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INFLUENCE OF PROCESSING PARAMETERS ON EYE SIZE AND ELASTICITY OF TEF-BASED *INJERA*

A Thesis in

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by

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ABSTRACT

Injera is a fermented and naturally-leavened flatbread indigenous to Ethiopia. It constitutes 70% of the diet of Ethiopians and is preferably made from the grain of *Eragrostis tef*. Elasticity and eye (pore) formation are important quality attributes of *injera*. The pliability of *injera* allows it to be used as a utensil to pick up *wot* (stew eaten with *injera*). The honey-comb like eyes help in the grasping of *wot* which soaks into the pores on the surface of *injera*. This study focused on determining the effects of fermentation time and viscosity on the elasticity and eye formation of *injera* and also understanding the mechanism by which eyes form. Viscosity and fermentation time were found to have a significant effect on elasticity and eye formation of *injera*. It was observed that *injera* baked from tef batters with low or high apparent viscosities had fewer eyes on their surfaces and hence an optimum range of apparent viscosity for baking *injera* with many evenly distributed eyes was determined to be approximately 1.1 to 1.4 Pa.s. Carbon dioxide was also shown to significantly influence the formation of eyes on the surface of *injera*. The higher the amount of CO_2 or gas bubbles in the fermented batter, the higher the number of eyes formed on the *injera*. Also, addition of sodium metabisulphite (a reducing agent) to tef batters significantly affected both elasticity and eye formation of *injera*. This suggested that disulfide bonds between proteins may contribute to elasticity and may have an influence on eye formation.

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CHAPTER 1

INTRODUCTION

1.1 Statement of the problem

Injera is a traditional Ethiopian sourdough flatbread that constitutes 70% of the diet of Ethiopians (Ekris and Gamboa, 2008). *Injera* is usually made with flour from the tef grain (*Eragrostis tef [Zucc.]* Trotter), but can also be made from other grains such wheat, sorghum, and maize. The elastic texture and pores (referred to as 'eyes') formed on the surface of *injera* are important quality attributes. According to Yetneberk *et al.* (2004), a good *injera* is soft, fluffy and able to be rolled without cracking. *Injera* is elastic despite the absence of gluten. The pliable texture and honeycomb nature of the surface of *injera* enables it to be used as a utensil in picking up '*wot*' (stew made with either vegetables and/or meat). As gluten in wheat makes wheat products spongy and elastic, the functional property of proteins in tef is of interest. Apart from proteins, other structural components such as starch, hemicelluloses in tef flour and bacterial exopolysaccharides in fermented tef batter may also contribute to this elastic quality.

In order to commercialize the production of *injera* in Ethiopia, it is important to produce good quality *injera* that will be acceptable to the Ethiopian consumer. According to Yetneberk (2004), tef is preferred to sorghum for producing the best quality *injera*. The reason tef is the preferred cereal for making *injera* has not been scientifically explored in detail. There is also limited scientific knowledge on the characteristics of the protein, starch, hemicellulose and bacterial exopolyssacharide fractions of tef or fermented tef batter. The focus of this study is to determine the influence of *injera* processing parameters such as viscosity of tef batter and fermentation time on the elasticity and eye formation of *injera*, and to understand the molecular mechanisms underlying these characteristic qualities. The tef grain is gluten-free and has the potential of replacing wheat in gluten-containing products such as bread. The use of tef in the production of *injera* may stimulate research in the development of quality gluten-free bread based on tef flour.

1.2 Rationale and Significance

Elasticity and eye formation are important characteristics that determine the quality of *injera*. To produce good quality *injera*, a standardized method of *injera* production will have to be put into place, and to do so requires knowledge of the effect of processing parameters on the quality of *injera*. This knowledge may also lead to improvements in the quality of gluten-free breads by incorporating tef flour in gluten-free formulations.

1.3 Hypotheses

The hypotheses tested were:

1. Fermentation time has a significant effect on the quality of *injera*.

2. Viscosity of the batter has a significant effect on eye formation and elasticity of *injera* and is influenced by the addition of water during *absit* making.

3. Eyes are formed from CO_2 produced during fermentation.

4. Protein contributes to injera structure through formation of disulfide bonds.

1.4 Objectives

1. To develop a scaled-down *injera* preparation protocol that accurately represents traditional *injera* baking,

2. To quantify the eye size and distribution of different brands of *injera* already on the U.S. market,

3. To measure chemical changes and viscosity of batter during a 72 h fermentation, and eye formation and elasticity of *injera* baked from that batter,

4. To alter the ratio of fermented batter-to-water during *absit* preparation in the range1:2 - 1:7 and determine the effect on viscosity of batter, eye formation and elasticity of *injera* baked from it,

5. To de-gas batter and determine the effect of dissolved gases (i.e. carbon dioxide) on eye formation,

6. To determine the effect of sodium metabisulfite on elasticity and eye formation of *injera*.

CHAPTER 2

LITERATURE REVIEW

2.1 The tef grain and its properties

The grain ‘tef’ (*Eragrostis tef* [Zucc.] Trotter) is cultivated as a major cereal in Ethiopia and is a staple food for the majority of Ethiopians (Bultosa *et al.*, 2002). In a country of over 80 million people, tef accounts for about 15% of all calories consumed in Ethiopia (Fufa *et al.*, 2011). According to Assefa *et al.* (2011) the crop has both its origin and diversity in Ethiopia, and plays a vital role in the country’s overall food security. Tef is a resilient crop that can withstand varying environmental and cultural conditions, including reasonable tolerance to both drought and waterlogging (Assefa *et al.*, 2011). The color of the tef grains can be ivory, light tan to deep brown or dark reddish brown purple, depending on the variety (Ekris and Gamboa, 2008). The composition of tef is similar to that of millet, although it contains generally higher amounts of the essential amino acids, including lysine, the most limiting amino acid (Jansen *et al.* 1962). According to Ekris and Gamboa (2008), the potential of this grain as an interesting raw material for new food product development is due principally to its protein composition – it is gluten free and has a high quality amino acid composition.

2.1.1 Nutritional quality of tef and *injera*

The principal use of tef is in the production of *injera*. The main components of tef are protein, ash, fat, fiber, moisture and carbohydrates. Prolamins are the major proteins found in the tef grain. Adebowale *et al.* (2011) compared tef prolamins to sorghum prolamins and concluded that tef prolamins are more hydrophilic, less polymerized and have lower thermal stability. They further stated that these differences probably make tef prolamins more functional in bread making. Jansen *et al.* (1962) indicated that tef has an excellent balance among the essential amino acids that makes it somewhat comparable to that of egg, except for its relatively low lysine content. Steinkraus (1996) observed that during the *injera* fermentation process, the nutritional value of protein decreased markedly. However, it is possible though unknown, that the overall effect of fermentation is to improve bioavailability of nutrients.

The tef grain is also rich in iron, calcium, magnesium and phosphorous (Taylor and Emmambux, 2008). According to Ekris and Gamboa (2008) tef has a high content of minerals such as: iron, calcium, zinc and magnesium compared to wheat, barley and oats. Forsido and Ramaswamy (2011) described tef as nutritious as major staple cereals like wheat, rice, oats, barley, and even better in some aspects, containing more calcium, zinc, iron and potassium. They also described tef as being high in dietary fiber. The properties of tef and *injera* have been reviewed by Yetneberk *et al.* (2004) and Gebremariam *et al.* (2012).

In the preparation of *injera*, tef flour undergoes fermentation by lactic acid bacteria and yeast. In a review by Ashenafi (2006), *Pediococcus cerevisieae*, *Lactobacillus brevis, Lactobacillus plantarum* and *Lactobacillus fermentum* were found to be the dominant microorganisms in fermenting tef batter. Other studies have isolated *L. plantarum, L. fermentum,* and *L. brevis* (Gashe, 1985) from *injera*. Fermentation serves as a means of preserving food. According to Mehta *et al*. (2012), fermentation alters food shape, texture, and flavor, increases its nutritional value, and promotes safety. Blandino *et al.* (2003) stated that, in general, natural fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly- and oligosaccharides. Also, certain amino acids may be synthesized and the availability of B group vitamins may be improved. Ramachandran and Bolodia (1984) evaluated the effect of fermentation on the bioavailability of iron, zinc, and phosphorous by dialysis of tef batter and reported increases in dialyzable portions of iron from 9 to 24%, phosphorous from 16 to 60%, and zinc from 2 to 43%. These findings suggest that fermentation plays a key role in reducing the phytic acid concentration of tef and in increasing the bioavailability of minerals (Umeta *et al*., 2005). Although it has been suggested that tef contains tannins which are considered an anti-nutritional factor, Bultosa and Taylor (2004) found that the testa of neither white nor brown varieties contained tannin.

In addition to its nutritional qualities, the tef grain is gluten-free. The demand for gluten-free foods is growing as more people are diagnosed with celiac disease and other types of gluten sensitivity (Bultosa and Taylor, 2004; Spaenij-Dekking *et al.*, 2005; Hopman *et al.*, 2008). Tef can be a valuable addition to a gluten-free diet of celiac disease patients (Ekris and Gamboa, 2008).

2.2 Characteristics 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). It constitutes 70% of the diet of Ethiopians (Ekris and Gamboa, 2008) and is preferrably made from the tef grain which is indigenous to Ethiopia. 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, tef *injera* is the most preferred because it can be stored for 3 days without losing its pliability (Steinkraus, 1996) . Pliability is related to the ability of *injera* to roll without tearing or cracking and this is a mark of a good quality *injera*. This pliability of *injera* enables it to be used as a utensil to pick up *wot* (meat or vegetable sauce eaten with *injera*). 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 (Fig. 2.1). *Injera* is unique in that despite the fact that it is not made from gluten-containing wheat, it is leavened.

Figure 2.1 *Injera* (source: http://mosob.com/menu/guide)

2.2.1 Preparation of *injera*

The sour taste of *injera* is a result of the fermentation of tef flour by lactic acid bacteria (LAB). The fermentation may be spontaneous (performed by naturally occurring bacteria) or performed by a selected starter culture. In Ethiopia, a portion of a previously fermented tef batter is used as an inoculum in the next fermentation. Apart from LAB, yeasts are also known to play a role in fermentation as they break down simple sugars to produce carbon dioxide. According to Stolz (2003), spontaneous fermentations of carbohydrate-rich and weak acid dough are usually characterized by the successive or contemporary growth of LAB and yeasts. In tef fermentation, the liquid layer that accumulates on top is usually poured away. In the process of pouring off this liquid layer, other soluble compounds (amino acids, sugars, and minerals) and a large portion of the microorganisms involved in the fermentation process are also removed (Alemayehu, 2001).

Primary fermentation of tef flour usually lasts between 48 to 72 hours. It is initiated by the mixing of tef flour, water and *irsho* (starter). After primary fermentation about 10% by weight of the fermented tef is mixed with 3 parts water (1:3 ratio) and cooked for 2-3 minutes until the mixture boils and the starch gelatinizes. This gelatinized mixture is referred to as *absit*. The *absit* is cooled and then added back to the fermented tef batter and mixed thoroughly. This initiates the secondary fermentation of the batter which usually lasts between 30 minutes to 2 hours (Ashenafi, 2006; Boka *et al.*, 2013; Parker *et al.*,1989). After secondary fermentation, *injera* is baked on a *mitad* or griddle for about 2-3 minutes at a temperature of 180 to 220 °C (Figure 2.2). During baking, the starch in the batter gelatinizes and the effect is to trap gas bubbles in the batter that turn into cells as the gas escapes (Belton and Taylor, 2004; Taylor and Emmambux, 2008). This makes the surface of *injera* look like that of a honeycomb. Yetneberk *et al.* (2004) stated that the bottom surface of *injera* is smooth and shiny. They described a good *injera* as being soft, fluffy and able to be rolled without cracking. *Injera* is also considered of good quality when the upper surface has numerous ‘eyes’ or pores on it.

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 attribute. The pliability of *injera* makes it good for folding or rolling and its eyes good for picking up *wot*.

With respect to taste, many Ethiopians prefer the sour nature of *injera*. According to Zegeye (1997)**,** the major quality attribute of a good *injera* is its slightly sour flavor. However, some prefer their *injera* sweet. This type of *injera*, called *aflegna*, is produced when the *injera* batter does not ferment for the recommended three days. *Komtata injera*, is very sour-tasting. While some have preference for it, normally this kind of *injera* is made by unskilled women, usually newly-weds, who have not learned the proper timing (Stewart and Getachew, 1962).

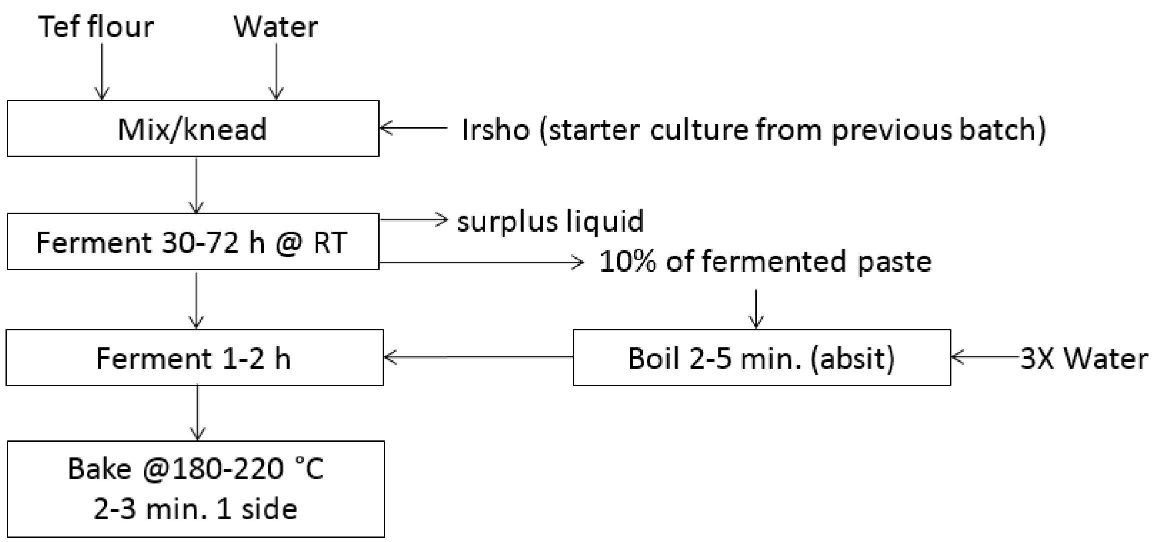


Figure 2-2 *Injera* preparation flow diagram

2.2.2 Eye formation on *injera*

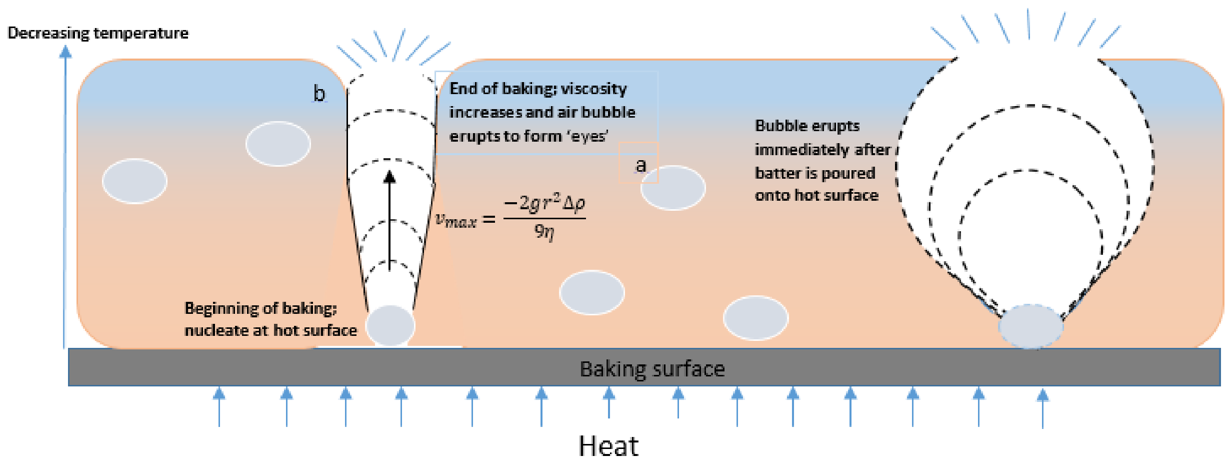
2.2.2.1 Characteristics of eyes on *injera*

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. Figure 2.3 shows some brands of *injera* with characteristic numerous eyes on their surfaces. A study by Cherinet (1988) on composite flour development for *injera* determined the appropriate number of eyes on the surface to be 11-15 per cm^2 .

Figure 2.3: Examples of *injera* on the market

2.2.2.2 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 tef 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. Figure 2.4 shows the proposed mechanism by which eyes are formed on the surface of *injera*. Viscosity may not only affect eye formation on *injera* but may also contribute to its elastic texture. 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*.



KEY:

a) Bubble trapped in tef batter during baking
b) Bubble erupts or escapes at surface to form 'eyes'

Figure 2.4: Model for the mechanism of eye formation

2.2.2.3 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 cm^2 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.

2.2.3 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 tef 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 that 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)**.**

2.3 Structural components of tef and fermented tef flour

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* are 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.

2.3.1 Proteins

Since tef flour does not contain gluten, its elasticity is not due to gluten. However, proteins in tef may have a function similar to that of gluten in wheat flour. Gluten is the primary structural component in wheat breads and provides structure and texture in other wheat-based bakery products. Its alteration by protease degradation or its complete absence often results in a viscous system or even a liquid bread batter rather than dough (Rosell *et al*., 2002). The gluten proteins in wheat contain gliadin and glutenin subunits. The fractions have functional significances as the glutenins are largely responsible for gluten elasticity and gliadins for viscosity (Shewry *et al*., 1995). According to Damodaran *et al*. (2008) and Dong and Hoseney (1995), the viscoelasticity of wheat dough is related to the extent of sufhydryl-disulfide interchange reactions. This view is supported by the fact that when reductants such as cysteine, or sulfhydryl-blocking agents, such as N-ethylmaleimide, are added to the dough, viscoelasticity decreases greatly (Damodaran *et al*., 2008). Interactions other than disulfide crosslinks, such as hydrogen bonding and hydrophobic interactions may also play a vital role in viscoelasticity of wheat dough. Belitz *et al*. (1986) stated that partial or total reduction of intermolecular disulfide bonds lowers both molecular weight and elasticity of gluten. Adebowale *et al.* (2011) observed in their study of tef proteins that polypeptide bonds in prolamins are disulphide bonds and that a large proportion of the storage proteins in cereals are bonded into large polymeric networks. Tef prolamins were however found to be less cross-linked by disulfide bonds than sorghum prolamins. A study by Vallons *et al.* (2011) showed that protein polymerization by thiol/disulfide-interchange reactions occurred in white rice and tef batters using capillary gel electrophoresis but there was no such cross-link mechanism in buckwheat proteins due to the absence of free sulfhydryl groups. Even though Adebowale *et al.* (2011) showed that there is crosslinking of disulfide bonds of prolamins in tef, it is still unknown to what extent this interaction may be responsible for elasticity of *injera*. Other studies (Renzetti and Arendt, 2009; Moore *et al.*, 2006) have used enzymatic manipulation to crosslink proteins in gluten-free flours in order to obtain similar textural characteristics of gluten-containing cereal flours.

Different strategies have been used in the absence of gluten in order to mimic its viscoelastic properties. According to Gallagher (2009), rice flour which is also gluten-free, is unable to form a dense gluten-like protein network when the rice flour is kneaded with water. In a study on roti, an unleavened bread made from sorghum, the authors stated that although sorghum grains do not contain gluten, when sorghum flour is mixed with water and kneaded it produces a sticky dough. They further stated that good quality dough should be sticky and easily rollable without breaking. Renzetti and Arendt (2009) improved the breadmaking performance of gluten-free flours from corn and sorghum that they attributed to protein polymerization, which can enhance elastic-like behavior of batters. A study by Edema *et al.* (2013) on fonio and sorghum flours showed that sourdough fermentation of these flours substantially changed the rheological behaviors of their doughs under kneading conditions, making the dough properties more similar to those of wheat flour and rice flour which were improved by addition of soy protein isolates and hydroxypropylmethylcellulose (HPMC) in a study by Marco and Rosell (2008). Traditionally in Ethiopia, tef dough is kneaded (Girma *et al.* 2013; Zegeye, 1997) and this forms a dense sticky mass. It is however unknown whether this dense mass is as a result of network formation by tef proteins.

The various proteolytic activities induced by fermentation hydrolyze cereal proteins to produce free amino acids (Spicher and Nierle, 1988; Thiele *et al*., 2002). Cereal flour, yeasts, and lactic acid bacteria contain proteases and peptidases that can contribute in different ways to these proteolytic events. According to Renzetti and Arendt (2009), lower molecular weight proteins resulting from hydrolysis by proteases affected the resistance of brown rice flour batters during proofing, and increased batter elasticity and paste stability. Higher proteolytic activities are encountered for brans and whole grain flour compared to white flour (Loponen *et al*., 2004), and this is likely to have an effect on *injera* prepared from tef flour since it contains bran. In a study conducted by Salmenkallio-Marttila *et al.* (2001) on wheat bran, they found that the positive effect of fermentation of bran on bread quality was evident when comparing the well-developed protein network structure of the breads baked with fermented bran and the control bread containing unfermented bran. Protein degradation in sourdough fermentation is among the key phenomena that affect overall quality of sourdough breads according to Ganzle *et al.* (2008). The functionality of the cross-linking enzymes such as transglutaminases and tyrosinases in gluten-free breadmaking is also comparable to that in wheat breadmaking. According to Renzetti and Arendt (2009), in the absence of other hydrocolloids, protein structures are important to ensure the textural quality of gluten-free breads**.** It has however not been proven that proteins contribute to the elastic texture in *injera*.

In a study on the proteins found in sorghum and millet, Belton and Taylor (2004) stated that full analysis and sequencing of the main storage proteins is yet to be carried out and very little work exists on their functional properties. The results from a study by Schober *et al.* (2007) suggested that proteases mainly degrade proteins that are already soluble at the beginning of fermentation, and degradation of those proteins soluble in the dough liquid (supernatant after centrifugation) to smaller peptides may well explain why they can no longer cross-link and therefore do not aggregate upon baking. According to Schober *et al.* (2007) , it remains unclear whether and according to which physicochemical mechanism protein degradation and bread quality are related in a gluten-free system. Parker *et al.* (1989) observed that tef storage proteins played no part in the structural integrity of cooked *injera*, although they may add to the texture. This statement is however not clear as structural integrity of a material is related to its texture**.** Parker *et al.* (1989) also found out that total protein levels decreased slightly during the preparation of *injera*. It is therefore necessary to understand the contribution of proteins to the quality of *injera*.

2.3.2 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 wall 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*.

2.3.3 Hemicelluloses

Since the grains of tef are extremely small, less than 1.5 mm in length (Parker *et al.*, 1989), the whole tef grain is milled into flour. Hemicelluloses are found in the bran or outer layer of grains. Hemicelluloses are generally classified according to the main sugar residue in the backbone, e.g., xylans, mannans, and glucans, with xylans and mannans being the most prevalent in plant tissues (Wyman *et al.*, 2005). Izydorczyk *et al.* (2001) stated that both beta-glucans and arabinoxylans may influence barley dough properties by affecting water distribution in the dough and may form elastic networks and contribute to overall elasticity and strength of the dough under conditions of restricted water availability. However, a study by Hung *et al*. (2005) showed that dietary fibers in baked products increase water absorption and decreased dough elasticity. According to Parker *et al.* (1989), the thin bran layers and endosperm cell walls in tef probably account for the lower levels of dietary fiber of less than 5% compared with 14% in wheat, however, these components appear to be unaffected by fermentation.

2.3.4 Exopolysaccharides

Lactic acid bacteria are responsible for the sour taste in sourdough fermentations like that of *injera*. Many lactic acid bacteria (LAB) can produce a wide variety of longchain sugar polymers called exopolysaccharides (EPS), which are varied in their chemical composition, structure and physical properties (Moroni *et al.*, 2011). According to Arendt *et al.* (2007), polymers produced from lactobacilli may be expected to beneficially affect a number of technological properties of bread, including water absorption of the dough, dough machinability, increased loaf volume, and retarded bread staling. These studies provide evidence that EPS produced by sourdough LAB have the potential to improve dough rheology and bread texture, and show that EPS produced by LAB may be used to replace or reduce more expensive hydrocolloids used to improve bread texture.

In a study by Rühmkorf *et al.* (2012), they observed that the higher the sucrose concentration in tef flour, the more EPS was produced. EPS production also correlated strongly to cell counts at beginning of fermentation but not to highest cell counts reached after 24 hour of fermentation (Rühmkorf *et al.*, 2012). The application of EPS forming starter cultures in sourdoughs is a promising approach to improve gluten-free breads, since EPS is produced in situ and can act as a hydrocolloid and does not have to be declared (Rühmkorf *et al.*, 2012).

2.4 The effect of other components in fermented tef on *injera* quality

2.4.1 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*.

2.4.2 Polyphenols

Antioxidant activity is a fundamental property important for life (Velioglu *et al.*, 1998). Polyphenols are known to have nutritional properties because of their antioxidant characteristics. Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants (Boka *et al.*, 2013). Tannins are known to cause bitterness in foods prepared from some cereals. A comparison of white tef, brown tef and red tef by Boka *et al.* (2013) reported that red tef contained the highest polyphenolic content while white tef contained the lowest.

In a study conducted by Yetneberk *et al.* (2005), tannins were observed to have an inhibiting effect on fermentation. Due to this phenomenon they decorticated sorghum by removing the bran, germ and testa (which contained tannins in some varieties of sorghum) in order to improve the color, taste and appearance of the *injera*. A study by Haslam (1974) observed that polyphenol-protein complex-formation which results in precipitation is caused by cross-linking of separate protein molecules by the phenol. McManus *et al.* (1981) also described a tendency of polyphenols to cross-link protein molecules at higher protein concentrations in his study. He stated that where the protein concentration is high the relatively hydrophobic surface layer is formed by complexation of the polyphenol onto the protein and by cross-linking of different protein molecules by the polyphenols. There has not been any research on tef, l*et al*one gluten-free grains to show whether this phenomenon occurs during fermentation or whether this contributes to the elasticity in *injera*.

2.4.3 Moisture

In 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.

2.5 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 breadmaking 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'. 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 gelatinised starch.

2.6 Viscosity of tef 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 Kamal-Eldin and Chiwona-Karltun (2008). The viscosity range 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:

$$\sigma = \sigma_o + k\gamma^n$$

Considering the effect that the consistency of batters have 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.

2.7 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*.

2.8 Summary

Physicochemical characteristics of tef and their effect on *injera* quality have not been well documented. The objectives of this study are to determine the effect of fermentation time and viscosity of tef batter on eye formation and elasticity of *injera* and, furthermore, to understand the mechanism of eye formation and elasticity development. The study of the effect of processing parameters on the quality of *injera* will help to determine how the components of the tef flour affect eye formation and elasticity of *injera*.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

Ivory tef flour was purchased from The Teff Company (Nampa, ID). All reagents were purchased from either VWR (Randor, PA) or Sigma Aldrich Chemical Company (St Louis, MO) and were of analytical reagent grade. The irsho was obtained from Ethiopia (NutrAfrica, Debre Zeit) and was maintained at 4°C by regular feeding with tef flour and water.

3.2. Fermentation of tef flour

Tef flour was fermented by mixing with tap water in a ratio of 1:1.6 (w/w) and 20% of starter from a previous fermentation batch (*irsho)* by weight of tef flour was added. The mixture was fermented for 72 hours at ambient temperature (22-25°C). After 72 hours of primary fermentation, the liquid that settled on the surface of the fermented batter was decanted and its volume measured. Ten (10) % by weight of the fermented tef was mixed with water at ratios from 1:2 to 1:7, but typically 1:3 (w/w). This mixture was heated to boiling in an aluminum saucepan over a hotplate until thickened (usually about 5 minutes) to form the *absit*. After boiling, the *absit* was cooled to below 50°C, and then mixed thoroughly with the rest of the batter to initiate secondary fermentation, which usually lasted for 4 hours at ambient temperature. Secondary fermentation may last for more than 4 hours. The end of secondary fermentation was determined by the appearance of bubbles in the tef batter.

3.3 Baking of *injera*

Baking of the *injera* was done after about 4 hours of secondary fermentation. Twenty (20) mL of the fermented tef batter was poured into 9 cm diameter tinplated steel crumpet rings placed on a non-stick griddle (Heritage Model 735, Bethany Housewares, Cresco, IA) set to 215°C. Baking was done for 2 minutes and 30 seconds. A lid was used to cover the *injera* during baking after eyes started forming on the surface. This was to allow steam to cook the upper surface of the injera and prevent it from drying out. The baking temperature was between 200 and 230 °C.

The Heritage griddle with a thermocouple connected to it (Figure 3.1) was used to bake *injera* in the experiment on the effect of fermentation time on elasticity and eye formation of *injera*. Because there were large variations in surface temperature, a new baking set up was subsequently built (Figure 3.2), comprising a 6 x 6 x 0.16 inch aluminum plate placed on a hot plate/stirrer (7X7 CER Hot/Stir, VWR, Radnor, PA). A thermocouple was inserted into a hole drilled in the plate at approximately the center in order to control the temperature of the plate using a temperature controller (Extech 48VTR, Nashua, NH). To minimize sticking of the *injera* to the plate, an oven liner (NORPRO, Everett, WA) was place on top of the plate before baking. The tinplated steel crumpet rings were placed at the center of the plate and 20 mL tef batter was poured into it. Baking of the *injera* samples was done for 2 minutes at a baking temperature of

200°C. A lid was used to cover the *injera* after 1 minute of baking to trap steam and prevent the surface of *injera* from drying out.

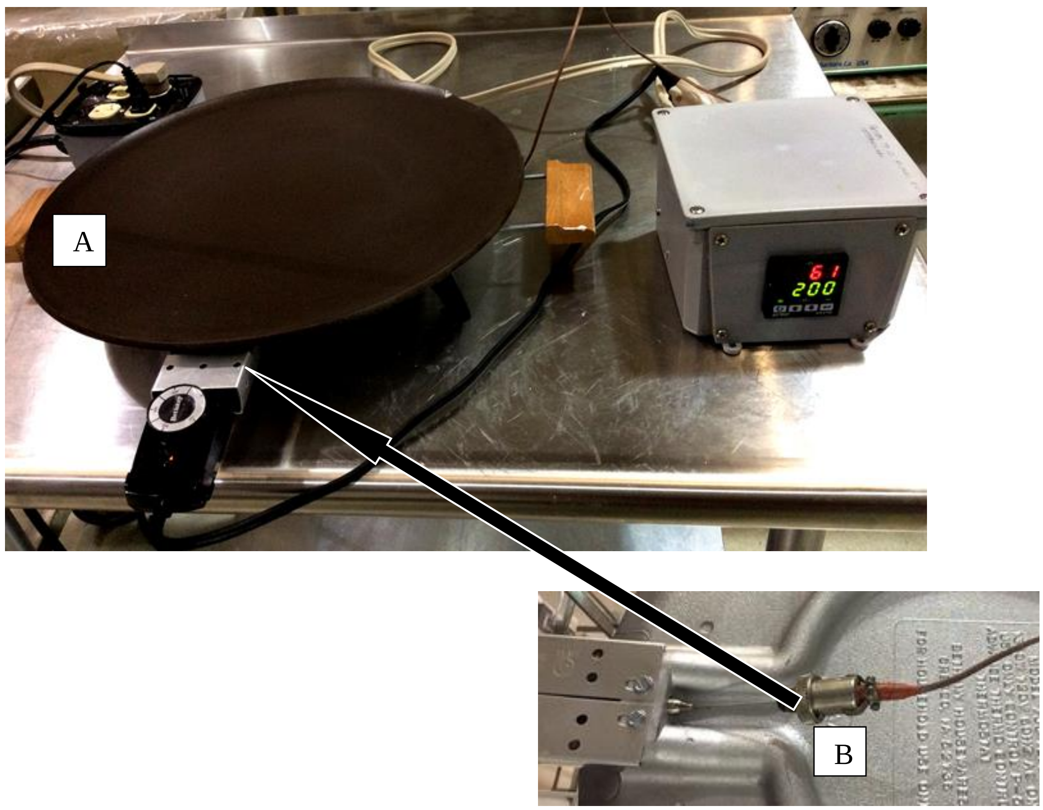


Figure 3.1. Heritage griddle (A) with thermocouple connected below the griddle controller (B).

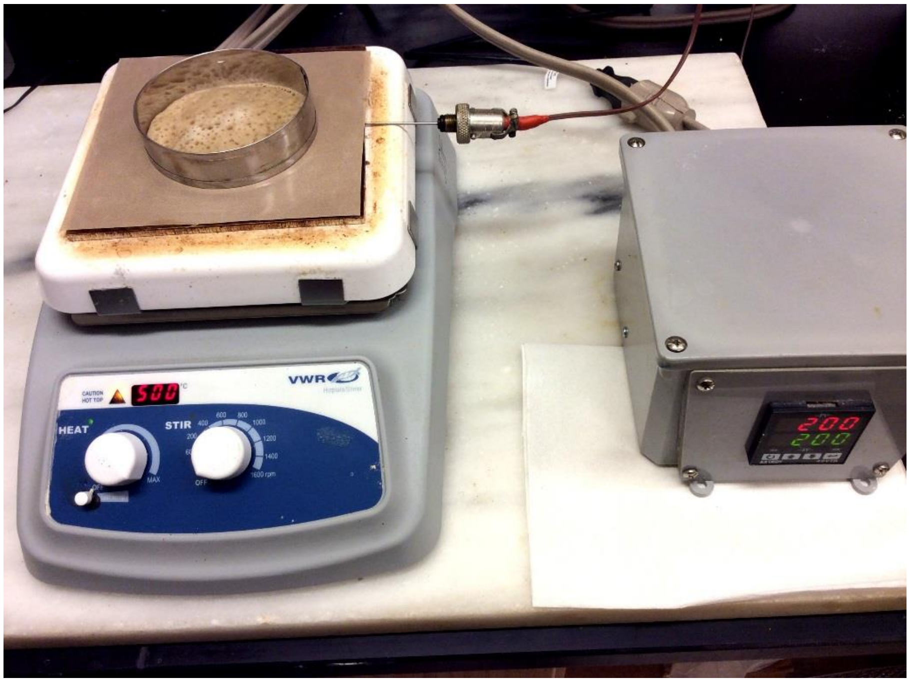


Figure 3.2 *Injera* baking setup consisting of a hotplate/stirrer, temperature controller and an aluminum plate with a thermocouple connected to the center of the plate.

3.4 pH

The pH of the fermented tef batter was determined directly using a glass electrode attached to a pH meter (SevenEasy S220, Mettler-Toledo Inc., Columbus, OH). The *injera* samples (10 g) were mixed with 100 mL of distilled water that was boiled and cooled, and the pH of the supernatant was immediately measured after decanting into a 250 mL beaker (AOAC, 1995).

3.5 Titratable acidity

Ten (10) g of the tef batter was mixed with 100 mL of distilled water and titrated with 0.1 N NaOH to an end point pH of 8.2 using an auto-titrator (Titroline Alpha Plus TA 20, S.I. GmbH, Mainz, Germany**)**. The volume of NaOH used for each titration was recorded and titratable acidity was expressed as % lactic acid (AOAC, 1995)) using the formula below:

$$\%\ Lactic\ acid = \frac{[ml\ of\ NaOH\ used]\ x\ [0.1N\ NaOH]\ x\ [0.09]\ x\ [100]}{g\ of\ sample}$$

3.6 Moisture content

The moisture content of the *injera* samples (1 g) were determined by drying for 10 minutes at a temperature of 115 °C using a moisture analyzer (model MB45, OHAUS Corporation, Parsippany, NJ).

3.7 Glucose concentration

The glucose concentration of tef batter samples was determined using the Glucose Oxidase test kit (Sigma-Aldrich Inc., Saint Louis, Missouri). One (1) mL of distilled water was added to 0.1 g of sample and 1 mL of the assay reagent was added to the sample at 37 °C. After 30 minutes, 1 mL 12 N H_2SO_4 was added to stop the reaction. The absorbance of the sample was measured at 540 nm using a spectrophotometer (Thermo

Spectronic Helios alpha, Thermo Electron Corporation, Cambridge, United Kingdom) and glucose content was obtained from the glucose standard curve (Appendix A).

3.7 Total phenolic content

Total phenolic content was assayed by the Folin–Ciocalteu colorimetric method adapted from Boka *et al.* (2013) with slight modification. Aliquots (0.5 mL) of appropriately diluted extracts or standard solutions were mixed with 0.5 mL 0.5 N Folin–Ciocalteu reagent and the reaction was neutralized with 0.5 mL of saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 725 nm using a spectrophotometer (Thermo Spectronic Helios alpha, Thermo Electron Corporation, Cambridge, United Kingdom**)** after incubation for 90 minutes in the dark. A calibration curve was prepared using gallic acid solution (Appendix B). Total phenolic content of the tef batter samples were expressed as milligrams of gallic acid equivalent per g of dry weight (mg GAE/g).

3.8 Flow behavior

In the experiment to understand the effects of fermentation time on elasticity and eye formation of *injera*, a digital viscometer (model DV 11, Brookfield Engineering Laboratories, Middleboro, MA) was used to determine the apparent viscosity in centipoise (cP) of the tef batters at a rotational speed of 10 rpm using a spindle # 2.

In order to determine the rheological behavior of the tef batter samples at low shear rates, a rheometer (ARES, TA instrument, New Castle, DE) was used in subsequent experiments. The steady shear rate sweep method with a cone and plate geometry (50 mm diameter and 0.0396 rad cone angle) was used at a temperature of 20 °C. The Herschel-Bulkley model was used for fitting the shear stress and shear rate data (Bhattacharya and Bhat, 1997). From this model, the yield stress (σ_o), consistency coefficient (k) and flow behavior index (n) of the different batters were obtained.

3.9 Modulus of elasticity

The *injera* samples baked from tef batter were analyzed using dynamic mechanical analysis (DMA) (Q800, TA Instruments, New Castle, DE). The samples were cut into 42 mm diameter discs using a specially made stainless steel punch for the analysis. The multi-strain compression mode with frequency of 1 Hz, amplitude of 1 to 50 μm and force of 0.1 N was used to determine the elastic modulus of the *injera* samples at 0.2% strain from stress-strain curves obtained from the analysis (Figure 3.3).

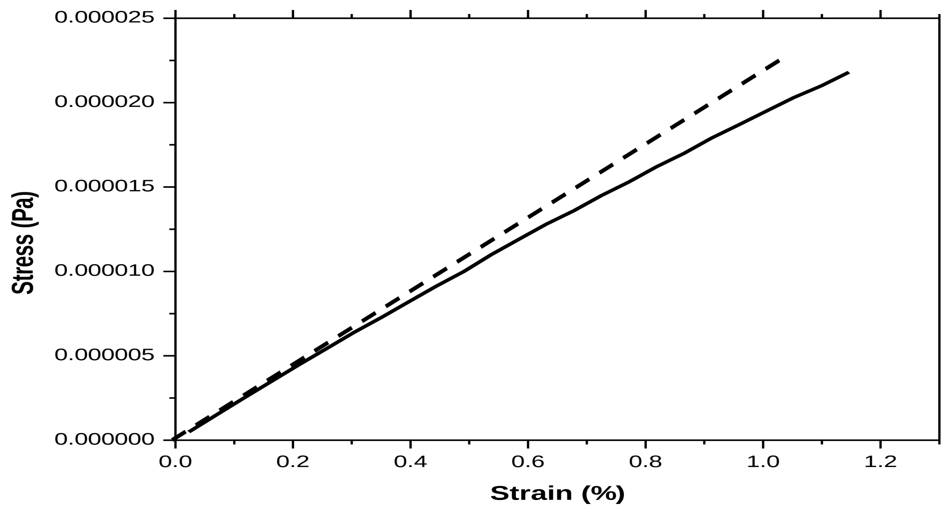


Figure 3.3 Stress vs. strain of *injera*

3.10 Eye size and distribution using image analysis

3.10.1 Image analysis of *injera*

The *injera* samples were scanned using a flatbed scanner (HP Scanjet 3970, Hewlett-Packard Development Company, Palo-Alto, CA) at a resolution of 300 dots per inch. Image analysis was performed on the scanned images using Photoshop® (Adobe Photoshop® C5, Adobe Systems Inc., San Jose, California) (Figure 3.4). The circular marquee tool in Photoshop® was used to crop the scanned image to 1020 pixels by 1020 pixels (width by height), and the cropped image was transformed using 'Bilevel thresholding', 'IP* Morphology' and 'IP* Measure features' plug-ins (Foveo Pro, Reindeer Graphics, Inc., Asheville, NC).

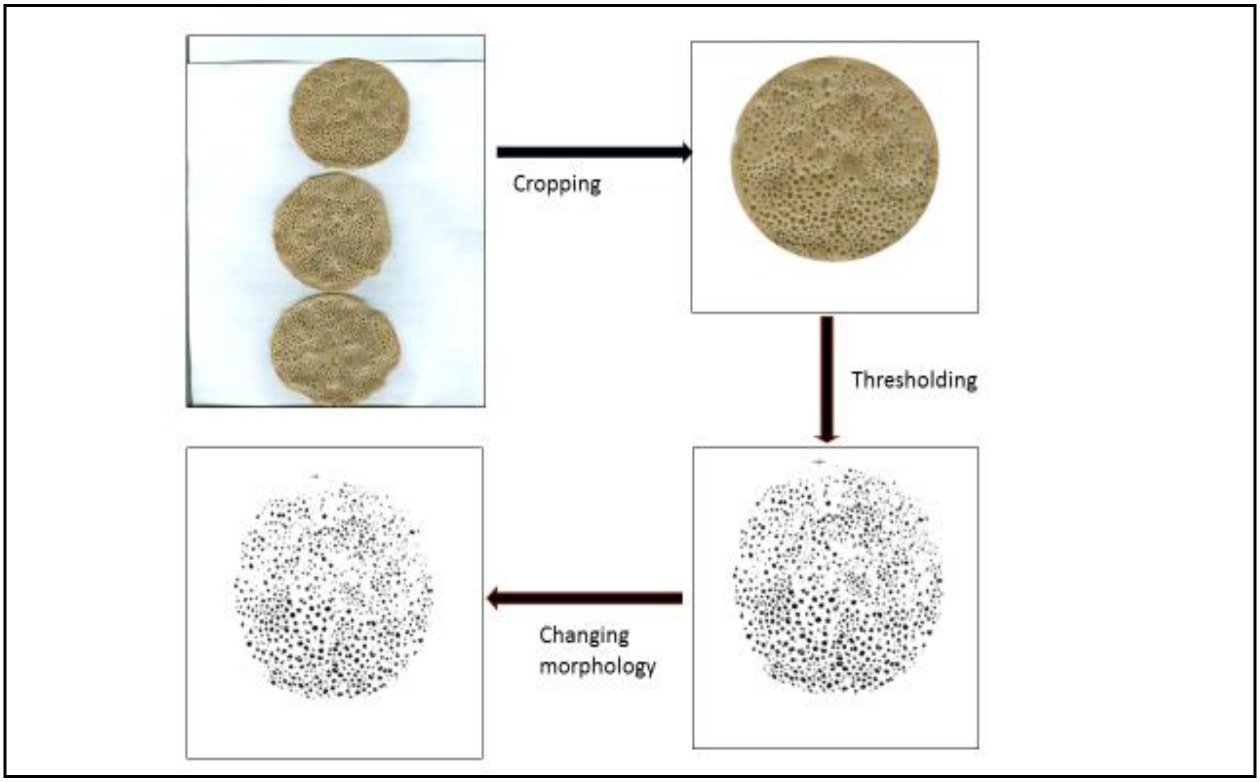


Figure 3.4 Steps used in image analysis

3.10.2 Number, mean equivalent diameter, % total area and distribution of eyes on *injera*

The number, mean equivalent diameter and % total area of 'eyes' on the injera were determined after excluding all features (eyes) with an equivalent diameter of less than 0.05 cm. The mean equivalent diameter of eyes on the surface of the *injera* samples was obtained by dividing the sum of equivalent diameters of all the eyes by number of eyes:

$$\bar{d} = \frac{\sum d^*}{n}$$

The % total area was determined by first calculating the total surface area cropped (58.6 cm^2) and then dividing the sum of the area of each of the features by the total surface area and multiplying by 100:

$$\%\ area\ eyes = \frac{\sum area\ of\ eyes}{total\ surface\ area**} \times 100$$

For example: for an *injera* scanned at a resolution of 300 dpi and a fixed ratio of 1020 px x 1020 px (width x height) of elliptical marquee tool, the % total area was calculated as follows:

Image resolution of 300 px = 2.54 cm

Hence, 118 px = 1 cm

The actual diameter of the image: 1020/118 = 8.64 cm

The actual radius of the image: 8.64/2 = 4.32 cm

The actual total surface area of *injera*: 3.142*4.32*4.32 = 58.6 cm^2

3.10.3 Effect of reducing agent, sodium metabisulphite, on elasticity and eye formation

After secondary fermentation, the tef batter sample was divided into two equal portions and 0.002% (by weight of tef flour) sodium metabisulphite (Oliver *et al.*, 1995) was added to one portion and both portions were mixed thoroughly before baking. Rheological measurements were done on the tef batters using a rheometer (Hybrid

Discovery HR-3, TA Instruments, New Castle, DE). Storage and loss moduli were measured on the tef batter samples using a 40 mm parallel plate geometry (gap = 1000 µm) at a frequency of 1 Hz. An oscillation sweep was used to measure change in storage and loss moduli with temperature and time. The tef batter was heated from 20 to 200°C (in approximately 200s), cooled to 20°C (in approximately 200s), and held at 20°C for 600 s. The number, mean equivalent diameter, and % total area of eyes formed on the surface of the *injera* samples were determined using image analysis described in section 3.10. The elastic moduli of the injera samples were determined using dynamic mechanical analysis (DMA) described in section 3.9.

3.11 Statistical analyses

All measurements were made in triplicate and analyzed using either regression, one-way, two-way or repeated measures (general linear model) analyses in Minitab 16 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. All experiments (except experiment on effect of fermentation time on eye formation and elasticity of injera) were replicated twice.

CHAPTER 4

RESULTS AND DISCUSSION

Processing parameters such as viscosity and fermentation time have an effect on the quality of *injera*. Experiments were conducted to determine how these parameters affect eye formation and elasticity of *injera* which are important quality attributes. The size and number of eyes on the surface of 4 brands of *injera* already on the market were compared in order to be used as a benchmark and also to determine the acceptable quality. Studies were also conducted on the effect of fermentation time and viscosity on the quality of *injera*. Lastly, in order to understand the mechanism of eye formation and elasticity of *injera*, two experiments were conducted to determine whether carbon dioxide has a significant effect on eye formation on *injera* and also to understand whether disulfide bond linkage of proteins contribute to the elasticity of *injera*.

4.1 Analysis of commercial *injera* products

Wub, Gebeta, Kare and Mena brands of *injera* (Figure 4.1) were obtained from Kare International Ethiopian market (Alexandria, VA) and stored at 4°C. These samples were analyzed to determine the number, mean equivalent diameter and % total area of 'eyes', pH and moisture content. Replicates of the injera samples were obtained from different pieces in the same package of each of the brands. Results obtained (Table 4.1) showed that the mean equivalent diameter and number of eyes on the surface of each of the *injera* were not significantly different ($p>0.05$) from each other. However the % total area of eyes for Wub *injera* was significantly different ($p<0.05$) from that of the other brands. From the frequency distribution graphs (Figure 4.2) it can be observed that Kare and Mena injera had the widest eye size distribution. There were 4-5 eyes/cm^2 on the surface of the commercial brands of injera and this is similar to what Gebrekidan and GebreHiwot (1982) reported.

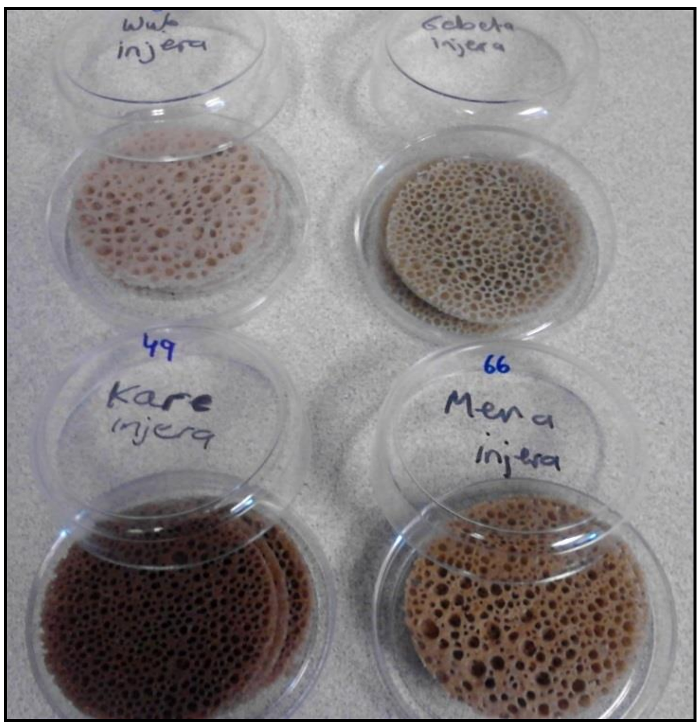


Figure 4.1. Wub, Kare, Gebeta and Mena *injera*

Table 4.1: Number, mean equivalent diameter and % total area of eyes on the surface of different brands of injera.

Brand	No. of eyes	Mean equiv. diam. (cm)	Total Area (%)
Gebeta	251.8 ± 44.7[a]	0.124 ± 0.017[a]	33.27 ± 6.50[a]
Kare	252.2 ± 41.1[a]	0.135 ± 0.016[a]	34.16 ± 6.68[a]
Mena	248.9 ± 70.6[a]	0.131 ± 0.024[a]	31.73 ± 5.09[a]
Wub	255.0 ± 77.9[a]	0.115 ± 0.016[a]	24.07 ± 3.04[b]

*Values (mean ± SD, n=9)

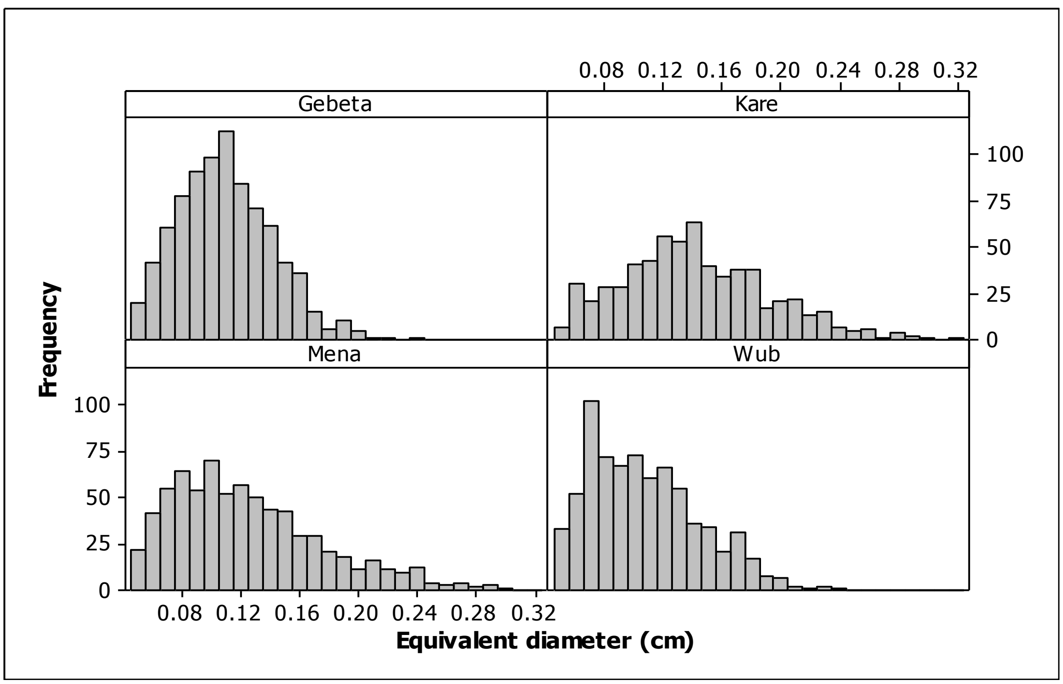


Figure 4.2: Eye size distribution of commercial brands of *injera*

The pH and moisture content of the *injera* were also determined. The sourness of *injera* is one of its unique qualities. The pH gives an indication of the amount of lactic acid produced during fermentation and hence it determines the sourness of the batter. ANOVA results showed that there was a statistically significant difference ($p<0.05$) in both pH and moisture content of the *injera*. From Table 4.2, it can be observed that Kare *injera* had the lowest pH but the highest moisture content and the inverse is observed in the Wub *injera*.

It can also be observed that for both pH and moisture content, the Wub *injera* and Kare *injera* were significantly different ($p<0.05$), however the Wub *injera* was not significantly different ($p>0.05$) from the Mena *injera*. The pH and moisture content of the Kare and Gebeta *injera* were also not significantly different ($p>0.05$) and the same observation is made for Gebeta and Mena *injera*.

Table 4.2.pH and moisture content of different brands of injera

Brand	**pH**	**% Moisture**
Kare	3.65 ± 0.06^{c}	60.40 ± 0.68^{a}
Gebeta	3.70 ± 0.07^{bc}	54.08 ± 2.91^{ab}
Mena	3.87 ± 0.08^{ab}	51.05 ± 1.35^{bc}
Wub	4.02 ± 0.02^{a}	44.68 ± 4.80^{c}

***Values (mean ± SD, n=3)**

4.2 Effect of fermentation time on physical and chemical properties of tef batter and *injera*

Fermentation is time dependent and known to cause changes in the physicochemical and structural properties of food materials. To observe whether there are any significant changes in pH, titratable acidity, viscosity, glucose content and total phenolic content of tef batter and elasticity, eye formation, and moisture of *injera*, it was necessary to monitor fermentation at different times. Tef flour was fermented for 72 hours at ambient temperature (22-25°C). Tef batter samples were collected every 4 hours and the pH, titratable acidity, viscosity and glucose content. Total phenolic content of the tef batter was determined at 0 and 72 hours. At 0, 24, 48 and 72 hours of primary fermentation, 300 g tef batter samples were collected and *injera* (3) prepared from them. Moisture content, elasticity, number, mean equivalent diameter and % total area of eyes for each of the *injera* were determined. It was hypothesized that fermentation time would have a significant effect on the physicochemical and structural properties of tef batter and *injera*.

4.2.1 pH and titratable acidity

The pH and titratable acidity give an indication of the sourness of a food product. A characteristic sensory quality of *injera* is its sour taste. Analysis of the different brands of *injera* in earlier experiments showed that the pH of the *injera* samples were between 3.65 and 4.02. In Figure 4.3, it can be observed that as lactic acid is produced during fermentation, the tef batter becomes more acidic leading to a decrease in pH. According to Sahlin (1999), the content of lactic acid at a certain pH is very much dependent on the raw material. Furthermore, environmental conditions such as the pH and moisture content may increase the activity of the flour amylases as well as starch hydrolyzing bacteria. This in turn increases the amounts of fermentable sugars and acid production causing a further decrease in pH. Urga and Narasimha (1997) conducted a 96-hour tef fermentation and observed that the pH of the tef batter decreased to a value of 3.83 with a corresponding increase in titratable acidity to a value of 1.31%. pH and titratable acidity values measured in this study were 3.73 and 2.33% respectively.

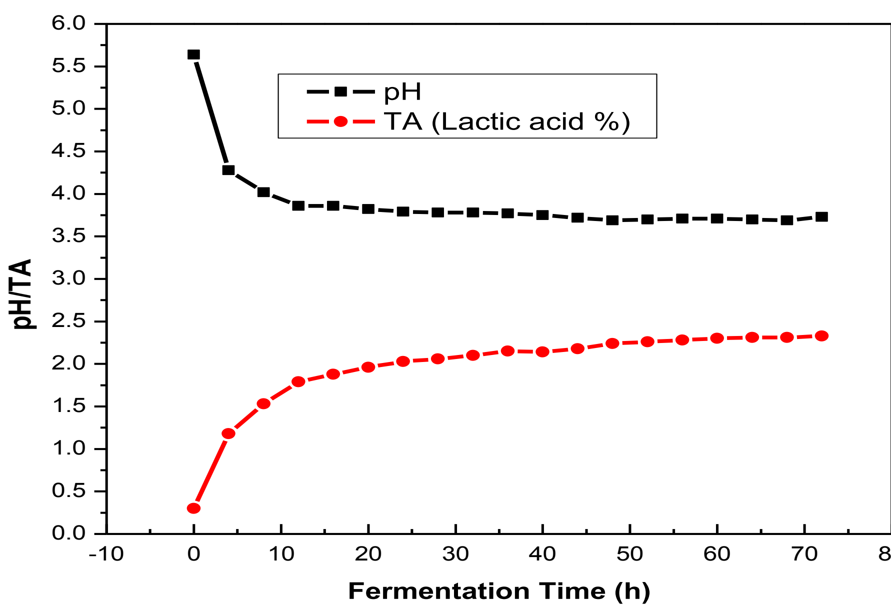


Figure 4.3. Effect of fermentation time on pH and titratable acidity of tef batters

4.2.2 Apparent viscosity

A single measurement of apparent viscosity of the tef batter samples was made at each sampling time. Figure 4.4 shows what appears to be a peak in apparent viscosity of tef batter at 24 hours of fermentation and then a gradual decrease in viscosity after 30 hours. A study by Wehrle and Arendt (1998) on wheat sourdough showed that after 2 hours of fermentation, viscosity of the sourdough decreased with ongoing fermentation. This peak is likely due to the higher gas evolution (increase in air bubbles) observed in the tef batter at 24 hours of primary fermentation. The increase in viscosity and gas evolution around 24 hours lead us to investigate the effect viscosity of tef batter would have on eye size and elasticity of injera in later experiments. Due to variability in the measurement of apparent viscosity of tef batter using the Brookfield viscometer in this experiment, an ARES rheometer was used in subsequent viscosity experiments.

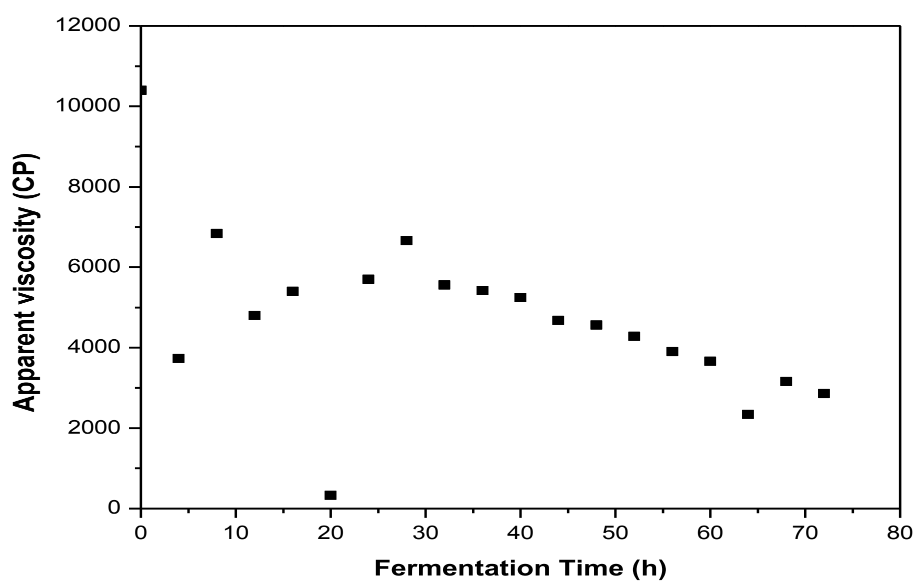


Figure 4.4: Effect of fermentation time on apparent viscosity of tef batters

4.2.3 Glucose content

Glucose concentration of tef batter decreases with increasing fermentation time (Figure 4.5). This is believed to be due to the breakdown of glucose to lactic acid as fermentation progresses. Baye *et al*. (2013) showed glucose to be the main fermentable sugar in a tef-white sorghum composite *injera*, and that glucose concentration increased in the first 6 hours of fermentation after which it started to decrease. This is similar to our findings which showed a decrease in glucose concentration after 4 hours of primary fermentation.

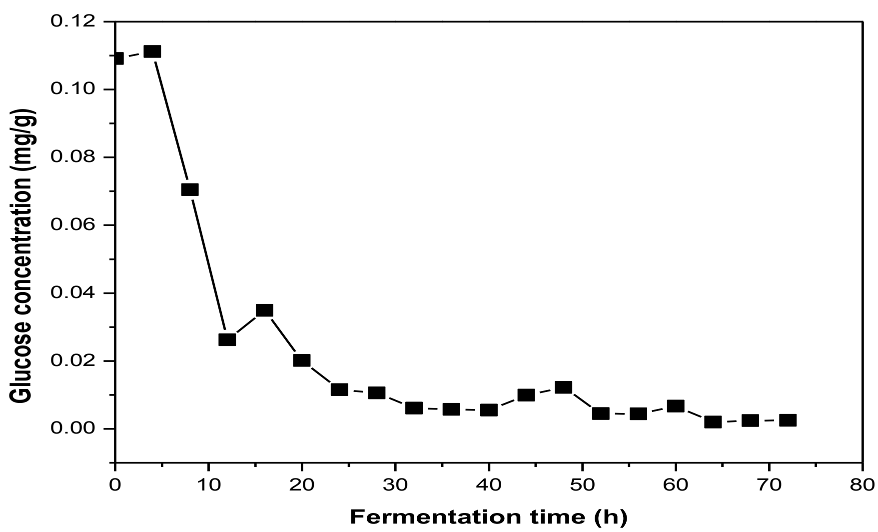


Figure 4.5. Effect of fermentation time on glucose content of tef batter samples (data points represent the average of two determinations).

4.2.4 Total phenolic content

The total phenolic content (TP) of the tef batter samples was determined at 0 and 72 h in order to observe whether there was a significant difference in polyphenolic content of the tef batter at the beginning and end of fermentation. TP at 0 h was 1.57±0.35 mg GAE/g while that at 72 h was 1.69±0.66 mg GAE/g. Forsido *et al.* (2013) reported the TP of tef flour to be 123.6 mg GAE/100 g DM (i.e. 1.24 mg GAE/g) of tef flour. Statistical analysis showed no significance difference ($p<0.05$) between TP tef batter at the beginning of fermentation and at 72 hours of fermentation. Fermentation is known to decrease phytic acid and polyphenols during fermentation of cereals (Oyewole, 1997). This was however not the case in our study as a slightly higher amount of total phenolic content was obtained for 72 hours. Kheterpaul and Chauhan (1991) also observed an increase in the polyphenol content of fermented pearl millet flour, which they attributed to natural lactic acid fermentation which has been known to increase the polyphenol content of pearl millet. The higher amount of TP may have been due to an easier extraction of the polyphenols and not an actual increase in the amount of polyphenols in the tef batter. Acidified methanol is used to extract bound polyphenols in food materials and because after fermentation the tef batter is acidic, it is likely that more of the bound polyphenols were extracted. Further research may be necessary in order to determine both the total bound and free phenolic content in tef batter samples at the beginning and end of tef fermentation.

4.2.5 Moisture content of *injera*

One of the most important ingredients in any gluten-free formulation is water. From Table 4.3, it can be observed that there was a significant difference ($p<0.05$) in moisture content of *injera* baked at 0, 24, 48 and 72 hours. During baking, the *injera* samples were covered with the lid of the griddle when eyes covered the whole surface of the *injera*. *Injera* baked at 48 hours was probably covered at a later time compared to *injera* baked at 24 hours. Due to this variability, the time of covering *injera* during baking was standardized in later tests (1 minute).

Table 4.3. Effect of fermentation time on moisture content of injera

Time (h)	Moisture content (%)
0	67.91 ± 1.37 ^{ab}
24	74.18 ± 6.40 ^{a}
48	46.74 ± 2.15 ^{c}
72	65.23 ± 0.69 ^{b}

***Values (mean ± SD, n=3)**

4.2.6 Elastic modulus of *injera*

There was a significant difference ($p<0.05$) in elastic modulus of *injera* baked at 0, 24, 48 and 72 hours (Table 4.4). *Injera* baked at 72 hours had the highest modulus and this may be due to more bacterial exopolysaccharides produced after 72 hours of fermentation compared to earlier fermentation times, though no measurements of EPS were made. Exopolyssachides can act as hydrocolloids in situ (Rühmkorf *et al.*, 2012) and hence may have contribute to the elastic texture of *injera*. Fermentation is also known to breakdown protein and starch and these changes may have resulted in the different elastic moduli at the different fermentation times.

Table 4.4. Effect of fermentation time on elasticity of injera

Time (h)	**Elastic modulus (Pa)**
0	2378.67 ± 807.67 [ab]
24	1339.33 ± 170.15[b]
48	1497.33 ± 90.96 [ab]
72	3181.67± 1265.76 [a]

***Values (mean ± SD, n=3)**

4.2.6 Eye formation of *injera*

4.2.6.1 Number of 'eyes' on the injera surface

There was a significant difference ($p<0.05$) in the number of eyes formed on the surface of *injera* baked at 0, 24, 48 and 72 h. It was observed that numerous eyes were formed on *injera* baked at 24 and 48 hours compared to those baked at 0 and 72 hours (Table 4.5). This is likely due to the vigorous gas evolution observed during fermentation at around 24 hours and 48 hours. This gas evolution then subsided towards the end of fermentation which explains the decrease in number of eyes on the *injera* from 48 to 72 hours. Pyle (2005) stated that the small bubbles of carbon dioxide (CO_2) resulting from fermentation play a crucial role in as nuclei for pore development and without these nuclei a porous structure in the final product will not be formed. The CO_2 nuclei formed during primary fermentation could possibly be the main determinant of the number of eyes that will be formed on the surface of *injera*. Also, the increase in gas bubbles at around 24 and 48 hours could have caused an increase in the viscosity of the batter. This was likely the cause of the peak in viscosity at 24 h of primary fermentation (Figure 4.4)

Table 4.5. Effect of fermentation time on number of eyes on the surface of injera

Time (h)	**Number of Eyes**
0	20.67 ± 9.84^{b}
24	326.67 ± 11.90^{a}
48	287.33 ± 81.37^{a}
72	64.33 ± 4.11^{b}

***Values (mean ± SD, n=3)**

4.2.6.2 Mean equivalent diameter of eyes on surface of injera

There was a significant difference ($p<0.05$) in mean equivalent diameters of eyes on *injera* baked at 0, 24, 48 and 72 hours. *Injera* baked from batters collected at 24 and 72 h primary fermentation had higher mean equivalent diameters of eyes on their surfaces.

Table 4.6. Effect of fermentation time on mean equivalent diameter of eyes on the surface of injera

Time (h)	**Mean equivalent diameter (cm)**
0	0.12 ± 0.01^{b}
24	0.16 ± 0.01^{a}
48	0.12 ± 0.01^{b}
72	$0.15 \quad 0.00^{a}$

***Values (mean ± SD, n=3)**

4.2.6.3 Percent (%) total area of eyes on surface of injera

The % total area represents the total area covered by 'eyes' on the surface of injera. There was a statistically significant difference in % total area of eyes of injera baked from tef batter samples collected at 0, 24, 48 and 72 h. There was a higher percentage of total area of eyes on injera baked at 24 compared to 0, 48 and 72 h. During fermentation, it was observed that there was vigorous gas evolution around 24 h and this may have resulted in higher number of eyes on the surface of the injera. Gas evolution became lower towards the end of primary fermentation and this could have accounted for the low % total area.

Table 4.7. Effect of fermentation time on % total area of eyes on the surface of injera

Time(hr)	**Total area (%)**
0	0.55 ± 0.38^{a}
24	14.98 ± 1.28^{b}
48	7.93 3.50 ^{c}
72	2.46 ± 0.33^{c}

***Values (mean ± SD, n=3)**

4.3 Effect of viscosity on eye formation and elasticity of *injera*

Absit added back to the fermented batter increases the viscosity of batter and provides the batter with a better gas-holding capacity (Zannini *et al.*, 2012). This experiment was conducted to determine the effect of different viscosities of tef batter on elasticity and eye formation of *injera***.** Tef batter was divided into 6 equal portions and 10 % by weight of each of the tef batter portions was used to prepare *absit* in different tef batter to water ratios: 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 (w/w). This experiment was replicated twice.

4.3.1 Flow behavior of tef batter

The viscosity of the batter and the amount of carbon dioxide produced during fermentation play a fundamental role in the formation of the cellular structure of leavened bread (Bloksma, 1990). The mean yield stress, consistency coefficient, flow index and apparent viscosity (shear rate of 1 s^{-1}) of each of the tef batter samples were determined (Table 4.8). Shear thinning behavior was observed in all samples except the 1:2 batter at a shear rate range of 0.1 to 10 s^{-1}.

Table 4.8: Apparent, viscosity, yield stress, consistency coefficient and flow index of tef batter samples

Batter-to-water ratio in *absit*	Apparent Viscosity at 1 s^{-1}(Pa.s)	Yield Stress (Pa)	Consistency Coefficient ($Pa.s^n$)	Flow Index
1:2	1.59 ± 0.17	0.81±0.12	0.74±0.06	1.18±0.33
1:3	1.47±0.13	0.78±0.14	0.69±0.04	0.93±0.10
1:4	1.37±0.09	0.67±0.07	0.71±0.04	0.81±0.06
1:5	1.27±0.06	0.55±0.06	0.72±0.05	0.71±0.027
1:6	1.13±0.08	0.39±0.09	0.74±0.11	0.63±0.07
1:7	1.06±0.09	0.26±0.07	0.80±0.05	0.50±0.04

***Values (mean ± SD, n=6)**

The Herschel-Bulkley model used to fit the shear stress data showed a high (0.986-0.997) coefficient of determination (R^2) for all samples. The apparent viscosity of the samples decreased with increasing amount of water used to prepare the *absit*. The magnitude of the consistency coefficient of a material is related to its viscosity (Mukprasirt *et al.*, 2000). According to D'Silva (2009), the consistency coefficient is an indication of viscous behavior. Hence, a batter that is thick will likely have a higher consistency coefficient compared to a thinner batter. It can be observed from Table 4.8 that the consistency coefficient was higher in the batter with lower amount of water (1:2) and decreased at the tef batter-to-water ratio of 1:3. The consistency coefficient increased again at the tef batter-to-water ratio of 1:4 to the highest water content (1:7). Consistency coefficient values are more sensitive to solid concentration in suspensions, and an increase in solid level markedly increases the consistency values (Bhattacharya and Bhat, 1997).The magnitude of the consistency coefficient is not directly comparable unless the flow index is constant, since the units of k are $Pa.s^n$. Since n changed with the water content in the *absit,* the values of k in Table 4.8 are not directly comparable. The batter with the lowest apparent viscosity exhibited high consistency, low yield stress and low flow index properties, and therefore was closer in character to a pseudoplastic fluid.

The flow behavior index (n) indicates the type of behavior in response to different shear rates (D'Silva, 2009). A value of $n< 1$ indicates shear thinning behavior, $n > 1$ indicates shear thickening behavior and $n = 1$ indicates Newtonian behavior. Hence, as the flow index tends towards 1, shear thinning properties become less pronounced. It can be observed that the flow index for all the samples except the batter with the highest viscosity (1.59 ± 0.17 Pa.s) were below 1 (Table 4.8), with this batter having the highest flow behavior index hence approaching shear thickening behavior. Kim *et al.*(2002) stated that under shear, starch from microscopic regions of highly concentrated clusters would be dispersed into solution raising the starch concentration and viscosity of the suspension. This results in the shear thickening of the concentrated starch suspension. A study by Bhattacharya and Bhat (1997) on rice-blackgram suspensions reported that the Herschel-Bulkley model yields slightly higher flow index values than the power law model. They stated that this is due to the existence of a yield stress term in the nature of Herschel-Bulkley model which predicts a lesser pseudoplastic nature of the suspensions.

From Table 4.8, it can also be observed that the yield stress values decreased with increasing water content. Bhattacharya and Bhat (1997) observed a decrease in yield stress with increasing moisture content in rice-blackgram suspensions. The yield stress of a material describes the force that must be exceeded to cause the material to flow. Hence, it shows that the thinner the batter the lower the force needed for it to flow. According to Bhattacharya and Bhat (1997), the high yield stress for concentrated suspensions occurs due to the crowding of the continuous phase**.** This implies that the lower the apparent viscosity of the suspension, the lower the crowding of the continuous phase hence more solvent or more free water in the continuous phase to facilitate movement of particles.

4.3.2 Eye formation on the surface of *injera*

The batters became less viscous with increasing amount of water used to prepare *absit*. All *injera* baked from the batters had eyes on their surfaces, with the viscosity of the tef batters having a significant effect on the number, mean equivalent diameter and % total area of eyes on the surface of the *injera*. This agrees with the proposed model for eye formation (in chapter 2). In the model of the effect of viscosity on eye formation, we hypothesized that the viscosity of the batter significantly affects the formation of eyes on the surface of *injera*. In a study on muffins by Baixauli *et al.* (2008), the viscous behavior of the batter system was the controlling factor in the final cake volume, due to its effects on bubble incorporation and movement. They stated that the rate at which bubbles rise due to buoyancy is inversely proportional to viscosity. Regression analysis showed that apparent viscosity had a significant ($p<0.05$) effect on the number of eyes formed on *injera*. Figure 4.6 shows a significant quadratic relationship ($p<0.05$) between viscosity and number of eyes. As apparent viscosity increased, the number of eyes on the surface of the *injera* also increased to a point (850 eyes) and then decreased. From this experiment it was observed that before the peak apparent viscosity, the lower the apparent viscosity of the fermented tef batter was before baking, the lower the number of eyes formed on the surface. This finding is attributed to the fact that a thinner batter is likely to cause bubbles to rise faster to the surface of *injera* during baking. However, once the bubbles rupture at the surface of the *injera* the batter flows and fills the pores formed, resulting in fewer eyes on the surface of the *injera*. It was also observed that thicker batters had fewer ‘eyes’ on their surfaces. This may be due to gelatinization of the batter surrounding the bubble thereby slowing down the rise of the bubble. This results in fewer bubbles rising to the surface and more bubbles getting trapped in the batter at the end of baking. According to Lakshminarayan *et al.* (2006), a sufficiently high batter viscosity in a cake batter might keep the air bubbles from rising out of the batter. Also it was observed from a cross-sectional view of cut *injera* (Figure 4.7) that these bubbles create tunnels in the batter during baking and these tunnels appeared to be more pronounced in thicker batters. Pyle (2005) also observed similar columnar structures in baked crumpets after 75 s of baking.

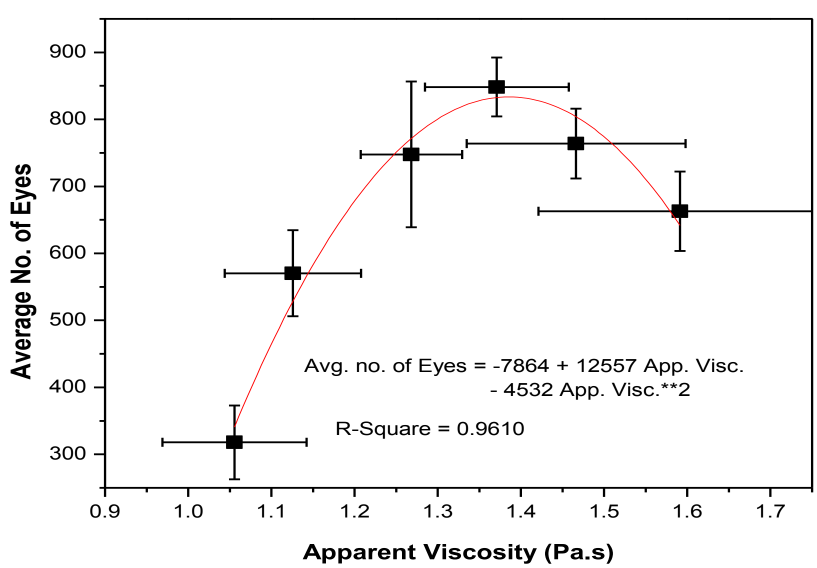


Figure 4.6. Effect of apparent viscosity on the number of eyes on the surface of *injera (error bars represent the standard deviation of the mean no. of eyes and apparent viscosity)*

Figure 4.7. Columnar structures (tunnels) in cross-sectional view of *injera*

Apart from the number of eyes formed, the size of the eyes was also affected. The mean equivalent diameter of the eyes formed on the surface of *injera* were significantly ($p<0.05$) affected by the viscosity of the batters from which they were baked. Lower viscosity batters had smaller eyes compared to batters with higher viscosities. Figure 4.8 shows a quadratic relationship between apparent viscosity and eye size on the surface of *injera*.

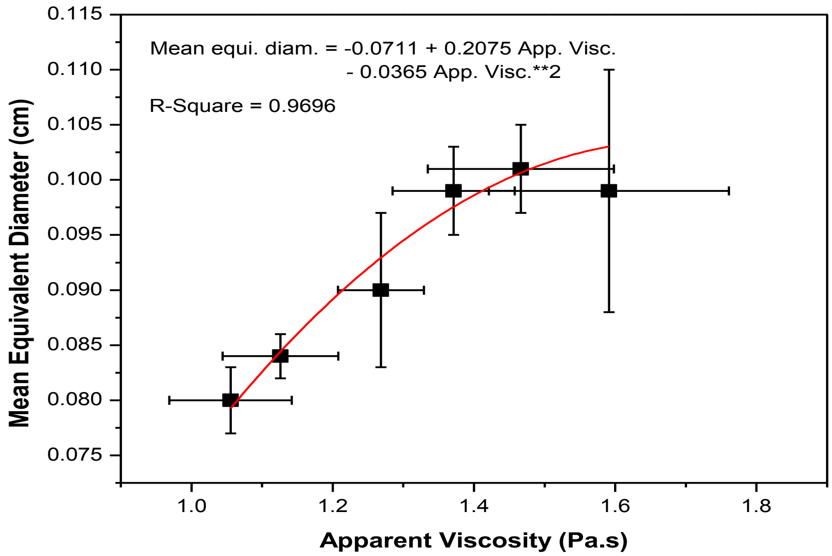


Figure 4.8. Effect of apparent viscosity on the mean equivalent diameter of eyes on the surface of *injera (error bars represent the standard deviation of the mean equivalent diameter and apparent viscosity).*

A statistically significant relationship ($p<0.05$) also existed between the viscosity of the tef batters and the % total area of eyes covering the surface of the *injera* baked from these batters. It can also be observed in Figure 4.9 that a quadratic relationship similar to what was observed for the number of eyes of *injera* (Figure 4.6) exists between the % total area of eyes and the apparent viscosity. According to Cauvain (2007), during the early stages of heating in an oven, the viscosity of the batter slows down the movement of the gases in the system so that the batter expands. It is believed that this same phenomenon occurs in tef batter during baking and explains why thicker batters have fewer eyes on the surface of *injera* baked from them. As the bubbles rise, the batters gelatinize and some of the bubbles become trapped in the batter leading to fewer bubbles rising to the surface and erupting to form eyes.

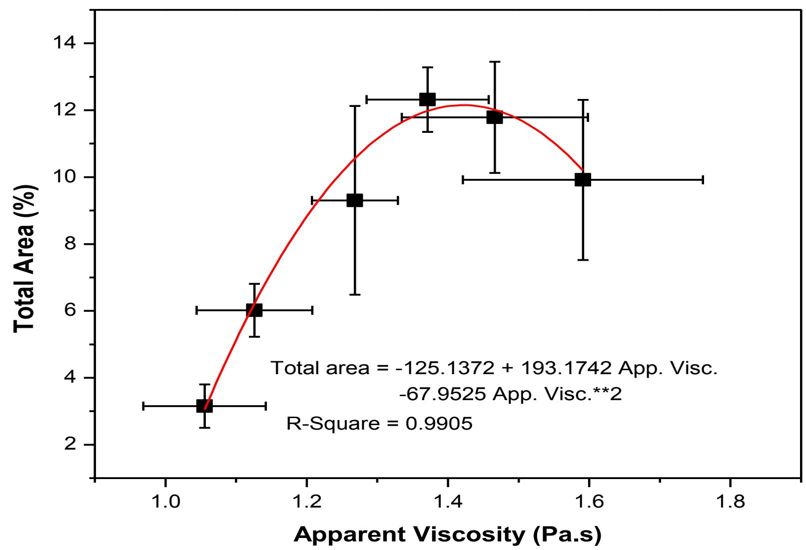


Figure 4.9. Effect of apparent viscosity on % total area of eyes on the surface of *injera (error bars represent the standard deviation of the mean % total area and apparent viscosity).*

4.3.3 Elastic modulus of *injera*

Apparent viscosity of the tef batters had a significant effect ($p<0.05$) on the elasticity and thickness of the *injera* baked from them. Regression analysis showed that there was a statistically significant relationship ($p<0.05$) between apparent viscosity and the elasticity of the *injera* samples analyzed. It was observed that the elastic modulus of the *injera* samples increased gradually with increasing apparent viscosity of the tef batters used to bake the *injera* (Figure 4.10). Thickness of the *injera* samples also increased with increasing apparent viscosity (Figure 4.11). The increase in elasticity may be due to the fact that a batter with a higher apparent viscosity will have a higher solids content and will be dense. The increase in thickness with increasing tef batter-to-water ratio was likely due to more air bubbles being trapped in the *injera* at higher viscosities**.**

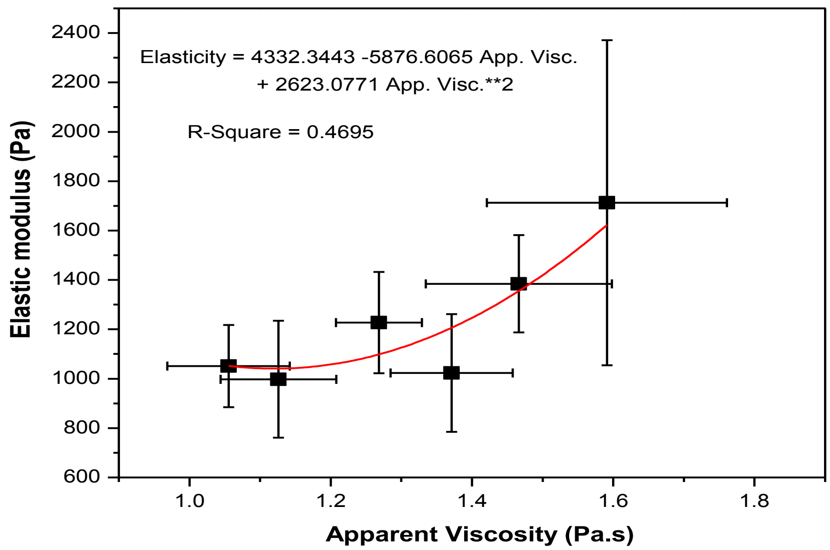


Figure 4.10. Effect of apparent viscosity on elastic modulus of *injera (error bars represent the standard deviation of the mean elastic modulus and apparent viscosity).*

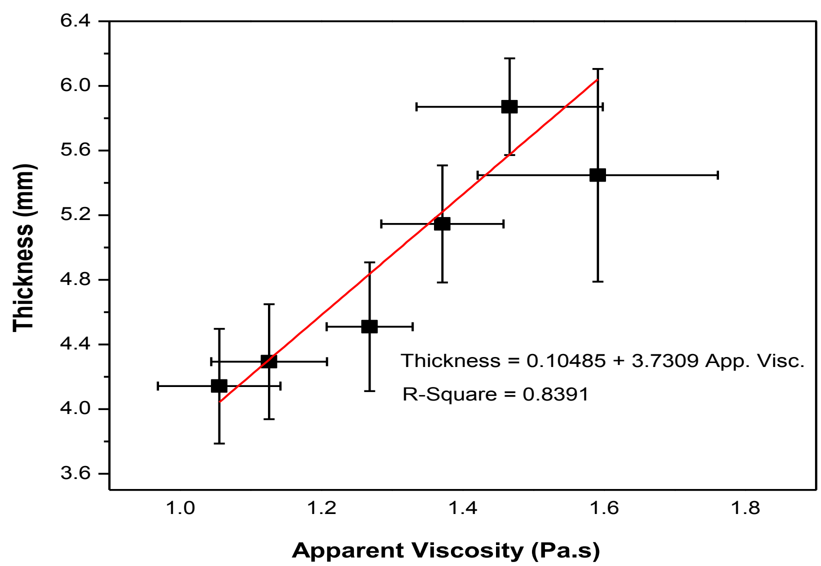


Figure 4.11. Effect of apparent viscosity on the thickness of *injera (error bars represent the standard deviation of the mean no. of eyes and apparent viscosity.)*

4.4 Influence of carbon dioxide on eye formation of *injera*

Pyle (2005) studied crumpet structures made from white wheat flour and concluded that carbon dioxide resulting from the fermentation stage plays a crucial role in pore development, and without these nuclei the final product would lack a porous structure. Pyle (2005) also stated that these nuclei are the source of the dominant pore structure in the final product, which results from the initial explosive release of water vapor from the batter together with the desorption of carbon dioxide. It is hypothesized that CO_2 produced during fermentation of tef batter significantly affects the number and size of eyes formed on the surface of *injera*. To test this hypothesis a fermented batter was degassed in a dessicator under vacuum (22-24 in Hg) for 30 minutes. The other samples were not degassed. Baking powder (0.7 % by weight of tef batter) was put in one of the undegassed portions. *Injera* samples were baked from each of the batters and the number, mean equivalent diameter and % total areas of the eyes formed on the surface of the *injera* samples were determined. Results from this experiment showed that CO_2 greatly affected eye formation on the surface of *injera*. The undegassed batter produced *injera* with evenly distributed average-sized eyes on their surfaces. However, the degassed batters resulted in injera with fewer eyes on their surfaces. Yetneberk *et al.* (2005) attributed the absence of eyes on sorghum *injera* to very little carbon dioxide produced during fermentation. The sample containing baking powder (positive control) also produced eyes on the surface of *injera* comparable to that of the control (undegassed batter).

Table 4.9: The effect of degassing, not degassing and not degassing with addition of baking powder of batters on the number, mean equivalent diameter and % total area of eyes on the surface of injera.

Type of Batter	No. of eyes	Mean equivalent diameter (cm)	% total area
Degassed (D)	43 ± 16.7^b	0.082 ± 0.000^c	0.43 ± 0.18^b
Not degassed (ND)	918.7 ± 173.0^a	0.102 ± 0.012^b	17.09 ± 4.98^a
Not degassed + baking powder (NB)	762 ± 4.7^a	0.139 ± 0.004^a	22.96 ± 1.1^a

***Values (mean ± SD, n=6)**

From Table 4.9, it can be observed that the number, mean equivalent diameter and % total area for both the undegassed batter with baking powder (NB) and the undegassed batter (ND) were greater than that of the degassed batters (D). The eyes formed on the injera baked from the degassed batter are as a result of pores created by water vapor during heating. Analysis of variance performed on the number of eyes and % total area of eyes on the *injera* showed that the ND and NB batters are not significantly different but are both significantly different from the mean no. of eyes and % total area of the D batters. However the average mean equivalent diameter for the *injera* baked from the D, ND and NB batters were significantly different. Eyes on the *injera* baked from the NB batters were bigger in size compared to eyes on the *injera* baked from D and ND batters with the eyes on the D batters having the smallest diameter among the 3 types of batters. According to Cauvain (2007), carbon dioxide gas is generated by the addition of baking powder and this will add to the inflation of the initial air bubbles in the case of cake batters. This could explain why the NB batter had a higher mean equivalent diameter compared to the other batters.

In conclusion, it is very clear from the results that CO_2 had a significant effect ($p<0.05$) on eye formation on the surface of *injera*.

4.5 Influence of a reducing agent, sodium metabisulfite (SMBS) on elasticity or storage modulus of *injera*

4.5.1 Rheological behavior of tef batter

Sodium metabisulfite (SMBS) acts as a source of sulfur (IV) oxoanions which modifies the rheology of the dough by breaking some of the disulfide bridges present in proteins (Oliver *et al.*, 1995). Pyle (2005) observed an increase in elastic modulus of crumpet batters with increasing temperature. The storage and loss moduli for both the tef batter with SMBS and tef batter without SMBS showed similar trends with increasing temperature. A short plateau was observed in both storage and loss moduli curves for tef batter without SMBS but not for tef batter with SMBS. This shows that there was some effect of SMBS on disulfide bonds of proteins in the tef batter with SMBS. None of the samples showed a significant elastic response during the dynamic shearing test at temperatures below 80°C (Figures 4.12 and 4.13). However, an increase in elastic modulus was observed for all batters from 80 to 120 °C. Bultosa *et al.* (2002) reported a starch gelatinization temperature of raw tef in the range 68-80°C. Xue and Ngadi (2006) studied the rheological properties of different gluten-free flour batters and attributed an increase in elastic modulus with increasing temperature of the batters to starch gelatinization. It can be observed from both graphs of storage modulus versus temperature (Figures 4.12 and 4.13) that batters without SMBS showed higher elastic modulus than the batters with SMBS.

The effect of storage modulus with respect to time at 20°C showed different trends in the samples containing the SMBS and samples not containing SMBS. For the tef batters with SMBS it was observed that the storage and loss moduli merged together resulting in a more viscous behavior of the batter towards the end of cooling. Biliaderis (1991) stated that amylose gels generally exhibit a rapid rise in storage modulus (G'), followed by a phase of much slower increase at longer times. This behavior was observed in tef batter without SMBS. At the end of cooling at 20 °C, the storage modulus of tef batter without SMBS was higher than that of tef batter with SMBS (Figure 4.12 and 4.13) meaning that the SMBS possibly had an effect on the disulfide bonds of the proteins resulting in a product of lower elasticity.

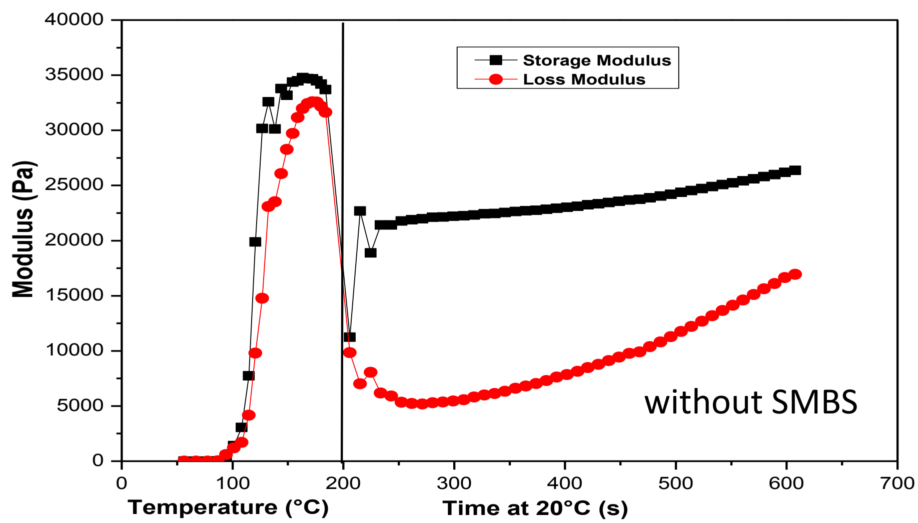


Figure 4.12. Effect of temperature and time (at 20°C) on storage and loss modulus of tef batter without SMBS

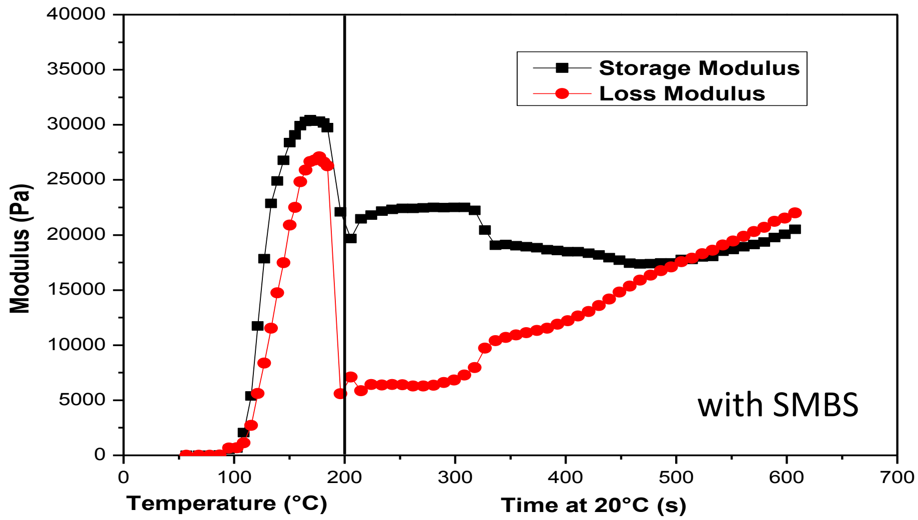


Figure 4.13. Effect of temperature and time (at 20°C) on storage and loss modulus of tef batter with SMBS

4.5.2 Determination of modulus of elasticity

There was a significant difference ($p<0.05$) in thickness and elastic modulus of injera baked from tef batter with SMBS and tef batter without SMBS (Table 4.10). This shows that the SMBS had an effect on the tef batter which caused *injera* baked from those batters to have a lower elastic modulus. SMBS is a reducing agent and known to break down sulfhydryl bonds of proteins resulting in a lower elasticity and softness of dough. Qarooni *et al.* (1989) observed a gradual softening and decrease in elasticity of wheat dough when SMBS was added to it and addition of a higher amount of the SMBS resulted in stickiness and handling difficulties of the dough. It was observed in the present study that *injera* baked from the SMBS containing batters were less viscous than those baked from the batters without SMBS. It is not clear whether the breakdown of the sulfhydryl bonds of tef proteins resulted in a thinner batter or whether the reduction in thickness and elastic modulus was due to a viscosity effect alone.

Table 4.10. Effect of SMBS on the thickness and elastic modulus of injera

Sample	Thickness (mm)	Elastic modulus (Pa)
Tef batter without SMBS (control)	6.59 ± 0.61[a]	1375.88 ± 395.76[a]
Tef batter with SMBS	4.54 ± 0.23[b]	1203.483 ± 388.81[b]

***Values (mean ± SD, n=6)**

4.5.3 Determination of the effect of SMBS on eye formation of *injera*

ANOVA results showed that there was a significant difference ($p<0.05$) in the number of eyes of *injera* baked from tef batter containing SMBS and tef batter without SMBS. The same observation was made for the mean equivalent diameter and % total area of the eyes formed on the surface of the *injera*. It is uncertain what may have caused a decrease in the number of eyes of *injera* baked from the SMBS containing batters. Sulfites are used as antimicrobial and antioxidants in food preservation (Schimz, 1980). Gesellschaft (1980) studied the effect of sulfites on *Saccharomyces cerevisiae* and observed that they inhibited colony formation of yeasts. Hence, the SMBS might have inactivated the yeasts in the batter and this prevented gas evolution in the batter. Because the SMBS batters were also less viscous than the batters without SMBS, it is possible that the tef batter covered the eyes that were being formed on the surface of the *injera* during baking thereby resulting in fewer eyes being formed on the surface.

Table 4.11. Effect of SMBS on number, mean equivalent diameter and % total area of eyes on the surface of injera

Sample	Number of eyes	Mean Equivalent Diameter (cm)	% total area
Tef batter without SMBS (control)	859.2 ± 65.5[a]	0.118 ± 0.010[a]	18.33 ± 2.29[a]
Tef batter with SMBS	587.5 ± 170.7[b]	0.090 ± 0.003[b]	7.50 ± 2.54[b]

***Values (mean ± SD, n=6)**

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Elasticity and eye formation are important quality attributes of *injera*. Hence, an *injera* which lacks these attributes is not acceptable to the Ethiopian consumer. The main objective of this study was to determine the effect of viscosity of tef batter and fermentation time on elasticity and eye formation of *injera*. From the results it was observed that viscosity and fermentation time have a significant effect on elasticity and eye formation. These are factors which should be controlled during processing of *injera* to ensure that good quality *injera* is produced.

From the results of this study, we were able to show that eyes are formed by dissolved gases, likely carbon dioxide produced during fermentation of tef batter. We were also able to show that *injera* baked from very low viscosity batters will have fewer eyes on their surfaces and those baked from higher viscosity batters will also have fewer but bigger eyes on their surfaces compared to the low viscosity batters. From this finding, we were able to obtain an optimal viscosity range of 1.1 to 1.4 Pa.s for baking *injera* with numerous, evenly distributed eyes. This fell within the tef batter viscosity range (0.2-1.5 Pa.s) stated by Admassu (2004). Results from rheological measurements to determine the effect of SMBS on the elasticity of *injera* showed that the tef batters containing SMBS had a lower storage modulus at the different heating temperatures compared to tef batters without SMBS. SMBS also had a significant effect on elasticity and eye formation of the *injera* indicating that proteins may have contributed to the elasticity of *injera* even though it is still uncertain whether they are the sole contributors to *injera* elasticity.

From the tests conducted we have been able to show that processing parameters such as viscosity and fermentation time have a significant effect on elasticity and eye formation of *injera*. These findings will play an important role in improving the quality of *injera* and gluten-free breads in general.

5.2 Recommendations

1. Sensory tests should be conducted on *injera* samples using the apparent viscosity range to determine acceptability among Ethiopian consumers

2. A study comparing different varieties of tef should be conducted to determine their effect on eye formation and of *injera* among other quality attributes.

3. A more in depth analysis on the role of proteins, starch, hemicellulose and exopolysaccharides for a better understanding of the structure-property relationships in *injera*.

REFERENCES

Abdel-Aal, E.-S.M., 2009. Functionality of Starches and Hydrocolloids in Gluten-Free Foods, in: Gallagher, E. (Ed.), Gluten-Free Food Science and Technology. Blackwell Publishing Limited, United Kingdom, pp. 200–224.

Abiyu, H.T., Zewdu, A.W., Haki, G.D., 2013. Preparation of injera from pre-fermented flour: nutritional and sensory quality. Int. J. Sci. Innov. Discov. 3, 165–175.

Abraha, A., Uhlen, A.K., Abay, F., Sahlstrøm, S., Bjørnstad, Å., 2013. Genetic variation in barley enables a high quality, the Ethiopian staple flat bread, comparable to tef. Crop Sci. 53, 2040.

Adebowale, A.-R.A., Emmambux, M.N., Beukes, M., Taylor, J.R.N., 2011. Fractionation and characterization of teff proteins. J. Cereal Sci. 54, 380–386.

Admassu, W., 2006. Injera manufacturing system. United States Patent US 7063008 B2.

Alemayehu, R., 2001. TEF : Post-harvest Operations. Holetta Genet, Ethiopia.

AOAC, 1995. Official Methods of Analysis of AOAC International. AOAC International, Arlington, Virginia.

Arendt, E.K., Ryan, L. a M., Dal Bello, F., 2007. Impact of sourdough on the texture of bread. Food Microbiol. 24, 165–74.

Ashagrie, Z., Abate, D., 2012. Improvement of injera shelf life through the use of chemical preservatives. African J. Food, Agric. Nutr. Dev. 12, 6409–6423.

Ashenafi, M., 2006. A review article on the microbiology of indigenous fermented foods. Ethiop. J. Biol. Sci. 5, 189–245.

Assefa, K., Aliye, S., Belay, G., Metaferia, G., Tefera, H., Sorrells, M.E., 2011. Quncho: the first popular tef variety in Ethiopia. Int. J. Agric. Sustain. 9, 25–34.

Autio, K., Laurikainen, T., 1997. Relationships between flour dough microstructure and dough handling and baking properties. Trends Food Sci. Technol. 8, 181–185.

Baixauli, R., Sanz, T., Salvador, A., Fiszman, S.M., 2008. Muffins with resistant starch: Baking performance in relation to the rheological properties of the batter. J. Cereal Sci. 47, 502–509.

Baye, K., Mouquet-Rivier, C., Icard-Vernière, C., Picq, C., Guyot, J.-P., 2013. Changes in mineral absorption inhibitors consequent to fermentation of Ethiopian injera: implications for predicted iron bioavailability and bioaccessibility. Int. J. Food Sci. Technol. 1–7.

Belitz, H.-D., Keiffer, R., Seilmeier, W., Weiser, H., 1986. structure and function of gluten proteins. Cereal Chem. 63, 336–341.

Belton, P.S., Taylor, J.R.N., 2004. Sorghum and millets: protein sources for Africa. Trends Food Sci. Technol. 15, 94–98.

Bhattacharya, S., Bhat, K.K., 1997. Steady shear rheology of rice-blackgram suspensions and suitability of rheological models. J. Food Eng. 32, 241–250.

Biliaderis, C.G., 1991. The structure and interactions of starch with food constituents. Can. J. Physiol. Pharmacol. 69, 60–78.

Blandino, A., Al-Aseeri, M.E., Pandiella, S.S., Cantero, D., Webb, C., 2003. Cereal-based fermented foods and beverages. Food Res. Int. 36, 527–543.

Bloksma, A.H., 1990. Rheology of the breadmaking process. Cereal. Foods World 35, 226–236.

Boka, B., Woldegiorgis, A.Z., Haki, G.D., 2013. Antioxidant properties of Ethiopian traditional bread (Injera) as affected by processing techniques and tef grain (Eragrostis tef (Zucc.)) varieties. Can. Chem. Trans. 1, 7–24.

Bultosa, G., 2007. Physicochemical characteristics of grain and flour in 13 tef [Eragrostis tef (Zucc .) Trotter] grain varieties. J. Appl. Sci. Res. 3, 2042–2050.

Bultosa, G., Hall, A.N., Taylor, J.R.N., 2002. Physico-chemical characterization of grain tef [Eragrostis tef (Zucc.) Trotter] starch. Starch - Stärke 54, 461–468.

Bultosa, G., Hamaker, B.R., BeMiller, J.N., 2008. An SEC-MALLS study of molecular features of water-soluble amylopectin and amylose of Tef [. Starch-Starke 60, 8–22.

Bultosa, G., Taylor, J.R.N., 2004. Paste and gel properties and in vitro digestibility of tef [Eragrostis tef (Zucc.) Trotter] starch. Starch - Stärke 56, 20–28.

Cauvain, S.P., 2007. Other cereals in breadmaking, in: Cauvain, S.P., Young, L.S. (Eds.), Technology of Breadbaking. Springer, United Kingdom, pp. 371–388.

Cherinet, H., 1988. Development of flours using traditional food crops for making injera. FAO Library AN:281301, Awassa.

Cherinet, H., 1993. Composite flour development for injera. Ethiop. J. Heal. Dev. 7, 71–77.

Cullen, P.J., Connelly, R.K., 2009. Rheology and Mixing, in: Cullen, P.J. (Ed.), Food Mixing-Principles and Applications. Wiley-Blackwell, Dublin, pp. 50–72.

D'Silva, T.V., 2009. Modification of the paste properties of maize and teff starches using stearic acid. MSc thesis, University of Pretoria, South Africa.

Damodaran, S., Parkin, K.L., Fennema, O.R., 2008. Fennema's Food Chemistry, fourth. ed. CRC Press/Taylor & Francis, Portland.

Dobraszczyk, B.., Morgenstern, M.., 2003. Rheology and the breadmaking process. J. Cereal Sci. 38, 229–245.

Dong, W., Hoseney, R.C., 1995. Effect of certain breadmaking oxidants and reducing agents on dough rheological properties. Cereal Chem. 72, 58–64.

Duncan, J., 2007. Principles and Applications of Mechanical Thermal Analysis, in: Gabbott, P. (Ed.), Principles and Application of Thermal Analysis. Wiley-Blackwell, New Jersey, pp. 120–163.

Edema, M.O., Emmambux, M.N., Taylor, J.R.N., 2013. Improvement of fonio dough properties through starch modification by sourdough fermentation. Starch - Stärke 65, 730–737.

Edwards, N.M., Dexter, J.E., Scanlon, M.G., 2002. Starch participation in durum dough linear viscoelastic properties. Cereal Chem. 79, 850–856.

Ekris, L., Gamboa, P., 2008. Teff (Survey on the nutritional and health aspects of teff (Eragrostis Tef)). Cartagena.

FAO, 1999. Fermented Cereals- A Global Perspective. FAO Agric. Serv. Bull. No 138 138.

Forsido, S.F., Ramaswamy, H.S., 2011. Protein rich extruded products from tef , corn and soy protein isolate blends. Ethiop. J. Appl. Sci. Technol 2, 75–90.

Forsido, S.F., Rupasinghe, H.P.V., Astatkie, T., 2013. Antioxidant capacity, total phenolics and nutritional content in selected ethiopian staple food ingredients. Int. J. Food Sci. Nutr. 7486, 1–6.

Fufa, B., Bhute, B., Simons, R., Tareke, B., 2011. Strengthening the Tef Value Chain in Ethiopia. Ethiopian Agricultural Transformation Agency.

Gallagher, E., 2009. Gluten-free Food Science and Technology. Blackwell Publishing Ltd., Iowa.

Ganzle, M.G., Loponen, J., Gobbetti, M., 2008. Proteolysis in sourdough fermentations: mechanism and potential for improved bread quality. Trends Food Sci. Technol. 19, 513–521.

Gashe, A., 1985. Involvement of lactic acid bacteria in the fermentation of tef (Eragrosfis tef), an Ethiopian fermented food. J. Food Sci. 50, 800–801.

Gebrekidan, B., GebreHiwot, B., 1982. Sorghum Injera Preparations and Quality Parameters, in: Rooney, L.W., Murty, D. (Eds.), International Symposium on Sorghum Grain Quality. J. V. Mertin, Patansheru, India, pp. 55–66.

Gebremariam, M.M., Zarnkow, M., Becker, T., 2012. Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review. J. Food Sci. Technol. 1–15.

Getenet, G., 2011. Heat Transfer Analysis during the Process of Injera Baking by Finite Element Method. MSc thesis, Addis Ababa University.

Girma, M., Gashe, B.A., Lakew, B., 1989. The effect of fermentation on the growth and survival of Salmonella typhimurium , Staphylococcus aureus , Bacillus cereus and Pseudomonas aeruginosa in fermenting tef (Eragrostis tef). M1RCEN Journal, 5, 61–66.

Girma, T., Bultosa, G., Bussa, N., 2013. Effect of grain tef [Eragrostis tef (Zucc.) Trotter] flour substitution with flaxseed on quality and functionality of injera. Int. J. Food Sci. Technol. 48, 350–356.

Goesaert, H., Gebruers, K., Courtin, C.M., Brijs, K., Delcour, J.A., 2006. Enzymes in breadmaking, in: Hui, Y.H. (Ed.), Bakery Products: Science and Technology. Blackwell Publishing Limited, Ames, Iowa, pp. 337–364.

Hamada, S., Suzuki, K., Aoki, N., Suzuki, Y., 2013. Improvements in the qualities of gluten-free bread after using a protease obtained from Aspergillus oryzae. J. Cereal Sci. 57, 91–97.

Haslam, E., 1974. Polyphenol-proetin interactions. Biochem.J. 139, 285–288.

Hopman, E., Dekking, L., Blokland, M.-L., Wuisman, M., Zuijderduin, W., Koning, F., Schweizer, J., 2008. Tef in the diet of celiac patients in The Netherlands. Scand. J. Gastroenterol. 43, 277–82.

Hung, P.V., Yamamori, M., Morita, N., 2005. Formation of enzyme-resistant starch in bread as affaceted by high-amylase wheat flour substitution. Cereal Chem. 82, 690–694.

Izydorczyk, M.S., Hussain, A., Macgregor, A.W., 2001. Effect of barley and barley components on rheological properties of wheat dough. J. Cereal Sci. 34, 251–260.

Jansen, G.R., DiMaio, L.R., Hause, N.L., 1962. Amino acid composition and lysine supplementation of teff. J Agric Food Chem 10, 62–64.

Kaletunc, G., Breslauer, K.J., 1993. Glass transition of extrudates - relationship with processing-induced fragmentation and end-product attributes. Cereal Chem. 70, 548–552.

Kamal-Eldin, A., Chiwona-Karltun, L., 2008. Products from traditional grains in Africa, in: Proceedings from the Second MISTRA Workshop on African Traditional Products for Health. SIK, Goteborg 23-24 September.

Keiffer, R., 2006. The Role of Gluten Elasticity in the Baking Quality of Wheat, in: Popper, L., Schäfer, W., Freund, W. (Eds.), Future of Flour-A Compendium of Flour Improvement. Verlag Agrimedia, pp. 169–178.

Kheterpaul, N., Chauhan, B.M., 1991. Effect of natural fermentation on phytate and polyphenolic content and in-vitro digestibility of starch and protein of pearl millet (Pennisetum typhoideum). J. Sci. Food Agric. 55, 189–195.

Kim, S., Willett, J.L., Carriere, C.J., Felker, F.C., 2002. Shear-thickening and shear-induced pattern formation in starch solutions. Carbohydr. Polym. 47, 347–356.

Lakshminarayan, S.M., Rathinam, V., KrishnaRau, L., 2006. Effect of maltodextrin and emulsifiers on the viscosity of cake batter and on the quality of cakes. J. Sci. Food Agric. 86, 706–712.

Lasekan, O., Khalil, S.K., 2010. The significance of glass transition temperature in processing of selected fried food products : a review. Mod. Appl. Sci. 4, 3–21.

Lawton, J.W., 1992. Viscoelasticity of zein-starch doughs. Cereal Chem. 69, 351–355.

Loponen, J., Mikola, M., Katina, K., Sontag-Strohm, T., Salovaara, H., 2004. Wheat dough fermentations. Cereal Chem. 81, 87–93.

Maloney, D., Foy, J., 2003. Yeast Fermentations, in: Kulp, K., Lorenz, K. (Eds.), Handbook of Dough Fermentations. Marcel Dekker, Inc., New York, pp. 43–60.

Marco, C., Rosell, C.M., 2008. Breadmaking performance of protein enriched, gluten-free breads. Eur. Food Res. Technol. 227, 1205–1213.

McManus, J.P., Davis, K.G., Lilley, T.H., Haslam, E., 1981. The association of proteins with polyphenols. J. Chem. Soc. Chem. Commun. 309b.

Mehta, B.M., Kamal-Eldin, A., Iwanski, R.Z., 2012. Fermentation: Effects on Food properties. CRC Press, Boca Raton, Florida.

Moore, M.M., Heinbockel, M., Dockery, P., Ulmer, H.M., Arendt, E.K., 2006. Network formation in gluten-free bread with application of transglutaminase. Cereal Chem. 83, 28–36.

Moroni, A. V., Bello, F.D., Zannini, E., Arendt, E.K., 2011. Impact of sourdough on buckwheat flour, batter and bread: Biochemical, rheological and textural insights. J. Cereal Sci. 54, 195–202.

Mukprasirt, A., Herald, T.J., Flores, R., 2000. Rheological characterization of rice flour-based batters food engineering and physical properties. J. Food Prot. 65, 1194–1199.

Mullineux, G., 2008. Non-linear least squares fitting of coefficients in the Herschel–Bulkley model. Appl. Math. Model. 32, 2538–2551.

Niranjan, K., Silva, S.F.J., 2008. Food Materials Science Principles and Practice. Springer Science+ Business Media, LLC, New York.

Oliver, G., Thacker, D., Wheeler, R.J., 1995. Semi-sweet biscuits : 1 . the influence of sodium performance. J Sci Food Agric 69, 141–150.

Oyewole, O.B., 1997. Lactic fermented foods in Africa and their benefits. Food Control 8, 289–297.

Parker, M., Umeta, M., Faulks, R.M., 1989. The Contribution of flour components to the structure of injera, an Ethiopian fermented bread made from tef (Eragrostis tef). J. Cereal Sci. 10, 93–104.

Petrofsky, K.E., Hoseney, R.C., 1995. Rheological properties of dough made with starch and gluten from several cereal sources. Cereal Chem. 72, 53–58.

Pyle, D.L., 2005. Crumpet structures-experimental and modelling studies. Food Bioprod. Process. 83, 81–88.

Qarooni, J., Woottonb, M., Mcmaster, G., 1989. Factors affecting the quality of Arabic bread - Additional Ingredients. J Sci Food Agric 48, 235–244.

Ramachandran, K., Bolodia, G., 1984. The effect of fermentation on the iron, phosphorous and zinc content of tef (Eragrostis tef). Ethiop. Med. J. 22, 45–48.

Renzetti, S., Arendt, E.K., 2009. Effects of oxidase and protease treatments on the breadmaking functionality of a range of gluten-free flours. Eur Food Res Technol 229, 307–317.

Renzetti, S., Arendt, E.K., 2009. Effect of protease treatment on the baking quality of brown rice bread: From textural and rheological properties to biochemistry and microstructure. J. Cereal Sci. 50, 22–28.

Roos, Y.H., 2010. Glass transition temperature and its relevance in food processing. Annu. Rev. Food Sci. Technol. 1, 469–96.

Rosell, C.M., Aja, S., Bean, S., Lookhart, G., 2002. Effect of Aelia spp and Eurygaster spp damage on wheat proteins. Cereal Chem. 79, 801–805.

Rühmkorf, C., Jungkunz, S., Wagner, M., Vogel, R.F., 2012. Optimization of homoexopolysaccharide formation by lactobacilli in gluten-free sourdoughs. Food Microbiol. 32, 286–94.

Russ, J.C., 2005. Image analysis of food microstructure. CRC Press LLC, Boca Raton, Florida.

Sahi, S.S., 1999. Influence of aeration and emulsifiers on cake batter rheology and textural properties of cakes, in: Campbell, G.M., Webb, C., Pandiella, S.S., Niranjan, K. (Eds.), Bubbles in Food. American Association of Cereal Chemists, St Paul, Minnesota, USA, pp. 263–271.

Sahlin, P., 1999. Fermentation as a method of food processing in fermenting cereals. Licentiate thesis, Lund Institute of Technology, Lund University, Sweden.

Salmenkallio-Marttila, M., Katina, K., Autio, K., 2001. Effects of bran fermentation on quality and microstructure of high-fiber wheat bread. Cereal Chem. 78, 429–435.

Schimz, K., 1980. The effect of sulfite on the yeast Saccharomyces cerevisiae. Arch. Microbiol. 125, 89–95.

Schober, T.J., Bean, S.R., Boyle, D.L., 2007. Gluten-free sorghum bread improved by sourdough fermentation: biochemical , rheological, and microstructural background. J. Agric. Food Chem. 55, 5137–5146.

Sciarini, L.S., Ribotta, P.D., León, A.E., Pérez, G.T., 2008. Influence of gluten-free flours and their mixtures on batter properties and bread quality. Food Bioprocess Technol. 3, 577–585.

Shelke, K., C., H., Faubion, J.M., Curran, S.P., 1992. Age-related changes in the properties of batters made from flour milled from freshly harvested soft wheat. Cereal Chem. 69, 145–147.

Shewry, R.P., Tatham, A.S., Barro, F., Barcelo, P., Lazzeri, P., 1995. Biotechnology of breadmaking: unraveling and Mmnipulating the multi-protein gluten complex. Biotechnology 13, 1185–1190.

Spaenij-Dekking, L., Kooy-Winkelaar, Y., Koning, F., 2005. The Ethiopian cereal tef in celiac disease. N. Engl. J. Med. 353, 1748–9.

Spicher, G., Nierle, W., 1988. Proteolytic activity of sourdough bacteria. Appl. Microbiol. Biotechnol. 28, 487–492.

Stanley, D.W., Baker, K.W., 2002. A Simple Laboratory Exercise in Food Structure/Texture Relationships Using a Flatbed Scanner. J. Food Sci. Educ. 1, 6–9.

Steinkraus, K.H., 1996. Handbook of indigenous fermented foods, second. ed, Handbook of indigenous fermented foods. Marcel Dekker, New York.

Stewart, R.B., Getachew, A., 1962. Investigations of the nature of injera. Econ. Bot. 16, 127–130.

Stolz, P., 2003. Biological fundamentals of yeast and lactobacilli fermentation in bread dough, in: Kulp, K., Lorenz, K. (Eds.), Handbook of Dough Fermentations. Marcel Dekker, Inc., New York.

Taylor, J.R.N., Emmambux, M.N., 2008. Gluten-free foods and beverages from millets, in: Arendt, E.K., Bello, F.D. (Eds.), Gluten-Free Cereal Products and Beverages. Academic Press, Burlington, Masachusettes, pp. 119–148.

Thiele, C., Ganzle, M.G., Vogel, R.F., 2002. Contribution of sourdough lactobacilli, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread flavour. Cereal Chem. 79, 45–51.

Tsegay, M., 2011. Design and manufacture of laboratory model for solar powered injera baking Oven. MSc thesis, Addis Ababa Institute of Technology.

Umeta, M., Faulks, R.M., 1988. The effect of fermentation on carbohydrates in tef (Eragrostis tef). Food Chem. 27, 181–189.

Umeta, M., Parker, M., 1996. Microscopic studies of the major macro-components of seeds, dough and injera from tef (Eragrostis tef). SINET Ethiop. J. Sci. 19, 141–148.

Umeta, M., West, C.E., Fufa, H., 2005. Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. J. Food Compos. Anal. 18, 803–817.

Urga, K., Narasimha, H.V., 1997. Effect of natural fermentation on HCL-extractability of minerals from tef (Eragrostis tef). Bull. Chem. Soc. Ethiop. 11, 3–10.

Valjakka, T., Kerojoki, H., Katina, K., 2003. Sourdough bread in Finland and Eastern Europe, in: Kulp, K., Lorenz, K. (Eds.), Handbook of Dough Fermentations. Marcel Dekker, New York, pp. 269–303.

Vallons, K.J.R., Ryan, L. a. M., Arendt, E.K., 2011. Promoting structure formation by high pressure in gluten-free flours. LWT - Food Sci. Technol. 44, 1672–1680.

Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46, 4113–4117.

Wang, F.C., Sun, X.S., 2002. Frequency dependence of viscoelastic properties of bread crumb and relation to bread staling. Cereal Chem. 79, 108–114.

Wehrle, K., Arendt, E.K., 1998. Rheological changes in wheat sourdough during controlled and spontaneous fermentation. Cereal Chem. 75, 882–886.

Welti-Chanes, J., Vergara-Balderas, F., Perez, E., Bermudex, D., Valdex-Fragose, A., Mujica-Paz, H., 2008. Phase transitions and hygroscopicity in chewing gum manufacture, in: Gutierrez-Lopez, G.F., Barbosa-Canovas, G.V., Welti-Chanes, J., Para-Arias, E. (Eds.), Food Engineering: Integrated Approaches. Springer, New York, pp. 139–153.

Wyman, C.E., Decker, S.R., Himmel, M.E., Brady, J.W., Skopec, C.E., 2005. Hydrolysis of Cellulose and Hemicellulose, in: Demetriu, S. (Ed.), Polysaccharides: Structural Diversity and Functional Versatility. Marcel Dekker, New York, pp. 1–39.

Xue, J., Ngadi, M., 2006. Rheological properties of batter systems formulated using different flour combinations. J. Food Eng. 77, 334–341.

Yetneberk, S., 2004. Sorghum injera quality improvement through processing and development of cultivar selection criteria. PhD thesis, University of Pretoria, South Africa.

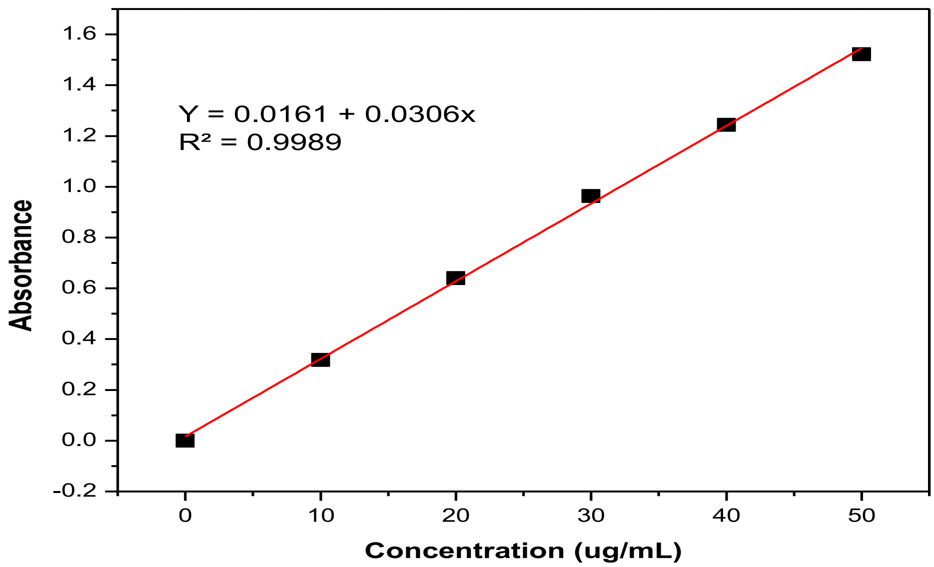
Yetneberk, S., de Kock, H.L., Rooney, L.W., Taylor, J.R.N., 2004. Effects of sorghum cultivar on injera quality. Cereal Chem. 81, 314–321.

Yetneberk, S., Rooney, L.W., Taylor, J.R., 2005. Improving the quality of sorghum injera by decortication and compositing with tef. J. Sci. Food Agric. 85, 1252–1258.

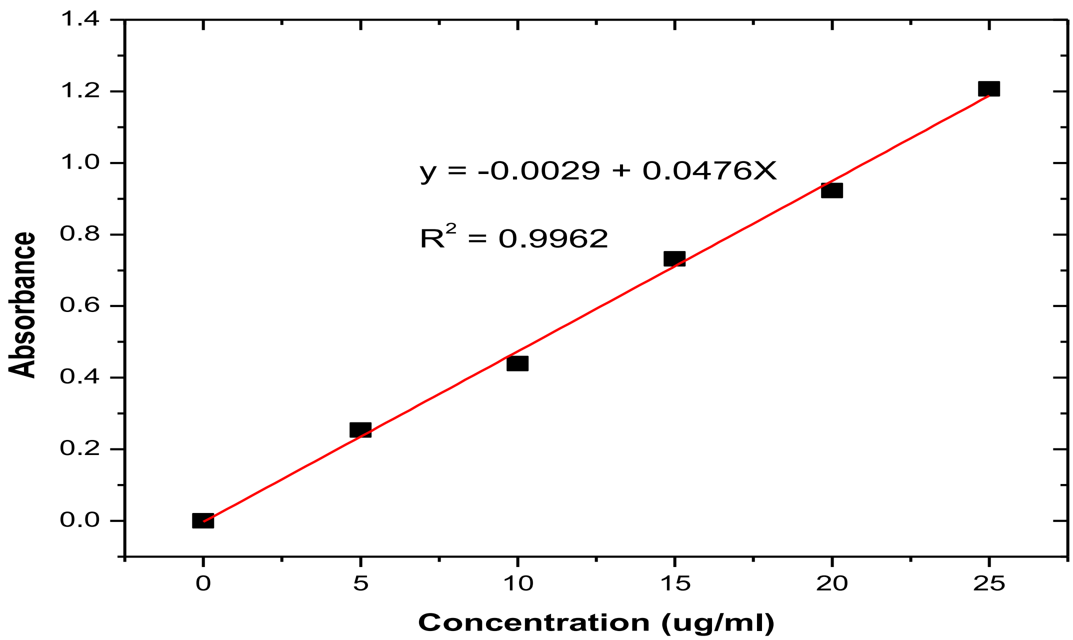
Zannini, E., Jones, J.M., Renzetti, S., Arendt, E.K., 2012. Functional replacements for gluten. Annu. Rev. Food Sci. Technol. 3, 227–45.

Zegeye, A., 1997. Acceptability of injera with stewed chicken. Food Qual. Prefer. 8, 293–295.

APPENDIX A: Glucose Standard Curve

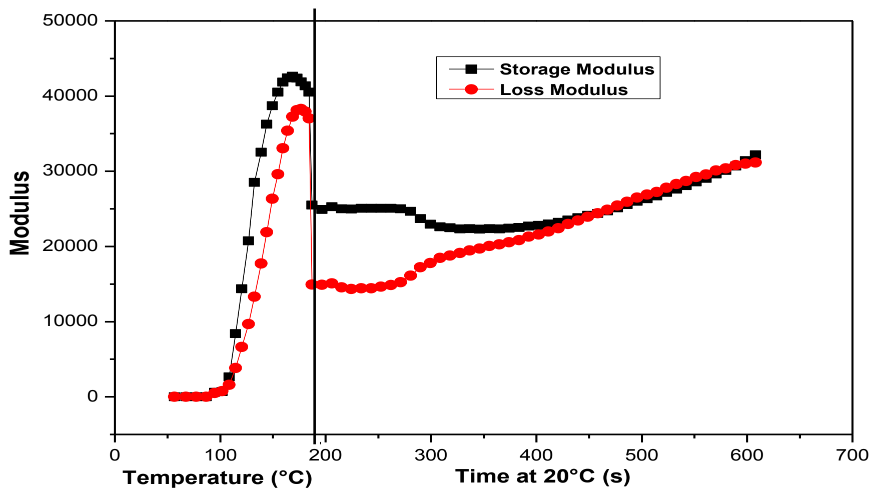


APPENDIX B: Gallic Acid Standard Curve



APPENDIX C- Effect of temperature and time (at 20°C) on storage and loss modulus of tef batter with and without SMBS

1. Tef batter with SMBS



2. Tef batter without SMBS