasing shear rates. A study by Bhattacharya and Bhat (1997) on rice-blackgram suspensions used to make 'dosa', a popular Indian dish similar to *injera*, showed that it exhibited shear-thinning behavior and the Herschel-Bulkley model fit the shear rate and shear stress data of the suspensions better than the power law model or the Casson model. The behavior of materials is described by three Herschel–Bulkley parameters (Mullineux, 2008): the consistency coefficient (k), flow behavior index (n) and yield stress (σ_o). This is represented by the equation:

$$\boldsymbol{\sigma = \sigma_o + k\gamma_n} \qquad \mathbf{2.1}$$

Considering the effect that the consistency of batters has on air bubble migration, it is believed that determining the viscosity of tef batter before baking is very crucial in ensuring that good quality injera is produced.

Dynamic viscosity is commonly reported in Centipoise (cP) and therefore the SI unit is the Pascal-second (Pa·s), which is equivalent to N·s/m2, or kg/(m·s). The cgs unit is the poise (P), named after Jean Louis Marie Poiseuille and more commonly used in many standards as centipoise (cP). **1 cP = 1 mPa·s = 0.001 Pa·s.** the relationship between SI units and CGS Units

2.10. Impact of pH and temperature on *injera* quality

The pH, fermentation temperature and baking temperature of fermented tef batter may also have an effect on the quality of *injera*. Cereal mashes with a pH of 5-6.2, which are rich in fermentable carbohydrates, will be preferentially fermented by LAB, at least to a pH below 4, and below this point, acid-tolerant yeasts dominate the fermentation (Stolz, 2003). Ashagrie and Abate (2012) determined the pH of injera to be 3.4. Rühmkorf *et al.* (2012) analyzed pH and titratable acidity (TA) in fermented rice, buckwheat and quinoa and stated that these parameters are an important control for contaminations. Also in their study, Rühmkorf *et al.* (2012) observed that the higher the inoculated cell counts, the faster the pH decreased and TA and lactate amounts increased. Fermentation temperature has been found to impact the pH of spontaneous tef fermentations and quality of *injera*.

According to Valjakka *et al.* (2003), temperature control is critical in sourdough production as changes in fermentation temperature may cause variation in microflora of sourdough and thus variation in sourdough and final bread quality and flavor.

The optimum temperature range for yeasts is 20-30 °C. Most lactic acid bacteria work best at temperatures of 18 to 22 °C but temperatures above 22 °C favor lactobacillus species (FAO, 1999). 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 desirable quality characteristics. During the 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 desirable characteristics (color, texture, and flavor) of the food (Getenet, 2011). 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. While most studies conducted on *injera* do not state the temperature at which *injera* was baked, a study by Tsegay (2011) observed that the baking of *injera* starts after the baking pan surface temperature reached 215 °C and dropped to about 92 °C when the batter was poured onto the pan surface. They measured the baking pan surface temperature in the experiment and registered a temperature of about 215 °C on the pan surface in order to make it possible to bake 'nice injera'.

According to Ashenafi (2006), the temperature in the middle of the *injera* during the baking process would reach around 90 °C. Because baking temperature of *injera* varies in the literature it will be necessary to obtain a standard temperature at which *injera* can be baked in order to obtain proper eye formation and elastic texture of *injera*.

2.11. Mathematical Models and Experimental Design

An experiment is a series of tests, called runs, in which changes are prepared in the input variables in order to recognize the reasons for changes in the output response (Montgomery). Design of Experiments (DOE) is a powerful technique used for exploring new processes; gaining increased knowledge of the existing processes and optimizing these processes for achieving world class performance (Jiju Antony, 2003).Engineering experimenters wish to find the conditions under which a certain process attains the optimal results. That is, by careful design of experiments, they want to determine the levels of the design parameters at which the response reaches its optimum. The optimum could be either a maximum or a minimum of a response (output variable) which is influenced by several independent variables (input variables). One of methodologies for obtaining the optimum results is response surface methodology.

Response surface methodology (RSM) involves mathematical and statistical techniques that are used for modeling and analyzing the problems in which a process response is influenced by several input variables and the research-objective is to optimize this response. For adopting RSM, selection of contributing parameters, their levels and proper experimental design are essential. RSM consists of a group of techniques used in establishing empirical study of the relationship between a response and several input variables. The main advantage of using RSM is to understand and evaluate the effect of multiple parameters and their interactions with each other in bringing out the response(s).

Response surface methodology also quantifies the relationship between the controllable input parameters and the obtained response surfaces. It is a well-known up to date approach for constructing approximation models based on physical experimented observations (Box *et al.*, Montgomery).

The main advantage of RSM is the reduced number of experimental runs needed to provide sufficient information for statistically acceptable results (Montgomery,2000).

- ✓ Designing of a series of experiments for adequate and reliable measurement of the response of interest.
- ✓ Finding the optimal set of experimental parameters that produce a maximum or minimum value of response.

RSM can be defined as a statistical method that uses quantitative data from appropriate experimental designs to determine and simultaneously solve multivariate equations, which specify the optimum product for a specified set of factors through mathematical models. These equations can be graphically represented as response surfaces which can be used in three ways:

- ➢ To describe how the test variables, affect the response;
- ➢ To determine the interrelationships among the test variables; and

CHAPTER THREE

3. Materials and Methods

3.1. Sample Analysis and Location of the study area

Teff flour and injera for physicochemical and sensory quality analysis was baked at Ethiopian Public Health Institute (EPHI), Food science and nutrition laboratory and the sensory and physicochemical analysis was conducted at Addis Ababa University, College of Natural and computational sciences at the Center for Food science and Nutrition.

3.2. Sample Collection and Preparation

Teff variety DZ-Cr-387(white teff, Quncho) that is under intensive production and commonly used by Ethiopians was collected from Debre Zeit Agricultural Research Centre (DZARC) which was grown and harvested in 2016/17 cropping season.

3.3. Experimental frame work of the research

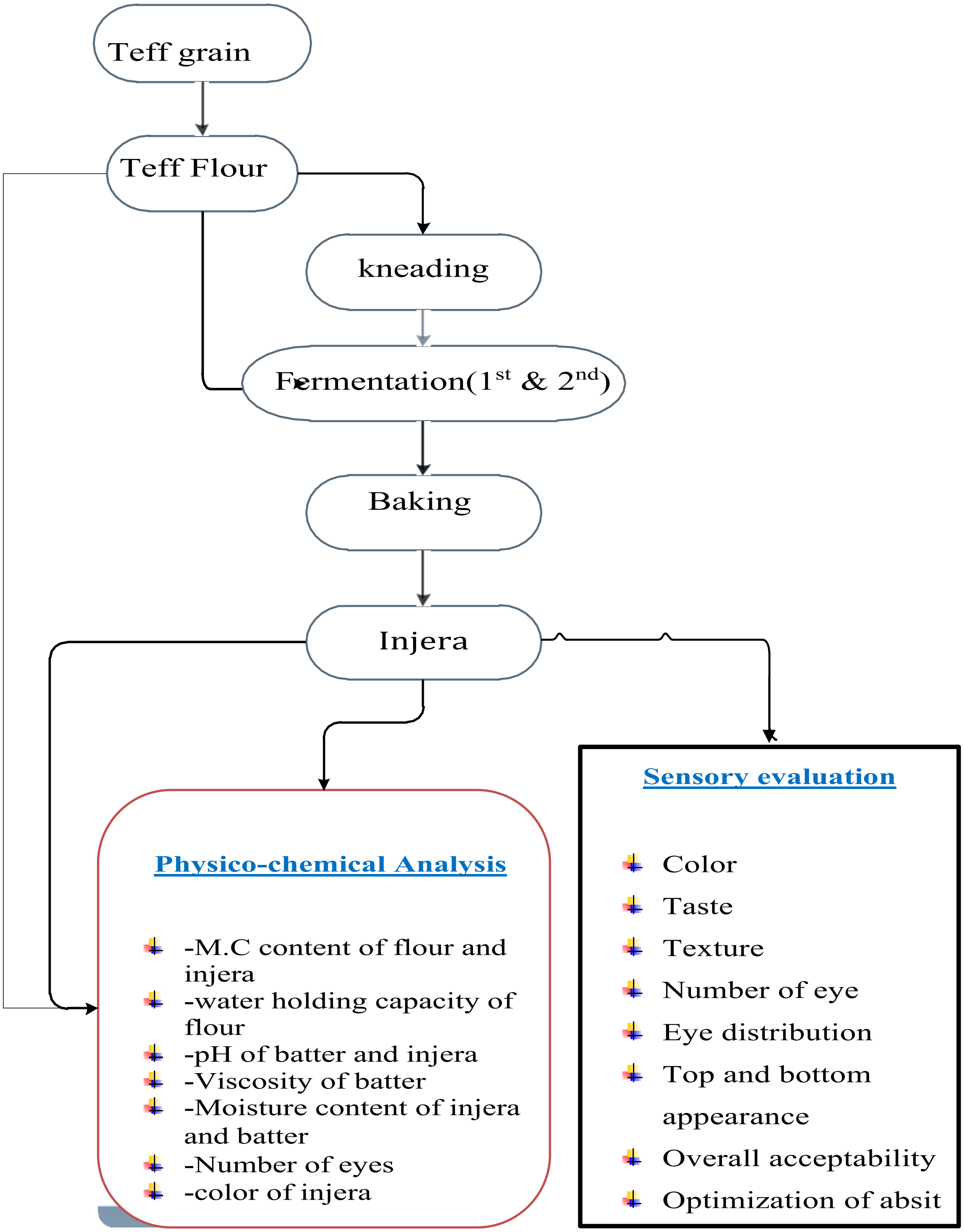


Figure 3.1 Experimental frame work of the research work

3.4. Raw Materials Preparation

Teff grain was first cleaned and milled to whole flour to fineness level traditionally used for injera making. Samples for laboratory analysis were kept in an air–tight, sealed plastic bag at room temperature until it is used.

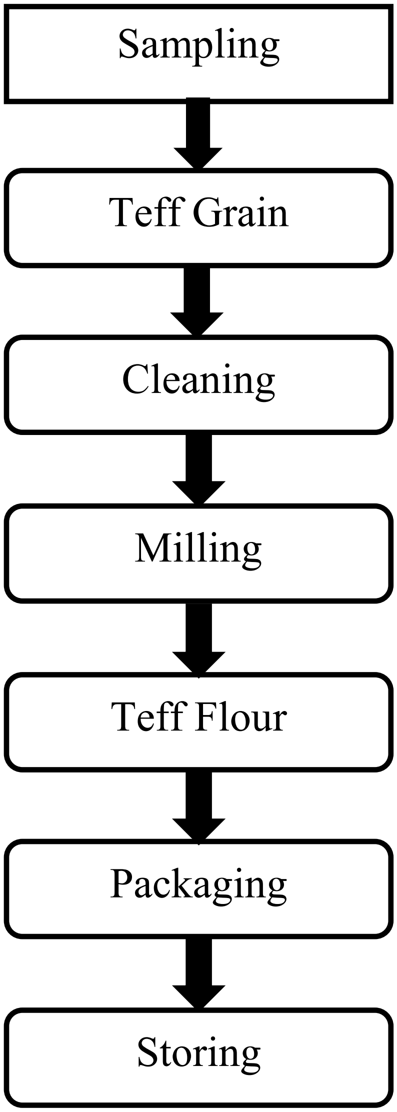


Figure 3.2: Process flow diagram for the preparation of Teff flour

3.4.1. Preparation of dough fermentation

Teff injera was prepared by mixing tef flour (1 kg) with about 2 L of water and about 160 mL of irsho (starter culture prepared previously) made into dough by hand kneading well as is done traditionally.

The kneaded dough was kept covered in different plastic bowl having coded each, at room temperature. Fermentation for injera making involves two phases that can last for about 96hrs.

The first phase starts spontaneously when appropriate combinations of teff flour, water and irsho is wetted, mixed and/or kneaded. The natural starter culture(irsho) for each experiment was prepared previously from the same flour and clean water.

Figure. 3.3: kneading process of the dough

After 72 hrs. of first fermentation of the dough, the yellowish liquid was slightly discarded and measured pH and viscosity for each of 15 experiments and recorded.

3.4.2. Absit preparation

Absit is a hot gruel prepared by mixing and cooking a part(5 to 20 % of dough) of the first fermented dough and water for about 3 minutes then cooled it at some temperature and added to the dough for second fermentation. Fifteen different absit were prepared based on the different combinations of processing factors.

Figure 3.4: Absit preparation

3.4.3. Injera baking process

Teff injera baking were conducted by following traditional teff dough preparation procedure as presented in the following figure.

Addition of absit and mixing with the agitator and put it for second fermentation in the vat, after 2hrs. the dough-rising and gas formation process were observed. The fermented dough was allowed to be thin enough to pour on to the hot flat pan, locally known as “mitad‟ .

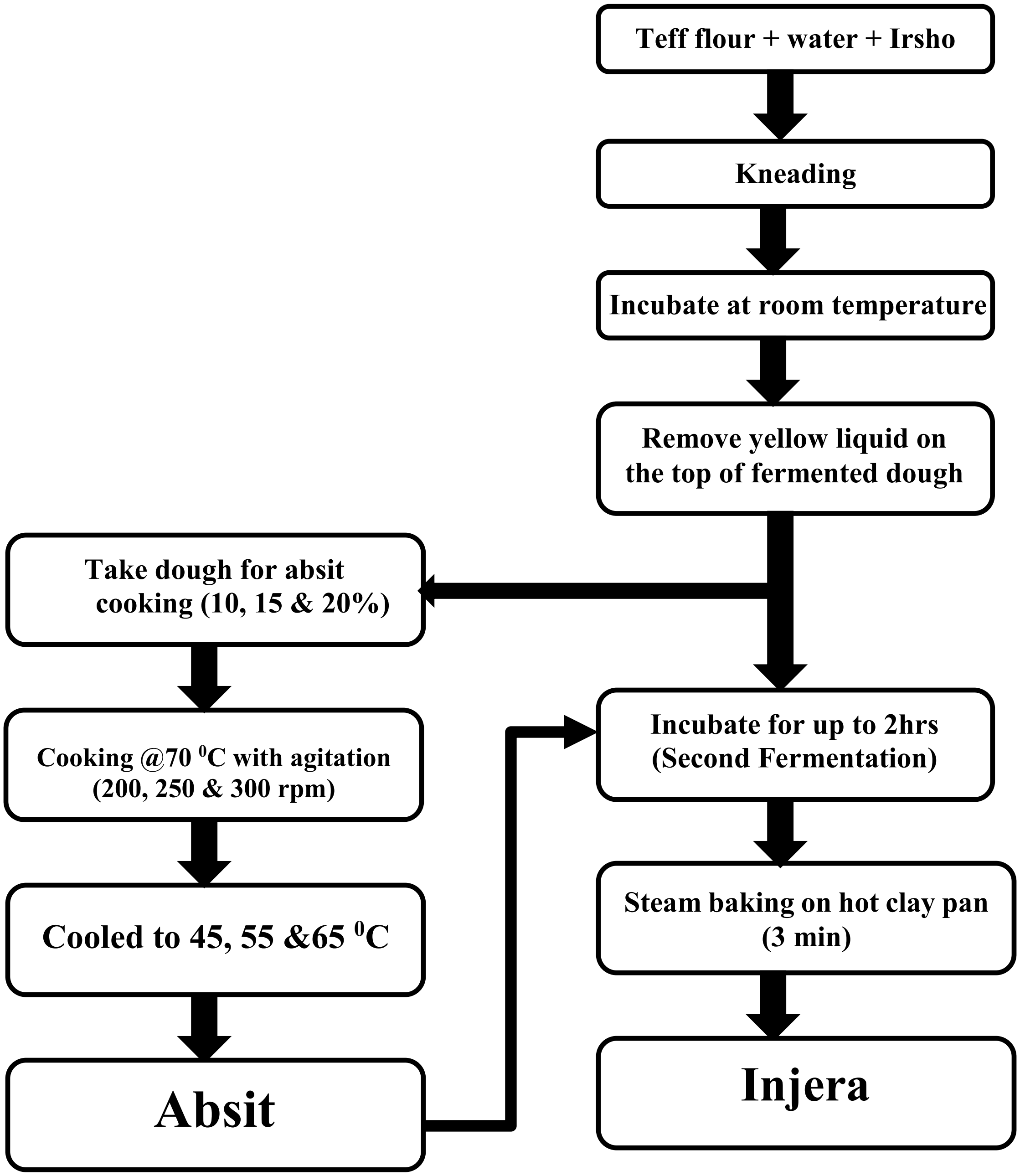


Figure 3.5: Flow diagram of Injera baking during the work

Figure 3.6: Dough and injera preparation: A) Dough fermentation, B) Baking process and C) Injera .

Note:

i. The same irsho were prepared previously as the starter culture for the main dough and was used for all experiments to avoid any variation due to the microorganisms present.
ii. One woman was doing all the preparation of dough making and baking injera.
iii. The time of first fermentation was held constants.
iv. All other processing factors and type of teff was the same.

3.5. Response Surface Methodology (RSM)

RSM is a collection of statistical and mathematical methods that are useful for the modeling and analyzing engineering problems. RSM also quantifies the relationship between the controllable input parameters and the obtained response surfaces. The design procedure of RSM is as follows:

- ✓ Designing of a series of experiments for adequate and reliable measurement of the response of interest.
- ✓ Developing a mathematical model of the second order response surface with the best fittings.
- ✓ Finding the optimal set of experimental parameters that produce a maximum or minimum value of response.
- ✓ Representing the direct and interactive effects of process parameters through two and three-dimensional plots.

3.5.1. Design of Experiments for RSM

RSM designs allow us to estimate interaction and even quadratic effects, and therefore give us an idea of the (local) shape of the response surface under investigation. Box-Behnken designs and central composite designs are efficient designs for fitting second order polynomials to response surfaces, because they use relatively small number of observations to estimate the parameters. Relatability is a reasonable basis for the selection of a response surface design. The purpose of RSM is optimization and the location of optimum is unknown prior to running the experiment, it makes sense to use a design that provides equal precision of estimation in all directions. For such purposes, Central Composite Design (CCD) - spherical or face centered and Box – Behnken design are the commonly used experimental design models for three level three factor experiments.

3.5.2. Box – Behnken design

Box and Behnken proposed three level designs for fitting response surfaces. These designs are formed by combining 2^k factorials with incomplete block designs. Table 3.1 illustrates the three variable Box – Behnken design. It can be noticed that the Box-Behnken design is a spherical design with all points lying on a sphere of radius $\sqrt{2}$. Also the Box –Behnken design does not contain any point at the vertices of the cubic region created by the upper and lower limits for each variable.

This could be advantageous when the points on the corners of the cube represent factor level combinations that are impossible to test due to physical process constraints or prohibitively expensive. Its "missing corners" may be useful when the researcher should avoid combined factor extremes. This property prevents a potential loss of data in those cases.

Box-Behnken designs require fewer treatment combinations than a CCD, in problems involving 3 or 4 factors. The Box-Behnken design is rotatable (or nearly so) but it contains regions of poor prediction quality like the CCD.

In this study, the experiments were planned and conducted according to a Box-Behnken type response surface design.

Table 3.1: Three factor Box-Behnken design

Run	X1	X2	X3
1	1	0	-1
2	-1	1	0
3	0	0	0
4	0	0	0
5	-1	-1	0
6	0	0	0
7	1	0	1
8	0	-1	-1
9	-1	0	-1
10	-1	0	1
11	1	-1	0
12	0	-1	1
13	0	1	-1
14	1	1	0
15	0	1	1

3.5.3. Mathematical Modeling

The second order response surface representing the surface taste, texture, appearance of injera, top and bottom surface and overall acceptability of injera can be expressed as a function of absit processing parameters (dough level, agitation speed and temperature),being the input variables of absit processing factors. A regression model can also be employed for this purpose.

3.5.4. Analysis of variance (ANOVA)

Analysis of variance, ANOVA, is a statistical decision making tool used for detecting any differences in average performances of tested parameters. It employs sum of squares and F statistics to find out relative importance of the analyzed absit processing parameters, measurement errors and uncontrolled parameters. Analysis of variance (ANOVA) was used to check the adequacy of the model for the responses in the experimentation.

3.6. Experiment Designs

❖ Selection of Process Parameters

Process parameters for the study had three levels as given in Table 3.2. The levels were fixed based on the preliminary experiment-trials and also the available literatures.

Table 3.2: Absit Process parameters with their values at 3 levels

Level	Dough level(%)	Agitation speed(rpm)	Temperature (ºC)
1	10	200	45
2	15	250	55
3	20	300	65

❖ Design of Experiment

RSM designs allow us to estimate interaction and even quadratic effects, and hence give us the idea of the (local) shape of the response surface under investigation. Box-Behnken design is having the maximum efficiency for an RSM problem involving three factors and three levels.

Also the number of runs required is less compared to a central composite design. The proposed Box-Behnken design requires 15 runs for modeling a response surface. The process parameters for the experimental runs are selected based on the standard design shown in Figure 1. Details of the experimental runs with the set of input parameters that were conducted are given in Table 2. Design expert software(Design-Expert®, version 7.0, Stat-Ease, SaMeep104 Inc.,2021 East Hennepin Ave., Suite 480 Minneapolis, MN 55413) was used to design the experiment and randomize the runs. Randomization ensures that the conditions in one run neither depend on the conditions of the previous runs nor predict the conditions in the subsequent runs. Randomization is essential for drawing conclusions from the experiment, in correct, unambiguous and defensible manner.

Most importantly, parameters corresponding to the central point (0,0,0) are repeated twice to establish that the experimental data is within the normal dispersion and repeatability is ensured.

Table 3.3: Box-Behnken design table for three parameters

Factors				**Responses**									
Run	X1:dough level	X2:agitation speed	X3:temperature	Viscosity	Color (%)	L* values (%)	Taste (%)	Texture (%)	No of eyes (%)	eye size (%)	eye distribution	Top & bottom	overall acceptability
	%	m/s	^{0}C										
1	20	300	55										
2	15	250	55										
3	10	250	45										
4	10	300	55										
5	20	200	55										
6	15	200	45										
7	15	300	65										
8	20	250	45										
9	10	200	55										
10	20	250	65										
11	15	300	45										
12	15	200	65										
13	15	250	55										
14	10	250	65										
15	15	250	55										

The experimental data for each response variable were fitted to the quadratic model as:

$$Y = \beta + X1 + X2 + X3 + X12 + X22 + X32 + X1X2 + X1X3 + X2X3 \text{ .... } (3.1)$$

Where,

Y denotes the responses; β=constant; X_1 is the dough level(%), X_2 is the agitation speed (rpm) and

X3 is the temperature of absit; X_1^2, X_2^2, X_3^2 = quadratic regression

X_1X_2, X_1X_3, X_2X_3 = interaction regression; X1, X2, X_3= linear regression.

3.7. Functional properties of flour

- **Moisture Content of Flour**

The moisture content of the teff flour samples (2gm) were determined by drying at a temperature of 115°C using instant moisture analyzer (AND model ML-50, made in Japan). The percentage of the moisture content were displayed on the LCD screen as the whole moisture released off with a peep sound at the end of the process. The sample was analyzed in triplicate.

- **Water Holding capacity (WHC)**

The water holding capacity (WHC) of the samples were determined according to Inglett et al., (2016). Each sample (2 g, dry weight) was mixed with 25gm of distilled water in 50 ml capacity of cupped falcon tube. Then the samples were mixed vigorously for 1min to form homogenous suspension using a Vortex stirrer. After mixing, the samples were held for 2 hour and centrifuged at 1,590rpm for 10min using a centrifuge model ROTOFIX-32A (made in Germany). Each treatment was triplicated. Water holding capacity (WHC) was calculated by the difference between the weight of water added (Wad) and water decanted (Wd) which as reported on dry basis (g of water absorbed /100 g of dry sample).

$$\mathrm{WHC} = (\mathrm{Wa} - \mathrm{Wd}) \qquad 3.2$$

Where, Wa = weight of water added

Wd = Weight of water decanted

3.8. Physicochemical analysis of the products

- **PH of batter**

The pH of the first and second fermented doughs were measured directly with scientific electronic bench top pH meter of Nine series (model 902 made in China) after calibrating with buffers at pH 4.0 and 7.0. The pH electrode was immersed in the beaker containing the teff batter (fermented), and a stable result was recorded.

- **PH of Injera**

Injera sample (10 g) was weighed and mixed with 100 mL of distilled and CO2 free water and stirred vigorously. The supernatant solution was decanted into a 250 mL beaker. The supernatant solution PH was measured using scientific electronic bench top pH meter of Nine series (model 902 made in China). The pH electrode was immersed in the beaker containing the supernatant solution, and a stable result was recorded.

- **<u>Viscosity of Batter</u>**

In the absit processing experiment to see the effect of viscosity on physicochemical and sensory quality of injera, a rotational digital viscometer made of Thermo Scientific (model HAAKE viscometer **E**, R-version V 1.1, made in Spain) was used to analyze the apparent viscosity of the teff batter before and after absit addition. The instrument was adjusted and the appropriate spindle number R7 with a suitable RPM and temperature set. The selected measuring feature was displayed on the LCD of the instrument. The selected and loaded spindle was immersed up to the mark into the beaker containing the sample. The viscosity of the batter was measured just before baking for all the samples by controlling the temperature using water bath at 25 ± 1°C.

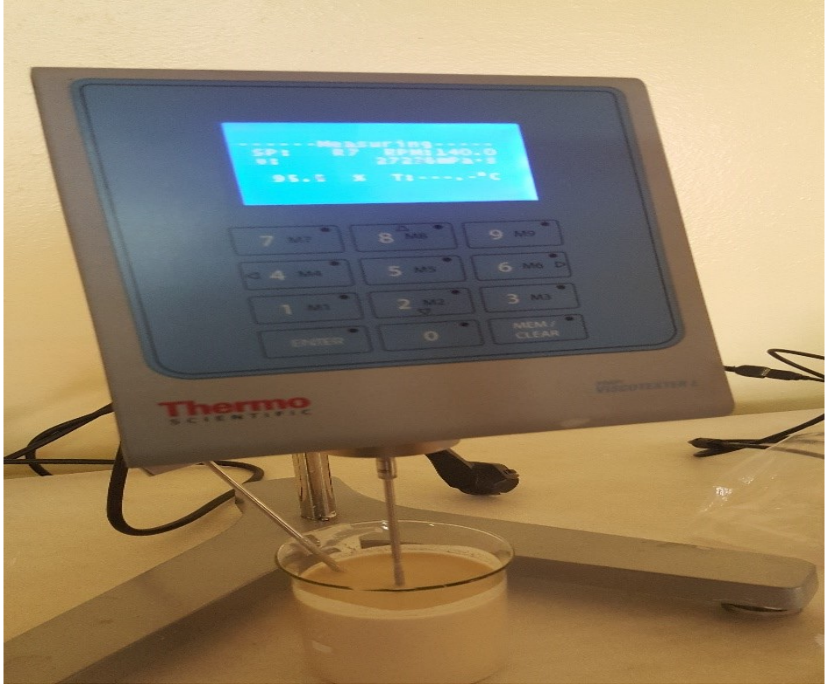


Figure 3.7: HAAKE Viscometer used for Viscosity measuring

- **Moisture Content of batter and Injera**

The moisture content of batter and injera samples (2gm) were determined by drying at a temperature of 115°C using instant moisture analyzer (AND model ML-50, made in Japan). The percentage of the moisture content were displayed on the LCD screen as the whole moisture released off with a peep sound at the end of the process. The sample was analyzed in triplicate and recorded.

Figure 3.8: Instant moisture analyzer

- **Number of eyes on Injera**

The number of eyes on the surface injera were determined by counting the number of eyes on a portion of sample 3cm x 3cm taken from four different parts from full injera. Therefore, the number of eyes was reported as No. of eye/cm^2 using the formula mentioned below (Cherinet, 1988).

$$\text{No. of eyes} = \frac{\text{total No. of eyes on injera}}{\text{3cmx3cm pieces of injera}} \qquad \mathbf{3.3}$$

Figure 3.9 Pieces of Injera (3x3 cm) Portion for eye counting

3.9. Sensory evaluation of Injera

Panelists were chosen from staff and students of the Food Science and Nutrition Department, Addis Ababa University. Injera prepared from the different absit process factors were evaluated for its sensory acceptability and preference by using 30 consumer participants. Evaluation of the injera samples was carried at 24 hours after the injera samples were baked. The attributes assessed included: visual color, taste, texture, appearance (eye size, number of eyes, eye distribution, top and bottom surface) and over all acceptance.

The nine point hedonic scale rated from 10% (extremely dislike), 50% (neither like nor dislike) to 100 %(extremely like) for evaluating the degree of liking and disliking were employed.

All the panelists were frequent consumers of Injera. The age ranges of the participants were 23-40 years old, so that they could fill the scorecard properly. Twelve (12) of the participants were female while eighteen (18) of the participants were male. At the beginning of the test, panelists were instructed about the objective of the research and how to fill the scorecard based on their evaluation. The panelists were asked to evaluate the different types of Injera on the bases of appearance, color, taste, texture, eye size, number and distribution, and overall acceptability based on a nine point hedonic scale(1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) (Abiyu et al., 2013). Here, the panelists were give points from 10 to 100% in terms of 1 to 9 to ease the work and to fill the scorecard appropriately.

3.10. Color Measurement of Injera by Image Analysis using Adobe Photo Shop™ Software

Color of the different injera was evaluated by the image analysis method according to Yam& Papadakis, (2004). It is a method that used a combination of digital camera, computer, and graphics soft wares (Adobe Photoshop) to measure and analyzes the color differences due to the different absit process factors of injera. Images were captured using an image acquisition system for a digital color camera similar to the method developed by Yam& Papadakis, (2004).

Samples were illuminated by using two parallel fluorescent lamps (length of 50cm). The two lamps were situated at 10 cm above the sample and at angle of 45^{o} of the sample plane to give a uniform light intensity over the food plane. The interior wall light of the room was switched off prior to acquiring images to avoid the light and reflection from the room and hence to stabilize the lighting system.

A Digital Color Camera (DCC) (SONY, Steady Shot DSC-S2100, 3.0 Clear Photo LCD 3.0"/7.5cm with 12.1 Mega Pixels) was located vertically at a distance of 45cm from the sample. The angle between the camera lens axis and the lighting sources was around 45°. The images of samples were captured on the camera settings: -manual mode, and no flash. A total of three images for each experimental injera samples were taken and stored in JPEG format for image processing and analyzing.

The photo images taken using a camera were transferred to a computer hard disc and opened with Adobe PhotoshopTM software (Vyawahare and Rao, 2011). CIE L *a*b* were measured on the digital image of the sample visualized on the monitor using a graphics software Adobe Photoshop CS3 by pointing the cursor on the surface area of the sample and by clicking on it. Three surface points from each injera sample were taken for the measurements.

3.11. Statistical analysis

All measurements were made in triplicate and analyzed using either regression, one-way, two-way or repeated measures (general linear model) analyses in Minitab 18 Statistical Analysis Package (State College, PA). Multiple comparisons of mean values were done using the Tukey's family error rate with a significance level of 0.05.

Design- Expert ®, version 7.0, Stat-Ease, (SaMeep104 Inc., Minneapolis, MN USA) was used to perform regression analysis on the sensory scores, and L*values. The adequacy of the model was evaluated based on model significance ($p<0.05$), lack of fit and adjusted coefficient of determination (R^2adj), which indicate the model fitness.

The model chosen was based on a significant model ($p<0.05$), insignificant lack of fit and highest adjusted R^2. A report by Okpala and Okoli, (2013) suggested that although determination coefficients of 80% and above are recommended, $R^2$of 60% can also be used for model prediction and if a model has a significant lack of fit; it is not a good indicator of the response and should not be used for the prediction.

3.12. Optimization

Both numerical optimization and graphical optimization technique were employed using the Design Expert TM version7.0 software (State Ease Inc.) and Minitab V.18 . The desired goal for each of the response attributes and ingredients were chosen.

The criteria for viscosity and sensory attributes (color, texture, no of eyes, eye distribution, top & bottom surfaces, and overall acceptability), and L* values were kept maximum for numerical optimization. Graphical optimization was carried out by superimposing the contour plots of sensory scores (color and overall acceptability) and L* value.

CHAPTER FOUR

4. Results and Discussion

In this study, absit process factors (dough level, agitation speed and temperature) were considered and see the responses of the physicochemical and sensory quality of teff injera. While conducting the experiment all the factors like type of teff, all the utensils and the woman who baked injera were all the same and controlled.

4.1. Functional Properties of flour

4.1.1. Moisture content of flour

Quncho teff flour moisture content was analyzed using instant moisture analyzer (model ML-50 brand of AND, made in Japan) and the mean result found was 10.39 %. A study by Bultosa, (2007) reported that the moisture contents of 13 different varieties of teff are in the ranges 11.22%-9.30%. On the work of Abebe and Ronda, (2014), Abebe, (2015), the moisture contents of Quncho teff was 10.4% .Therefore ,the moisture content Quncho teff flour is not significantly different from the above mentioned previous studies.

4.1.2. Water holding capacity (WHC) of teff flour

The water holding capacity of Quncho (DZ-Cr-387) teff flour was found 178.38g/100g. The fiber contents of whole grain teff and particle sizes are some of the reasons which influence to increase the water binding capacity of flours (Abebe,2015). On the other hand, the mineral contents (Ca & Mg) and protein affects the hydration properties of teff flours due to their interaction and formation of coagulation.

These interferences on WHC and other factors of the flours might determine the amount of flour to be hydrated. The flours with less hydration capacity requires more flours than the one with high hydration capacity to make dough. The flour with high WHC value might be preferable from economical point of view of the flour. Based on the above mentioned factors the hydration properties of cereal grains could be different.

4.2. Physicochemical Analysis of batter and injera

4.2.1. pH of batter and injera

A study by Yigzaw et al., 2004 and Uraga and Narasimha, 1997 showed that sourness test of traditionally fermented Ethiopian injera is one of the sensory attributes impacted by pH due to changes in Lactic acid concentration during fermentation . The pH of the first fermented bulk batter was measured and found to be 3.75 at 72 Hrs. which is not significantly different from a study by Uraga and Narasimha, 1997 on the 96 hrs. fermentation conducted the pH value is reported to be 3.83 . **Table 4.1: pH values for Batter and Injera**

Runs	PH of batter before absit addition	PH of batter after absit addition	PH of Injera
1	3.75	3.95	4.01
2	3.75	3.92	4.00
3	3.75	3.92	4.01
4	3.75	3.98	4.01
5	3.75	4.02	4.05
6	3.75	4.05	4.08
7	3.75	3.78	3.89
8	3.75	3.88	4.01
9	3.75	4.03	4.04
10	3.75	4.02	4.05
11	3.75	3.89	4.01
12	3.75	4.03	4.06
13	3.75	3.96	4.00
14	3.75	4.01	4.03
15	3.75	4.04	4.05

After the addition of absit, prepared from different processing factors the pH values found were different. The pH readings of the respective baked injera were measured and presented in (figure

4.1) bellow. The pH readings of the fully fermented bulk dough at 72 Hrs, after absit addition and respected baked injera are significantly different and presented below the graph .

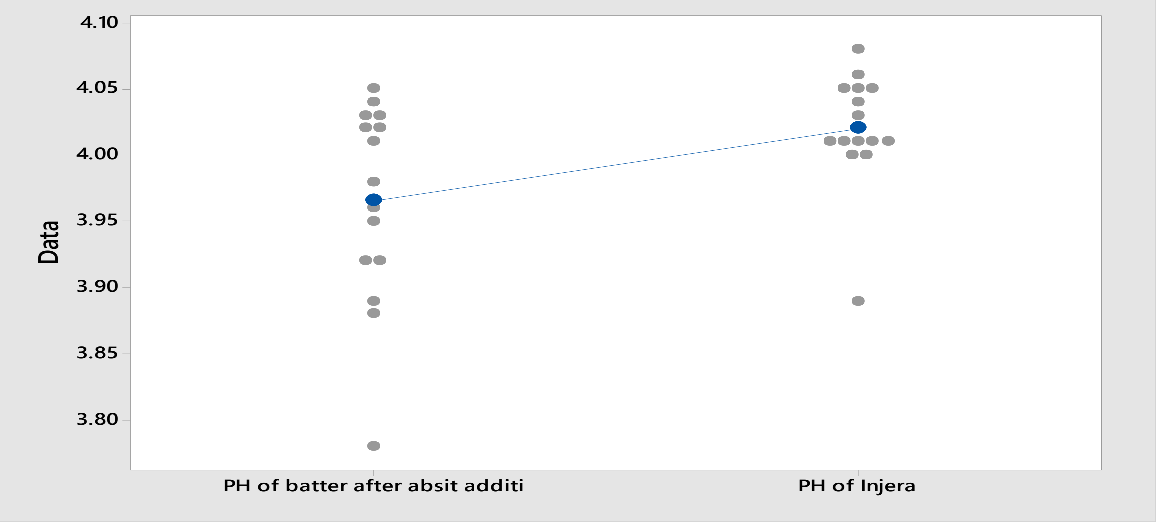
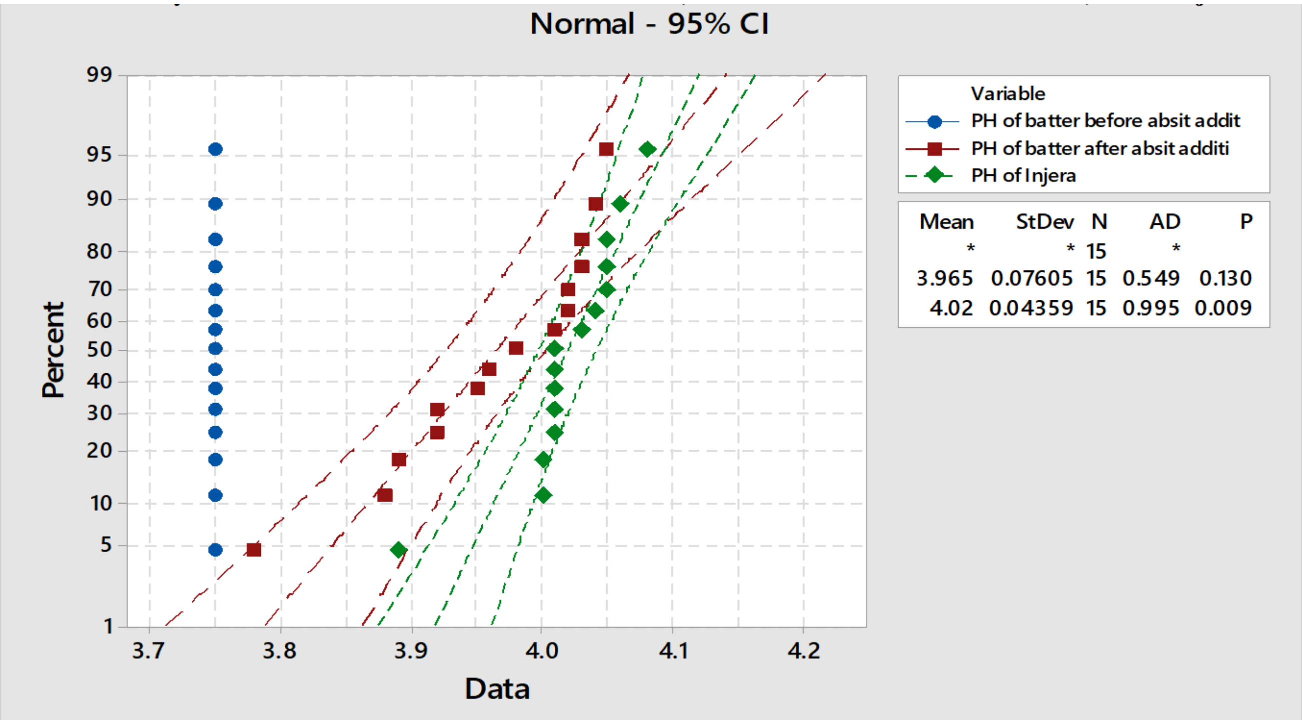


Figure 4.1: Plot of pH of batter before and after absit addition



* NOTE: Distribution could not be fit. The number of distinct rows of data must be greater than or equal to the number of estimated distribution parameters.

Figure 4.2: Probability plot of pH of batter before, after absit addition and PH of Injera

In different literatures the pH readings of injera is reported with different figures for variable reasons like type of flour, fermentation time, the removable supernatant liquids remedies and the amount of backslope (irsho**)** used (Yigzaw et al., 2004, Urga et al., 1997). In general, the ESA (2013) has set the pH of teff injera has to be in the range 3.45 to 4.0. As a result, the values of pH obtained was not significantly different from the set values of pH by (ESA, 2013) except run 7 which is 3.89. as we can see the above distribution plot, there were a significant difference of the PH readings and it is because of the absit addition and have a significant effect on the quality of injera.

4.2.2. Viscosity of Teff Batter

The single apparent viscosity of the batters before and after absit addition were measured and recorded. The viscosity values recorded for the fermented batter were different and influenced by the absit addition and processing factors like agitation speed, temperature and dough level, brought from the bulk .

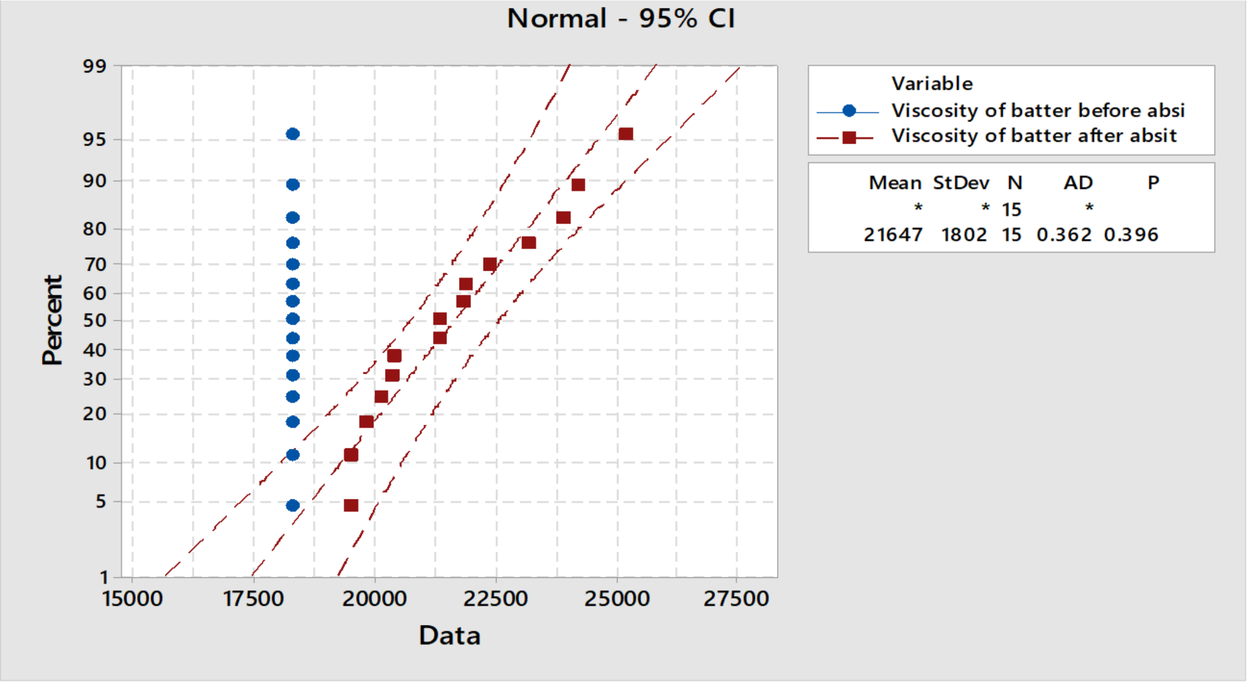


Figure 4.3 : probability of viscosity of batter before absit addition, viscosity of batter after absit addition

4.2.3. Moisture Content of Batter and Injera

The moisture content of batter and injera were determined by instant moisture analyzer (AND model ML-50, made in Japan). The moisture content of the batter of different runs immediately before baking were 72.04% .The moisture content of injera for each runs of experiment were measured and found to be ranged 61.04 % to 62.32%. In some literatures the moisture content is reported to be 65.23% for injera and on the other literature it was found in the range 62-65% (Ashagrie and Abate, 2012; Attuquayefio, 2014). Studies by (Gebrekidan and Gebrehiwot, 1982; Yetneberk *et al*., 2004) reported that the moisture content of injera made from different cereals is above 50%. The moisture content of injera should be in the range of 58% to 63% (ESA, 2013) is set. There was no significant different of moisture content of first fermented batter among the fifteen runs because the amount of water added for each were equal. Generally, the moisture content of baked injera was much less than the moisture content of the respected batter.

4.2.4. Number of eyes on Injera

The number of eyes on injera were determined and reported in this work were done by counting the number of eyes per unit area of the eyes on 3cm x 3cm piece of injera. Four portion from different parts of injera were taken for the determination of the number of eyes. The number of eyes for the different samples were found and ranges from 11.32 to 13.65 eyes per cm2.There was some variation on the size of the eye nuclei observed for injera obtained from the different baked injera samples due to the absit and other factors.

4.3. Sensory Acceptability of the baked injera

The sensory acceptability (visual color, taste, texture, appearance which includes no of eyes, eye size, eye distribution and top & bottom surfaces, and overall acceptability of all samples of injera baked and analyzed at day 2 were presented and discussed below independently.

4.3.1. Visual color of injera

The visual color of the baked injera was found and ranged from 56.63 to 72.58 %. There is no significant difference ($p<0.05$) in the preference of the panelists among the 15 experimental trials. All the 15 experimental trial were accepted greater than 56.63% (like slightly) by the panelists.

The multiple regression analysis, the model for color data was not significant (p=0.8892) and the lack of fit test (p= 0.9548) showed that the model is not suitable for prediction purposes. Therefore, the color of the baked injera could not be affected by the absit processing factors and it is mainly due to the color of the flour of the teff variety.

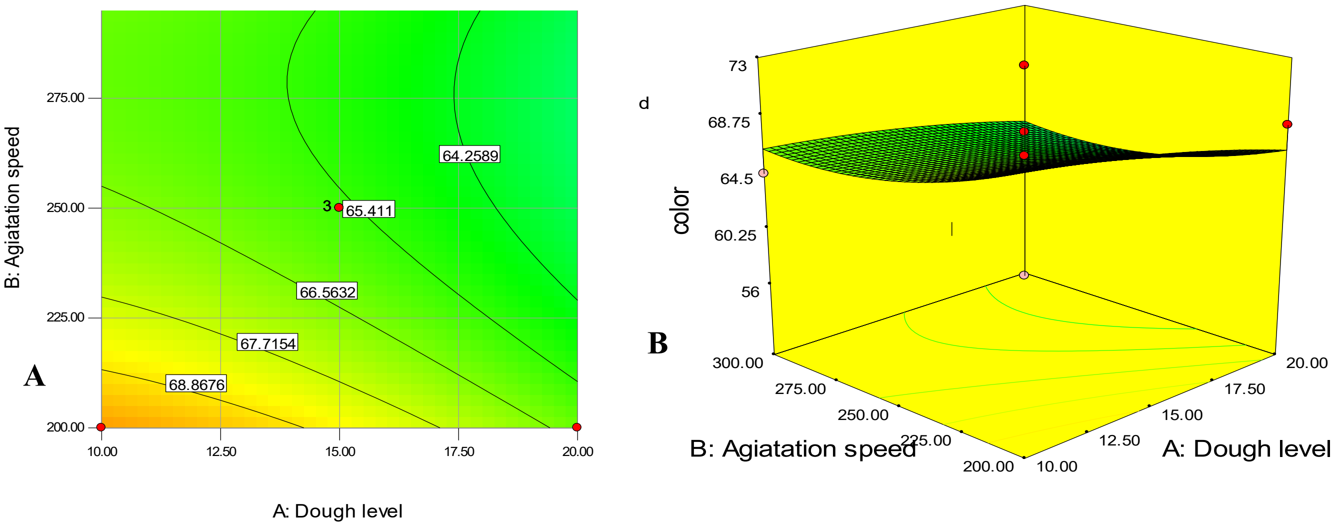


Figure 4.4: A) Contour) and B) 3D surface plots for color obtained using actual-components.

The contour plot showed that there is a slightly preference when the dough level and agitation speed is 11% and 200 rpm respectively when the temperature is hold at 55ºC.

4.3.2. Taste

The taste of the baked injera was ranged from 47.47 to 65.05% by the panelists. There is a significant difference ($p<0.05$) in the preference of the panelists among the 15 experimental runs. R1(Injera made from the absit process factor combinations (10,250 and 65) of dough level, agitation speed and temperature respectively)) was the most preferred taste by the panelists. The minimum value was obtained in R10((15,200 and 65) of dough level, agitation speed and temperature respectively)).

The multiple regression analysis showed that there is a significant model term in predicting the sensory score for taste in this case A, BC, A^2 are significant model terms where, A= dough level, B=. agitation speed and C=temperature.

The following is the model equation developed from the data for taste.

$$\mathbf{Taste} = -\mathbf{4.05} * \mathbf{A} + \mathbf{4.38} * \mathbf{A}^{2} + \mathbf{5.66} * \mathbf{BC} \qquad \mathbf{4.1}$$

The contour plot bellow showed that there is a considerably preference when the dough level and agitation speed is 11% and 200 rpm respectively when the temperature is hold at 55°C.

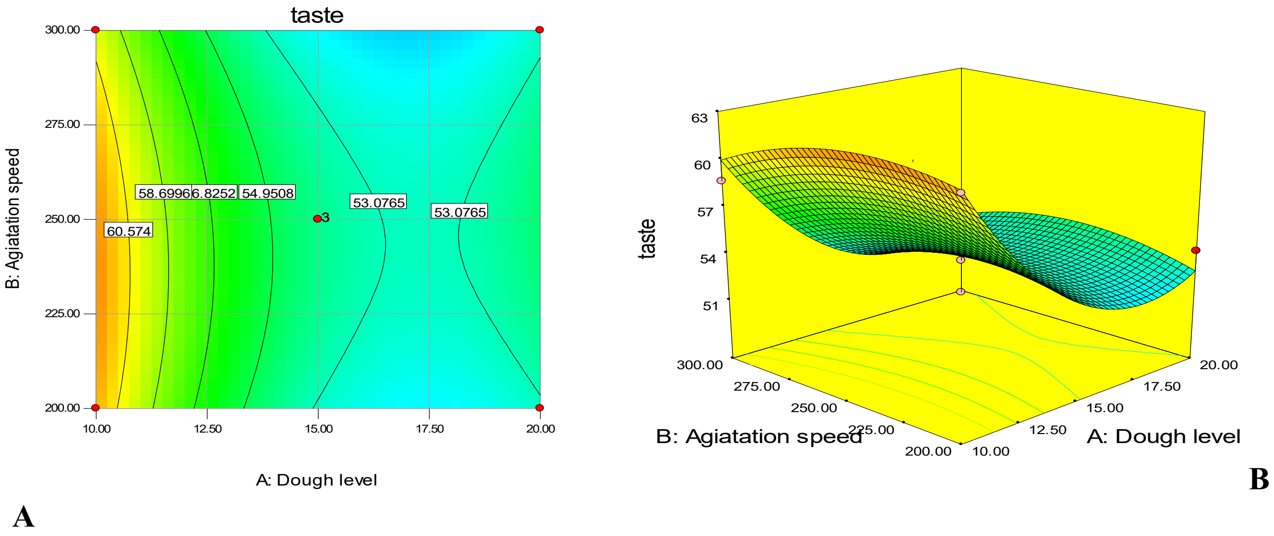


Figure 4.5:A) Contour, B) 3D surface plots for taste obtained using actual-component

4.3.3. Texture

The texture of teff injera is soft and pliable to wrap and catch the wot and it is the most preferable by Ethiopian consumers (Gebrekidan and Gebrehiwot,1982). The sensorial texture value for the baked injera scored by the panelists were found to be 57.11 to 74.88 %. Panelists texture response varies among the15 experimental runs at $p<0.05$ and maximum response (74.88%) was obtained. Minimum value for texture was obtained in R14(15,250 and 55 of absit process factors). The relative softness of teff injera could be related to starch granule size and this starch granule might be utilized due to the absit preparation and fermentation of dough.

The RSM application on texture scores showed that the linear model was significant ($p<0.035$), no lack of fit was obtained since the lack of fit is not significant ($p=0.608$) with relative to the pure error.

The model obtained for the texture of the samples was:

$$\boldsymbol{Texture} = -\mathbf{4.09} * \boldsymbol{A} + \mathbf{5.88} * \boldsymbol{A}^{2} + \mathbf{4.61} * \boldsymbol{BC} \qquad 4.2$$

The processing factors of absit have effects in the model of the equations.

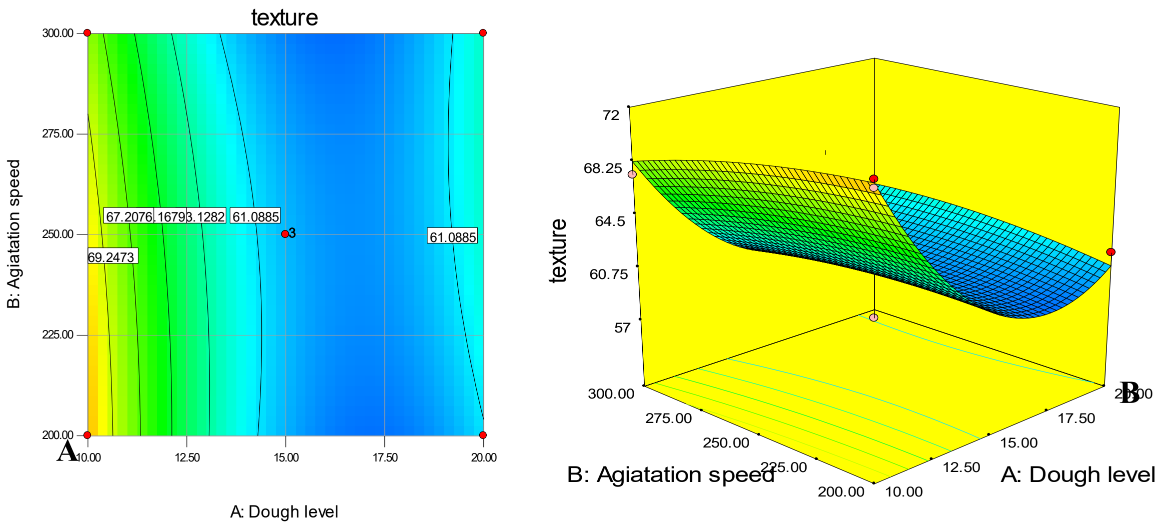


Figure 4.6:A) Contour, B) 3D surface plots for texture obtained using actual-components.

4.3.4. Number of Eyes

There is a significant difference ($p<0.05$) in the sensory acceptability test result for no of eyes of the baked injera samples. The test result from counter plot showed that no of eyes is higher when the dough level is between 12 and 15 % and agitation speed is around 200 rpm while the temperature is held at 55°C. Starch plays a major role in the formation of surface gas holes for injera made from teff (Parker et al., 1989) and the starch could possibly be utilized when absit is added and fermentation processes undergoes and finally the gas bubbles create and ceases . There were significantly different in the model terms, linearly except agitation speed and in all the terms.

Design Expert™ application on the data of no of eyes indicates that the linear model is significant ($p= 0.006$), and lack of fit was obtained with no significant level ($p=0.157$).

The model obtained for the no of eyes of the samples was:

$$\textbf{No of eyes} = -5.79 * A - 2.68 * C - 10.44 * A^2 - 4.47 * C^2 - 4.43 * AB + 3.21 * AC + 7.27 * BC \qquad 4.3$$

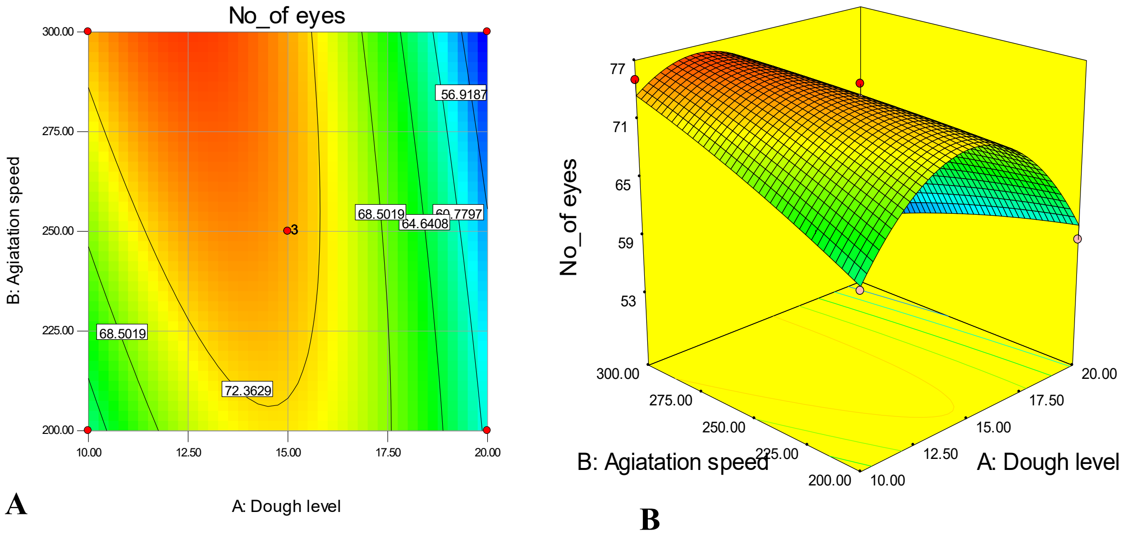


Figure 4.7:A) Contour, B) 3D surface plots for no of eyes obtained using actual-components.

4.3.5. Eye Size

Eye size of the baked injera was found and ranged from 41.26 to 66.26 %. Maximum scores for eye size was obtained in R12 (15,300 and 65 of the absit processing factors) and minimum scores was found in R13(20,250 and 65). The multiple regression analysis showed that there is a significant model term (p=0.0021) in predicting the eye size scores of injera samples. There were significantly different models terms in the following cases A, AB, BC, A^2, C^2. The Lack of Fit F-value of 3.92 implies the Lack of Fit is not significant relative to the pure error and R-Squared value were 97.29%. The model obtained for the eyes size of the samples was:

$$\mathbf{Eye\ size} = -5.68\mathbf{A} - 10.26 * \mathbf{A}^2 - 4.81\mathbf{C}^2 - 5.57 * \mathbf{AC} + 8.51 * \mathbf{BC} \qquad 4.3$$

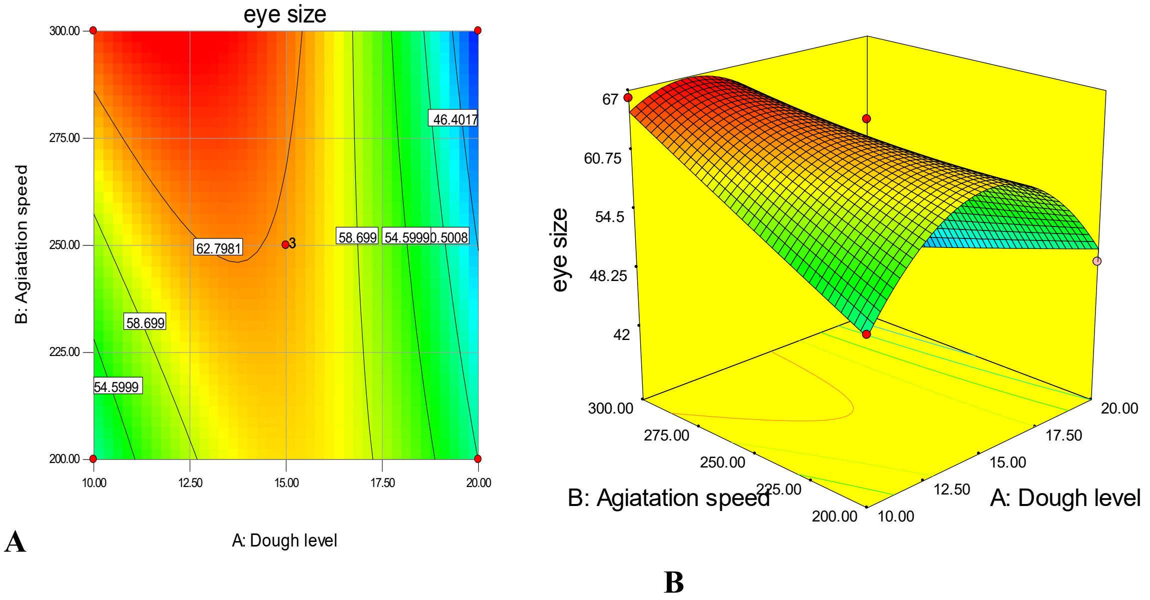


Figure 4.8:A) Contour, B) 3D surface plots for eye size obtained using actual-components.

4.3.6. Eye distribution

The sensory response for eye distribution of the baked injera was found and ranges from 46.76% to 71.71% and are significantly different ($p<0.05$). The eye distribution of R8(10,300 and 55)is most liked than other absit process factor combinations. The contour plot showed that the eyes of injera were more evenly distributed and liked when the dough levels were range between 12 to 15 % and the agitation speed were above 275 rpm at 55°C. According to the probability of F and lack of fit values, the predicted model of eye distribution could be developed.

The model obtained for the eye distributions of the injera was:-

$$ED = -5.67 * A - 10.43 * A^2 - 4.77 * A^2 - 5.63 * AB + 8.36 * BC) \qquad 4.4$$

ED= *Eye distributition*

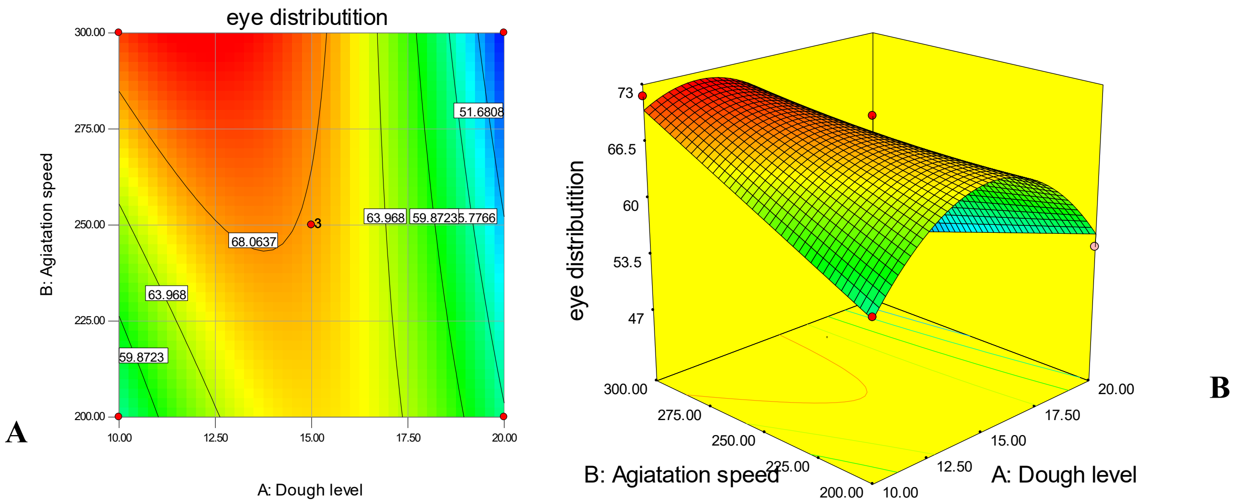


Figure 4.9:A) Contour, B) 3D surface plots for eye distribution obtained using actual-components

4.3.7. Top and bottom surfaces

The top and bottom surfaces of the baked injera were significantly($p<0.05$) among the experimental runs of baked injera. The values reported by the panelists for top & bottom surfaces ranged from 51.34 to 77.80%. The top and bottom surfaces of R7(15,250 and 55) of the absit processing factors is more preferred when compared with other combinations.

The application of the Design Expert^{TM} showed that there is a significant model term (p=0. 0106) in predicting the top & bottom surfaces scores of injera samples and in this case A, AB, AC, BC, A^2, C^2 are significant model terms for top & bottom surfaces of the baked injera and the equation obtained from the data is as follows:

$$\mathbf{TBS = -4.24 * A - 10.32 * A^2 - 4.78C^2 - 5.33 * AB + 4.81 * AC + 5.72 * BC} \qquad \mathbf{4.5}$$

TBS = Top and bottom surfaces

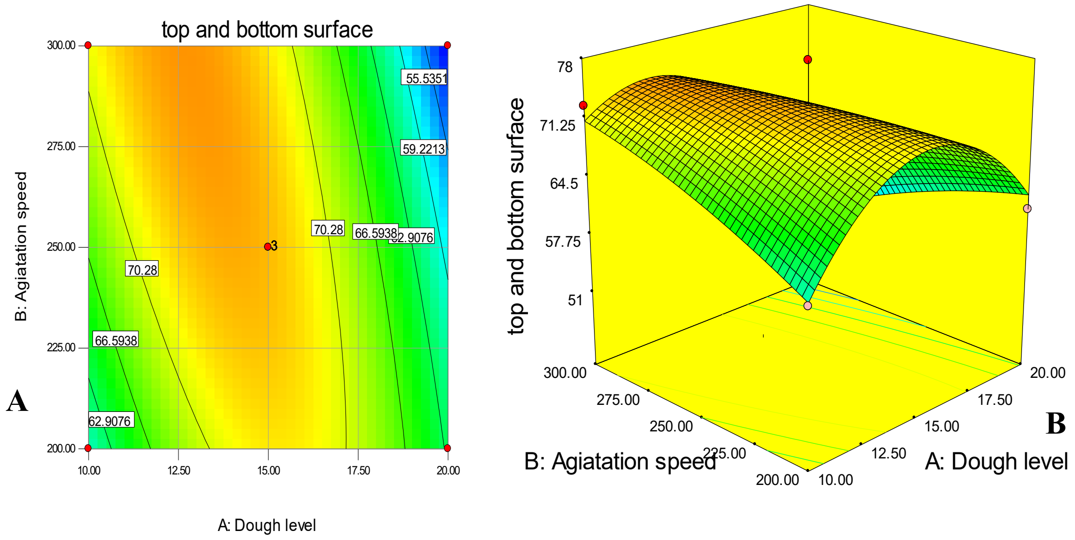


Figure 4.10:A) Contour, B) 3D surface plots for top and bottom surfaces obtained using actual-components

3.1.1. Overall Acceptability

The overall acceptability of the 15 experimental runs of the baked injera were found to be in the ranges of 50.03 to 68.38%. Injera made from absit process factors combinations R1(10,250 and 65) is the most liked experimental injera and R10(15,200 and 65) is the least liked injera of all samples. The regression model obtained for the overall acceptability of the injera samples was:

$$\boldsymbol{OA = -3.69 * A + 5.24 * A^2 + 6.04 * BC} \qquad \mathbf{4.6}$$

Statistical analysis indicated that the model was significant in predicting the overall acceptability of the injera (p= 0.0173). The model could explain about 93.46% of the observed variations and did not present significant lack of fit (p=0.6890). There were are significant model terms in case of A, BC, A^2.

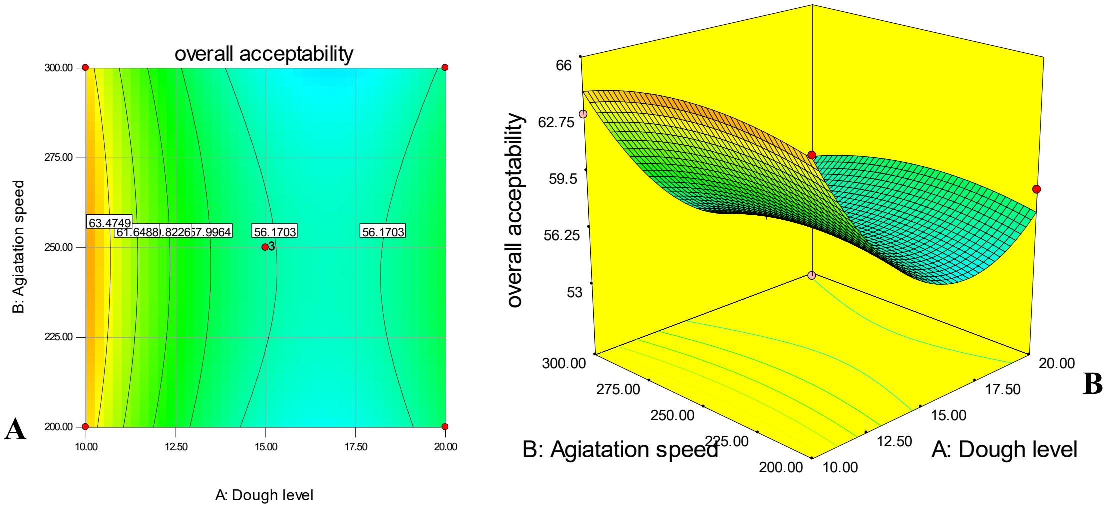


Figure 4.11: A) Contour, B) 3D surface plots for overall acceptability obtained using actual-components

3.2. Color (L* Values) of the baked Injera

Color of baked products, together with texture and aroma contributes important characteristic to consumer preference (Mahmoud al at., 2013) is an important characteristic. There is no significant difference ($p<0.05$) in L* values (lightness) among the injera samples. The mean L* values (lightness) ranged from 60 – 75.78%. Highest L* value (75.78%) was found in runs at R2(15,250 and 55) of absit process factors. In a study by Cherie et al., (2018), there is a significant difference ($p<0.05$) in L* values(lightness) between the composite injera samples as the blending ratios varies. Here, the color L*values of the baked injera depends mainly by the different variety of flours not by the processing factors of the preparation.

The application of the Design Expert$^{\text{TM}}$ showed that there was no significant influence in all the model terms on the L* values of injera samples. Therefore color (L* values) of the baked injera did not influenced by the absit processing factors considered for a Quncho teff variety.

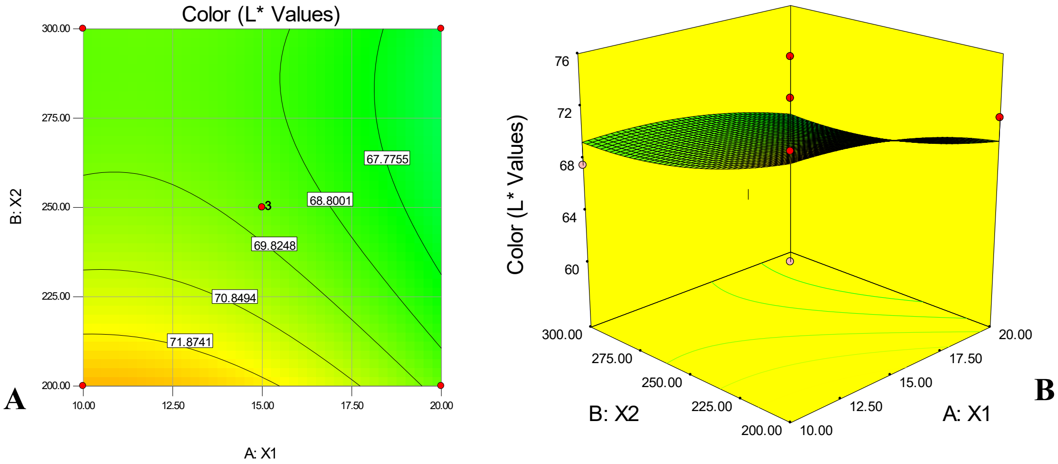


Figure 4.12: A Contour, B) 3D surface plots for color L*values obtained using actual-components

Table 4.2 bellow shows the coefficients estimates, adjusted regression coefficients (R^2adj), the results of model significance and lack of fit for sensory scores (color, taste, texture, n<u>o</u> of eyes, eye size, eye distribution, top & bottom surfaces and overall acceptability) and L* values of the produced injera. The multiple regression analysis of sensory scores showed that the model terms are significant for prediction and the model presented no significant lack of fit. Therefore the model terms are adequate to create regression equation for prediction for these attributes.

Table 4.2 :Coefficient estimates, model significance, adjusted regression coefficient (adj $R^{2)}$ and lack of fit values for sensory attributes of injera samples

Dependent variables	Predictive models									Model (Prob>F)	Adj R^2	R^2	Lack of fit
	$Yi=a1X_1+a2X_2+a3X_3+a4X_1^2+a5X_2^2+a6X_3^2+a7X_1X_2+a8X_1X_3+a9X_2X_3$												
(Yi)	a_1	a_2	a_3	a_4	a_5	a_6	a7	a8	a_9				
Taste	-4.05	_	_	4.38	_	_	_	_	5.66	0.016*	0.8249	0.9375	0.714 (ns)
Texture	-4.09	_	_	5.88	_	_	_	_	6.61	0.035*	0.7499	0.9107	0.608 (ns)
NE	-5.79	_	-2.68	-10.44	_	-4.47	-4.43	3.21	7.27	0.001*	0.9559	0.9842	0.157 (ns)
ES	-5.68	_	_	-10.26	_	-4.81	-5.57	_	8.51	0.002*	0.9240	0.9729	0.210 (ns)
ED	-5.67	_	_	-10.13	_	-4.77	-5.63	_	8.36_	0.002*	0.9201	0.9715	0.221 (ns)
TBS	-4.24	_	_	-10.32	_	-4.38	-5.33	4.81	5.72	0.011*	0.8513	0.9469	0.826 (ns)
OA	-3.69	_	_	5.24	_	_	_	_	6.04	0.017*	0.8168	0.9346	0.689 (ns)

ai = L-pseudo-component value, (X1) = dough level , (X2) = agitation speed, (X3) = temperature,

* = Significant at $P < 0.05$, (ns) = not Significant

NE=No of eyes ,ES= Eye Size , ED=Eye Distribution ,TBS=Top & Bottom Surface and

OA=Overall Acceptability

3.3. Optimization of absit process parameters

The numerical optimization technique (design expert 7.0 software) was used to get the optimum values of the independent variables i.e. dough level, agitation speed and temperature of absit process factors. The response variables selected for optimization were taste, texture ,appearance(no of eyes, eye size and eye distribution) ,top and bottom surface and overall acceptance of the baked injera.

Multi-objective optimization was aimed at to achieve better absit quality for better teff injera which is acceptable in its sensory and physico-chemical quality.

Table 4.3:Response Optimization

Response	Goal
Taste	Maximum
Texture	Maximum
No of eyes	Maximum
Eye size	Maximum
Eye distribution	Maximum
Top and bottom	Maximum
overall acceptance	Maximum

Accordingly optimization criteria for each response were selected as given in
Table 4.4 bellow which is the best Solution satisfying the above criteria was obtained using the 'Design Expert' software, which is given below and it has the overall desirability of 0.910.

Table 4.4: optimized values of the absit process parameters with the respected baked injera.

Dough level (%)	**Agitation speed(rpm)**	**Temperature (^{O}C)**	**Taste**	**Texture**	**No of eyes**	**Eye size**	**Eye distribution**	**Top and bottom**	**overall acceptance**	**Desirability**
11.06	**200**	**45**	**64.84**	**74.88**	**74.61**	**62.79**	**68.16**	**70.41**	**68.27**	**0.91**

Contour plot given in Figure 4.13, shows the variation of Desirability with change in dough level and agitation speed when temperature is kept constant at optimum level of 45 ^{o}C.

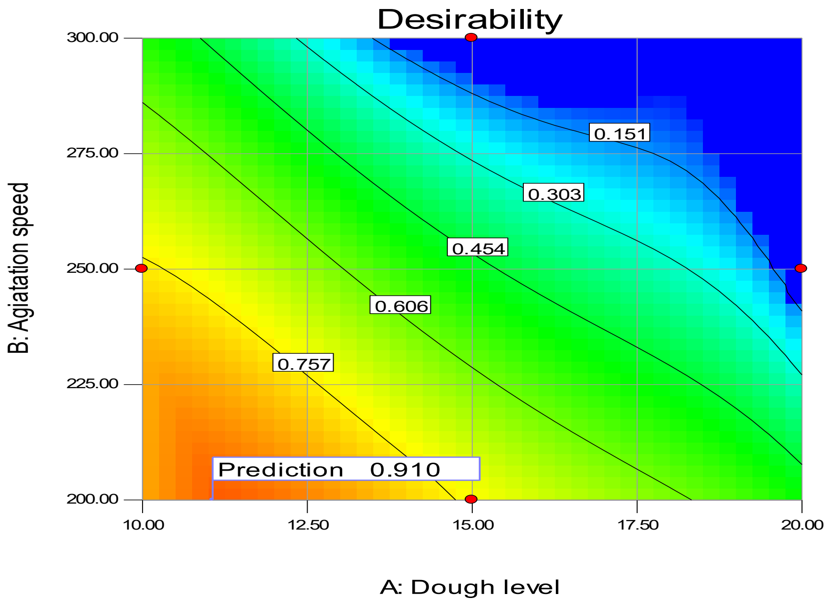
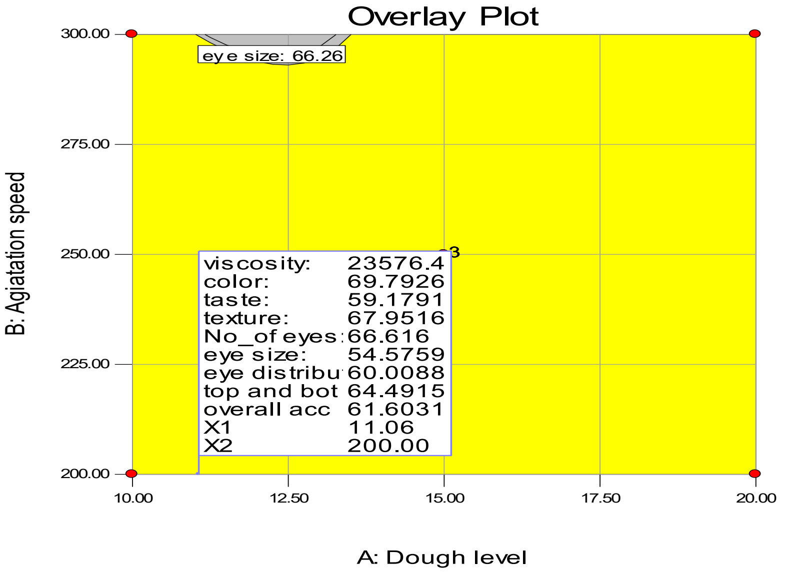


Figure 4.13: Contour plot of the desirability for multiple response function.



viscosity: 23576.4
color: 69.7926
taste: 59.1791
texture: 67.9516
No_of eyes: 66.616
eye size: 54.5759
eye distribu 60.0088
top and bot 64.4915
overall acc 61.6031
X1 11.06
X2 200.00

Figure 4.14: Overlay plots illustrating the optimum responses using graphical optimization.

The optimum range (dark shaded area) of injera samples that has the acceptability ratings of sensory score of greater than 64% on a 9-point hedonic scale. Graphical optimization suggested that the absit processing factors to produce teff injera that would hit targeted sensory scores of like slightly ranges between 11.06-12 % dough level and 200 rpm of agitation speed while the absit temperature is held at 45°C.

CHAPTER FIVE

4. Conclusion and recommendations

4.1. Conclusions

The study showed that all the absit process parameters were important parameters affecting the physicochemical properties, sensory scores except absit viscosity and color(both the visual and L*values).

Box Behnken design was successfully adopted and the experiments were designed choosing the input variables for the levels selected. With minimum number of experiments, data was collected and the models were developed. Response Surface Models evolved for responses show the effect of each input parameter and its interaction with other parameters, illustrating the trend of responses. Verification of the Fitness of each model using ANOVA technique, shows that all the models can be used with confidence level of 0.95, for navigating the design space. Further validation of the models done with the additional experimental data collected demonstrates that the models have high reliability for adoption within the chosen range of parameters.

The optimum responses parameters of absit (dough level, agitation speed and temperature) in terms taste, texture, N<u>o</u> of eyes , Eye size , Eye distribution , Top and bottom and overall acceptance of baked teff injera were 11.06%,200rpm and 45ºC respectively with a desirability of 0.910.

The optimized injera had taste, texture, N<u>o</u> of eyes , Eye size , Eye distribution , Top and bottom and overall acceptance values 64.84,74.88,74.61,62.79,68.16,70.41 and 68.27 % respectively.

Surface plots produced shows the trend of different responses by varying the 2 input parameters keeping the 3rd parameter constant with reduced number of experimental runs, fairly convincing, logical and acceptable results have been obtained, which can be followed for getting solution to the appropriate absit cooking requirements. This has resulted in saving of considerable amount of time and money.

4.2. Recommendations

More researches on the application of RSM to food science and technology (food process, formulation and optimization, recipe formulation and optimization) need to be done.

Households, entrepreneurs, and injera making industries can prepare injera using this absit cooking parameters so that they can bake consistent Teff Injera..

This study focuses how absit cooking parameters could possibly affect the physico-chemical and sensory quality of teff injera(Quncho-white teff) therefore further study needs to be done with the use of Design Expert^{TM} software for other varieties of teff to make injera with comparable quality in the physico-chemical and sensory acceptance of teff injera.

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Appendices

Appendix I: Sensory evaluation score sheet and questionnaire

Health status: ok not ok	**Age: Sex: Male Female**
Panelist No:- Sample Code:	**Date:**

Instruction:-Please mark your degree of preference for the given sample on the scale below

1. Color

Much too dark

Much too light

2. Taste

Not sour enough

Much too sour

3. Texture

Much too brittle

Much too soft enough

4. Appearance

4.1.Number of eyes

Many too few

Many too many

4.2.Eye size

Much too big

Much too small

4.3.Eye distribution

Much too irregular

Much too regular

4.4.Top and bottom surfaces

Much too powdery, sticky

Much too non powdery, non-sticky

5. Overall acceptability

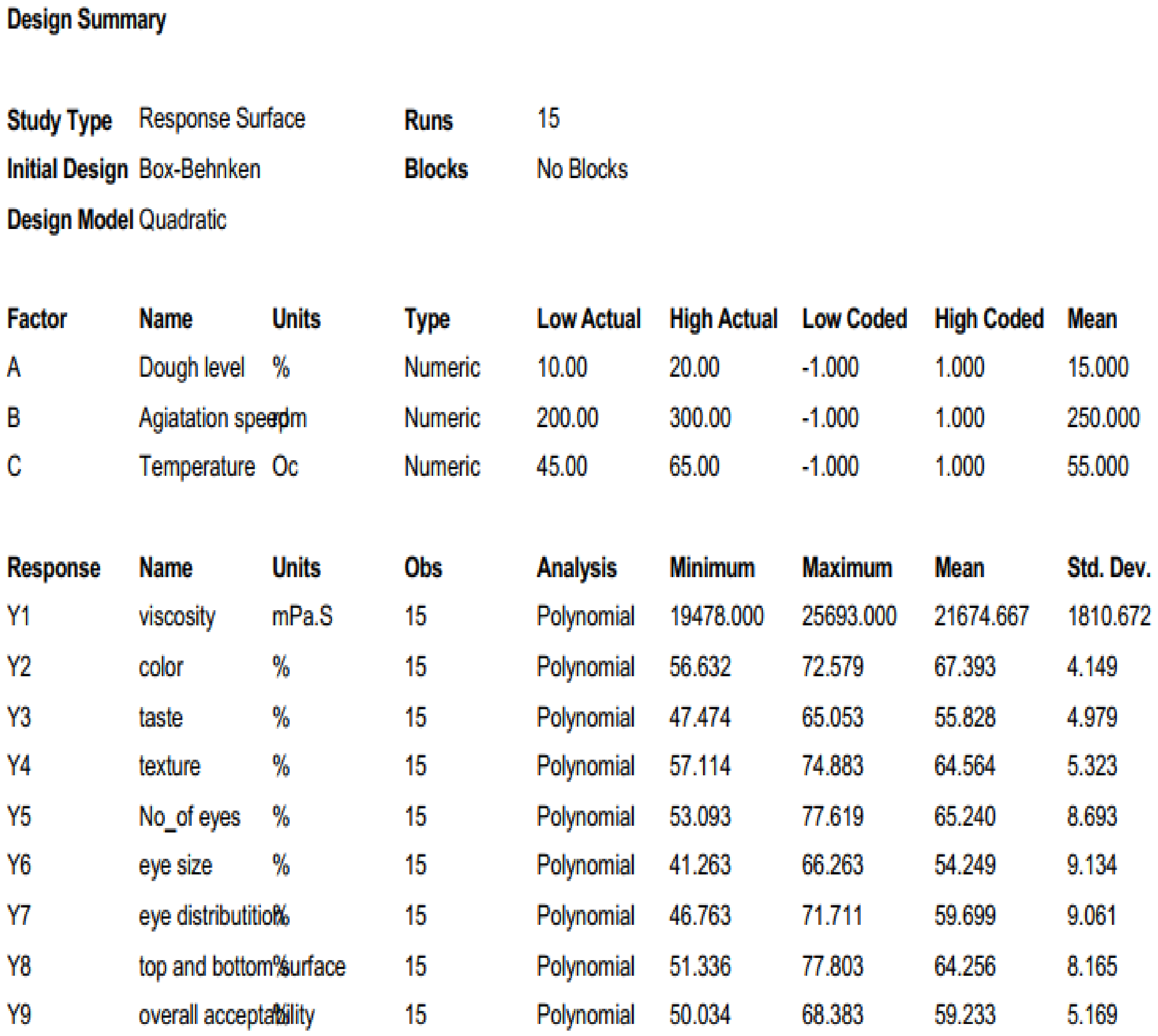
Dislike extremely

Like extremely

Questionnaire: Please fill the following few inquiries after you finish marking the scale, thank you in advance

1. Please specify your work place:_______________________________, and living place:_____________________________________
2. From your perspective, how do you describe a good injera in terms of color: __________________, thickness: __________________ and eye size: ________________________________?

Appendix I I: Design Summary of experimental data



Design Summary

Study Type	Response Surface	**Runs**	15
Initial Design	Box-Behnken	**Blocks**	No Blocks
Design Model	Quadratic		

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded	Mean
A	Dough level	%	Numeric	10.00	20.00	-1.000	1.000	15.000
B	Agiatation speed	rpm	Numeric	200.00	300.00	-1.000	1.000	250.000
C	Temperature	Oc	Numeric	45.00	65.00	-1.000	1.000	55.000

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.
Y1	viscosity	mPa.S	15	Polynomial	19478.000	25693.000	21674.667	1810.672
Y2	color	%	15	Polynomial	56.632	72.579	67.393	4.149
Y3	taste	%	15	Polynomial	47.474	65.053	55.828	4.979
Y4	texture	%	15	Polynomial	57.114	74.883	64.564	5.323
Y5	No_of eyes	%	15	Polynomial	53.093	77.619	65.240	8.693
Y6	eye size	%	15	Polynomial	41.263	66.263	54.249	9.134
Y7	eye distributition	%	15	Polynomial	46.763	71.711	59.699	9.061
Y8	top and bottom surface	%	15	Polynomial	51.336	77.803	64.256	8.165
Y9	overall acceptability	%	15	Polynomial	50.034	68.383	59.233	5.169