



PROCESS OPTIMIZATION AND QUALITY CHARACTERIZATION OF TEF FLOUR FOR THE INDUSTRIAL MANUFACTURING OF INJERA

A Dissertation

By

YOSEPH LEGESSE ASSEFA

Submitted to the school of Chemical and Bio Engineering in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY (FOOD ENGINEERING STREAM)

Advisors: Dr.Eng. Shimelis Admassu (Associate professor)
Professor Felicidad Ronda
Dr.Workineh Abebea (Senior Researcher)

Addis Ababa Institute of Technology, Addis Ababa University
Addis Ababa, Ethiopia
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Abbreviations

AACC	American Association of Cereal Chemists
a	Exponent from fitting power law to G' data from a frequency sweep
a*	Chromatic color coordinate
b	Exponent from fitting power law to G'' data from a frequency sweep
b*	Chromatic color coordinate
BD	Bulck density
BM	Blade mill
BMF	Flour from blade mill
BMI	Injera from blade milled flour
BV	Breakdown viscosity
c	Exponent from fitting power law to $\tan \delta$ data from a frequency sweep
C*	Chroma of the color
DM	Disk mill
DMF	Flour from disk mill
DMI	Injera from disk milled flour
FC	Foaming capacity
FS	Foam stability
FV	Final viscosity
G1'	Elastic modulus
G1''	Viscous modulus
h	Hue of the color
HM	Hammer mill

HMF	Flour from hammer mill
HMI	Injera from hammer milled flour
L*	Luminosity color coordinate
OAC	Oil absorption capacity
PT	Pasting temperature
PV	Peak viscosity
RAG	Rapidly available glucose
RDS	Rapidly digestible starch
RS	Resistant starch
SDRI	Starch digestion rate index
SDS	Slowly digestible starch
SEM	Scanning electron microscope
SV	Set back viscosity
(tan δ)1	Loss tangent
TS	Total starch
TV	Trough viscosity
WAC	Water absorption capacity
WAI	Water absorption index
WSI	Water solubility index

Abstract

Tef [*Eragrostis tef* (Zucc.)Trotter] is an Ethiopian indigenous tropical cereal crop and it has been cultivated for many years in Ethiopian highlands. Tef injera making in industry scale holds a significant economic and social interest but requires a thorough study of how the process variables affect the product quality. Tef is the main staple in the country mostly used to make *injera*. *Injera* is Ethiopian flat bread which is mostly made from tef flour. The general objectives of this thesis was optimizing injera making process (milling, kneading and Absit or scolded dough preparation) and identify the quality characteristics (tef flour and dough which includes flour functional and textural properties, dough rheological properties, injera sensorial quality and starch digestibility of injera) of tef flour, dough and injera for industrial manufacturing by 1) investigating the effects of mill type (hammer, disc, and blade), kneading conditions and injera absit preparation on injera sensorial quality; 2) assessing the effect of mill type and kneading conditions on starch digestibility, tef dough fermentation kinetics and phytate to mineral molar ratio;3) studying tef flour characteristics and its dough rheological properties as affected by mill type; and 4) introducing software based inhere quality evaluation technique. Standard methods were adopted for all analysis and determinations. Injera made with disc mill flour had higher overall sensorial acceptability (6.6) than that obtained from hammer mill (4.2) and blade mill (4.1) flours. The injera made with blade mill flour obtained the lowest rapidly available glucose (52.6) and rapidly digestible starch (47.3). Based on its higher injera overall acceptability and moderate starch digestibility, DM flour was preferred.

Tef flour from blade mill had contained higher BD (0.77 g/mL) and FC (12.67 mL). On the other hand, flours from disk mill had higher WSI (7.94 g/100g) while lower was for hammer mill (4.17 g/100g). A lower value (64%) of dispersibility was recorded on flour from disk mill. The highest

hardness (2.09 N) and gumminess (0.75N) was recorded tef gels from blade mill. Tef flour prepared from hammer mill showed significantly lower values of G'_1 and G''_2 and higher values of $\tan \delta$ than other mill type flours. Tef flours from disk mill had significantly higher peak and breakdown viscosity but lower trough viscosity values. Tef dough stickiness and forward extrusion was significantly ($p < 0.05$) affected by mill types. Tef flour from disk mill yielded with the highest percentage of WSI indicates suitability to produce higher quality tef dough for injera making than other mill types.

Changes in kneading conditions (time/speed) showed important variation on injera RAG and RDS content. Flavonoid, total phenolic content and phytate were also significantly varied at different kneading time-speed combinations. Injera sensory quality was also significantly varied due to the changes in kneading conditions. Tef dough kneading method was optimized at kneading speed of 6 (800 rpm) with 3 minutes of kneading time which has higher desirability to give 8-9 point hedonic scale of injera overall acceptability. In addition to kneading conditions, absit preparation method (water to fermented dough ratio) was found to affect the quality of tef injera. Absit preparation method was also optimized with a combination of 100 ml of batter to 900 ml of water to gives the higher desirability in terms of injera overall acceptability.

The effect of mill type and kneading speed-time combinations on fermentation kinetics and phytate/mineral molar ratio were significant. The outcome of introducing software for the determinations of injera number of eyes was found effective; its result difference to human eye determination was insignificant.

In general, transformation of traditional injera making process to industry level requires an intense investigation on mill type, dough kneading conditions and absit preparation methods, as these significantly affects tef flour characteristics, dough rheological properties and injera quality. On the other hand, software based injera quality evaluation is an important aspect to be considered in injera making industries to assess product quality and improve production yield.

Chapter 1-*Introduction*

Introduction

1.1 Background

Tef [*Eragrostis tef*] is an Ethiopian indigenous tropical cereal crop and it has been cultivated for many years in Ethiopian highlands. It is the main staple in the country mostly used to make injera, traditional fermented flat bread. The whole tef grain is ground to flour for making injera, local beverage, porridges and soup and sweet unleavened bread (Bultosa & Taylor, 2004). According to central statistical agency of Ethiopia (2014/15), from the total grain crop area (12,558,444.55 hectares), 80.78% (10,144,252.30 hectares) was under cereals. Tef took up 24.02% (about 3,016,053.75 hectares) of the grain crop area. As to production, tef made up 17.57% (47,506,572.79 quintals) of the grain production. Tef is also cultivated outside Ethiopia mainly as forage for livestock feed in countries like South Africa, Australia and United States (Baye, 2014). The sizes of the seed are very small, ranging from 0.6–1mm diameter and 1–1.7mm long with 1000 seed weight averaging 0.3–0.4 grams and 150 grains of tef has comparable weight with almost one seed of wheat (Dijkstra et al., 2008). Seeds of the tef plant are very minute in size, perhaps the smallest among cereals, and the existence of genotype differences is equivocal (Assefa et al., 2001).

Products of tef grain are nutritionally well packed because they are always consumed as whole grain with high content of carbohydrate and fiber (USDA, 2007), with more iron, zinc and calcium than other cereal grains, including sorghum, wheat and barley (Abebe et al., 2007). Due to the absence of gluten and gluten-like proteins, tef has recently been receiving global attention, particularly as a “healthy food”, making it right for celiac disease patients (Spaenij-Dekking et

al., 2005), and also because of other dietary benefits such as slow-release of carbohydrate constituents useful for diabetic patients (Abebe & Ronda, 2014).

In Ethiopia, there are three main categories of tef: white, red and mixed. In general, white tef grows only in the highlands and it needs relatively good growing conditions. The higher consumer preference for white tef, may answer why this tef type is the most expensive. However, in recent years, red tef, believed to be highly nutritious, is also getting popularity between health conscious consumers (Baye, 2014).

Tef grain has complex carbohydrates which make up to 80 percent, approximately with 73 percent of starch content. In the small intestine, the extent to which carbohydrate is digested and absorbed and the speed at which this digestion takes place affects a person's blood glucose level. Tef provides recognizable health benefits like a slowly digestible starch, (Baye, 2014). The dietary fiber content of tef is several folds higher than that of maize, sorghum, wheat and rice. The reasons for this may be that whole grains contain higher fiber content than decorticated ones and those small grains, like tef, contain a relatively high proportion of fiber bran (Bultosa, 2007). Compared to the common cereals like rice, wheat and maize, less is known about tef. Besides, technological limitations in processing tef, has long limited its further widespread consumption outside Ethiopia (Baye, 2014). Consequently, additional researches on the processing qualities and nutritional composition of tef have been done and development of the new tef-based products has accelerated (Baye, 2014).

Though injera is a major food for majority of Ethiopians, its preparation takes several days as it involves a longer fermentation process and the process in general is not well standardized yet.

Preparations of traditional fermented foods are usually time consuming and made under primitive conditions which lead to inconsistent quality (Achi, 2013). Tef injera making process

can be categorized in this group which led people to buy homemade injera from shops rather than baking at home (Abiyu et al., 2013). But, injera from different shops were reported with its inconsistent quality and lack uniformity. According to Hamaker (2007) as industrialization expands and civilization achieved, people will be busy and experience time constraints to cook foods with longer process. Due to these, they prefer to use semi processed or processed foods from different industries. Because of the changes in life style in urban areas of the country, these interests are growing in Ethiopia. However, baking injera in industry level is not well established.

In addition, it is noted that injera processing firms making injera for export markets are emerging. Beside the contribution on the minimization of postharvest loss, such value addition is being encouraged by the Ethiopian government as it contributes for income and job creation as well as foreign currency earning (Bekabil et al., 2011). However, it was seen that injera baking firms are using traditional injera baking method. Beside its time consuming nature, traditional injera baking method has no identified quality control points and parameters throughout the process leading to complaints by their customers on inconsistent quality and lack of uniformity. On the other hand Ethiopian standard agency has developed injera standard which focused on physical parameters of injera quality which includes size and weight of the tef injera. However, nutritional and sensorial specifications of injera quality are still lacking from the standard.

Transformation of traditional food processing into a modern way of processing technologies is highly required to standardize the quality of the end products and to save time and energy without affecting their desirable properties (Nout, 1992). Accordingly, transformation of injera making process into industrial level of processing is mandatory to guarantee efficiency, and high product quality and uniformity.

Nowadays, a number of studies are published on different features of tef which include its techno-functional characteristics, nutritional and health benefits, different aspects of injera batter fermentation and product formulation (Gebremariam et al., 2012; Baye, 2014). However, engineering processing aspects of injera making process that optimize tef milling, kneading, batter preparation with fermentation and baking methods helping the process optimization for modernization are not addressed yet. Therefore, research and studies are mandatory in food engineering sector to address the gap.

Research question in this study was “is injera processing which includes milling, kneading and absit preparation affects the final quality of injera or not” and it was hypothesized that, injera quality is significantly influenced by those milling, kneading and absit preparation differences.

1.2 Objectives of the thesis

The general objectives of this thesis was optimizing injera making process (milling, kneading and Absit or scolded dough preparation) and identify the quality characteristics of tef flour for industrial manufacturing of injera. In order to accomplish the general goal, the following five specific objectives were developed:

- 1) Determine the influence of mill type on tef injera quality and introduce software-based injera quality evaluation technique.
- 2) Characterize the textural and rheological properties of dough and the functional properties of tef flour as affected by milling type.
- 3) Optimize mechanical tef injera dough preparation through studying the effect of kneading time, beater speed on tef injera quality.
- 4) Investigate the effect of ‘*absit*’ preparation difference on tef *injera* quality and optimize dough to water ratio.

- 5) Assess the influence of mill type and mechanical kneading conditions on fermentation kinetics of tef dough during injera making process; and phytate to mineral molar ratio of tef injera.

1.3 Thesis organization

This PhD thesis is organized as follows:

Chapter two presents the literature review that covers a brief description about tef grain, its nutritional facts, phytochemicals and starch digestibility, which is followed by a presentation of the health benefits of tef based products. Tef injera processing, and engineering properties of tef flour are discussed. Finally, brief descriptions on quality characteristics of tef flour are presented.

Chapter three gives a description about sample variety and the experimental designs used.

Chapter four presents the findings from the investigation of influence of mill type on tef injera quality and the application of software on injera quality evaluation. The paper published in *Food Chemistry*, 2018, and presents the effect of mill type on flour particle size and damaged starch level which influence sensorial injera quality, and injera starch digestibility. The findings from the study on the application of software to evaluate injera number of eyes, eye size and eye distribution is presented and discussed.

Chapter five presents the result from the study of the characterization of rheological and textural properties of dough and the functional properties of tef flour as affected by milling type. The paper submitted in *Journal of Food Process Engineering*, 2018.

Chapter six presents the results from the investigation of the effect of kneading time, and beater speed on tef injera quality during mechanical kneading. The result from the investigation of how ‘absit’ preparation difference can influence tef injera sensorial quality is also presented and

discussed with a brief introduction. Finally, Kneading and absit preparation method optimized. The paper is published in African Journal of Food Science in 2018.

Chapter seven presents the results from the investigation of the influence of mill type and mechanical kneading conditions on fermentation kinetics of tef dough during injera making process. The results from the study of the influence of mill type and mechanical kneading conditions on mineral to phytate molar ratio of tef injera are presented and discussed with a brief introduction. The paper is published in Research and Reviews: Journal of Food Science and Technology in 2018.

Chapter eight gives a general conclusion on the findings of the study.

Chapter 2-*Literature Review*

Chapter 2

Literature Review

2.1 Structure of tef grain

Tef seeds are oval in shape. The cross-sectional view of the grains shows that the germ is larger in ratio to the rest of the kernel (McDonough and Rooney 2000; Umeta and Parker 1996; Parker et al., 1989) compared to other cereal grains. The thin outer layer, pericarp, forms a bran, which is a protection of the seed. In the red and brown (mixed) varieties of tef, the inner surface of bran has pigmented material with higher polyphenols and tannins similar to those in finger millet and sorghum (McDonough and Rooney 2000; Umeta and Parker 1996). The endosperm represents the largest proportion of the tef seed, with inconsistently distributed protein bodies mainly in the outer part and starch granules originate in the central part of the endosperm (McDonough and Rooney 1985). The starch granules found in tef are 2-6 μm in diameter and smooth and polygonal in shape, are the biggest proportion of the carbohydrate fraction in tef (Bultosa et al., 2002). The granule size is comparable to oat (Zheng and Sosulski, 1997), rice (Juliano, 1992), buckwheat (Zheng and Sosulski, 1997) and small millet (Kumari and Thayumanavan, 1998) starch granules. The tef starch granule is smaller compared to rye (Jane et al., 1994), barley (Tang et al., 2001) and wheat (Evers, 1973) starch granules, but is slightly larger than the starch granules of quinoa (Zheng and Sosulski, 1997) and amaranth (Jane et al., 1994).

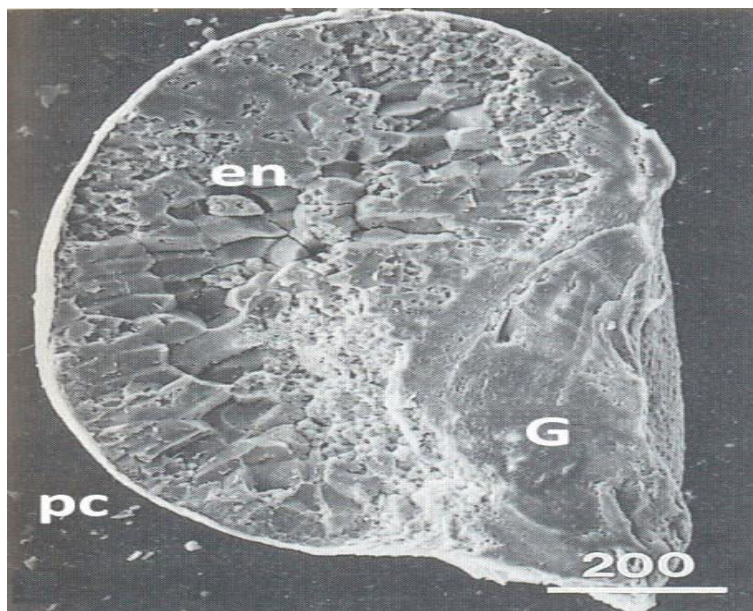


Figure 2.1 Cross-sectional view of tef grain showing endosperm (en), germ (G) and pericarp (pc) X200 (Parker et al., 1989).

2.2 Nutritional compositions of tef

Although tef is one of the preferred cereal crops for wider health consumption, but because of the lack of familiarity by consumers and limited interests in tef causes the Ethiopians to think for centuries that their crop is lesser in quality (Yetneberk, Rooney and Taylor, 2005). On the other hand, for the last ten years the discovery of the gluten-free property of tef grain has encouraged researchers in nutrition, agronomy, breeder, and food science to exert more endeavors to improve the historically neglected crop. Consequently, numerous studies have been made on the post harvest values and composition of tef nutrition. Now days, the development of new tef-based food products have accelerated outside Ethiopia initially in Denmark (Baye, 2014).

The nutritional value of tef compares with some of the main staple crops and in fact, it is superior to some of these cereal crops in mineral content especially copper, zinc, and manganese (Ketema, 1993). The nutritional profile of tef showed the highest amount of protein compared to

usually consumed staples in Ethiopia and its calorie status is solely exceeded by maize. It is considered to contain excellent amino acid contents, and it also said to have higher lysine levels than barley and wheat and a little lower than oats and rice (Stallknecht, 1997).

Carbohydrates

Eighty percent of the tef grain is complex carbohydrates (Baye, 2014). It contains approximately 73 percent of starch making tef a starchy cereal (Baye, 2014). Bultosa (2007) reported that amylose content of 13 tef varieties tested ranged from 20 to 26 percent which is comparable to other cereals such as sorghum.

In general, the level of carbohydrate, which is digested and absorbed in the small intestine, determines its health effect. Rapidly digested and absorbed carbohydrates (glycemic carbohydrates) have a greater effect on blood glucose levels, as they result to greater metabolic perturbation (Lafiandra et al., 2014). Such perturbations have been related to metabolic diseases such as cardiovascular diseases and type-2 diabetes (Ludwig, 2002). Hence, from a health point of view, slow digesting carbohydrates are chosen over rapidly digesting ones.

Harris and Geor (2009) said that the rate of carbohydrate digestion of a food can be characterized by its glycemic index (GI). The GI of a food depends on endogenous factors of the food matrix such as protein and lipid content, starch susceptibility to α -amylase and the macroscopic structure of the food (Fardet et al., 2006). Starch susceptibility to α -amylase is in turn determined by its structure, crystal structure, encapsulation, degree of gelatinization, the proportion of damaged granules and the retro gradational properties of starch granules (Fardet et al., 2006). By using a scanning electron microscope (SEM), the size of tef starch was found to be 2-6 μm (Wolter et al., 2013; Bultosa et al., 2002). This makes tef starch granules smaller than those of

sorghum (20 μm), maize (20 μm) and wheat (A type 20-35 μm) (Delcour et al., 2010). Given their large surface area, enzymatic attack is more susceptible on smaller starch granules (Tester et al., 2004). Nonetheless, the in vitro starch digestibility of tef was found to be considerably lower in comparison to wheat, which has larger starch granules (Wolter et al., 2013). In line with this, the predicted glycemic index of tef (74) was considerably lower than that of white wheat (100) but comparable to that of oats (71) and sorghum (72) (Wolter et al., 2013).

This somewhat lower GI for tef than expected may be justified by its lower starch damage, amylose content, and the possible formation of amylose-lipid complexes which can hinder enzymatic access and thus starch digestibility (Singh, et al., 2010). Moreover, the high (68-80 °C) gelatinization temperature of tef (Wolter et al., 2013; Bultosa, 2007) has the capacity to hinder gelatinization and decrease enzymatic attack susceptibility by α -amylase (Fardet et al., 2006).

Protein

On average, the crude protein content of tef is in the range of 8 to 11 percent, which is much more similar to other more common cereals such as wheat. Tef's fractional protein composition indicates that albumins (37 percent) and glutelins (45 percent) are the major protein storages, while prolamins are of small constituent (~ 12 percent) (Tatham et al., 1996; Bekele et al., 1995). In contrast, most of the recent studies reported that prolamins are the major protein storages in tef (Adebowale et al., 2011). The different methods of extraction between these studies may explain the clashing findings. Based on the examination of the amino acid profile of tef, the higher contents of leucine, glutamine, alanine, and proline and the relatively lower content of lysine further indicate that prolamins are the major storage proteins (Adebowale et al., 2011).

According to Baye (2014) the amino acid composition of tef is well-balanced. Relatively, high concentration of lysine, a major limiting amino acid in cereals grain, is found in tef. In the same way, compared to other cereals grain, higher contents of tyrosine, isoleucine, valine, threonine, leucine, methionine, arginine, alanine, phenylalanine and histidine are found in tef.

Another important aspect of tef is that it has no gluten (Hopman et al., 2008). Spaenij-Dekking, Kooy-Winkelaar, and Koning (2005) found out that the presence or absence of gluten in pepsin and trypsin digests of 14 tef varieties and the digests were analyzed for the presence of T-cell–stimulatory epitopes. In contrast to those known gluten containing cereal grains, no T-cell stimulatory epitopes were identified in the protein digests of all the tef varieties assayed, thus assuring the absence of gluten in tef. This makes tef a valuable ingredient for functional foods intended for celiac patients who are gluten intolerant.

Fat

In general, cereals are not the best source of fat; however, as they are frequently consumed in large quantities, cereals can contribute a considerable amount of essential fatty acids to the diet (Michaelsen et al., 2011). According to Simopoulos (2001) fatty acids are potentially helpful for growth, development and long-term health. Consequently, there has been higher interest in recent years in their inclusion in diets. For instance, increased intake of n-3 fatty acids (α -linoleic acid) were found to minimize biological markers associated with cardio-vascular disease, inflammatory and autoimmune diseases.

The crude fat content of tef is greater than that of rice and wheat, but smaller than maize and sorghum. Wheat, rice, and maize contain insignificant amounts of linoleic acid (LA) and only

traces of α -linoleic acid (ALA). Furthermore, these widespread cereals are consumed after decortications and further refining reduces their amount of crude fat and n-3 and n-6 polyunsaturated fatty acids. According to Baye (2014) by maintaining whole cereal grains, as in the case of tef, this provides a better source of fatty acids than refined ones. Tef grains have a high amount of unsaturated fatty acids, mainly oleic acid (32.4 percent) and linoleic acids (23.8 percent) (El-Alfy et al., 2012). Although a clear consensus has not been reached on the optimal proportion of LA and ALA fatty acids, the standards of Codex recommended for infants formula a LA:ALA ratio in the range of 5 to 15 (Koletzko et al., 2005). In this regard, the LA: ALA ratio of 7:1 for tef is considered favorable and is comparable to legumes that are good sources of fatty acids, such as soybean.

Fiber

According to the American Association of Cereal Chemists definition, dietary fiber is “edible parts of plant or analogous carbohydrates resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine” (DeVries, 2003). The recent Codex definition further added that dietary fibers should have “proven physiologic effects of benefit to health” (Cummings et al., 2009). Lowering blood glucose levels after eating, fecal bulking (laxation) and lowering plasma LDL-cholesterol are some of the physiologic effects of the fiber (Champ et al., 2003).

The crude fiber, total and soluble dietary fiber content of tef is many folds higher than that found in common cereals, maize, rice, wheat and sorghum (Baye, 2014). There may be many reasons for this. According to Bultosa (2007), first, whole grains have the larger fiber content than decorticated ones. Second, small cereal grains have comparatively high proportion of bran,

which are high in fiber. Therefore, higher dietary fiber intake and the associated health benefits are expected with increased consumption of tef.

Starch

The starch of tef has been subjected to limited study, but tef starch has been shown to have some unusual and potentially beneficiary properties. The granule size of starch is small, does not seem to have pores and is from compound granules (Bultosa et al., 2002). Tef starch also has lower peak and better than maize starch has setback viscosities and shows some resistance to breakdown during pasting (Bultosa et al., 2002).

In another study, from the total aggregate of chemicals 60% of tef is starch with 20% of rapidly digestible that convert into blood glucose within about half an hour. Whereas the remaining 50% of the starch is slowly digestible starch that changes into blood glucose within 3 to 5 hours while 30% is resistant starch, which is resistant to be converted into glucose, but it has advantage for the bacterial flora in the colon (The Netherlands, 2007). Studies showed the postprandial physiological responses to the ingestion of rapidly digestible starch and slowly digestible starch in healthy subjects and type 2 diabetics (Ells et al., 2005; Seal et al., 2003). Considerably greater and more rapid changes of blood glucose, insulin and non-esterified fatty acids concentrations were observed after consumption of rapidly digestible starch compared to slowly digestible starch. A reduction of potential risk factors for the metabolic syndrome by exchange of rapidly digestible starch by slowly digestible starch was proposed (Ells et al., 2005).

Minerals

Tef grain has a high concentration of different nutrients with very high calcium content and significant amount of the minerals iron, copper, phosphorus, magnesium, aluminum, zinc, boron,

barium, and thiamin (Stallknecht, 1997). The difference in mineral content between and within different tef varieties is wide ranging. Red tef contain higher iron and calcium content than white and mixed tef (Abebe et al., 2007). On the other hand, higher copper content than red and mixed tef were seen on white tef (Baye, 2014).

The bioavailability of zinc and iron is reduced by the tannins present in cereals (Derman et al., 1977; Gillooly et al., 1984) but the tannin content of foods can vary widely (Umeta et al., 2005). Umeta et al. (2005) reported injera prepared from tef, sorghum and maize, the tannin content was 2–3 times that of wheat injera. However, the tannin content of sorghum porridge was more than 4 times that of maize porridge, possibly because the sorghum porridge prepared from varieties richer in tannins than the sorghum injera. The sorghum samples of injera and sorghum porridge collected for the most part from different areas of the Ethiopia

Umeta et al. (2005) found 3–4-fold reduction in the molar ratio of [calcium x phytate]: [zinc] in fermented injera. For tef, the ratio was 1.3 and 0.3 in unfermented and fermented injera, respectively, while for sorghum, the ratio was 3.0 and 1.8 in unfermented and fermented injera, respectively. Phytate: zinc molar ratios >15, indicative of poor zinc bioavailability (Morris and Ellis, 1989; Turnlund et al., 1984), were found in many of the food consumed in Ethiopia, except for fermented injera (9–11) (Umeta et al., 2005).

Cereal processing is advantageous, as it can highly affect mineral bioavailability (Hurrell, 2003). Food processing mechanisms increase the activity of native enzymes (e.g. phytase), and degrading absorption inhibitors, such processing methods includes soaking, germination,

cooking and fermentation (Lestienne et al., 2005). Soaking of millet, whole maize, sorghum and rice seeds for 24 hours significantly minimized phytate content, however, only slightly decreased phytate: zinc molar ratio (Lestienne et al., 2005). The result suggests that soaking alone would not improve bioavailability of minerals; however, in combination with other treatments, it may be useful in improving bioavailability of nutrients.

Fermentation during tef injera preparation showed a reduction of phytic acid and tannin content of 75% and 55%, respectively, after 96 hours of fermentation (Urga et al., 1997). Furthermore, according to the study of Ramachandran and Bolodia (1984) fermentation of tef has resulted in the rise of the dialysable portion of iron, phosphorus and zinc content from 9%, 16% and 7%, respectively, to 24%, 60% and 43%, which may account for the enhancement of this mineral bioavailability in tef injera.

2.3 Phytochemicals in tef

For minerals to be utilized for normal metabolic functions (bioavailable), their absorption is done through the small intestine (Fairweather Tait, 2002). According to Hurrell and Egli (2010) subject/host and dietary factors affect the bioavailability of minerals. Between dietary factors, phytochemicals such as phytates and polyphenols, named major mineral absorption inhibitors and for long time, referred as ‘anti-nutritional’ factors. However, the recognition of their health beneficiary effects including anti-diabetic, anti-cancer and antioxidative behaviors (Shamsuddin, 1995) have made the term anti-nutritional factor obsolete (Schlemmer et al., 2009).

2.3.1 Phytates

Phytates are commonly found in many legumes and cereals (Schlemmer et al., 2009) it is the primary form of phosphorus storage in seeds with 60-90 percent of the total phosphorus. It also contributes up to 1.5 percent of the dry weight of cereals (Loewus, 2001; Bohn et al., 2008).

Tef has high amounts of phytate with a wide range of variability, probably due to the differences in varieties and growing conditions. Tef phytate content comparable to the values reported for wholegrain cereals by Schlemmer et al. (2009). These high values in phytate affect the absorption of zinc and iron (Hurrell and Egli, 2010). The mechanism of phytate by which it inhibits mineral absorption is due to the formation of phytate-mineral which is insoluble or peptide-mineral-phytate complexes in the gastrointestinal tract (Weaver and Kannan, 2002). Phytates also form complexes with endogenously secreted minerals such as zinc (Sandström, 1997; Manary et al., 2002) and also calcium (Morris and Ellis, 1985), which makes these minerals unavailable for re-absorption into the body.

Phytate can be degraded by endogenous phytases which can possibly be initiated by food processing techniques like soaking, fermentation, and germination and in less extent during cooking (Baye, 2014). Positively, phytates have been shown to prevent kidney stones by working as inhibitor (crystallisation) of calcium salts in biological fluids (Curhan et al., 2004). Phytates also have anti-cancer (Singh, Agarwal, and Agarwal, 2003) and glucose lowering properties (Lee et al., 2005, 2006) and this increase the value of tef based products. Fischer et al. (2014) studied phytic acid degrading lactic acid bacteria in thirteen different tef-injera fermentation and found Injera pancakes prepared with *L. buchneri* MF58 fermented tef contained substantially less pytic

acid (68%) than traditional fermentation preparation (42%) that could help to improve zinc absorption. The final molar ratios of phytic acid to iron of 4 and to zinc of 12 achieved with *L. buchneri* MF58 were decreased by ca. 50% compared to the traditional fermentation. They recommended that phytic acid levels are still relatively high and further PA degradation studied for increased iron absorption. Baye et al. (2015) investigated the effect of removing phytate (IP6), iron-binding polyphenols, and dietary fibers on iron bioaccessibility in wheat-red sorghum and tef-white sorghum flour blends used in Ethiopia to make injera through the application of exogenous enzymes. Phytase treatment resulted in IP6 reduction but iron bioaccessibility not improved. Phytase, xylanase and cellulase treatment increased iron bioaccessibility in tef-white sorghum and wheat-red sorghum flour blends, whereas phytase + polyphenol oxidase treatment only showed improvement in the tef-white sorghum blend. Although responses to enzyme treatments and iron bioaccessibility were matrix dependent, a positive effect of dietary fiber hydrolysis with xylanase and cellulase obtained, irrespective of the blend. Dietary fibers had a negative effect on iron bioaccessibility independent of phytates.

2.3.2 Polyphenols

Polyphenols are plant defense against pathogens or ultraviolet radiation (Manach et al., 2004). Polyphenols similarly protect cell from oxidative damage, positively affect the risk of diseases associated with oxidative stress (Scalbert et al., 2005). According to Baye (2013) red sorghum has high amount of total polyphenols gallic acid equivalents (GAE) per 100 g of flour, followed by barley, wheat, tef and white sorghum. However, only one tef variety, *sergegn*a was analyzed. Polyphenols can hamper iron utilization from plant-based foods (Hurrell and Egli, 2010). Consequently, reducing polyphenol amounts in predominantly plant-based diets has been encouraged (Matuschek and Svanberg, 2002). Iron binding properties of polyphenols are related

with the galloyl (tri-hydroxy-benzene) and catechol (ortho-dihydroxy benzene) functional groups. According to Brune et al. (1989) not all polyphenols have inhibitory effects. Tef galloyl content is similar with that of wheat, white sorghum, and barley. In other way, the catechol content was comparable with that of barley, greater than that of wheat and white sorghum (Baye, 2013). Kotaskova' et al. (2016) identified free phenolics in brown tef flour prepared from Combi-Star mill grinder (trans-p-coumaric, protocatechuic, ferulic and Gallic acids) and bound phenolics (ferulic and Gallic acids, quercetin and catechin) while the major free phenolics in white tef (rutin, protocatechuic and ferulic acids) and bound phenolics (ferulic acid, rutin, catechin and quercetin). Cooked tef showed very high level of in vitro organic matter digestibility (80.5–85.1%), whereas brown tef was more digestible than white tef.

The effect of fermentation on soluble and bound phenolic profiles and antioxidant potential of 0, 24, 72 and 120 h fermented injera from brown and white color 4 tef flour varieties that passed through sieve opening 1.19 mm evaluated by Shumoy et al. (2017). Results indicated that fermentation for 72 h showed the highest increase in total phenolic and antioxidant contents. Brown seed colored varieties (Zagurey and Zezew) obtained superior total phenolic and antioxidant contents compared to the white varieties (Quncho and Tseday). Chlopicka et al. (2016) investigated the effect of adding (in two different doses 15% and 30%) pseudo cereal (buckwheat, amaranth and quinoa) flour on the antioxidant properties of breads. Buckwheat based bread had the highest phenolic content (7.25 mg/g), 2-4 fold higher total flavonoids, 2.36 fold and 3.64 fold antioxidant activity by means of FRAP and DPPH respectively when compared to amaranth and quinoa added breads.

According to McDonough, Rooney, and Derna Saldivar (2000), Ferulic acid is the major constituent of phenolic acid in tef. Cinnamic, vanillic and coumaric are also important parts of the phenolic acids. These major constituents of phenolic acids in tef do not contain galloyl and catechol functional groups and thus are less possible to hamper iron absorption. According to Alaunyte et al. (2012) supplementing wheat bread with 30 percent tef flour, it was possible to increase considerably the total antioxidant capacity.

2.4 Starch digestibility

Dietary carbohydrates are the main source of plasma glucose (Alaunyte, 2013). Starchy food product, especially refined or white bread, have high glycemic index (GI) as a result of their usually porous structure and high amount of gelatinized starch (Fardet et al., 2006). Due to these, processed starches are more rapidly digested when compared to native starches. Several methods were proposed for the favorable effect of sourdough technology to minimize the glycemic response. The formation of acids during sourdough processes was shown to minimize starch hydrolysis, hence, to lower the rate of starch digestion in bread (Liljeberget al., 1995).

A wide range of methods is being used by the industry for processing various food materials. Processing result of an alteration in the food structure and also affect the nutritional characteristics of the food including starch digestibility (Anguita et al., 2006). Processing has been observed to result in an increase in the level of starch hydrolysis, with values more than 90% (at the end of incubation with pancreatine) for wheat, oats and barley (Anguita et al., 2006).

Particle size played an important role in analyzing the rate of hydrolysis (Abebe et al., 2015). Cooking made the starch much more readily available for enzymatic digestion presumably by gelatinizing it. According to Heaton et al. (1988) in vitro starch digestion by pancreatic amylase

was faster with decreasing particle size with all three cereals (oat, wheat and maize). Reduction in the size distribution of the granule results in an increase in the surface area. As a result, a process like grinding leads to a higher percentage of digestion. Anguita et al. (2006) noted that extrusion provoked a decrease in particle size compared to raw samples and affected the digestibility.

After milling of tef using two different mills Cyclotech Sample mill fitted with a 0.5 mm opening screen size and disk attrition mill, Abebe et al. (2015) found out that the disc attrition mill led to higher starch digestibility rate index and rapidly available glucose. According to Abebe et al (2015) the smaller particle size has the higher starch.

The in vitro starch digestibility of flour from the milling of cereal grains (Level 6 structure) also increases with the decrease in flour particle size (Mahasukhonthachat et al., 2010; Al-Rabadi et al., 2009). It has been postulated that the starch digestibility of flour is controlled by the diffusion of enzymes into the flour particles. The starch digestion rates were, however, different between barley and sorghum flours with similar particle sizes (Al-Rabadi et al., 2009) and between the sorghum flours from cryogenic milling and hammer milling with similar particle sizes (Mahasukhonthachat et al., 2010). In addition, the in vivo starch digestibility of wheat grains fed to pig was higher with finer particle size, and the supplementation of enzymes degrading cell-wall polysaccharides improved the starch digestibility of large particles (Kim et al., 2005). Therefore, the structures of non-starch components in grain/flour and the alterations of these structures by milling can greatly affect starch digestibility.

The impact of dough hydration level (70%, 90% and 110%) and particle size (fine and coarse) distribution of the rice flour on the gluten free bread quality and in vitro starch hydrolysis studied

by de la Hera et al. (2014). They found slowly digestible starch and resistant starch increased in the coarse flour breads. The coarse fraction complemented with a great dough hydration (90–110%) was the most suitable combination for developing rice bread when considering the bread volume and crumb texture. However, the lowest dough hydration limited starch gelatinization and hindered the in vitro starch digestibility.

2.5. Sensory properties of tef and tef incorporated products

The study by Ghebrehiwot et al. (2016) assessed acceptability of injera made of tef [*Eragrostis tef* (Zucc.) Trotter] and *Eragrostis curvula* (Schrad.) Nees (underutilized grass) flours each mixed with 0, 5, and 10% of sorghum flour produced from independently stone milled to a fine powder using junior mill and found insignificant differences in taste, texture, appearance and overall acceptability. Zegeye (1997) evaluated injera acceptability made from tef, maize, sorghum and barley for sensory panel responses with and without stewed chicken (doro-wot) by preference and difference tests, respectively. He found no significant difference between fresh sorghum and maize injera in flavor. However, tef injera preferred over other injera types. Injera from tef substituted with two flaxseed forms at 3%, 6% and 9% studied by Girma et al. (2013) results indicated that with an increase in the flaxseed substitution, most sensory acceptance increased, whereas injera eyes and color decreased and appeared superior for control (100% tef injera).

Tef grain has also been incorporated into leavened bread (Ben-Fayed et al., 2008; Mohammed et al., 2009). Tef breads, containing 10, 20 and 30% tef had significantly lower specific volumes and firmer crumb compared to white wheat bread (Ben-Fayed et al., 2008). Furthermore, only 10% tef bread had an acceptable flavor and was comparable to wheat bread, whereas breads containing higher levels of tef were judged significantly less acceptable. Similar results were

found by Mohammed et al. (2009). In their study, all tef breads (incorporation at 5, 10, 15 and 20% flour blends) had significantly lower sensory acceptability when compared to white wheat bread. Chlopicka et al. (2012) found acceptable sensory quality of breads from buckwheat added up to 15 g/ 100 g or 30 g/100 g levels than amaranth and quinoa breads.

2.6 Health benefits of tef and tef based product

Celiac disease, iron deficiency and diabetes

Globally, 0.6-1.0 percent of the population is affected by celiac disease (Gujral et al., 2012). According to the study of Dubé et al. (2005) the dominance of the disease in populations at risk of CD is as follows: 3 to 6 percent on type 1 diabetic patients, up to 20 percent in first-degree relatives, 10 to 15 percent on those with symptomatic iron-deficiency anemia (IDA), 1 to 3 percent in individuals with osteoporosis and 3 to 6 percent on those with asymptomatic IDA. Celiac disease is caused by aberrant T-cell responses to gluten and gluten-like proteins found in rye, wheat, barley and possibly oats (Arentz-Hansen et al., 2004; Vader et al., 2003). The symptoms include abdominal pain, diarrhea and disturbances in nutrient absorption caused by histological alterations of the small bowel (Alaedini and Green, 2005).

The only treatment for those with celiac disease available to date is to go after a strict gluten-free diet (Fasano et al., 2001). This in practice is difficult given the abundance of food products which have wheat or other gluten containing cereals. Corn, rice, millet and sorghum are cereals commonly used for coeliac patients (Campbell, 1982). Consequently, insufficient intakes of essential nutrients such as vitamin B12 and folate (Hallert et al., 2002), calcium, iron, and fiber have been observed in those with celiac disease (Thompson et al., 2005). Also, a higher percentage of energy intakes in such patients were noted to be from fat instead of carbohydrates.

This has a negative effect on their nutritional status (Bardella et al., 2000). Therefore, nutrient rich gluten-free alternatives are needed.

Tef has a good amount of fiber, minerals and phytochemicals. Compared to gluten-free cereals and pseudo cereals such as amaranth, quinoa, buckwheat, maize, sorghum and brown rice, tef is more nutrient-rich (Gebremariam et al., 2014; Alvarez-Jubete et al., 2010). In addition, the low glycemic index of tef may help on maintaining good glycemic control. This is very crucial given the high incidence of diabetes in those with celiac disease (Viljamaa et al., 2005).

According to Zimmermann and Hurrell (2007) iron deficiency is the most widespread micronutrient deficiency in the world, affecting more than 2 billion people. Child and maternal morbidity and mortality, and decreased immunity and work performance, Growth retardation, impaired mental and psychomotor developments are between the adverse effects of iron deficiency (Georgieff, 2011). The etiology of iron deficiency includes diseases which induce unnecessary loss or cause malabsorption of dietary iron, low intakes of bioavailable iron, or increased requirements due to physiologic status (e.g. pregnancy, infants and young children) (Pasricha et al., 2013).

Insufficient iron intake is common in low and middle income countries, specifically among pregnant women (Clark, 2008) and infants and young children (Gibson et al., 2010). Food fortification and nutritional supplements may have constituted effective strategies to prevent iron deficiency (Stoltzfus, 2011). However, these strategies have side effects, especially when applied to areas where malaria and infections are prevalent (Zimmermann et al., 2010; Sazawal et al., 2006). Therefore, optimizing iron intakes with iron-rich foods may be preferred. Tef can be a good option (Adish et al., 1999; Gebre-Medhin et al., 1976). The study of Alaunyte et al. (2012)

showed that by supplementing wheat bread with 30 percent of tef flour, the iron content of the bread was being more than doubled. By the assumption of the average daily consumption of 200g of tef-enriched bread, on the study of Alaunyte et al. (2012) it was achievable to cover between 42 and 81 percent and 72 and 138 percent of daily intake requirements for iron in women and men, respectively. This encourages the development of tef based products and increases the value and acceptability of tef.

The world incidence of diabetes is increasing and has become a main public health problem (Danaei et al., 2011). According to the study of Shaw et al. (2010) in 2010, an estimated 285 million people worldwide were diabetic, a figure seen to rise to 439 million by 2030. The health implication and socio-economic effect of this disease, particularly in low and middle-income countries, are massive. The onset and progression of diabetes can be prevented by adjusting lifestyle factors, of which diet constitutes a great part (Hu, 2011).

Numerous features of tef suggest that its consumption may prevent or control diabetes. Diets which have higher whole grains have been linked with a 20 to 30 percent reduction in the risk of developing type II diabetes (Hu, 2011). Known that tef is consumed as a whole grain, similar impact can be estimated from the consumption of tef. Although the method by which whole grains help in the prevention of type II diabetes is not evidently elucidated, it is thought to be through the synergistic effects of the essential macro and micronutrients, and phytonutrients (Jonnalagadda et al., 2011).

Between the macronutrients, the type of carbohydrate and its digestibility play an essential role in glucose levels after consumption, and hence on the risk to diabetes (Sheard et al., 2004). Comparing to wheat, tef has a low glycemic index and thus better suitable for diabetic patients

(Wolter et al., 2013). In addition, the relatively high dietary fiber amount in tef relative to other common cereals grains, can minimize fasting blood glucose levels and, thus, contribute to the prevention and management of diabetes (Post et al., 2012). The situations of impaired antioxidant status and inflammation have been linked to the development of insulin resistance and type 2 diabetes (Folli et al., 2011; Wellen and Hotamisligil, 2005). Regarding this, the high polyphenol and phytate content in tef (Abebe et al., 2007, Baye et al., 2014) and the related anti-oxidative property which is seen on tef, is likely to prevent and control diabetes (Lee et al., 2006; Munir et al., 2013).

2.7 Tef injera processing

Injera is pancake-like fermented flat bread mostly made from tef flour. It is the Ethiopian traditional common food and used with most of the meals. The typical injera is described as porous, thin, soft, round, flexible, with honeycomb-like ‘eyes’ and sour-tasting flat bread (Stewart and Getachew, 1962). Although it can be prepared from tef, sorghum, maize, barley or a combination of these, tef flour is much more preferred than any other cereals (Zegeye, 1997; Stewart and Getachew, 1962; Gifawesen and Bisrat, 1982).

Injera is prepared from batter-like dough, which is pre-fermented for 2-3 days. Fermentation is most of the time initiated spontaneously by addition of water to tef flour, allowing the naturally existing microorganisms to grow (Gashe, 1985). Primary or first stage fermentation can also be started by the addition of the starter, *Ersho*, which is a small amount of batter kept from previous dough (Parker et al., 1989). After the primary stage of fermentation, yellow liquid settles over the top of dough, which is discarded and used for the next batch as *Ersho*.

In the second stage of fermentation, some of the dough is diluted, boiled (*'absit'*) and returned to fermented dough. At this stage, gelatinization occurs due to boiling, swollen and misshapen of some starch granules, and attached to ungelatinised starch from the first stage fermentation dough (Parker et al., 1989). During fermentation stages, the main fermenting microorganisms are lactic acid bacteria (*Lactobacillus* species) (Gashe, 1985) and yeast (*Saccharomyces* species) (Gifawesen and Bisrat, 1982). These microorganisms result in the fall of pH to 4.0, gas production and dough rising and are responsible for desired final product flavor and acidity (Umeta and Faulks, 1988). Consistency of batter is seen on fermented tef dough. It is poured onto the hot oiled surface of the *metad* which is clay griddle, and baked covered with a lid to keep the steam (Alaunyte, 2013).

The result of microscopic evaluation of a baked injera structure shows that, tef starch is completely gelatinized into a matrix, with protein bodies, embedded fragments of bran layers, microorganisms, outer layers of endosperm and gas bubbles (Parker et al., 1989; Umeta and Faulks, 1988). The spongy and soft structure of tef injera is essential for its keeping qualities and acceptance and it is preferred because of the freshness, flavor and better keeping qualities than other injera types which are prepared from sorghum or maize (Zegeye, 1997).

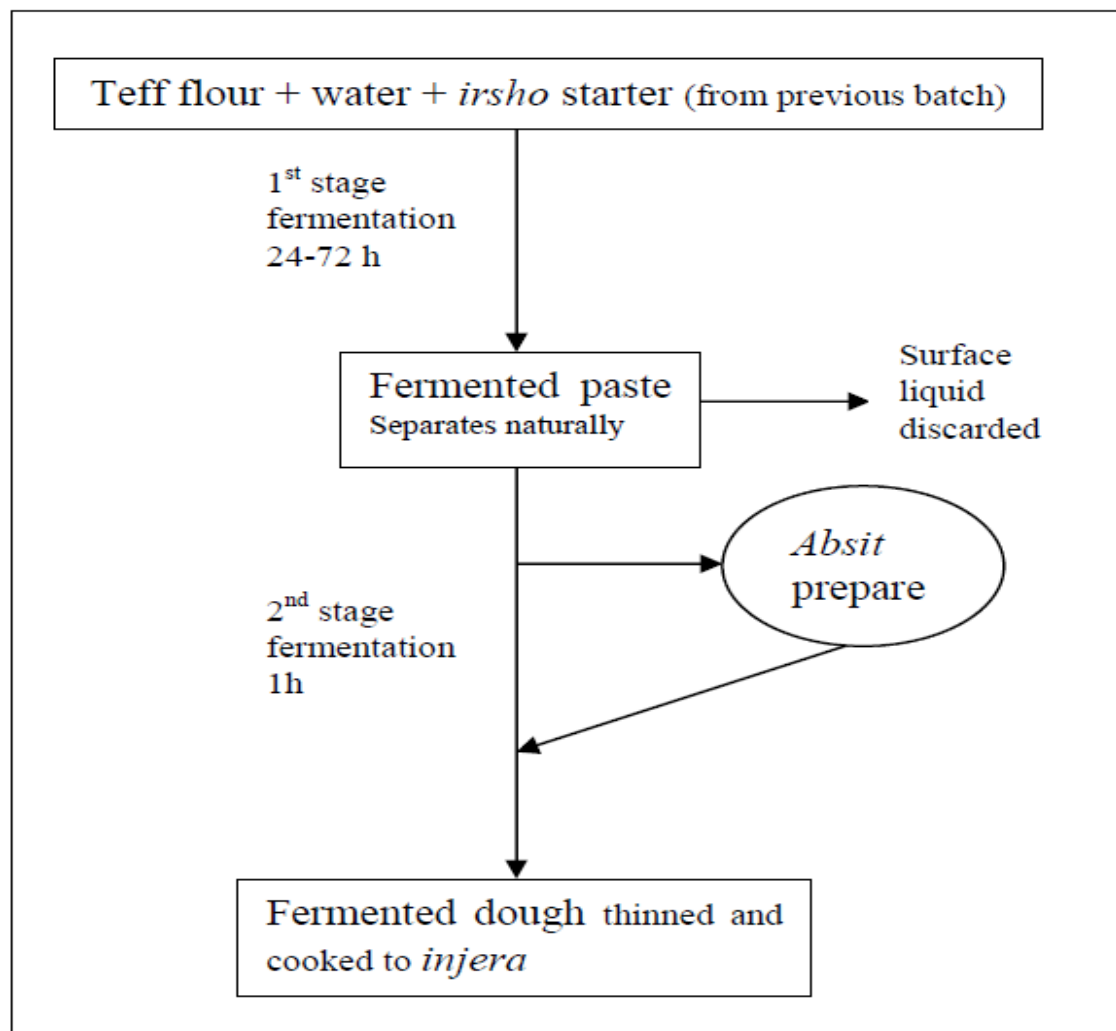


Figure 2.2 Traditional method of making tefinjera (adapted from Parker et al., 1989).

2.7.1 Fermentation and its effects

Several studies have reported the beneficial influence of fermentation in improving both nutritional and sanitary qualities of foods (Nout, 2009; Svanberg and Lorri, 1997). According to Nout and Motarjemi (1997) Production of organic acids with low molecular weight, such as acetic and lactic acid, reduces pH and may thus limit contamination by foodborne pathogens. Greiner & Konietzny (2006) also reported that fermentation can be a reason for the activation of several endogenous enzymes including phytases and may thus result in products with decreased

anti-nutritional factors. Hammes et al. (2005) stated that the level to which enzymes like phytases are activated depends on the fermentation kinetics, which in turn, depends on the condition of raw materials used. Baye et al. (2013) showed the influence of cereal flour blends (tef–white sorghum, barley–wheat and wheat–red sorghum) on dough fermentation kinetics at different fermentation time. Mono and disaccharide and organic acid (Acetic and lactic acid) concentrations were significantly varied among different blends. The kinetics of the decrease in pH followed the same pattern in all cereal blends. Significant phytase activities were noted among the samples. Beside flour blends, information is lacking on fermentation kinetics of tef flour dough as affected by mill type and kneading conditions.

2.7.2 Industrial injera making and process optimizations

The art of traditional processes needs to be transformed into a modern way of processing technologies to standardize the quality of the end products and to save time and energy without affecting their desirable traits (Nout, 1992). In Ethiopia although there are few injera exporting firms, their injera making methods still follow the traditional ways. This traditional injera making process should be transformed into industry level in order to make it suitable for production with having different in-process quality control points and giving product with predetermined quality and uniformity. Difference in tef grain milling and particle size distributions, kneading and absit preparation were mentioned by injera exporters as a reason for having different injera qualities and shows optimization of these injera making process is mandatory.

2.8 Paste clarity of tef

The paste clarity of most of the tef starches is similar to maize starch (Bultosa & Taylor, 2004). This is probably because of the difference in amylose and lipid contents of the starches not being

substantial, because the paste clarity is affected, in part, by amylose/amylopectin ratio and lipid contents of the starches (Zobel, 1988). The starch pastes from the red grain tef varieties seen less white than the pastes from white tef varieties and maize starch (visual observation) (Bultosa & Taylor, 2004). The availability of polyphenols reported in the red tef varieties (Urga et al., 1997) might have affected the starch color, as occurs with sorghum starch (Beta et al., 2001). Starch paste clarity is reported to be influenced by the interplay of the degree of swelling, junction zone formation of starch molecules in the paste, existence of amylose-lipid complexes, granular remnants in the paste, cross-linking, pH and trace non-starch components (Craig et al., 1989). In previous studies, the order of starch paste clarity was reported: potato>cassava>wheat>waxy maize>maize (Craig et al., 1989). Based on this and the work reported by Bultosa and Taylor (2004) the paste clarity of tef starch can be categorized as the same or slightly less than that of maize starch and it may have effect on color of injera.

2.9 Engineering properties of tef flour

2.9.1 Milling

On the study of Bayram and Oner (2005) the effects on the milling quality of bulgur were compared using disc, hammer mills and stone mill. For each milling system bulgur particles were examined for, surface structure, appearance, dimensions, bulk density, 1000 particle weight and particle size distribution. Regular and smooth shaped bulgur particles were obtained using the abrasive disc and stone mills. Stone milled bulgur particles, were not split or glassy, but were opaque. Hammer milling considerably influenced the surface characteristics and appearance of the bulgur particles and gave the highest deviation and variance in dimensions and the lowest minimum dimensions. Milling yields of stone (97.4%) and hammer (98.3%) mills were higher than for the disc mill (96.5%). Each mill type gave a different distribution between coarse,

medium and fine fractions. Stone mill gave the highest yield of the coarse fraction (83.1%). The result shows that, due to quality loss, hammer milling is not suitable for bulgur preparation but its suitability for injera preparation need to be studied.

In other study, effect of milling methods on the chemical, rheological and bread making characteristics of whole wheat flour were studied by Indrani and Venkateswara (1992). Whole wheat flour obtained by milling wheat in stone, hammer, disc and roller mills showed considerable variation in distribution of particle size. The damaged starch content and diastatic activity of flour shows variation in degree of severity of grinding in different mills. Dough raising capacity was also being influenced with the values between 53.4 and 79.6%. Dough properties of flours indicate significant variation and the water absorption varied from 64.9 to 72.6%. Samples of bread prepared from hammer and roller milled flours were better in quality than those from disc and stone milled flours.

The effect of milling technique on the sensory attributes of whole wheat pan bread was studied by Kihlberg et al. (2004). Six samples of wheat from field trials were roller and stone milled. Sensory evaluations were achieved through a descriptive profiling test, was conducted with 8 trained assessors using 19 sensory attributes for 48 different bread types. Image analysis was used to establish the slice area of the breads. Technique of milling had a greater influence on bread sensory qualities and on the slice area than did baking technique and farming system. Extensographic values (R_m, E), damaged starch and farinographic values for water absorption, dough stability and dough development time were higher for roller milled samples than for the stone milled. Breads (Whole meal) of roller-milled wheat were dominated by juiciness,

compactness attributes and sweetness, whereas those from stone milled wheat were characterized by roasted cereal attributes, saltiness and deformity.

In other study, chapati making quality of flour (*Atta*) from wheat (whole) obtained by different techniques of processing was studied by Inamdar et al. (2015). Wheat was processed in, pin (PM), hammer (HM), disk (DM), roller (RM) and *chakki* (CM) mill with the aim of quality characterization of whole wheat flour (*atta*) in relation to *chapati* making quality. Results showed that *atta* produced from RM was cooler and had retained more moisture. Variation in damaged starch was seen which was 15.99, 13.76, 11.76, 10.16 and 9.1% for CM, HM, DM, PM and RM ground *atta*, respectively. Water absorption (Farinograph) of CM ground *atta* was highest at 85% and least for RM ground *atta* (71.5%). Overall quality of *chapati* made from CM *atta* scored higher and had better texture and desirable wheaty aroma. Research revealed that *atta* quality parameters and its *chapati* making quality varied with processing techniques.

Different milling or grinding processes produce flours with different particle sizes and consequently the resulting in to different surface area available for reactions like water binding, solubility, heat transfer, and swelling that dictate flour quality and functionality (Brou et al., 2013; Chen, Lu & Lii 1999) and end use. Particle size also affects the physical properties of flour such as density, flow ability and packing characteristics which influence the flour handling characteristics (Landillon et al., 2008). The rheological and cooking properties of gluten-free dough and noodles prepared with dry- and wet-milled rice flours characterized by Heo et al. (2013) and reported dry-milled (roller mill followed by air-jet milling) rice flour with a higher degree of starch damage exhibited greater water hydration properties than wet-milled rice flour

(roller mill +air-jet milling) at room temperature. Similar study by Asmeda and Noorlaila (2015) also found rice flours produced from wet grinding technique (hammer mill) exhibited significantly finest average particle size distribution ($9.32\mu\text{m}$), with significantly lowest damaged starch (4.08%) and highest L^* value (93.55) than dry milling technique (hammer and stone mill).

Heo et al. (2013) found higher value of peak viscosity in wet-milled (roller mill +air-jet milling) rice dough than dry-milled rice (roller mill +air-jet milling) dough due to its great swelling power upon starch gelatinization. However increased cooking loss observed in dry-milled rice noodles which were attributed to great water solubility derived from a higher degree of starch damage. The study by Turkut et al. (2016) evaluated the effect of quinoa flour produced from hammer mill on gluten-free bread batter rheology and bread quality and found batter formulations (rice, potato starch and buckwheat) independent of the quinoa amount (12.5, 25, 37.5 and 50%) exhibited pseudoplastic behavior, and G' values higher than G'' values in expressing the solid like characteristics of the batter. however, 25% quinoa flour bread displayed softer texture and higher sensory scores.

Kadan et al. (2008) and Araki et al. (2009) observed that the milling system affected the damaged starch and particle size of rice flour and therefore the volume of breads was also affected. They also found a high negative correlation between damaged starch and bread specific volume when added with wheat gluten to the recipe, thus these results would not be completely extrapolated to gluten-free breads. In fact, de la Hera et al. (2013) reported that as the particle size of rice flour was reduced by sieving, the specific volume of gluten-free breads, made with 80% of water flour basis (f.b), decreased. This effect was attributed to the behavior of dough during fermentation, since dough made with fine flours were scarcely able to retain gas produced

in this step, which in turn could be due to the structural differences observed among the different dough's. Nonetheless, in the case of flours made with higher hydration (110% f.b), these effects became less clear and only a noticeable reduction of the gas retention and bread volume in the case of the finest flour (<80 μ m) was reported.

2.9.2 Kneading

Dough processing is an essential factor which affects the quality of bread. Kneading is one of most important mechanical step in industrial dough processing (Esselink et al., 2003). In all of bread processing steps, significant changes in the structure and properties of the dough can occur. On both a laboratory-scale and industry-level, these (structural) effects are well characterized and studied for bread making (Esselink et al., 2003). However, so far, no systematic study has been performed on both laboratory and industrial scale tef flour dough processing especially kneading. The molecular and micro-structural changes that can take place during industrial tef flour dough processing were not studied.

On the study of Angioloni and Rosa (2005) thermo-mechanical properties of dough's prepared from common wheat flour were investigated under different kneading conditions and with different amounts of sodium chloride addition. Dynamic mechanical thermal analysis indicates that high-speed mixing and the addition of sodium chloride to dough slowed heat-induced reactions such as protein coagulation and starch gelatinization. The influence of dough mixing technology was more significant than the amount of sodium chloride in modifying dough rheological characteristics.

In another study, effects of cysteine and mixing conditions on white/whole dough rheological properties were studied by Angioloni and Rosa (2007). The effects of cysteine and processing parameters (time and mixing speed) of white/whole dough viscoelastic properties were evaluated. Similar amount of cysteine (20 mg/kg) was added for each mixing time (10, 15, 20 s) in order to assess its effect on dough rheological properties. Fundamental and empirical rheological measurements have indicated that dough viscoelastic properties were influenced by kneading conditions. In traditional injera preparation, kneading is done manually however; its effect on injera quality is not studied yet.

Elgeti et al. (2017) studied interrelation between mechanical and biological aeration in starch based gluten-free bread from mixture of quinoa white flour and corn starch in a ratio of 3:1 produced from Quadrumat Junior mill. The density and temperature monitored in mixing experiments without yeast, aiming at maximum mechanical aeration and subsequent biological aeration with yeast fermentation and baking. Results showed that the gas volume fraction was elevated to 21%, instead of 6% with conventional kneading. Reducing the water content from 120% to 90% (flour/starch weight base) raised dough viscosity and temperature without affecting the state of aeration. The bread volume strongly influenced by the dough temperature after mixing ($R^2 = 0.98$), since it depended on yeast activity. The implemented process is suitable to aerate starch-based dough systems mechanically and enables the production of gluten-free bread with high volume and fine pores.

The work by Sadot et al. (2017) aimed to study cereal dough mixing in a prototype spiral mixer able to modulate the pressure between -960 and +500 mbar. A model was used to determine the

kinetic parameters of entrainment and disentrainment of air during mixing with pressure step-changes. It was found that the greater the pressure reduction was, the slower the degassing speeds was. Moreover, the final volumetric air content was only dependent on the final mixing pressure. Gómez et al. (2013) studied the effect of mixing on two different gluten-free bread formulas (80 and 110% hydration). In less hydrated breads, no significant differences found depending on the mixing arm (flat beater or dough hook), but mixing time influenced the specific volume of bread, being these higher while mixing time increased. Both mixer arm and mixing speed found to have a significant effect on bread volume and texture in more hydrated dough, achieving higher specific volumes and softer breads with the wire whip compared with the flat beater, with lower mixing speeds and longer mixing time. In more hydrated breads, proofing time improved bread specific volume, but in less hydrated breads the effect was the opposite. This effect was remarked in longer mixing times.

2.9.3 Technological functional properties

Techno-functional properties of tef flour were studied by Abebe et al. (2015) and results were compared with rice and wheat. According to the study, cultivar and mill type did not show significant effect on foaming capacity and foaming stability of the tef flours. However, foaming capacity values seen by the flours from tef cultivars were 1.7 times lower than wheat flours and 1.8 times higher than rice flours. Foaming of flour occurs mainly due to a continuous cohesive film formed around the air bubbles in the foam. The foaming capacity score of tef flours could indicate their better suitability than rice in gluten-free food systems that require aeration for leavening and textural properties. The foaming stability of tef flours was higher than wheat and rice indicating their ability to maintain the foam. Flour hydration properties were significantly affected by both type of tef cultivar and mill type. Among the tef cultivars, DZ-Cr-387 (Qouncho

tef) had relatively higher water absorption index, swelling volume, water solubility index, swelling power and water holding capacity while it scored lower mean water absorption capacity. Rice and wheat flours had significantly lower swelling volume and water holding capacity than tef flours. The higher fiber content in tef flours, as whole meal (Collar & Angioloni, 2014b), could also explain its higher water binding capacity with respect to refined rice and wheat flours (Santos et al., 2008). The water absorption capacity values of tef flours were apparently lower than rice and higher than rice flour.

According to Mason and Hosney (1985) the water absorption index measures the volume occupied by denatured protein and gelatinized starch. Compared to rice and wheat flours, the values of the water absorption index of the flours from three tef varieties were apparently lower. Water solubility index of tef was apparently higher than that of rice and wheat flours. According to Yetneberk et al. (2005) in tef and sorghum composite flours the water solubility index increased progressively with increasing proportion of tef, giving injera better quality. The increase in water solubility index agreed with the observation that, during mixing, compared with sorghum, tef dough tended to be stickier and water-soluble components in the tef flour could have modified the dough rheology and the texture of injera positively (Yetneberk et al., 2005). In the evaluation of injera making potentials of sorghum varieties higher water solubility index gave softer, fluffier, and rollable injera (Yetneberk et al., 2004). According to Qarooni et al. (1993) in flat breads high quality is associated with wheat flours with water absorption and high damaged starch content.

Martínez et al. (2014) reported changes in the emulsifying capacity and stability of rice and wheat extruded flours as a function of the extrusion conditions. These effects were attributed to

the protein unfolding and aggregation along with starch gelatinization that increases the number of hydroxyl groups available to form hydrogen bonds with the proteins. Regarding foaming capacity, extrusion did improve foaming capacity and stability of rice flours. These different emulsifying and foaming properties could make extruded flours suitable for some gluten-free products. Asmeda and Noorlaila (2015) investigated significantly highest water absorption index, flour swelling volume and solubility in dry ground rice flour as compared to semi-wet and wet grinding machines and concluded that different grinding methods (hammer and stone mill) significantly affected chemical and functional properties of rice starch.

2.9.4 Rheological and textural properties of tef dough

The science of rheology has many benefits in the area of food acceptability, food processing, and food handling (Barbosa-Ca'novas et al., 1996). Foods, however, are complex materials rheologically and structurally and, in many cases, they have mixtures of solids as well as fluid structural components (Finney, 1972). These components have an impact on rheological and textural properties of foods (Tabilo-Munizaga, 2005).

Rheological properties are important in determining the properties of wheat flour dough during mechanical handling in addition to their influence on the quality of the finished product. Knowledge of dough properties and rheological behavior is becoming more mandatory as the baking industry becomes more automated (Mani, 1992). Mixing highly alters the rheological behavior of dough. Mixing quickly hydrates the flour particles. The high shear rate in a dough mixer helps hydration by removal of the outer layer of flour particles as they become hydrated and exposing a new surface for hydration (Spies, 1990).

Through processing, manufacture, and consumption of foods, gels are formed and the gelled systems are subjected to large deformations that can cause the food material either to be deformed irreversibly or to fail in a fracture (Tabilo-Munizaga & Barbosa-Cànovas, 2005). Hence, for development of new foods such as gel-like products from tef and/or to include it in existing formulations for modifying their functional and nutritional quality, an in depth research on gel properties was necessary. Abebe and Ronda (2014) studied the rheological and textural properties of tef grain flour gel. Gel viscoelastic properties of three varieties of tef (two white and one brown) at different concentrations (14, 12, 10, 8 and 6 % w/w) were analyzed at 25°C and 90°C. All tef flour suspensions preheated to 95°C led to gels with a solid-like behavior, both at 25°C and 90°C, with higher uniformity than wheat gels at the same concentration. The reliance of viscoelastic model with concentration fulfilled the power law. The Avrami model was fully fitted to the textural evolution of tef gels. Significant differences were seen among rice, tef and wheat flours, most likely, contributed by their differences in starch, protein, lipid and fiber constituents. The characterized gelling properties suggest that, tef flours would be suitable ingredients in gel type food formulations.

According to Bultosa and Taylor (2004), at 10% starch, the pastes of tef starches were short textured similar with maize starch, and on cooling the pastes became gels. DZ-01-99 (red) and South African Brown formed firmer gels than maize starch whereas starch gel of DZ-01-196 (White tef) is softer than that of maize starch. The highest gel firmness was seen for South African Brown and the lowest was for DZ-01-196. Gel firmness of DZCr-37 and DZ-01-1681 tef starches was similar to maize starch gels. The gel of normal cereal starches of sufficient concentration is a mixture of leached continuous amylose matrix with swollen granular remnants

acting as fillers in the continuous phase (Tsai et al., 1997). The gel firmness of normal cereal starch has been positively correlated with starch granule associated proteins (Han et al., 2002) and leached amylose (Tsai et al., 1997), negatively with starch lipids complexes with amylose (Takahashi & Seib, 1988), and positively with free starch lipids, because free lipids will not restrain the leaching of amylase (Hibi, 1998) and phosphorus contents (Lin & Czuchajowska, 1998). Comparison of starch gel texture parameters obtained from different studies is difficult because of differences in experimental conditions (sample size and shape, ratio of compressing probe size versus sample, replicates per mean value, extent of deformation, number of bites and cross-head speed) (Pons & Fiszman, 1996). However, under uniform experimental condition, gel firmness of normal native wheat starch is higher than that of normal native maize starch (Takahashi & Seib, 1988) but the native maize starch higher than normal native oats in gel firmness (Zhou et al., 1998) and rice starches (Tsai et al., 1997). Based on this, the gel firmness of tef starches at 10% concentration can be placed between wheat and maize starches in gel firmness.

According to Mezaize et al. (2010) the effect of unfermented frozen dough process on the properties of gluten-free dough and the quality of bread. Rheological oscillation tests indicated that viscoelastic properties were unchanged for fresh and thawed dough. However flow tests exhibited an effect of freezing on consistency index and flow behavior index. Regarding the quality of bread, gluten-free breads obtained by frozen dough process had lower specific volumes and harder crumbs than conventional gluten-free breads (unfrozen breads). Distribution of gas cells was more homogeneous with a freezing step. Crust color characteristics also modified by the freezing step. Empirical and fundamental rheological measurements made on fresh and frozen dough (stored at two different temperatures, -18 °C and -30 °C for 1, 7 and 28

days) to study the effects of freezing and frozen storage conditions using four dough formulations (standard wheat dough, a fiber-enriched wheat dough, a standard gluten-free dough and a gluten-free dough containing amaranth flour) without yeast addition by Leray et al. (2010). The wheat dough is more affected by freezing and by the first days of storage whereas the gluten-free dough is more affected by a longer storage time. A storage temperature of -30 °C alters dough rheological properties more than -18 °C. The addition of dietary fibers to the wheat dough increases its resistance to freezing and frozen storage. The addition of amaranth flour to gluten-free dough also increases its resistance to freezing but decreases its resistance to storage conditions.

Although researches on tef undertaken so far were more focused on physico-chemical or functional characterization of starch extracted from the flour (Bultosa et al., 2008; Bultosa and Taylor, 2004), baking quality (Mohammed et al., 2009; Bultosa, 2007) and nutritional attributes (Hager et al., 2012), research on rheological and textural properties of tef flour dough are very important for the establishment of injera industry.

2.10 Quality characteristics of tef flour

Different quality characteristics of tef flour were reported by many researchers in past years. Rheological, textural and pasting properties, flowability, color, falling number, starch digestibility, thermal properties, starch damages, foaming capacity and foam stability, particle size distribution, water holding capacity, water absorption capacity, water absorption index and water solubility index and nutritional values (Abebe et al., 2015, Bultosa and Taylor, 2004, Bultosa 2007, Adebawale et al., 2011, Michaelsen et al., 2011). However study on the effects of processing on quality characteristics of tef flour is not sufficient.

2.11 Concluding remark

In summary, compared to other more common cereals, tef is better in its nutrient composition. Its starch is slowly digestible and has a low GI; it also has a favorable amino acid composition and contains no gluten. Tef is also a good source of unsaturated fatty acids. Tef is high in minerals, especially iron and calcium. Furthermore, its dietary fiber along with phytochemicals makes tef a good option for a functional food for health promotion and disease prevention.

Mill type exhibited important effect on flour granulation and uniformity of particle size and starch damage. These parameters are important factors affecting the processing performance of the flours by determining the absorbed water and dissolved flour components and the pasting properties of tef flour. Particle size has significant effect on starch digestibility of tef.

Kneading has significant effect on the rheological and textural properties of dough and bread.

However, during injera making process the effects of tef processing on rheological, textural and functional properties of flour and injera quality are not addressed sufficiently. Although injera is consumed in high rate by Ethiopian people, injera making method is not optimized based on injera quality. The quality characteristics of injera as affected by processing methods (Milling, kneading and absit preparation) and usage were not fully studied.

Chapter 3-*Materials and Methods*

Chapter 3

Materials and Methods

The techniques and equipment employed and analytical approaches followed in the four different studies undertaken are presented in the respective material and methods section of each study. The material and method which are common throughout all studies were presented.

3.1 Sample preparation and storage

Based on its popularity among Ethiopian tef farmers and users, Qouncho tef variety (DZ-Cr-387) was selected and obtained from DebreZeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR) which is located 42km away from Addis Ababa, Ethiopia. Tef sample was hermetically stored in cool and dry place (21°centigrade) using polyethylene bag. Before milling, tef grain was cleaned by sifting. This research was conducted in Addis Ababa University, Ethiopia (For sensorial quality evaluation, functional properties, polyphenols, particle size distributions), and University of Valladolid labs, Spain (the remaining analysis).

3.2 Experimental design and statistical analysis

Full factorial experimental design was used to conduct this research and analysis of variance was performed on the data to establish significant ($p < 0.05$) differences between the samples. All analysis was done in duplicate and the descriptive categories were converted to numerical scores. The scores were then subjected to analysis of variance using SPSS statistical software (Version 16) and means of duplicate results were compared by Tukey's Honestly significant difference test.

Chapter 4-Influence of Milling Type on Tef Injera Quality

Chapter 4

Influence of Milling Type on Tef Injera Quality

Abstract

Injera is an Ethiopian flat bread that is mostly made from tef flour. Injera making on an industrial scale holds a significant economic and social interest but requires a thorough study of how the process variables affect the product quality. The aim of this work was to investigate the effects of mill type (hammer, disc, and blade) on injera sensory quality and starch digestibility. The application of software for the determination of injera quality descriptors and its comparison with visual human eye evaluation was also established. Injera made with disc mill flour had higher overall acceptability (6.6) than that obtained from hammer mill (4.2) and blade mill (4.1) flours. The injera made with blade mill flour obtained the lowest rapidly available glucose and rapidly digestible starch. The outcome of introducing software for the determinations of injera number of eyes was found effective; its difference with human eye determination was insignificant.

Keywords: Tef; Milling; Injera quality; Sensory quality evaluation software

4.1. Introduction

Injera is leavened, flat round Ethiopian traditional bread made from cereals such as tef and sorghum (Pasqualone, 2018). Its surface has essentially evenly spaced gas holes that make up a honeycomb-like structure formed due to the production of gas during fermentation and baking. Injera has a shiny and smooth bottom surface. As stated by the work of Yetneberk et al. (2004), good injera is expressed as soft and roll-able. A slight sourness is a characteristic taste of injera. Because injera is leavened bread made from natural gluten-free flour, it has great potential for commercial production internationally.

Injera prepared from the flour of tef [*Eragrostis tef* (Zucc.) Trotter], a tiny, millet-like grain, is the most preferred (Yetneberk et al., 2004). Tef is an Ethiopian indigenous tropical cereal crop, and it has been cultivated for many years in the Ethiopian highlands (Viswanath, 2012). It is the main staple in the country and is mostly used to make *injera*. Tef represents 24% of the grain crop area in Ethiopia and 17.6% of the grain production (Central Statistical Authority, 2015).

The whole tef grain is ground to flour for making injera, local beverage porridges and soup and unleavened bread (Bultosa and Taylor, 2004). The sizes of the seed are very small, ranging from 0.6–1 mm diameter and 1–1.7 mm long with 1000 seed weight averaging 0.3–0.4 g and 150 grains of tef have comparable weight with almost one seed of wheat (Diskstra, 2008). Tef grain products are nutritionally rich because they are eaten as whole grain with the significantly higher content of fiber, carbohydrate (USDA, 2007), iron, zinc and calcium than wheat, barley and sorghum (Abebe et al., 2007). Due to the absence of gluten and gluten-like proteins, tef has recently been popular globally particularly, as a “healthy food”, making it right for celiac disease patients (Spaenij-Dekking et al., 2005), and in addition, because of other dietary benefits such as the slow-release of carbohydrate constituents, it is useful for diabetic patients (Abebe and Ronda, 2014).

Depending on the mechanical forces and temperature during the grinding process, various milling or grinding methods have been introduced to produce different flours with different particle size and starch damage level (Kadan, Bryant & Miller, 2008). In Ethiopia, mostly disk mill is used to grind tef grain in the homemade injera process. However, other types of mills have not been checked for their better quality of injera. The effect of milling technique on the sensory attributes of whole-wheat pan bread was studied by Kihlberg et al. (2004). The result showed that technique of milling had a greater influence on bread sensory quality and on the

slice area than did the baking technique and farming system. However, techniques of milling on sensory attributes of tef injera remain lacking.

The number, size, and distribution of holes (commonly called *eyes*) on the injera surface represent one of the most important quality attributes of injera (Yetneberk et al., 2005). However, determinations of these injera quality descriptors are very difficult due to the nature of its uncountable eyes, which lead to subjective quality evaluation. Due to this, the injera standard, which was developed by the Ethiopian standard agency, lacks these quality attributes in measurable form. Currently, as injera industrialization is emerging, a systematic way of injera quality determination (injera number of eyes, eye size, and eye distributions) is mandatory for maintaining uniform quality.

Therefore, the objective of this research was to investigate the effects of three different mill types (Hammer, Disc, and blade) on injera sensorial quality and starch digestibility and to compare a software-based evaluation of injera quality descriptors with visual evaluation.

4.2. Materials and methods

4.2.1 Materials

Based on its popularity among Ethiopian tef farmers and users, the qouncho tef variety (DZ-Cr-387) was selected and obtained from the DebreZeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). The tef sample was hermetically stored in a cool and dry place using polyethylene bag. Before milling, the tef grain was cleaned by sifting.

4.2.2 Tef milling

Tef grain was milled using three types of mills to obtain the whole flour of the tef sample. The first one was the Hammer mill (HM) (Perten 120, Finland) with a 0.8 mm sieve fitted inside as part of the mill, the second mill was the stone-disk mill (DM) (cottage tef grain-milling, Denmark) and the third mill was the blade mill (BM) (Nutri Bullet NB-101B, China). One kg of

sample was milled by HM and DM for 7 minutes, and five kg of samples were milled by BM for 7 minutes at ambient temperature.

4.2.3 Injera preparation

The tef injera samples were prepared according to Parker et al. (1989) and Zegeye (1997). An amount of starter (*Ersho*) equal to 60 ml was initially added for each kg of flour. *Ersho* is a small amount of batter kept from previous dough to start first stage fermentation (Parker et al., 1989). The tef flour was mixed 2:3 (w/w) with potable water and kneaded by hand in a bowl until obtaining a homogenous mixture in the traditional way. The dough was allowed to spontaneously ferment for 60 hours at room temperature (21 ± 5) °C in an injera baking household in Addis Ababa, Ethiopia. After this primary fermentation, 10% of the dough was mixed 1:3 (v/v) with boiling water and heated for 15 min with continuous stirring. The hot cooked dough (*absit*) was then mixed back into the fermenting dough, and sufficient potable water was added to make a batter. The batter was left covered for 2 hours for secondary fermentation. Additional water was added to thin and form the right consistency of the batter. Finally, half a liter of batter was poured onto the hot clay griddle in a circular form. After 2-3 min of cooking using traditional electric injera baking equipment, the injera was removed and placed in a basket.

4.2.4 Flour characterization

4.2.4.1 Proximate analysis

Tef grain was milled using a stone-disc mill and flour proximate composition (Crude protein, fat, ash, fiber) was determined using AACC methods (AACC, 2000). Total carbohydrate was determined by difference to 100% (FAO, 2003).

4.2.4.2 Flour color

Flour color was evaluated according to the methods of Abebe, Collar & Ronda (2015). The spectrophotometer was used for flour color measurements. CIE L*a*b coordinates were used to obtain the result by using the D65 standard illuminant and the 2° standard observer. The hue (h) and the chroma (C*) were calculated from Eqs. (1) and (2) respectively.

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \dots\dots\dots (1)$$

$$C^* = \left((a^*)^2 + (b^*)^2 \right)^{1/2} \dots\dots\dots (2)$$

4.2.4.3 Particle size distribution

According to Sivaramakrishnan, Senge, and Chattopadhyay (2003), the particle size distribution was evaluated by passing the tef flour through an automatic standard sieve shaker (Retsch, Germany) that contains 5 sieves. Sieves with the sizes of 710, 500, 250, 125 and 90µm were used. The percentage fraction of the sample retained on each sieve was measured by weighing.

4.2.4.4 Damaged starch evaluation

The damaged starch level of the tef flour samples was determined according to the AACC method (AACC, 2012) using a Megazyme starch damage kit (Megazyme International Ireland Ltd., Co., Wicklow, Ireland). Absorbance was read at 510 nm in a microplate reader from BIOTEKEPOCH (Izasa, Barcelona, Spain). The damaged starch level of the tef flour was determined as a percentage of the flour weight on a dry basis.

4.2.4.5 Scanning electron microscopy (SEM)

Scanning Electron Microscope (SEM) model Quanta 200-F (FEI, Oregon, USA) equipped with an X-ray detector was used to examine the three tef flours. Samples were directly placed on stubs, and observations were done by accelerating voltage of 1.5 keV.

4.2.5. Injera quality analysis

4.2.5.1 Starch fractions analysis

The method by Englyst, Kingman, and Cummings (1992) was used to measure in vitro starch digestibility of tef injera with the modifications by Englyst et al.(1999); Englyst et al. (2000). The hydrolyzed glucose at 20 min (G20) and 120 min (G120) and the total glucose (TG) were measured by the glucose oxidase colorimetric method. The free sugar glucose (FGS) content was measured by a separate test according to the procedure proposed by Englyst et al. (2000). Rapidly digested starch (RDS) = $0.9 * (G20 - FGS)$, slowly digestible starch (SDS) = $0.9 * (G120 - G20)$, resistant starch (RS) = $0.9 * (TG - G120)$, for total starch, (TS) = $0.9 * (TG - FGS)$ and rapidly available glucose of the sample (RAG) = G20 were calculated. As used by Abebe, Collar, and Ronda (2015), the starch digestibility rate index (SDRI) was computed from the percentage of RDS in TS in the flours.

4.2.5.2 Descriptive sensory analysis

The sensory evaluation was conducted by a panel trained according to Einstein (1991). The selected panelists were tested for their ability to detect basic tastes (Jellinek 1985). The selected panel comprised 10 people, as recommended by Stone and Sidel (1985). They were females and males, who were students at Addis Ababa University. Nine *injera* quality descriptors were used for evaluation: color, taste, odor, texture (degree of softness), injera number of *eyes*, *eye* size, *eye* distribution (*eye* uniformity), and top and bottom surface (degree of being powdery and sticky); overall acceptability was also evaluated. A score sheet was prepared using the selected descriptors. Each one of the attribute was evaluated using a 10-point numerical scale (0–9) anchored on both sides with verbal descriptions (i.e., 0 = unpleasant, 9 = pleasant) to allow the panel to score the intensity on a framed common scale. Good sensory practices were followed

according to Lawless and Heymann (1999). *Injera* samples were presented to the panelists on a tray at ambient temperature ($\approx 25^{\circ}\text{C}$) within 3-4 hr after baking. A glass of drinking water was used for rinsing between samples.

4.2.5.3 Software based injera quality evaluation

Photos of injera samples were acquired and imported into ImageJ software (version 2) determining injera number of eyes, eye size, and eye distributions. Injera photos were converted to gray scale using ImageJ software, and the software converted the image into detectable form to easily recognize the injera eyes.

4.2.6 Statistical analysis

Analysis of variance was performed on the data to establish significant ($p < 0.05$) differences between the samples. All analyses were done in duplicate, and the descriptive categories were converted to numerical scores. The scores were then subjected to analysis of variance using SPSS statistical software (Version 16), and the means of duplicate results were compared by Tukey's Honestly significant difference test.

4.3. Results and discussion

4.3.1. Flour characteristics

4.3.1.1 Proximate composition

Tef sample has $10.9 \pm 1.2\%$ of moisture and $2.13 \pm 0.01\%$ of ash content. Baye (2014) presented a similar result of ash content (2.8). Wheat (1.6) and sorghum (1.6) have lesser amounts of ash than that of the tef sample. The higher ash content of the sample could be due to its higher fiber content, as tef flour comes from whole grain (Abebe & Ronda 2014). However, the fat ($2.53 \pm 0.29\%$), protein ($10.99 \pm 0.29\%$) and carbohydrate ($81.35 \pm 0.21\%$) contents of the sample agreed with those of the report that was compiled by Baye (2014) with 2.5% of fat, 11.0% of

protein and 80% of carbohydrate in dry matter. The higher contribution of bran to the whole tef grain flour composition could be explained by the smaller size of the tef grain (Bultosa, 2007), which gives a higher surface area of bran per unit amount of grain, in comparison with whole wheat. However, the crude protein content of the sample is slightly higher than sorghum (8.3) and comparable to wheat (11.7).

4.3.1.2 Flour color

The color coordinates of the tef flours obtained from the three mills are summarized in Table 4.1. Although the values of the coordinates show significant differences, the color differences, ΔE , that resulted were low enough to be appreciated by the human eye (<5), except for the blade mill flour, which depicted significantly lower lightness than the flours from the two other mills. The effect of mill type on the color of the tef flours varied significantly; this variation may come from the difference in milling principles among the three mills. Lightness (L^*), hue angle (h^*) and chroma (c^*) of samples varied significantly among HM, DM, and BM (Table 1). DMF (87.7) had significantly higher lightness, which was followed by HMF (85.3) and BMF (71.0). Such an effect of mill type could probably be related to the degree of breaking and pulverization of the bran of the tef grains. The hue angle of the three flour varied significantly in the order BMF (68.9) $<$ HMF (71.4) $<$ DMF (72.7). This seems slightly different from the work of Abebe et al. (2015) in the case of cyclotech sample mill (L^* 83.2, h^* 85.3) and disk mill (L^* 81.7, h^* 85.2). However, the chroma of flours varied significantly in the order DMF (15.1) $<$ HMF (17.1) $<$ BMF (18.5). However, the chroma of flour from the disk mill agreed with the report by Abebe et al. (2015) for cyclotech and disk mill (14.6 and 15.4).

Table 4.1 Tef flour color

Mill	L*	a*	b*	h*	c*
HMF	85.3 ± 0.0 ^b	5.45 ± 0.0 ^b	16.2 ± 0.0 ^b	71.4 ± 0.04 ^b	17.1 ± 0.0 ^b
DMF	87.7 ± 0.0 ^c	4.50 ± 0.0 ^a	14.5 ± 0.01 ^a	72.7 ± 0.03 ^c	15.1 ± 0.0 ^a
BMF	71.0 ± 0.02 ^a	6.68 ± 0.01 ^c	17.3 ± 0.01 ^c	68.9 ± 0.04 ^a	18.5 ± 0.0 ^c

HMF, DMF, and BMF stand for flour from hammer, disk and blade mills, respectively. Data are expressed as the mean ± standard deviations; different superscripts in the same column indicate statistically significant differences ($P < 0.05$). L*, a*, and b* are CIE coordinates, h = hue, and C* = chroma.

4.3.1.3 Particle size distribution

Particle size distributions of the three tef flour (HMF, DMF, and BMF) showed a significant difference in percent retention at all sieve sizes used, except at 710 μ m (Table 4.2). In particular, at 500 and 250 μ m sieve, 6.7 and 56.2% of BMF was retained, respectively, which was significantly higher than that of HMF (0.2 and 18.9%) and DMF (0.2 and 2.9%). However, DMF was retained significantly in a lower amount than that of HMF on 250 μ m sieve. Flour retention on 125 μ m sieve varied significantly in the order BMF (31.1%) < DMF (35.4%) < HMF (44.5%). For sieve size (90 and < 90 μ m), flour particles varied significantly in the order BMF (5.9 and 0%) < HMF (10.9, 25.4) < DMF (28.9, 32.6), respectively. The reason for having different particle size distributions is perhaps due to the difference in milling procedures among the three mills. The hammer mill crushed the grain repeatedly until it passed through the sieve that was fitted inside the mill. Tef grain was ground between two stone-discs in the case of disc mill. The blade mill had rotary blades to grind the grain.

4.3.1.4 Damaged starch

The damaged starch level of tef flour obtained from the three mills varied significantly in the order HMF (1.50%) < BMF (2.66%) < DMF (5.11%) (Table 4.2). The inverse relationship

between flour particle size and damaged starch was noted on DMF: as particle size decreased ($<90\mu\text{m}$), the damaged starch level increased (5.11%). This agrees with a report by Abebe, Collar and Ronda (2015). However, due to the difference in milling principle among the three mills (HM, DM, BM), this relationship was not seen on HMF. The reason for having different starch damage levels was a result of grinding conditions such as milling force (Nowakowski et al., 1986 and Tran et al., 2011) and temperature and grain moisture content (Li et al., 2014).

Table 4.2 Particle size distribution and damaged starch level of tef flour

Mill	Particle size distribution (% retained)						Damaged Starch
	710 (μm)	500 (μm)	250 (μm)	125 (μm)	90 (μm)	<90 (μm)	
HMF	0.1 ± 0^a	0.2 ± 0.1^a	18.9 ± 1.1^b	44.5 ± 0.6^c	10.9 ± 0.4^b	25.4 ± 0.3^b	1.50 ± 0.05^a
DMF	0.0 ± 0^a	0.2 ± 0.1^a	2.90 ± 0.3^a	35.4 ± 0.1^b	28.9 ± 1.3^c	32.6 ± 0.7^c	5.11 ± 0.14^c
BMF	0.1 ± 0^a	6.7 ± 0.3^b	56.2 ± 0.3^c	31.1 ± 0.1^a	5.90 ± 0.1^a	0.00 ± 0.0^a	2.66 ± 0.01^b

HMF, DMF, and BMF stand for flour from hammer mill, flour from disk mill and flour from blade mill, respectively. Data are expressed as the mean \pm standard deviations; different superscripts in the same column indicate statistically significant differences ($P < 0.05$).

4.3.1.5 Scanning electron microscopy (SEM)

The effects of mill type on tef grain were seen by scanning electron microscope (SEM) (fig. 4.1). Starch granule pulverizations and the release of individual starch granules were seen on flour from HM and DM. However, the extent of pulverization on DMF was more as its average particle size is smaller than that of HMF and BMF. This result agrees with a report by Abebe et al. (2015). As to the release of individual starch granules on BMF seen less than DMF and HMF, its larger particle size explains the difference. In case of disc mill, the milling discs were the most effective in releasing individual starch granules than the rotary blades of blade mill. Less release of individual starch granules was noted during the use of rotary blades compared to the

hammer. According to Jane et al. (2003), the damage, which forms pinholes on granule surface and channels inside the granules, is probably from the effect of endogenous enzymes hydrolysis inside the grain.

4.3.2 Injera characteristics

4.3.2.1 In vitro starch digestibility

The three tef injeras had similar content of resistant starch (RS) and total starch (TS) (Table 4.3). The effects of mill type on starch hydrolysis of tef injera were significantly varied; HMI leads to higher FSG, RAG, and RDS than that of BMI. However, there was no significant difference in RAG, RDS and SDS content between DMI and HMI except for FSG, and DMI and BMI. Tef grain milled repeatedly until the flour pass-through the 0.8 mm sieve size that was fitted on the hammer mill. This difference in milling principle between blade and hammer mill justifies the results. However, this principle was not reflected on DMI, which had higher damaged starch with smaller particle size. This agreed with Li et al. (2014), who asserted that cereal flour starch digestibility is highly influenced by the degree of damaged starch and flour particle size. Due to starch fragmentation, damaged starch granules have larger relative surface area for enzyme attack than intact native starch granules. However, it has not yet been proven whether the same principle applies to the digestibility of gelatinized starch in cooked cereal flour, but the SDS of BMI (24.9) was significantly higher than that of HMI (6.3). The inverse correlation between flour particle size and RAG, RDS and SDS were seen on BMI, as its flours (BMF) have a higher mean particle size lead to the lower content of RAG and RDS, with the higher content of the SDS of BMI. These agree with the reports by Mahasukhonthachat et al. (2010) and Al-Rabadi et al. (2009). It has been understood that the flour starch digestibility is controlled by enzyme diffusion inside flour particles. However, with similar particle sizes, the

rate of starch digestion was seen to be different between barley and sorghum flours (Mahasukhonthachat et al., 2010) and between sorghum flours from the hammer and cryogenic mill (Al-Rabadi et al., 2009). As reported by Ronda et al. (2015) the RAG (82.7) and the RDS (74.3) content of wheat bread was significantly higher than that of HMI (65.7, 58.9), DMI (61.5, 55.3) and BMI (52.6, 47.3). According to Miao et al. (2015), the health implication of moderate postprandial glycemic and insulinemic response due to slowly digestible starch (SDS) implies that foods with a high amount of SDS may provide wide health advantages by reducing common chronic diseases related to diet, such as diabetes and pre-diabetes, cardiovascular diseases, and obesity (metabolic syndromes). The SDRI of BMI (64.6) varied greatly from HMI (75.8). This may be a result of the difference in particle size of the tef flour; however, the same principle was lacking in justifying DMI (72.3), which was not significantly different from HMI and BMI.

Table 4.3 Starch fractions, FSG, RAG, and SDRI, expressed in percentage referring to the dry matter

Mill	FSG	RAG	RDS	SDS	RS	TS	SDRI
HMI	0.30±0.04 ^b	65.7±2.6 ^b	58.9±2.3 ^b	6.3±2.3 ^a	8.8±5.2 ^a	74.0±5.2 ^a	75.8±3.0 ^b
DMI	0.05±0.07 ^a	61.5±3.6 ^{ab}	55.3±3.2 ^{ab}	15.5±3.2 ^{ab}	5.8±1.3 ^a	76.6±1.3 ^a	72.3±2.9 ^{ab}
BMI	0.07±0.01 ^a	52.6±0.0 ^a	47.3±0.0 ^a	24.9±0.0 ^b	1.0±1.7 ^a	73.2±1.7 ^a	64.6±1.5 ^a

HMI, DMI, and BMI stand for injera from hammer mill, injera from disk mill and injera from blade mill, respectively. FSG= free sugar, RAG = rapidly available glucose, RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistant starch, TS = total starch, and SDRI = starch digestion rate index. Data are expressed as the mean ± standard deviations; different superscripts in the same column indicate statistically significant differences (P < 0.05).

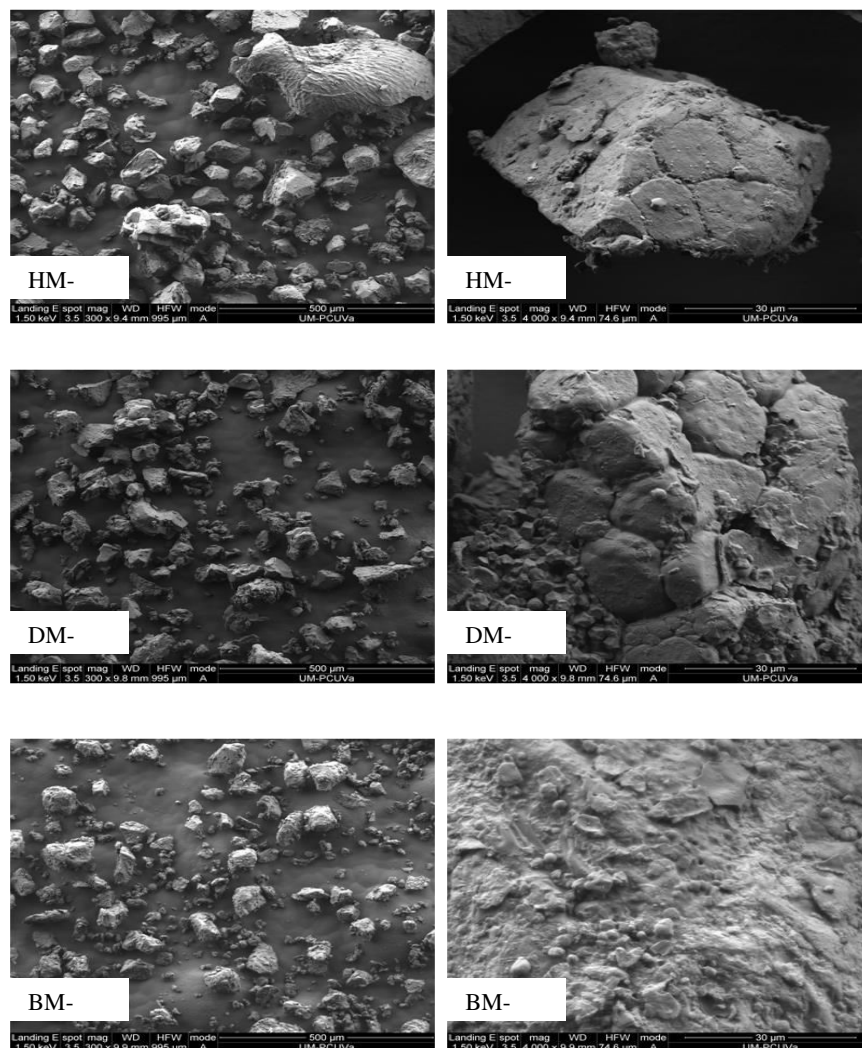


Fig. 4.1 SEM pictures of tef flours studied. HM: Hammer mill, DM: Disc mill, BM: Blade mill

4.3.2.2 Sensory result

The impacts of mill type (HM, DM, and BM) on sensorial quality of tef injera (HMI, DMI, and BMI) are presented in table 4.4. There was no significant difference between HMI, DMI and BMI in color, odor, taste, number of eyes, eye distribution and the top and the bottom surface of injera. The three injeras were described as white in color, acceptable in odor, slightly sour in taste with many eyes and regular eye distribution with a non-sticky and non-powdery top and bottom surface. However, a significant difference in the intensity of texture (degree of softness), eye size and overall acceptability were found. DMI (6.9) had a significantly softer texture than

that of HMI (4.10) and BMI (3.5). However, HMI and BMI had significantly higher scores of eye size, 6.0 and 6.1 respectively, than DMI (1.6). The difference in injera texture and eye size may come from the difference in tef flour particle size and its impact on fermentation (De la Hera et al., 2014). This implies that the fermentation kinetics may be affected by the flour particle size distribution and level of starch damage. Hence, the overall acceptability of injera was found to be influenced by the injera texture. There was no significant difference in the intensity of injera texture, eye size and overall acceptability between HMI and BMI.

Table 4.4 Sensory panel responses of injera prepared from tef flour using hammer, disc and blade mill.

Descriptors	Injera intensity		
	HMI	DMI	BMI
Color	7.1±1.6 ^a	7.2±1.8 ^a	6.2±0.8 ^a
Odor	6.6±1.2 ^a	5.6±1.9 ^a	4.3±1.1 ^a
Taste	5.3±1.5 ^a	6.4±1.7 ^a	5.3±0.7 ^a
Texture	4.1±1.5 ^a	6.9±1.7 ^b	3.5±1.1 ^a
Number of Eyes	6.1 ±1.4 ^a	7.8±1.9 ^a	6.2±1.1 ^a
Eye size	6.0 ±1.5 ^b	1.6±2.2 ^a	6.1±1.0 ^b
Eye distribution	4.4±1.5 ^a	6.4±1.9 ^a	5.5±1.0 ^a
Top and bottom surface	6.0±1.2 ^a	5.4±1.8 ^a	5.9±1.0 ^a
Overall acceptability	4.2±1.1 ^a	6.6±1.8 ^b	4.1 ± 1.2 ^a

HMI, DMI, and BMI stand for injera from hammer mill, injera from disk mill and injera from blade mill, respectively. Data are expressed as the mean ± standard deviations; different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

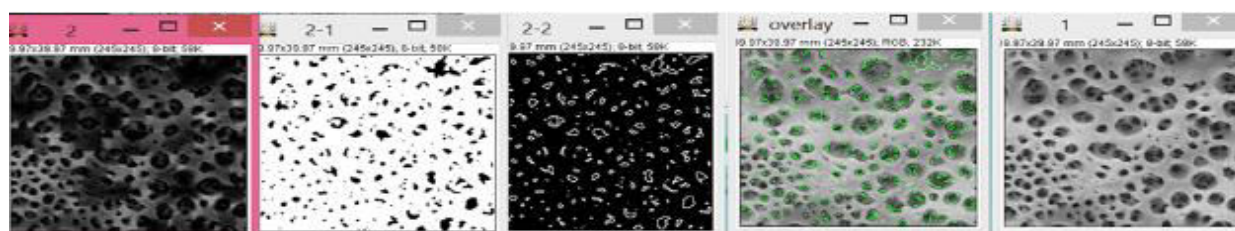
3.2.3 Application of Software in injera quality evaluation

ImageJ software was used for the quantification of the injera number of eyes, eye size and distribution and compared with human performance on three injeras (HMI, DMI, and BMI) (Table 4.5). There was no significant difference in the quantification of the injera number of eyes between human eyes and the software. The software results of the injera number of eyes (HMI-224 < BMI-271 < DMI-298) agrees with the sensory panelists' response (HMI-6.1 < BMI-6.2 < DMI-7.8). It is very difficult and takes much time to analyze the injera number of eyes, eye size, and eye distribution visually by human eye; however, the software analyzed it very quickly without any problem. The software easily converted the image of the injera sample into other forms and analyzed and reported it (Fig. 4.2). The software application required a quicker determination time than did visual determination. This may save time and help in manufacturing a uniform quality of injera in the manufacturing industry. In addition, the use of this software can give additional benefits to injera manufacturing countries to improve injera standards and requirements by including important injera quality attributes (number of eyes, eye size, and distribution) in measurable ways.

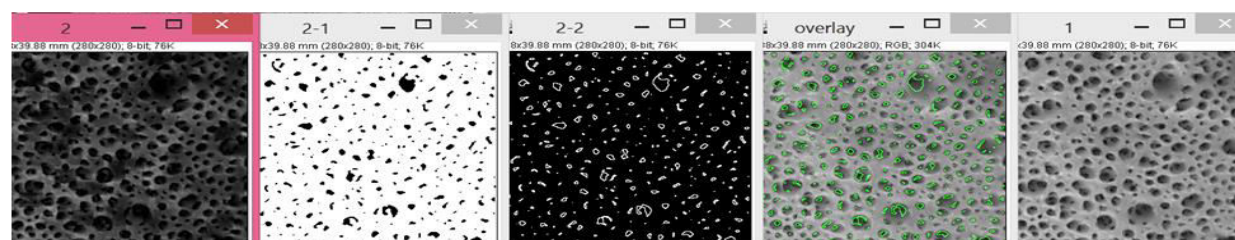
Table 4.5 Application of software in injera quality evaluation

Injera	Total number of eyes (Human Eye)	Total Number of eyes per square (Software)	Eye distribution and size			
			<1mm (%)	1-4mm (%)	4-7mm (%)	7-10mm (%)
HMI	224±3.4	224±4.0	41.6±1.2	55.2±1.4	3.2±0.1	0±0.0
DMI	298±2.4	298±2.4	74.3±0.9	20.2±1.0	5.5±0.1	0±0.0
BMI	271±3.6	271±3.8	47.8±1.7	49.1±1.1	3.1±0.3	0±0.0

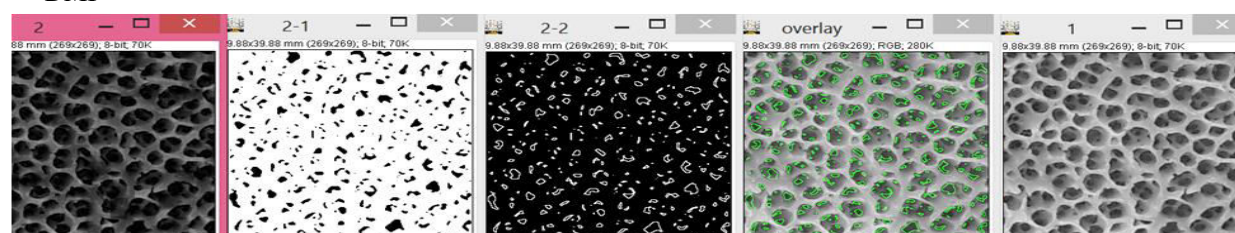
HMI, DMI, and BMI stand for injera from hammer mill, injera from disk mill and injera from blade mill respectively.



HMI



DMI



BMI

Fig. 4.2 Software based injera quality evaluation. HMI, DMI and BMI stand for injera from hammer mill, injera from disk mill and injera from blade mill respectively.

4.4 Conclusions

Differences in the mill type used to grind tef grain during injera preparation influenced the particle size distribution and damaged starch level of the tef flour and hence the final quality of injera. Although mill differences affect the color of the tef flour, the final color of the injera was not affected. This may be a result of the involvement of other unit operations that are mandatory in injera making. The variation in injera sensorial quality and starch digestibility were due to the differences in milling type and its effect on particle size distribution and the damaged starch level of tef flour. The use of DM gives smaller flour particle size with a high level of starch damage and leads to better sensorial injera quality than that of BM and HM. The use of BM leads to a larger flour particle size, which could be responsible for having lower RAG and RDS.

However, it is highly important to study the relationship between damaged starch and flour particle size on cooked flour. The influence of different tef cultivars and mill types on injera quality needs to be studied.

The application of software-based injera quality evaluation to determine the number of injera *eyes* gave effective results, which thus can replace human manual determination. Determinations of injera eye size and distribution are the additional advantage of using software-based injera quality evaluation.

Chapter 5-Influence of Mill Types on Tef Flour Characteristics and Injera Dough Rheological Properties

Chapter 5

Influence of Mill Types on Tef Flour Characteristics and Injera Dough Rheological Properties

Abstract

This study aimed at evaluating the effect of mill type - hammer (HD), disk (DM), and blade (BM) - on tef flour characteristics and gels and injera dough rheological properties. The flour obtained from BM had the highest bulking density (0.77 g/mL) and foaming capacity (12.67 mL) while those from DM showed the highest Water Solubility Index (7.94 g/100g that doubled the value of HM flour) and the lowest dispersibility (64%). Tef flours from DM had significantly higher peak and breakdown viscosities and lower trough viscosity. HM and BM flours exhibited higher final and setback viscosities while those from BM recorded higher pasting temperature. The highest hardness (2.09 N) and gumminess (0.75N) were recorded for gels made from BM flour while the lowest values (1.40N and 0.44N respectively) corresponded to DM flour. The type of mill showed an important effect on injera dough's rheological properties obtained from fundamental and empirical tests. The less consistent injera dough's, with the lowest viscoelastic moduli and the highest $\tan \delta$, were obtained for dough's made from HM flour. DM led to the stickiest dough's, with adhesive values of 0.94 N, while the minimum forward extrusion force was obtained for dough's made from BM tef flour. The disk mill yielded functional properties that indicate its better suitability to produce higher quality tef dough for injera making than other mill types.

Practical Applications

The findings from this study are needed for the transformation of traditional injera making process to an industrial level. This requires identifying the effect of different mill types and its influence on functional, textural, and rheological properties of tef flour and their gels and dough.

It is necessary to set-up industrial unit operations and during machinery selection process to get the required tef injera quality.

Keywords

Tef, mill type, rheology, texture, functional properties, pasting properties

5.1. Introduction

Food products developed from tef [*Eragrostis tef* (Zucc.) Trotter] are recently receiving global attention because they are rich in nutrient content, free from gluten and/or functional foods (Abebe & Ronda, 2014). Tef is an Ethiopian indigenous tropical cereal crop and the main staple in the country mostly used to make injera (Abebe & Ronda, 2014). Injera is leavened, flat round Ethiopian traditional bread made preferably from tef (Yetneberk et al., 2004). Its surface has essentially evenly spaced gas holes, that make up a honeycomb-like structure formed due to the production of gas during fermentation and baking, while the bottom surface is smooth and shiny (Yetneberk et al., 2004). The quality of injera is also expressed in its softness, fluffiness and its ability to be rolled without cracking (Yetneberk et al., 2004). As it is leavened bread made from non-gluten containing flour, it has great potential for commercial production internationally.

According to Achi (2013) traditional fermented foods are usually made under primitive conditions and time consuming, which result in inconsistent quality such as its sensory properties. Tef injera making process can be categorized in this group forcing many people to buy homemade injera from supermarkets and small injera shops. In addition, entrepreneurs processing injera for export markets are emerging. However, these firms are still using the same traditional injera baking method. Nout (1992) stated that the art of traditional processes requires being transformed into a modern way of processing technologies to standardize the quality of the end products and to save time and energy without affecting their desirable traits. Accordingly, the traditional injera making process should be transformed in order to make it suitable for home, cottage and industrial level of processing that guarantee efficiency and product with predetermined quality and uniformity.

However, most of the past studies related to injera were focused on studying the effect of fortifying or replacing tef with other cereals or characterizing the fermentative bacteria and studying their effect on the nutritional and antinutritional composition of the product (Baye, 2014). On the other hand, with the recent global attention is given to tef, a number of studies were published on tef and these works mostly dwelt on starch characterization, flour techno-functional properties, and nutritional and health benefits in wheat-based supplementation and/or gluten-free formulations of the western type products (Bultosa et al., 2002; Hager and Arendet, 2013; Renzetti et al., 2008 Gebremariam et al., 2012, Ronda et al., 2015).

Milling or grinding of cereal grains is commonly performed to produce flour, which is then used as an ingredient in many food products (Li et al., 2014). In Ethiopia, almost all tef grain is dry milled (whole floured) to make injera using Danish type stone-disk mill. In comparison to other milling operations, power use efficiency in these types of mills is generally low and yet they are preferred in many developing areas because of low costs and high durability (Bayram and Oner, 2005; Singh et al., 2005). However, in the modern food industry, there are many milling techniques like ball milling, hammer milling, and pin milling, which employ different types of mechanical forces to break the grains into smaller fragments or fine particles (Tran et al., 2011; Liu et al., 2011). These different milling or grinding processes produce flours with different particle sizes and density which consequently determine surface area available for reactions like water binding, solubility, heat transfer, and swelling that dictate flour quality and functionality (Brou et al., 2013; Chen et al., 1999) and end use. The damaged starch content is inevitably influenced by milling process and equipment utilized. Starch damage level can largely affect the functional flour characteristics mentioned above and starch digestibility (Hasjim et al., 2013; Mahasukhonthachat et al., 2010).

The impact of milling process on flour physical and functional properties, damaged starch content, and end product quality for common cereals like wheat and rice is well documented (Al-Rabadi et al., 2009; Kadan et al., 2008; Tran et al., 2011). Regarding tef, a report by Abebe and Ronda (2014) indicated some visible effects of concentration, temperature and storage time on tef flour gel rheological and textural properties. On tef flours obtained from two types of mills, influences of tef variety type and flour particle size distribution were significant on flour functional properties, starch damage and starch enzymatic hydrolysis (Abebe et al., 2015). Therefore, in looking for different options of milling to produce suitable flour from tef assessing the performance of different mill types is mandatory. In thriving for improved injera processing, studying dough rheology and structure to relate it with the baking process and injera quality is critical. Evaluation of dough mechanical handling attributes like stickiness and extrusion is also important to avoid disruption and product loss (Armero & Collar, 1997).

Therefore, the present study evaluates the effect of three different mill types on tef flour characteristics (functional and pasting properties, gel rheological and textural characteristics) and dough rheological properties intended for injera making.

5.2. Materials and methods

5.2.1. Materials

Based on popularity among Ethiopian tef farmers and users, DZ-Cr-387 or Qouncho (white tef) was selected for this study and the grain was obtained from DebreZeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR).

5.2.2. Milling process

The tef grain was manually cleaned by winnowing and sifting to remove stones, soils, weeds, large chaff and other unwanted materials. Three types of mill were used to get the whole flour: A hammer mill (HM) (Pertin 120, Finland) fitted with 0.8 mm sieve, a stone-disk mill (cottage tef grain-mill, Denmark) (DM) and a blade mill (BM) (Nutri Bullet NB-101B, China). In the case of the blade mill, the grain sample was milled for 7 minutes.

5.2.3. Flour characteristics

5.2.3.1. Particle size distribution

According to Sivaramakrishnan et al. (2004) flour particle-size distribution was evaluated by passing the tef flour through an automatic standard sieve shaker (Retsch, Germany) which contains 5 sieves. Determination of flour particle size distributions was done when the sieves were shaken using electric power. The percentage fraction of the sample retained on each sieve was measured by weighing.

5.2.3.2. Bulk density

Bulk density (BD) (g/ml) was determined according to the method described by Wang and Kinsella (1976). A known amount of sample was weighed into 50 ml graduated measuring cylinder. The sample was packed by gently tapping the cylinder on the bench top from a height of 5 cm. The volume of the sample was recorded.

5.2.3.2. Functional properties

Foaming capacity (FC) and foam stability (FS) were determined as described by Collar and Angioloni (2014a, 2014b) based on the methods used by Alu'datt et al. (2012). Briefly, about 2 g of flour sample was mixed with 40 mL distilled water at 30°C in a 100 mL measuring cylinder. The suspension was stirred and shaken manually for 5 min to produce foam. The volume of foam was measured after 0 min (VT) and 60 min (V1). FC was calculated directly from VT while FS were calculated from $100 \cdot (V1/VT)$.

Water absorption index (WAI) (g/g) and water solubility index (WSI) (g/100g) were determined according to Solusulski (1962). 1g of sample (w_o) was weighed into tared centrifuge tube, added 10ml of distilled water, stirred gently with a stirring rod for 30min, cooked at 90°C for 10 min and cooled to room temperature. The tube was centrifuged at 4000rpm for 15min. The supernatant was decanted into tared crucible, dried in oven at 105°C until total water evaporation and weighed (w_{sd}). The sediment in the centrifuge tube was also recorded. WAI was obtained from w_{ss}/w_o and WSI from $100 \cdot w_{sd}/w_c$.

Water absorption capacity (WAC) (ml/g) was determined according to Vioque et al. (2000). About one gram of sample was weighed into 25 mL pre-weighed centrifuge tubes. For each sample, de-ionized water was added in small increments and mixed with stainless steel wire until the sample was saturated with water. The samples were allowed to stand for 30 min at room temperature (20 °C) and then centrifuged at 2200xg for 30 min. The water released by centrifugation was drained and the tube weighted. WAC was calculated from the weight of the moistened flour divided by the initial weight of sample.

Oil absorption capacity (OAC) (ml/g) was determined according to Adeleke and Odedeji (2010). About 1.0 g sample was mixed with 10ml of oil for 5min using glass rod, the mixture was

centrifuged at 3.500 rpm for 30 min. The oil released by centrifugation was drained and the tube weighted. OAC was calculated from the weight of the moistened flour divided by the initial weight of sample.

Dispersibility (%) was determined according to the method described by Kulkarni et al. (1991). About 10 g of flour was suspended in 100 ml measuring cylinder and distilled water was added to reach a volume of 100ml. The setup was stirred vigorously and allowed to settle for three hours. The volume of settled particles was recorded and subtracted from 100. The difference was reported as percentage dispersion.

5.2.3.3. Pasting properties

Viscometric profiles of flours were obtained with a Kinexus Pro+rheometer (Malvern Instruments Ltd, Marlvern, UK) supplied with starch pasting cell and controlled by rSpace software. Samples of tef flours (3 g, 14% moisture-based) were transferred to the cylindrical container where 25 mL \pm 0.1 mL of distilled water was added. Each sample was analyzed at least in duplicate. Parameters calculated from the pasting profiles were: peak viscosity (PV), trough viscosity (TV), breakdown (BV), final viscosity (FV), setback (ST), pasting temperature (PT) and Peak time (Pt).

5.2.4. Gel texture

Gels were prepared from 16% (w/w) suspensions of flours (28.5 g total weight), the minimum concentration that led to self-standing gels (Abebe & Ronda, 2014), by using Kinexus Pro+rheometer (Malvern Instruments Ltd, Marlvern, UK). The suspensions were stirred with a constant rotating paddle at 960rpm for the first 10 seconds and then 160 rpm, heated from 50 to 95 °C at a rate of 6°C/min, followed by rapid cooling. The samples in canisters were hermetically closed to prevent moisture loss and equilibrated to room temperature for 1 h before analysis. The

texture properties including firmness, adhesiveness, springiness, cohesiveness, and resilience and gumminess of flour gels were evaluated using a TA-XT2 Texture Analyzer (Stable Microsystems, Surrey, UK) equipped with the software Texture Expert. Measurements were done on gel samples with 2 cm-diameter and 2 cm-high. A double compression test (TPA) with an aluminum 75 mm diameter (SMSP/75) probes and recorded at a crosshead speed of 1 mm/s. A 50% deformation was chosen to avoid the total destruction of the gel structure in the first compression of TPA tests. Larger deformations usually applied (75-80%) could crush the samples and lead to invalid textural results (Huang et al., 2007). All measurements were taken in duplicate.

5.2.5. Dough rheology

5.2.5.1. Dough preparation

Injera dough was prepared according to the method described by Zegeye (1997) from 2:3 flour-water ratios using kneading machine (Auto bakery FAB-1800, China) for 8 minutes. In order to minimize the changes in the dough rheological properties with time and get a stable measurement, starter culture ('ersho') was not added in the dough samples (Ronda et al., 2017).

5.2.5.2. Oscillatory measurements

Dynamic oscillatory rheometry of the dough samples was carried out according to the method described by Abebe and Ronda (2014) using rheometer (Kinexus Knx5210, United Kingdom) with a serrated surface parallel plate (40 mm diameter) and with 1 mm gap at 25 °C. Samples were left for five minutes to allow relaxation before starting the assay. Frequency sweeps were carried out in the previously established linear viscoelastic zone, from 10 to 0.1 Hz. Stress sweeps were conducted from 0.01 to 500 Pa at 1 Hz of frequency. The dough was prepared twice and measured in duplicate. Frequency sweep data were fitted to the power law model as in

previous works (Ronda et al., 2011): The coefficients G'_1 , G''_1 , and $(\tan \delta)_1$, stand for the elastic modulus, viscous modulus, and the loss tangent at a frequency of 1 Hz. Fittings were done in the frequency range (1-10 Hz), where a linear double logarithm curve was systematically obtained. The a, b and c exponents quantify the degree dependence of these moduli and the loss tangent with the oscillation frequency, ω , expressed in Hz.

5.2.5.3. Forward extrusion test

Dough consistency measured from forward extrusion tests were carried out according to Ronda et al. (2013) using a TA-XT2 Texture Analyzer (Stable Microsystems, Surrey, UK) equipped with a 25 kg load cell and operating at 0.5 mm/s head speed. The test measures the compression force required for a piston disk to extrude the dough through a specific size outlet (5 mm) in the base of the sample container. Samples were carefully scooped into acrylic cylindrical containers (20 cm³ volume) with help of a spatula. The complete sample container was located into a centralizing insert fitted into the Heavy Duty Platform, and the plunger was attached to the load cell using a probe adaptor. Compression force time curve allowed evaluating maximum force, determined as the force at which the slope changed. The change of slope was visually detected, and the force at this point calculated using the Texture Analyzer software. The curve plateau representing the force necessary to continue with the extrusion process and the area under the curve were both used to define the sample consistency. All measurements were performed in triplicate.

5.2.5.4. Dough stickiness

This assay was conducted by following the procedure proposed by Grausgruber et al. (2003) and used by Ronda et al. (2011). A texturometer TA-XT2 from Stable Microsystem (Surrey, UK) provided with a SMS/Chen-Hoseney device where the sample was placed and a methacrylate 25-

mm cylinder (P/25P) as compression cell were used. The stickiness of the dough was determined at pretest and test speeds of 0.5 mm/s, a post-test speed of 10.0 mm/s and 40g force. Three parameters were used to define stickiness: the positive maximum force or adhesive force, which is the measure of stickiness, the positive area under the curve or the adhesive energy, which is the work of adhesion, and the distance the sample is extended on probe return, which is an indication of sample cohesion/dough strength. Six replicates were carried out for all dough samples.

5.2.6. Experimental design and statistical analysis

The experiment consisted of one factor (the type of mill) at three levels. SPSS was used to test for significant variations between means of tef flours from mill types. Duncan's multiple range tests were used to identify significant differences. Significance was accepted at $p < 0.05$. The sample was run at two replications and the results were reported as mean \pm standard deviation (SD).

5.3. Results and Discussion

5.3.1. Flour particle size, bulk density and functional properties

Flour fraction in $<0.09\text{mm}$ had significantly varied in the order of disk mill (32.6) > hammer mill (25.4) > blade mill (0.0). Flour from blade mill (0.29mm) had the highest average flour particle size while lowest was disk mill flour (0.11mm). The reason for having different particle size distribution maybe from the difference in milling principle's among the three mills. Hammer mill hammered the grain repeatedly until it passes through the sieve which was fitted inside the mill as the part of the mill. Tef grain was ground between two stone-discs in case of disc mill. Blade mill had rotary blades to grind the grain.

The BD of the tef flours obtained from the three mill types varied significantly ($p < 0.05$) (Table 5.2) in the order: disk mill (0.63g/ml) < hammer mill (0.73g/ml) < blade mill (0.77 g/ml). This could be related to the size of flour particles which varied in similar order (table 5.1). Disk mill led to flours with the smallest average particle size. The observation, in agreement with the statement of Brown and Richards (1970) corroborated by Abebe et al., (2015) can be ascribed to the loosely packed structure in fine particles versus denser aggregated granules found in samples with larger particle size.

The impact of mill type was important ($p < 0.05$) on flour FC while its effect on FS was not as such visible (Table 3.2). The tef flour milled from blade mill exhibited the highest FC (12.67%) followed by the flour from the disk mill (10.50%) which was apparently in the range reported for DZ-Cr-387 (Abebe et al., 2015). Therefore, the suitability of these flours in food systems that require aeration for textural and leavening may vary accordingly. Among the flour hydration properties WSI and dispersibility were significantly ($p < 0.05$) affected by the mill type whereas WAI and WAC remained equivalent. The flour from disk mill had the highest WSI (7.94 g/100g) while lowest score was for hammer mill (4.17 g/100g). The higher value of WSI from disk mill could be due to the smaller particle size of flour from this mill, the greater surface area for binding water molecules and higher damaged starch content as corroborated by Abebe et al. (2015).

There was no significant ($p > 0.05$) effect of the mill type on the WAI, WAC and OAC of tef flours which averaged values were 2.3 g/g, 0.9 mL/g and 2.4 mL/g respectively in agreement with results found by Asmeda and Noorlaila (2015).

A lower dispersibility (64%) was recorded in tef flour obtained from the disk mill while higher dispersibility was shown by the tef flours from the hammer and blade mills with no significant ($p>0.05$) difference between themselves. This could also be related to the finer flour particle size in the disk milled tef flours causing clump formation and may make it takes more time and energy during dough preparation.

Table 5.1 Average flour particle size as affected by mill type

Mill type	Average size (mm)	Variation coefficient	
		(%)	Fraction < 0.090 mm
Hammer	0.16	69	$25.4 \pm 0.3b$
Disk	0.11	64	$32.6 \pm 0.7c$
Blade	0.29	49	$0.00 \pm 0.0a$

5.3.2. Pasting properties

The pasting properties of tef flours were significantly ($p<0.05$) influenced by mill type (Table 5.3). Significantly higher ($P<0.05$) PV and BV values were scored by the tef flours from the disk mill than the flours from the hammer and blade mills which have no significant difference among them. This may be due to the smaller flour particle size in the flours from the disk mill. PV was strongly negatively correlated with an average particle size of the granules (Asmeda et al., 2016). Abebe et al. (2015) reported PV values 1304-1618 mPas and 1317-1676 mPas for three tef flours prepared from cyclotech and disk mill respectively which supports this study. They also noted that tef flours with higher WSI and WAI tend to have higher PV and BV ($p < 0.05$, $r \geq 0.6$) which agreed with current results. The lower BV results from hammer and blade mill implied the higher thermo-stability and lower shear thinning and disintegration of swollen systems (Abebe et al., 2015).

Effect of mill type on TV of tef flours was significant ($p < 0.05$) where the TV values varied in the order: hammer mill (1011 mPas) > blade mill (968 mPas) > disk mill (926mPas). In this study, the lower TV values for the flours from the disk mill could be contributed by the smaller particle size it has a higher percentage of <0.09 (mm) (table 5.1). Asmeda et al. (2016) suggested that average particle size of the granules were strongly negatively correlated with TV.

Tef flours obtained from hammer and blade mill, which did not score significantly ($p > 0.05$) different FV and SV among them, exhibited higher FV and SV values than the tef flour obtained from disk mill. The higher SV scored by the tef flours from the hammer and blade mill indicates the higher recovering of viscosity in them during cooling period and the presence of relatively higher amylose retrogradation.

The PT of the tef flours was significantly influenced by the mill type and it varied in the order: disk mill (78.3°C) < hammer mill (80.1 °C) < blade mill from the blade mill (83.4 °C). Abebe et al. (2015) suggested tef flours with higher WSI tend to have lower pasting temperature ($p < 0.05$, $r \geq -0.6$) and this is in agreement with the current finding.

Table 5.2 Flour bulk density and functional properties

Mill type	BD (g/ml)	FC (ml)	FS (%)	WSI (g/100g)	WAI (g/g)	WAC (ml/g)	OAC (ml/g)	Dispersibility (%)
Hammer	0.73±0.01 ^b	6.83±0.29 ^a	78.02±7.21 ^a	4.17±1.04 ^a	2.37±0.04 ^a	0.93±0.12 ^a	2.40±0.17 ^a	71.00±0.0 ^b
Disk	0.63±0.00 ^a	10.50±0.50 ^b	85.70±8.25 ^a	7.94±0.91 ^c	2.37±0.07 ^a	0.87±0.06 ^a	2.50±0.00 ^a	64.00±0.0 ^a
Blade	0.77±0.00 ^c	12.67±0.29 ^c	76.41±10.13 ^a	7.49±0.54 ^b	2.30±0.03 ^a	0.97±0.06 ^a	2.30±0.17 ^a	71.00±0.0 ^b

Note: BD= bulk density, FC= foaming capacity, FS= foam stability, WSI=water solubility index, WAI= water absorption index, WAC= water absorption capacity and OAC= oil absorption capacity. Data are expressed as mean ± standard deviations; Different superscripts in the same column indicate statistically significant differences (P < 0.05).

Table 5.3 Pasting properties of tef flour influenced by mill types

Mill type	PV (mPas)	TV (mPas)	BV (mPas)	FV (mPas)	SV (mPas)	PT (°C)
Hammer	1547±26 ^a	1011±75 ^c	535±72 ^a	1951±15 ^b	991±9 ^b	80.11±1.33 ^b
Disk	1768±93 ^b	926±19 ^b	841±47 ^b	1917±13 ^a	929±22 ^a	78.28±1.39 ^a
Blade	1544±15 ^a	968±19 ^a	576±33 ^a	1940±23 ^b	983±16 ^b	83.42±0.99 ^c

Data are expressed as mean ± standard deviations; Different superscripts in the same column indicate statistically significant differences (P< 0.05). Note: PV = peak viscosity, TV = trough viscosity, BV = breakdown viscosity, FV = final viscosity, SV = set back viscosity, and PT = pasting temperature.

Table 5.4 Gel textural properties of tef flour as influenced by mill types

Mill type	Hardness (N)	Gumminess (N)	Adhesiveness (N)	Springiness (N)	Cohesiveness (N)	Chewiness (N)	Resilience (N)
Hammer	1.77±0.02 ^b	0.52±0.04 ^b	-0.55±0.12 ^a	0.50±0.02 ^a	0.25±0.02 ^a	0.26±0.03 ^a	0.14±0.02 ^a
Disk	1.40±0.11 ^a	0.44±0.00 ^a	-1.50±0.63 ^a	0.48±0.02 ^a	0.38±0.03 ^c	0.21±0.01 ^a	0.10±0.01 ^a
Blade	2.09±0.13 ^c	0.76±0.02 ^c	-1.47±0.11 ^a	0.54±0.02 ^a	0.31±0.03 ^b	0.41±0.03 ^b	0.14±0.02 ^a

Note: Data are expressed as mean ± standard deviations; Different superscripts in the same column indicate statistically significant differences (P < 0.05).

5.3.4. Flour gel textural properties

The texture is a very important quality attribute of injera that affects consumers' perception. Softness, fluffiness and its ability to be rolled without cracking and bottom surface smoothness are the critical factors determining injera quality because injera is normally consumed by hand with a stew which is made from plant and animal products (Zegeye, 1997; Yetneberk et al., 2004). Difference in mill type had significantly ($p < 0.05$) affected important TPA parameters like hardness, and gumminess, cohesiveness and chewiness (Table 5.4). The gel prepared from the tef flour obtained from the disc mill scored the lowest hardness, gumminess, and chewiness and it was found to be the most cohesive than the flours from the other mills. The probable reason for this could be the higher pulverization by the disc mill which gave a higher proportion of small-sized flour granules which has the highest starch damage and water-soluble components (WSI) that led for the formation of softer tef flour gels. However, the influence of the mill type on the remaining TPA parameters, which include adhesiveness, springiness, and resilience, was not as such important. The findings of Abebe and Ronda (2014) on tef flour gels from three tef varieties indicated the presence of some variation in gel textural properties by variety type and evolution of the texture properties of the tef gels varied notably during storage, taking a longer time to level off.

5.3.5. Viscoelastic properties of tef dough

The results of fitting the frequency sweeps data to power law are presented in Table 5.5. The values of G' and G'' exhibited by the dough's in this study were much lower than usual for wheat flour dough (Dobraszczyk & Morgenstern, 2003) and they are still lower than wheat-tef composite dough samples with high amount of water in the *giabatta* formulation (Ronda et al., 2015) which decreased with tef flour incorporation level. This is due to the absence of gluten in tef flour, which is often termed as the structural protein for bread making, that when hydrated

forms a viscoelastic network responsible for the retention of the gas produced during fermentation, proofing and for the dough development as a result of its expansion during baking. However, dough with higher elastic moduli, G' , may not be suitable for injera making because the carbon dioxide in the batter has to escape easily facilitating the formation of the required evenly spaced injera eyes.

The coefficients G'_1 , G''_1 and $(\tan \delta)_1$ and the exponents a , b and c showed a significant difference ($p < 0.05$) with the mill type indicating the dependence of the viscoelastic behavior of the dough samples on the mill type utilized. The viscoelastic moduli of dough's prepared from the tef flour obtained from blade mill were very much lower than those obtained from the hammer and blade mills. In addition, the viscous modulus (G''_1) of the dough obtained from the blade mill tef flour was nearer the elastic one (G'_1) which can be evaluated from the loss tangent (G''_1/G'_1) that was particularly high (0.74) than when DM or HM mills were used (0.30 and 0.27 respectively) denoting the dough still have elastic-like behavior. The dough from the blade mill had structures most dependent on frequency (higher a and b exponents) and higher $\tan \delta$ (Ronda et al., 2013, 2015).

Table 5.5 Effect of mill type on dough rheological properties

Mill type	G'_1 (elastic module)	a	G''_1 (viscous module)	b	$\tan \delta (G''_1/G'_1)$	c
Hammer	327.5±136.88 ^c	0.11±0.01 ^a	86.31±29.04 ^c	0.31±0.02 ^a	0.27±0.04 ^a	0.21±0.02 ^b
Disk	182.69±62.07 ^b	0.12±0.01 ^a	53.09±13.36 ^b	0.34±0.02 ^b	0.30±0.03 ^a	0.22±0.01 ^b
Blade	12.40±3.22 ^a	0.63±0.09 ^b	8.90±1.08 ^a	0.65±0.06 ^c	0.74±0.11 ^b	0.03±0.01 ^a

Data are expressed as mean ± standard deviations; Different superscripts in the same column indicate statistically significant differences ($P < 0.05$).

5.3.6. Tef dough stickiness

Tef dough stickiness was significantly ($p < 0.05$) affected by mill types (Table 5.6). Tef flours produced from disk mill exhibited the highest adhesive force, (0.94 N) and adhesive energy (0.04

mN·s), significantly above (more than 50% higher) the stickiness of dough's made from BM and HM tef flours. This could be associated with the presence of a higher percentage of smaller sized flour particles which may have higher starch damage level in the disc milled flours (Yildiz et al., 2012). Earlier work by Abebe et al. (2015) revealed that incorporation of tef flour in wheat-based dough increased dough stickiness and the values increased with the increased proportion of tef flour. Distance to return, the distance the samples extended on probe return (mm) indicating the sample cohesion/dough strength, of the samples, did not reach significant differences.

Table 5.6 Tef dough stickiness influenced by mill types

Mill type	Adhesive force (N)	Adhesive energy (Positive area) (mN s)	Distance on return (mm)
Hammer	0.59±0.08 ^b	0.03±0.01 ^b	1.95±0.45 ^a
Disk	0.94±0.04 ^c	0.04±0.01 ^c	2.29±0.68 ^a
Blade	0.40±0.06 ^a	0.02±0.01 ^a	1.62±0.52 ^a

Data are expressed as mean ± standard deviations; Different superscripts in the same column indicate statistically significant differences (P< 0.05).

5.3.7. Dough forward extrusion

It was not possible to undertake the forward extrusion test on dough made from blade mill flour; this was due to the very low dough consistency that passed through 0.5mm (the smaller hoe size available) outlet by gravitational force (without applying extrusion force) in coherence with rheological behavior already presented from fundamental/oscillatory rheological tests. Forward extrusion results of tef dough from the disk mill and hammer mill showed no significant (p<0.05) differences in forward extrusion force and energy with averaged values of 1.45N and 123 mN·s respectively between disk mill and hammer mill flour in force required to push the dough to pass through 0.5mm outlet (Table 5.7).

Table 5.7 Influence of mill type on dough Consistency (forward extrusion test)

Mill type	Force (N)	Adhesive energy (Positive area) (mN s)	Distance on return(mm)
Hammer	1.50±0.07 ^a	123.45±2.30 ^a	41.25±2.73 ^a
Disk	1.35±0.05 ^a	122.47±15.73 ^a	42.66±3.20 ^a
Blade	NA	NA	NA

Data are expressed as mean ± standard deviations; Different superscripts in the same column indicate statistically significant differences (P< 0.05). NA: Not applicable

5.4. Conclusion

This research showed that different mill types have a substantial impact on functional characteristics of tef flours and the rheological and textural properties of the gels and injera dough's derived from them. Tef flour produced from disk mill yields with the highest percentage of WSI that indicates suitability to produce higher quality flour based products like Injera than other mill types.

***Chapter 6-Effect of Mechanical
Kneading and Absit Preparation
Difference on Tef Injera Quality and Its
Parameters Optimization***

Chapter 6

Effect of Mechanical Kneading and Absit Preparation Difference on Tef Injera Quality and Its Parameters Optimization

Abstract

Processing contributes for the quality of baked goods. Injera, the common and staple Ethiopian food needs deep study on its inherent characteristics related to processing for its use in various food applications across the globe. Therefore, the present study was to investigate the effect of mechanical kneading and ‘*absit*’ preparation difference on tef *injera* quality. Standard methods were adopted to determine the starch fraction, total phenol, flavonoid, phytate and tannins. Sensory injera quality was assessed using nine point hedonic scales. Change in kneading conditions (time/speed) did not significantly affect the FSG, SDS, RS, TS and SDRI. On the other hand statistically significant variation was observed on RAG and RDS. Flavonoid, total phenolic content and phytate were significantly varied at different kneading time- speed combinations. Injeras sensory quality were also significantly affected due to change in kneading conditions. Tef dough kneading method was optimized at kneading speed 6 with 3 minutes of kneading time which has higher desirability to give 8-9 point hedonic scale of injera overall acceptability. In addition to kneading conditions, absit preparation (water to fermented dough ratio) was also found to affect the quality of tef injera. Absit preparation method was optimized with a combination of 100 ml of batter to 900 ml of water to gives the higher desirability in terms injera overall acceptability. In conclusion, both kneading and absit preparation method have significantly influence starch hydrolysis, sensory, flavonoid, total phenolic and phytate content of injera. Further studies are also required on different tef varieties.

Keywords: Kneading, Sensory quality, Absit preparation, Starch fractions, polyphenols

6.1. Introduction

More than 70% of Ethiopian populations rely on injera for their diet, which is a traditional Ethiopian sourdough flatbread (Dijkstra et al., 2008). Injera is a fermented and naturally leavened flatbread indigenous to Ethiopia, ~50cm in diameter with a honeycomb-like texture, rather like a giant crumpet (Belton and Taylor, 2004). It is mostly made from flour obtained from the tef grain (*Eragrostis tef* [Zucc.] Trotter), in addition, injera can be made from different cereals such as wheat, sorghum, and maize having different quality (Yetneberk et al., 2004). 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. 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 with uniformly spaced honeycomb-like “eyes” on the top.

The fermentation of injera begins with adding water to tef flour and mixing or kneading it with a starter (back-slopped culture) called *Ersho*. This process commences the ‘primary fermentation’ (Attuquayefio, 2014). 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 *Ersho* are kneaded into a thick paste or dough (Ashagrie and Abate, 2012; Girma et al., 2013).

Kneading in bread making is known to aerate the dough and according to Maloney and Foy (2003), gas retention depends on the development of the proper dough structure which requires

adequate enough 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.

Injera with much and evenly spread eyes, soft texture, easily roll able and bland after taste is rated as excellent (Yetneberk et al., 2004). Intrinsic tef flour quality factors which favor these quality aspects include starch granule characteristics and the higher water solubility index of tef flour which positively influence injera quality (Yetneberk, Rooney, & Taylor, 2005). Dough processing is also an important factor determining the quality of baked goods. The most important mechanical steps in industrial dough processing are kneading, extrusion, and molding. In all of these processing steps, considerable changes in the structure and properties of the dough can occur (Amjid et al, 2013). Moreover, the polyphenolic content of foods will also be affected due to processing like kneading and mixing (Chlopicka et al., 2012). Li et al., (2015) also described as food processing including mixing, kneading, and heating affects the antioxidant properties of foods for which polyphenols are responsible.

Banu (2000) described that kneading is one of the most important operation in the manufacturing of bread. The main purpose of the kneading operations is to obtain a homogeneous mixture of the raw and auxiliary materials and at the same time obtain dough with viscous-elastic structure and properties. In addition, while kneading, in dough it is included a quantity of air, which is very important for rheological properties of the dough, and for the quality of the final product. During kneading, frictional heat causes the rise of the dough temperature. To control the desired

dough temperature the water temperature has to be adjusted. The formation of the dough with its specific structure and rheological properties occurs because of several processes such as physical, colloidal, biochemical, and the main role are being held by the physical and colloidal processes (Bordei, 2004).

Once fermentation has been takes place, 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’ (Zegeye, 1997). Zanniniet al. (2012) stated that the functionality of *absit* in the injera flat bread 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 reported that the *absit* is a dough enhancer (improves the texture of the dough) and Girmaet 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. Yetneberket 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 et al. (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.

Tef can be a valuable addition to a gluten-free diet of celiac disease patients (Dijkstra et al., 2008). However, as a relatively new raw material for most countries other than Ethiopia, a deep study on its inherent characteristics related to processing is still needed for its use in various food applications across the globe. Therefore, the present study was to investigate the effect of mechanical kneading and ‘*absit*’ preparation difference on tef injera quality.

6.2. Materials and methods

6.2.1. Materials

Among the different varieties released by DebreZeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR), farmers predominantly prefer the qouncho tef variety (DZ-Cr-387). This variety was selected and obtained from DebreZeit Agricultural Research Center. Tef sample was hermetically stored in cool and dry place using polyethylene bag. Before milling, tef grain was cleaned by sifting. The kneading conditions (time/speed) (Table 6.1) was chosen based on preliminary assessment of injera exporters, kneading machine capacity, and based on other works on bread dough kneading, similarly the ratio of water to fermented dough for absit preparation was based on previous work and traditional practices. Based on the sensory results, Kneading # 1 (have shorter kneading time with slower kneading speed and gives moderate overall injera acceptability), #5 (have moderate kneading time with moderate speed and gives the highest injera acceptability) and #9 (have longer kneading time with fastest kneading speed and gives the lowest injera acceptability) were selected for starch hydrolysis, flaonoids, total phenolic contents, phytate and tannins analysis.

Table 6.1. Kneading and absit variables

Kneading variables			Absit variables		
Kneading	Time (min)	Speed (speed)	Absit	Fermented dough (ml)	Water (ml)
1	1	1	1	100	100
2	1	6	2	100	300
3	1	12	3	100	900
4	3	1	4	300	100
5	3	6	5	300	300
6	3	12	6	300	900
7	7	1	7	0	100
8	7	6	8	0	300
9	7	12	9	0	900

6.2.2. Injera Preparation

Tef dough samples were prepared according to Parker et al. (1989) and Zegeye (1997) with little modification. Amount equal to 60ml of starter (*ersho*) was initially added for each kg of flour. Accordingly, the tef flour (from stone-disc mill) was mixed 2:3 (w/w) with potable water and kneaded by kitchen aid (Moulinex Masterchif 720, France). The dough was allowed to ferment for 60 hours at room temperature (21 ± 5) °C After this primary fermentation, different dough-water combinations (table 6.1) were used to prepare *absit* and the *absit* was heated for 15min with continuous stirring. The hot cooked dough (*absit*) was then mixed back into the fermenting dough, and sufficient potable water was added to make a batter. The batter was left covered for 2 hours for secondary fermentation. Additional water was added to thin and form the right consistency of batter. Finally, half a liter of batter was poured onto the hot clay griddle in a circular form. After 2-3 min of cooking using electric injera baking equipment, injera was removed and placed in a basket.

6.2.3. Starch fractions analysis

Method by Englyst et al. (1992) were used to measure in vitro starch digestibility of tef injera with the modifications by Englyst, et al. (1999); Englyst et al. (2000). The hydrolyzed glucose at 20 min (G20) and 120 min (G120) and the total glucose (TG) were measured by the glucose oxidase colorimetric method. The free sugar glucose (FGS) content was measured by a separate test according to the procedure proposed by Englyst et al. (2000). Rapidly digested starch (RDS) = $0.9 * (G20 - FGS)$, slowly digestible starch (SDS) = $0.9 * (G120 - G20)$, resistant starch (RS) = $0.9 * (TG - G120)$, for total starch, (TS) = $0.9 * (TG - FGS)$ and rapidly available glucose of the sample (RAG) = G20 were calculated. As used by Abebe et al. (2015), starch digestibility rate index (SDRI) was computed from the percentage of RDS in TS in the flours.

6.2.4. Sample extraction

Samples were extracted based on the procedures outlined by Barros et al., (2007) and Ferreira et al. (2007). About five gram of injera sample was extracted by stirring with 100 ml of methanol at 25°C at 150 rpm for 24 h using temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 ml portions of methanol as described above. The combined methanolic extracts were evaporated at 40°C to dryness using rotary evaporator (Stuart R3300) and re-dissolved in methanol at the concentration of 50 mg/ml and stored at 4°C for further use.

6.2.5. Determination of total phenolic content

Phenolic compounds concentration in the injera methanolic extracts was estimated based on procedures described by Ferreira *et al.* (2007). One milliliter of sample (2000 µg) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water.

The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to construct the standard curve (0.5–100 µg/ml).

6.2.6. Determination of total flavonoids

Total flavonoid was determined by a colorimetric method as described by Xu and Chang (2007). Briefly, 0.25 ml of sample (50 mg) was mixed with 1.25 ml of deionized water and 75 µl of a 5% NaNO₂ solution. After 6 min, 150 µl of a 10% AlCl₃.6H₂O solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the pink color was measured at 510 nm against the blank. For the calibration curve, (+)-Catechin was used with a concentration range of 10–1000 µg/ml. Results were expressed as mg (+)-catechin equivalent (CE)/g of extract.

6.2.7. Phytate

The phytate content in the sample was determined according to the method described by Oyaizu, (1986). About 0.1 g of fresh samples was extracted with 10 ml 2.4% HCl in a mechanical shaker for 1 hour at a room temperature. The extract was centrifuged at 3000 rpm for 30 minute. The clear supernatant was used for phytate estimation. One ml of Wade reagent (containing 0.03% solution of FeCl₃.6H₂O and 0.3% of sulfosalicylic acid in water) was added to 3 ml of the sample solution (supernatant) and the mixture was mixed on a vortex for 5 seconds. Absorption readings at 500 nm were taken against a blank sample consisting of 3 ml extract solution with 2 ml of 2.4% HCl without wade reagent. Sodium salt of phytic acid (4.5-36 mg/ml) was used as standard for construction of calibration curve.

6.2.8. Condensed Tannins

Tannin was determined by Burns, (1971) as modified by Maxson and Rooney, (1972). One gram of sample was weighed and mixed with 10 ml 1% HCl in methanol in a screw cap test tube. Then

the tube was shaken for 24 hr at room temperature on a mechanical shaker. The solution was centrifuged at 1000 rpm for 5 minute. One ml of supernatant was transferred to another test tube and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8%concentrated HCl in methanol and 4% vanillin in methanol). After 20 minutes, the absorbance of the solutions and the standard solution were measured at 500 nm. Blank sample consisted 1ml of extract solution with 5 ml of 1 % HCl without vanillin-HCl reagent. (+) catechin (0.5-12mg /100ml) was used as standard for construction of calibration curve.

6.2.9. Sensory quality Analysis

The sensory evaluation was carried out by trained panelists according to Einstein (1991). The selected panelists were tested for their ability to detect basic tastes (Jellinek 1985). The selected panel consisted of 10 people as recommended by Stone and Sidel (1985). They were female and male, who were student in Addis Ababa University. Nine injera quality descriptors were used for evaluation: color, taste, odor, texture (degree of softness), injera number of eyes, eye size, eye distribution (eye uniformity), top and bottom surface (degree of being powdery and sticky); overall acceptability was also evaluated. A score sheet was prepared using the selected descriptors. Each one of attribute was evaluated using a 10-point numerical scale (0–9) anchored on both sides with verbal descriptions (i.e., 0 = much too dark, 9 = much too light) to allow the panel to score the intensity on a framed common scale. Good sensory practices were followed according to Lawless and Heymann (1999). Injera samples were presented to the panelists on a tray at ambient temperature ($\approx 25^{\circ}\text{C}$) within 3-4hr after baking. A glass of drinking water was used for rinsing between samples.

6.2.10 Optimization

Expert design (Version 7) with full factorial design was used to optimize kneading and absit preparation methods.

6.2.11. Statistical analysis

Analysis of variance was performed on the data to establish significant ($p < 0.05$) differences between the samples. The descriptive categories were converted to numerical scores. The scores were then subjected to analysis of variance using SPSS statistical software (Version 20) and means of duplicate results were compared by Tukey's Honestly Significant Difference Test.

6.3. Results and Discussions

6.3.1. Effect of kneading (time/speed) on injera quality

6.3.1.1. Starch fractions at different kneading conditions

Different quality parameters of injera were analyzed in order to evaluate the effect of kneading. Process conditions (time/speed combinations) were optimized and the optimum combinations were chosen based on sensory evaluation for further analysis of the quality. Table 6.2 present the effect of kneading at different time/speed combinations on starch fractions.

Table 6.2 Effect of kneading conditions on starch fraction of tefinjera (mean \pm SD)

Kneadin g	FSG	RAG	RDS	SDS	RS	TS	SDRI
1	0.13 \pm 0.0 ^a	69.3 \pm 0.0 ^b	62.3 \pm 0.0 ^b	5.4 \pm 1.3 ^a	13.0 \pm 4.5 ^a	77.1 \pm 0.8 ^a	80.8 \pm 0.9 ^a
5	0.13 \pm 0.0 ^a	70.6 \pm 0.0 ^c	63.4 \pm 0.0 ^c	9.23 \pm 5.5 ^a	6.4 \pm 0.0 ^a	76.9 \pm 0.9 ^a	82.3 \pm 1.0 ^a
9	0.13 \pm 0.0 ^a	68.0 \pm 0.1 ^a	61.0 \pm 0.1 ^a	10.0 \pm 5.0 ^a	4.3 \pm 5.9 ^a	74.8 \pm 4.1 ^a	81.8 \pm 4.6 ^a

FSG= free sugar, RAG = rapidly available glucose, RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistant starch, TS = total starch, and SDRI = starch digestion rate

index. Data are expressed as mean \pm standard deviations; Different superscripts in the same column indicate statistically significant differences ($P < 0.05$)

The analysis revealed that changes in kneading conditions (time/speed) did not significantly affect the FSG, SDS, RS, TS and SDRI. On the other hand statistically significant variation was observed on RAG and RDS which were kneaded at different time/ speed combinations. Though no statistical difference was observed with increasing the time and speed of kneading, the SDS value increased with increasing the time and speed. The digestion of starch is an important process with respect to dietary requirements (Sujka and Jamroz, 2013). Factors which influence the digestibility of starch are the compositional and morphological properties and the physical access of enzymes to the starch (Bechtel et al., 1990; Singh et al., 2010). Alonso et al. (2000) and Altan et al. (2009) mentioned the effect of processing on starch digestibility. According to their finding, starch loses structural integrity due to shearing and kneading, making it more susceptible towards enzymatic attacks; increased hydrolysis; faster digestion. Kneading #5 (3 min at speed 6) has higher RAG (70.6) and RDS (63.4) while the lower RAG (68.0) and RDS (61.0) were observed on Kneading #9 (7 min at speed 12). According to Canja et al., (2014), the formation of dough and its rheological properties may be affected by some factors like flour quality, the quantity of water, electrolytes (NaCl) and the kneading conditions (intensity of kneading, the amount of energy transmitted to the dough and time of kneading). The kneading conditions influence profound the properties of the dough and they can lead to an optimal growth, an incomplete development or to extra-kneaded dough. The end of kneading is appreciated through sensorial analyses. Well-kneaded dough should be homogeneous, tight, consistent, may be elastic and easy to come down from the mixer's arm and from the walls of the kneading

container. The dough must become a thin strip, transparent and flexible without breaking (Rus et al., 2008).

6.3.1.2 Effect of kneading conditions on total phenolic content, flavonoids, phytate and tannin

Different phenolic compounds were analyzed for injera obtained from different kneading conditions. **Table 6.3** presented the effect of kneading conditions on flavonoids, total phenolic contents, phytate and tannins. The finding showed that changing kneading conditions have no significant effect on the tannin contents of the final product. Unlike tannin, flavonoids, total phenolic content and phytate showed significant variation due to change in kneading conditions. Chlopicka et al. (2012) observed losses of antioxidants during dough mixing and kneading. According to their explanation antioxidant activity of breads could be modified by active oxidative enzymes presented in ingredients of compounds used in breads production, or oxidized by ambient oxygen. The addition of water will initiate enzyme activities, while a substantial incorporation of oxygen occurs during the initial dough mixing and the remolding into smaller pieces. To the contrary of the observation of Chlopicka et al. (2012), the total phenolic content increased with increasing the time and speed of kneading. The bound phenolics may be released with elongated kneading time and speed of kneading as a result of heat induced due to friction. This might be the other possible explanation for the increment in flavonoid and total phenol content of injera. Phytate content degraded significantly as kneading time and speed increased. It decreased in the order Kneading #1 (94.1mg/100g) > Kneading #5 (70.0mg/100g) > Kneading #9 (46.5mg/100g). According to Baye (2014), Phytate can be degraded by endogenous phytases which can be activated by food processing techniques. According to Hurrell and Egli (2010) high values in phytate are likely to impair the absorption of iron and zinc. Moreover, phytates can

form complexes with minerals which are secreted endogenously such as calcium (Morris and Ellis, 1985) and zinc (Manary et al., 2002; Sandström, 1997) and, making these minerals unavailable for re-absorption into the body. Increasing the kneading time and speed can degrade phytate and minimize these effects. On the other hand, according to Curhan et al. (2004) phytate can prevent kidney stones by serving as crystallization inhibitor of calcium salts. They also have anti-cancer properties (Singh, Agarwal, and Agarwal, 2003) and glucose lowering effects (Lee et al., 2005, 2006).

Table 6.3 Effect of kneading conditions on total phenolic content, flavonoids, phytate and tannins (mean \pm SD)

Kneading	Flavonoid (mg/100g)	Total Phenol (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)
1	0.12 \pm 0.00 ^a	0.13 \pm 0.00 ^a	94.1 \pm 0.16 ^c	198.5 \pm 6.8 ^a
5	0.15 \pm 0.00 ^b	0.32 \pm 0.00 ^b	70.0 \pm 0.31 ^b	177.6 \pm 0.0 ^a
9	0.15 \pm 0.02 ^b	0.38 \pm 0.00 ^c	46.5 \pm 0.31 ^a	172.4 \pm 7.4 ^a

Means with different superscripts in the same column are statistically different ($\alpha < 0.05$)

6.3.1.3. Effect of kneading conditions on sensory quality of injera and its parameter optimization

The effect of kneading conditions on the sensory quality of injera is presented on **Table 6.4**. From the analysis of variance it was observed that no significant difference was existed on the color, number of eyes, taste and odor of injera made from dough obtained from different kneading conditions. On the other hand, the remaining sensory attributes like texture, eye size, distribution and top and bottom surfaces were significantly affected due to changing of kneading conditions. This might be explained based on the relationship between kneading and formation of gas. Kneading or remixing of the dough favors the release of large gas bubbles, resulting in a more even distribution of the bubbles within the dough which finally contribute for the quality of the product (Rosell, 2011). The sensory qualities texture, eye size and distribution and top and

bottom surfaces rated more with kneading time of 3 min and speed 6 (Kneading #5). Gómez et al. (2013) concluded that dough mixing parameters will always need to be optimized for each formulation and system, taking into account the speed and duration of mixing and the type of stand mixer. The size, distribution, growth, and failure of the gas bubbles released during proofing and baking have a major impact on the final quality of the bread in terms of both appearance (texture) and final volume (Cauvain, 2003).

Design-Expert® Software

sensory

X1 = A: Speed

X2 = B: Time

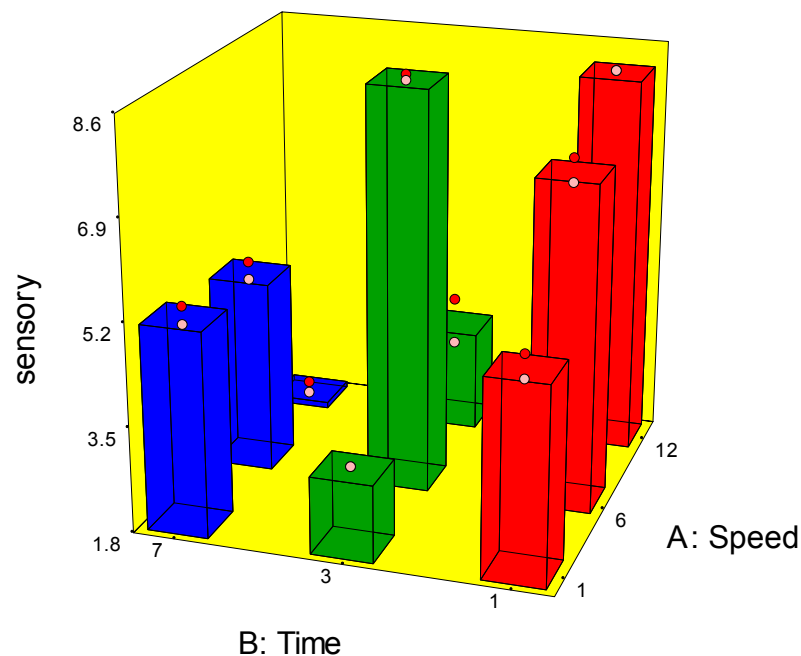


Fig. 6.1 Interaction effects of kneading speed and time (min).

The overall acceptability of injera samples was affected by texture, eye size, distribution and top and bottom surfaces of the injera. The interaction effects of kneading speed and time on injera overall acceptability was significant (fig 6.1). Increasing of kneading speed at one minute

keeping time showed improved injera overall acceptability. However, when kneading time increased to 3 and 7 minutes similar trends of increment in injera overall acceptability was not seen. Based on the interaction effects ($r^2=0.9931$) of kneading speed and time on injera overall acceptability, dough kneading method was optimized to give higher injera overall acceptability (fig. 6.2).

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Desirability

X1 = A: Speed

X2 = B: Time

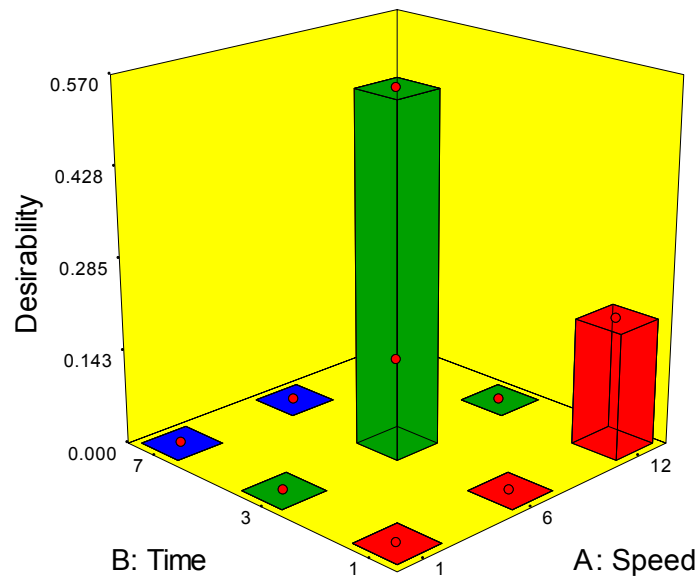


Fig 6.2 Optimized graph for tef dough kneading time (min) and speed

Tef dough kneading method was optimized at kneading speed 6 with 3 minutes of kneading time which has higher desirability to give 8-9 point hedonic scale of injera overall acceptability, and the equation for optimized kneading model is as follow:

Overall acceptability

$$= 5.33 - 0.84A1 + 1.64A2 + 1.54B1 - 0.28B2 - 0.92A1B1 - 1.21A2B1 - 1.11A1B2 + 1.86A2B2 \quad (6.1)$$

Where, A is kneading speed and B is kneading time

Table 6.4 Effect of kneading conditions on the sensory quality of injera (mean \pm SD)

Kneading	Sensory attributes							
	Color	Taste	Texture	Number of eyes	Eye size	Eye distributio n	Top and bottom surfaces	Odor
1	5.3 \pm 1.3 ^a	5.5 \pm 0.1 ^a	4.1 \pm 0.7 ^{ab}	6.7 \pm 1.4 ^a	1.4 \pm 0.4 ^a	5.6 \pm 1.1 ^{ab}	5.6 \pm 0.9 ^b	5.5 \pm 0.4 ^a
2	6.0 \pm 0.0 ^a	5.4 \pm 0.6 ^a	6.5 \pm 1.0 ^{bc}	6.1 \pm 0.1 ^a	3.6 \pm 0.1 ^b	3.7 \pm 0.1 ^{ab}	6.2 \pm 0.2 ^b	6.3 \pm 0.3 ^a
3	7.4 \pm 0.1 ^a	6.2 \pm 0.3 ^a	6.3 \pm 0.4 ^{bc}	5.2 \pm 0.2 ^a	6.3 \pm 0.4 ^c	4.4 \pm 0.8 ^{ab}	5.8 \pm 0.6 ^b	5.4 \pm 2.1 ^a
4	6.0 \pm 0.5 ^a	5.1 \pm 1.5 ^a	2.8 \pm 1.8 ^a	4.2 \pm 1.1 ^a	4.7 \pm 0.9 ^{bc}	3.1 \pm 1.8 ^{ab}	3.9 \pm 0.1 ^{ab}	4.2 \pm 2.1 ^a
5	6.3 \pm 1.6 ^a	5.3 \pm 1.8 ^a	6.7 \pm 0.1 ^{bc}	5.3 \pm 0.8 ^a	5.2 \pm 0.1 ^{bc}	6.9 \pm 0.4 ^b	6.7 \pm 1.4 ^b	6.1 \pm 1.1 ^a
6	4.8 \pm 0.3 ^a	4.9 \pm 0.8 ^a	3.1 \pm 0.4 ^a	5.5 \pm 0.4 ^a	5.9 \pm 0.3 ^c	5.9 \pm 0.3 ^{ab}	6.2 \pm 0.7 ^b	5.1 \pm 0.5 ^a
7	5.4 \pm 0.8 ^a	5.2 \pm 0.8 ^a	6.8 \pm 0.6 ^{bc}	6.0 \pm 0.4 ^a	6.0 \pm 1.0 ^c	3.3 \pm 0.7 ^{ab}	5.2 \pm 1.1 ^b	6.2 \pm 0.1 ^a
8	4.6 \pm 1.2 ^a	4.5 \pm 0.2 ^a	4.3 \pm 0.1 ^{ab}	6.0 \pm 0.1 ^a	3.5 \pm 0.6 ^{ab}	4.8 \pm 1.3 ^{ab}	1.8 \pm 0.2 ^a	6.1 \pm 0.4 ^a
9	6.3 \pm 0.1 ^a	6.3 \pm 0.1 ^a	8.7 \pm 0.2 ^c	5.4 \pm 0.6 ^a	4.5 \pm 0.3 ^{bc}	2.0 \pm 0.7 ^a	1.3 \pm 0.2 ^a	6.4 \pm 0.5 ^a

Means with different superscripts in the same column are statistically different ($\alpha < 0.05$)

6.3.2. Influence of *absit* preparation method on injera quality and its parameter optimization

The impacts of water to fermented batter ratio during *absit* preparation on the sensory quality of injera are presented on **Table 6. 5**. For different sensory attributes the analysis of variance showed that the ratio of water to fermented dough did significantly affect the quality of injera. Texture, number of eyes, top and bottom surface and odor of injera obtained from *absit* #3 which prepared from a ratio of 900:100 ml of water to fermented dough gained higher sensory score. *Absit* #6 (300 ml batter with 900 ml of water) has less overall acceptability (**Fig. 6.3**) than that of *Absit* # 3 (100ml batter with 900 ml water) but, the individual sensory attribute had gained the higher value except injera texture and eye size. The possible reason for the difference in the sensory score of the different injera from different *absit* preparation might be due to the amount of gelatinized *absit* which used primarily to bring about cohesiveness of the dough and secondly to provide easily fermentable carbohydrate to leaven the injera (Yetneberk et al., 2004). Abiyu *et al.* (2013) stated that *absit* used to activates yeasts responsible for CO₂ production 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”.

sensory

X1 = A: Batter

X2 = B: Water

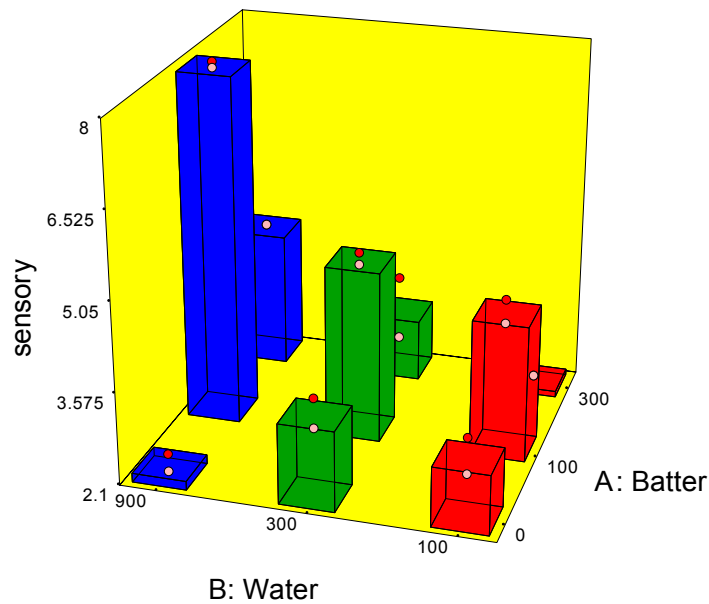


Fig 6.3 Interaction effects of the amount of batter (ml) and water (ml) of absit on injera overall acceptability

The interaction effects between the amounts of batter and water used during *absit* preparation on injera overall acceptability was significant ($r^2=0.9793$) (fig. 6.3). Increasing trends of sensory quality (overall acceptability) of injera was noted when, 100 ml of batter used with 100, 300 and 900 ml of water. Similar trends were noted at 300 ml of batter. Different findings reported the percentage of fermented dough needed to be used for preparation of *absit*. According to Ashenafi (2006) and Girma *et al.* (2013) 10% 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. On the other hand, it was understood from the traditional injera making process that the amount of *absit* and the ratio of water to fermented dough have significantly depend on tef varieties and required optimization. Based on the interaction effect between the amount of batter and water used during *absit* preparation, *absit* preparation method was optimized to give higher injera overall acceptability (**Fig 6.4**).

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Desirability

X1 = A: Batter

X2 = B: Water

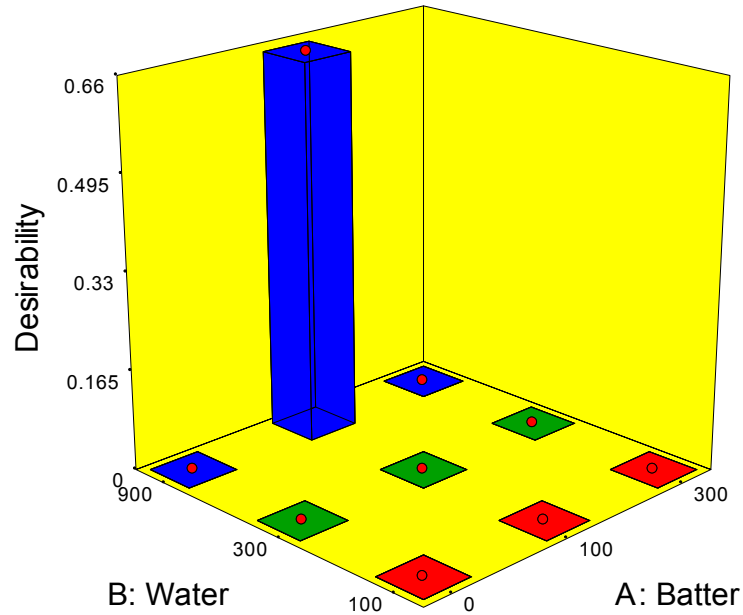


Fig 6.4 Optimized graph for the amounts of batter (ml) and water (ml) during absit preparation

Absit preparation method was optimized with a combination of 100 ml of batter to 900 ml of water to gives the higher desirability in terms of injera overall acceptability, and the following equation is given for the optimized model,

Overall acceptability

$$\begin{aligned} &= 3.99 - 1.06A1 + 1.79A2 - 0.76B1 - 0.12B2 + 0.92A1B1 - 0.63A2B1 \\ &+ 0.64A1B2 - 0.66A2B2 \end{aligned} \quad (6.2)$$

Where A is the amount of batter and B is the amount of water in absit preparation.

Table 6.5 Effect of different water to fermented dough proportions on the sensory quality of injera (mean \pm SD)

Sensory attributes								
Absit	Color	Taste	Texture	Number of eyes	Eye size	Eye distribution	Top and bottom surfaces	Odor
1	5.4 \pm 0.0 ^a	4.1 \pm 0.9 ^a	4.9 \pm 0.8 ^{ab}	5.8 \pm 0.4 ^a	3.9 \pm 1.3 ^{bc}	4.8 \pm 0.8 ^{ab}	5.3 \pm 0.6 ^b	5.7 \pm 0.2 ^a
2	6.0 \pm 0.2 ^a	4.3 \pm 0.1 ^a	6.0 \pm 0.2 ^{ab}	6.0 \pm 0.2 ^a	3.2 \pm 1.3 ^{bc}	6.1 \pm 1.9 ^{ab}	5.4 \pm 0.0 ^b	6.8 \pm 0.1 ^{ab}
3	6.6 \pm 0.7 ^a	4.8 \pm 0.0 ^a	7.5 \pm 0.4 ^b	6.2 \pm 0.0 ^a	5.2 \pm 0.4 ^b	6.9 \pm 0.0 ^{ab}	6.3 \pm 0.9 ^b	7.3 \pm 0.2 ^b
4	5.9 \pm 0.4 ^a	5.1 \pm 0.1 ^a	3.9 \pm 1.1 ^a	4.8 \pm 0.4 ^a	2.9 \pm 0.6 ^{bc}	2.4 \pm 0.4 ^a	5.5 \pm 0.5 ^b	5.7 \pm 0.1 ^a
5	6.9 \pm 0.7 ^a	4.3 \pm 0.2 ^a	4.1 \pm 0.5 ^a	5.2 \pm 1.5 ^a	1.9 \pm 1.0 ^a	4.5 \pm 2.1 ^{ab}	5.7 \pm 0.0 ^b	6.3 \pm 0.8 ^{ab}
6	6.9 \pm 0.8 ^a	4.1 \pm 0.0 ^a	7.5 \pm 0.3 ^b	5.5 \pm 0.2 ^a	8.7 \pm 0.1 ^c	8.4 \pm 0.3 ^b	6.3 \pm 0.2 ^b	6.6 \pm 0.2 ^{ab}
7	5.5 \pm 0.1 ^a	4.8 \pm 1.3 ^a	5.1 \pm 1.4 ^{ab}	6.0 \pm 0.6 ^a	1.5 \pm 0.0 ^a	5.1 \pm 0.4 ^{ab}	5.1 \pm 0.3 ^b	6.3 \pm 0.4 ^{ab}
8	5.7 \pm 0.4 ^a	4.1 \pm 0.2 ^a	4.4 \pm 0.1 ^a	5.9 \pm 0.5 ^a	1.2 \pm 0.1 ^a	5.3 \pm 1 ^{ab}	5.3 \pm 0.6 ^b	5.8 \pm 0.2 ^a
9	6.3 \pm 0.3 ^a	4.3 \pm 0.3 ^a	4.9 \pm 0.6 ^{ab}	4.7 \pm 0.3 ^a	2.2 \pm 0.3 ^a	3.0 \pm 1.5 ^a	3.2 \pm 0.5 ^a	5.7 \pm 0.1 ^a

Means with different superscripts in the same column are statistically different ($\alpha < 0.05$)

6.4 Conclusion

The objective of this study was to assess the effect of kneading conditions (time and speed) and the ratio of water to fermented dough on the quality of injera. Kneading conditions considerably affect the starch fractions (RAG and RDS) and sensory quality of injera. Kneading time of 3 min and speed of 6 (800 rpm) used to optimize kneading method based on the highest injera overall acceptability. flavonoid, total phenolic content and phytate of injera were also affected by kneading conditions. On the other hand absit making method (the ratio of water to fermented dough) also affects the quality of injera. Absit preparation method was optimized with a combination of 100 ml of batter to 900 ml of water to gives the higher desirability in terms of injera overall acceptability, and further studies are required on different tef varieties.

Chapter 7-Effects of Mill Type and Mechanical Kneading Conditions on Fermentation Kinetics of Tef Dough

Chapter 7

The Effects of Mill Type and Mechanical Kneading Conditions on Fermentation Kinetics of Tef dough

Abstract

Injera is Ethiopian traditional fermented flatbread which made mostly from tef flour. Tef grain is milled and the flour kneaded to have batter-like dough which will ferment before injera baking. The influence of mill type: hammer mill (HM), disc mill (DM) and blade mill (BM) and mechanical kneading speed-time combinations (K1, K5, and K9), on fermentation kinetics was investigated. Phytate to mineral molar ratio (Fe, Zn and Ca) of tef injera was also investigated as affected by mill type and different kneading conditions. In both milling and kneading levels, maltose was the highest sugar concentration initially, which followed by glucose and fructose. As fermentation continued, a similar trend in maltose break down was seen among HM, DM, and BM. However, different patterns of glucose and fructose break down were seen on HM than DM and BM. Similarly, HM had a different pattern in increment of lactic acid concentration than DM and BM. Similar trend in maltose concentration was seen between K1, K5, and K9. Again glucose breakdown and the increment of lactic acid in K9 were different than that of K1 and K5. Phytate/mineral molar ratio of BM was significantly different ($p < 0.05$) from HM and DM. There was also a significant difference ($p < 0.05$) in phytate/mineral molar ratio between K1, K5, and K9. Decreased phytate/mineral molar ratio was seen with increasing of kneading speed (rpm) for a longer period of time. The effect of mill type and kneading speed and time combinations on fermentation kinetics and phytate/mineral molar ratio were significant.

Keywords: Tef, milling, kneading, fermentation kinetics, phytate/mineral molar ratio.

7.1. Introduction

Tef [*Eragrostis tef*] is an Ethiopian indigenous tropical cereal crop and it has been cultivated for many years in Ethiopian highlands (Demissie, 2001; Viswanath, 2012). Products of tef grain are nutritionally well packed because they are always consumed as whole grain with a high content of carbohydrate and fiber (USDA, 2007) with more iron, zinc, and calcium than other cereal grains, including sorghum, wheat and barley (Abebe et al., 2007). Due to the absence of gluten and gluten-like proteins, tef has recently been receiving global attention, particularly as a “healthy food”, making it right for celiac disease patients (Spaenij-Dekking et al., 2005), and also because of other dietary benefits such as slow-release of carbohydrate constituents useful for diabetic patients (Abebe and Ronda, 2014). Tef is the main staple in the country mostly used to make *injera*, traditional fermented flatbread (Bultosa and Taylor, 2004).

Injera is prepared from batter-like dough, which is pre-fermented for 2-3 days. Fermentation is most of the time initiated spontaneously by addition of water to tef flour, allowing the naturally existing microorganisms to grow (Gashe, 1985). Primary or first stage fermentation can also be started by the addition of the starter, *ersho*, which is a small amount of batter kept from the previous dough (Parker et al., 1989). During fermentation stages, the main fermenting microorganisms are lactic acid bacteria (*Lactobacillus* species) (Gashe, 1985) and yeast (*Saccharomyces* species) (Gifawesen and Bisrat, 1982). These microorganisms result in the fall of pH, gas production and dough rising and are responsible for desired final product flavor and acidity (Umeta and Faulks, 1988).

Several studies have reported the beneficial influence of fermentation in improving both nutritional and sanitary qualities of foods (Nout, 2009; Svanberg & Lorri, 1997). According to Nout & Motarjemi (1997) Production of organic acids with low molecular weight, such as acetic and lactic acid, reduces pH and may thus limit contamination by foodborne pathogens. Greiner

and Konietzny (2006) also reported that fermentation can be a reason for the activation of several endogenous enzymes including phytases and may thus result in products with decreased anti-nutritional factors. Hammes et al. (2005), stated that the level to which enzymes like phytases are activated depends on the fermentation kinetics, which in turn, depends on the condition of raw materials used.

Depending on the mechanical forces and temperature during the grinding process, different milling or grinding methods have been seen to produce flours with different particle size and damage starch level (Kadan et al., 2008). A report by Abebe et al. (2015) are also stated that flour particle size and degree of starch damages are influenced by mill types which are used to grind cereal grain. On the other hand, Li et al. (2014) stated flour particle size and damaged starch extent affect enzymatic hydrolysis of flour. De la Hera et al. (2014) stated the particle size of flour also affect the amount of carbon dioxide which retained during fermentation. However, information is lacking on the effects of mill type on fermentation kinetics of tef dough during injera making process and its effect on mineral to phytate molar ratio since tef possessed both significantly.

Dough processing is an essential factor which affects the quality of bread or injera. Kneading is one of the most important mechanical steps in industrial dough processing (Esselink et al., 2003). Different studies were made on the effect of dough mechanical kneading, on thermo-mechanical properties of dough's (Angioloni and Rosa, 2005), dough rheological properties (Angioloni and Rosa, 2007) and bread quality (Kim & De Ruiter, 1968). All studies showed that influences of dough kneading conditions are significant. However, the effect of mechanical kneading on fermentation kinetics and mineral to phytate molar ratio during tef dough fermentation is still lacking. Therefore, the aim of this study was to investigate the influence of mill type and

mechanical kneading conditions on fermentation kinetics of tef dough during injera making process; and mineral to phytate molar ratio of tef injera.

7.2. Materials and methods

7.2.1 Materials

Based on popularity among Ethiopian tef farmers and users, qouncho tef variety (DZ-Cr-387) was selected and obtained from DebreZeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Tef sample was hermetically stored in cool and dry place using polyethylene bag. Before milling, tef grain was cleaned by sifting.

7.2.2 Milling process

Hammer mill (HM) (Perten 120, Finland) fitted with 0.8 mm sieve, stone-disk mill (DM) (cottage tef grain-milling, Denmark) and blade mill (BM) (Nutri Bullet NB-101B, China) were used to get the whole flour of tef sample. The sample was milled for about 7 minutes in case of blade mill.

7.2.3 Dough preparation, fermentation and injera making

Tef dough was prepared according to Parker et al. (1989) and Zegeye (1997) with little modification. Amount of starter (*Ersho*) equal to 60ml was initially added to each kg of flour. The tef flour (from stone-disc mill) was mixed 2:3 (w/w) with potable water and kneaded by kitchen aid (Moulinex Masterchif 720, France). Dough kneading times (1, 3 and 7 min) and kneading speed (Speed 1, 6 and 12) were selected based on injera exporters practice and kneading machine capacity. Nine different tef dough were prepared using kneading machine by varying time and speed of kneading machine. Based on the sensory results of injera, Kneading # 1 (kneading by speed 1 for 1min and gives moderate overall injera acceptability), #5 (kneading

by speed 6 for 3 min and gives the higher injera acceptability) and #9 (kneading by speed 12 for 7 min and gives the lower injera acceptability) were selected for further studies.

To study the milling effects, flour from HM, DM, and BM was mixed similarly with water and kneaded by hand in a bowl until obtaining a homogenous mixture in the traditional way. The dough was allowed to ferment for 60 hours at room temperature (30 ± 5) °C.

After the primary fermentation, 10% of the dough was mixed 1:3 (v/v) with boiling water, and heated for 15 min with continuous stirring. The hot cooked dough (*absit*) was then mixed back into the fermenting dough, and sufficient potable water was added to make a batter. The batter was left covered for 2 hours for secondary fermentation. Additional water was added to thin and form the right consistency of the batter. Finally, half a liter of batter was poured onto the hot clay griddle in a circular form. After 2-3 min of cooking (traditional electric injera baking equipment) injera was removed and placed in a basket.

7.2.4 Flour characterization

7.2.4.1 Determination of phytates

The colorimetric method as described by Hang and Lantzseh (1983) was used to determine the phytic acid content of the samples.

7.2.4.2 Mineral determination

Iron, zinc and calcium were determined using methods 999.11, 968.08 and 985.35 of AOAC (Latimer, 2016).

7.2.5 Fermentation kinetics

7.2.5.1. Change in pH

As used by Baye et al., (2013), during fermentation the pH of the slurry was recorded using a pH meter (Oakton-Eutech Instruments PH6, France). The rate of change in pH ($\Delta \text{pH}/\text{dt}$) was

calculated for each sample as follows: $-(dpH/dt) = pH(t+1) - pH(t)/(t+1) - t$, where “t” stands for time (hours). The maximal value of $-(dpH/dt)$ for each sample observation was then averaged to give the maximal rate of change in pH ($-(dpH/dt)_{max}$).

7.2.5.2 Extraction procedures

The extraction method was that of Bervas (1991) in which a 10 g of sample was homogenized with 90 mL distilled water using magnetic bar stirrer. Five milliliters of 1 mol/L HClO₄ solution was added to a 10 mL aliquot of the homogenate. The mixture was centrifuged for 15 min at 4000 g at 15 °C, the supernatant was neutralized (pH 7.070.1) with 2 mol/L KOH and the volume was adjusted to 25 mL with distilled water. After 30 min precipitation on the ice, the solution was filtered on 0.45 mm cellulose filter (Millipore).

7.2.5.3 Determination of sugars, organic acids, and ethanol

HPLC analyses were performed with an Agilent 1200 model equipped with a 20 mL automatic injection loop. Detection was performed with a refractive index detector (RID 156 Beckman) connected to a Shimadzu STANG-ST3A integrator. Chromatographic separation was performed using an ICsep ICE-COREGEL 87H3 column (Transgenomic) (300 mm × 7.8 mm, Interaction Chromatography, France) under the following conditions: mobile phase 0.001 N H₂SO₄, flow rate 0.6 mL/min, column temperature 35 °C.

7.2.6 Statistical analysis

Analysis of variance was performed on the data to establish significant ($p < 0.05$) differences between the samples. The scores were then subjected to analysis of variance using SPSS statistical software (Version 16) and means of duplicate results were compared by Tukey's Honestly Significant Difference Test.

7.3 Results and discussion

7.3.1 Fermentation kinetics

7.3.1.1 Changes in pH

The kinetics of decrease in pH showed the same pattern in all the three types of dough's for both milling and kneading variables (Fig. 7.1 & 7.2). However the mean pH at the start of fermentation for BM, was higher (6.41) than that of DM (6.32) and HM (6.23). On the other hand, the initial dough pH was not affected by kneading resulting in the pH of (6.34) for K1, K5 and K9. During the first 10h of fermentation, pH decreased from 6.23 to 4.22 for HM, and from, 6.32 to 4.13 and 6.41 to 4.36 for DM and BM dough respectively. Decrease in pH was also observed due to change in kneading conditions i.e.; for K1 from 6.34 to 4.16, from 6.34 to 4.1 and from 6.34 to 4.23 for K5 and K9, respectively. According to Nout et al. (1989) the common characteristic among the different dough's is a rapid drop in pH this may be due to back-slopping effects. The maximum rate of pH decrease was similar in both milling and kneading levels. However, the fermentation time to see higher pH rate for K5 was 2h, which was different than that of K1 and K9 (10h) (Table 2). This difference is may be from the difference in kneading speed/time combination which may affect the fermentation kinetics. In all dough's, the rate at which pH was decreased when the fermentation extended from 10 to 24h. In both milling and kneading, the pH was decreased at slower rate when fermentations extended from 24 to 48h. Similar result was reported by Baye et al. (2013). The final pH of HM, DM, and BM were 3.47, 3.22 and 3.32, respectively. Final pH of 3.53, 3.51 and 3.80 was noted for K1, K2 and K3, respectively. Lefebvre et al., (2002) stated that, when the utilization of fermentable carbohydrates and the LAB population increase, the pH value of the inoculated sourdough decreased. Lefebvre et al. (2002) reported the time necessary to obtain the minimum pH value was about 13 h though the total titratable acidity continued to increase until 19 h and the pH

decrease was correlated with the production of organic acids. According to Kingamkono et al. (1994) and Nout et al. (1989) the pH rapidly reached values below 4.5, promoting better hygienic conditions.

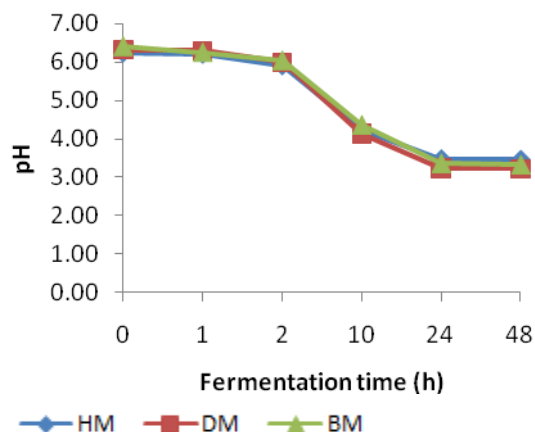


Figure 7.1 Changes in pH during injera sourdough fermentation. HM, DM and BM stand for dough from hammer, disc and blade mill. Error bars represent the standard deviation of means.

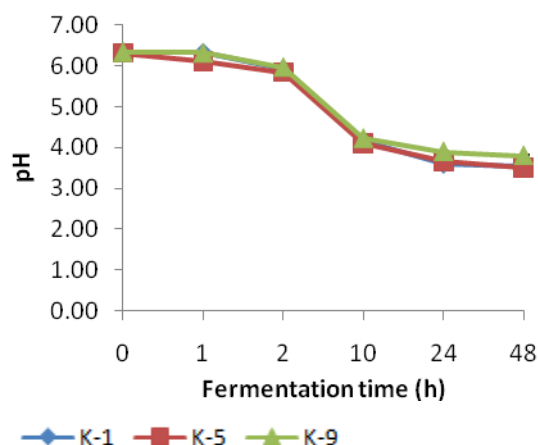


Figure 7.2 Changes in pH during injera sourdough fermentation. K1, K5, and K9 stand for dough with three-time/rpm kneading combinations.. Error bars represent the standard deviation of means.

7.3.1.2 Kinetics of substrate consumption and product formation

After tef milled using three different mills, the initial sugar content of dough before adding starter culture (*Ersho*) was investigated (Fig 7.3). In all dough's obtained from (HM, DM, BM), the dominant sugar was maltose which was followed by glucose and fructose. This agrees with a

report by Baye et al (2013) for barley-wheat and wheat-red sorghum dough in injera making. The concentration of maltose at the start of fermentation was 535.23 ppm for HM, 615.68 ppm for DM and 501.36 ppm for BM. After which, it starts decreasing to the final values (zero) in all dough's (HM, DM, BM). This agrees with Kulp & Lorenz (2003) report which stated, maltose breaks down into glucose molecules during sourdough fermentation. For the dough from HM, decrease in glucose (22.64 ppm) and fructose (56.1 ppm) concentration was observed during 2h of fermentation. Kulp & Lorenz (2003) explain glucose and fructose are the first sugars to be used during fermentation. These sugars showed increment at 10h of fermentation, after which, it starts decreasing to reach the final values of glucose (18.23 ppm) and fructose (0 ppm). Kulp & Lorenz (2003) stated that, sucrose is rapidly broken down to glucose and fructose by yeast enzymes already present outside the cell membrane; this may justify an increment shown for glucose and fructose during fermentation. The increment of fructose in the absence of sucrose in the sample could be due to a glucose isomerase activity on the non-fermented glucose (Gobetti et al., 1995) or could have originated from the degradation of the fructosans fraction of the flour (Rocken et al., 1992).

On the other hand, at 2hr of fermentation, DM and BM were seen with the increased value of glucose and fructose and then start decreasing to the final concentration of 32.44 ppm and 21.15 ppm of glucose and fructose consecutively in DM and 19.83 ppm and zero in BM. Similar to Kulp & Lorenz (2003) This result in line with a report by Rogers and Langemeier (1995) as invertase enzymes on the surface of yeast cells rapidly hydrolyze sucrose to glucose and fructose at mixing and the early stages of fermentation. Glucose and fructose uptake systems have much in common: uptake rates of either sugar alone are very similar (Serrano and De la Fuente, 1974; D'Amore et al., 1989), uptake of one sugar is affected by the presence of the other (Waley 1981;

D'Amore et al., 1989), there is no lag between uptake of glucose followed by fructose (Orlowski and Barford, 1987).

Similar to HM, DM, and BM, maltose (615.68 ppm) was the dominant sugar for kneading conditions K1, K5 and K9 before adding starter culture (*Ersho*) which was followed by glucose and fructose (Fig. 3.4). An increase in maltose concentration was seen during 2h of fermentation. After which, the values were decreased unto the final concentration of 34.57 ppm for K1, 25.27 ppm for K5 and zero for K9. The maltose content might be increased during the sourdough fermentation by the hydrolytic activity of indigenous amylases on the starch fraction damaged during the milling process (Mathewson, 2000). Similar results were obtained when using a *L.plantarum* strain as starter (Gobetti et al., 1994). An increment in glucose concentration was seen for K1 and K5 until 10 hr of fermentation. After 10 hrs of fermentation, a decrease in glucose concentration was noted until it reached to the final value (20.79 ppm in K1 and 42.98 ppm in K5) at 48h of fermentation. K9 had different trends of glucose break down than that of K1 and K5. For K1 and K9, the lower initial concentration of fructose (173.03 ppm) showed increment during 2hr of fermentation and end up at 23.45 ppm (K1) and 116.8 ppm (K9) at 48h of fermentation. Both glucose and fructose increment was in agreement with Rogers and Langemeier, (1995) report. However, a different trend was seen in K5 than K1 and K9 which showed increment in fructose concentration during 2hr of fermentation, then decreased until 24hr of fermentation and end with slight increment. The difference in mill type and kneading conditions (speed to time combination) may contribute to have different concentration of sugars during sourdough fermentation.

Similar trends of acetic acid were seen between HM, DM, and BM. However, HM had a different pattern of lactic acid concentrations than that of DM and BM. This may relateto the

different pattern of glucose concentration in HM. Lactic acid concentration showed an increment starting at 2hr of fermentation. Similarly, the acetic acid concentration was also increased at 2hr of fermentation in HM and DM. However, this was seen at 10hrs of fermentation for BM. This result agrees with Axelsson and Ahrné (2000) who stated, lactic acid bacteria convert starch to lactic acid. Kulp and Lorenz (2003) also stated that fructose usually found in flour; partially push the metabolism of hetero fermentative lactic acid bacteria toward the acetate kinase pathway, producing traces of mannitol and increase of acetic acid. On the other hand Kulp & Lorenz (2003) stated fructose is used in part to produce mannitol and acetate, and in part to be metabolized to lactate and ethanol. The difference in lactic acid concentration between samples may be a result of having different particles size distribution and damaged starch level which caused by the three different mill type.

Acetic acid concentration for K1, K5, and K9 showed similar trends like HM, DM, and BM. However, K9 had different trends of lactic acid concentration as it had a different pattern of glucose breakdown than that of K1 and K5. The highest concentration rate was seen between 2hrs and 24hrs of fermentation. The difference in lactic acid concentration between samples may be a result of having different kneading speed/time combination which may have an impact on fermentation time.

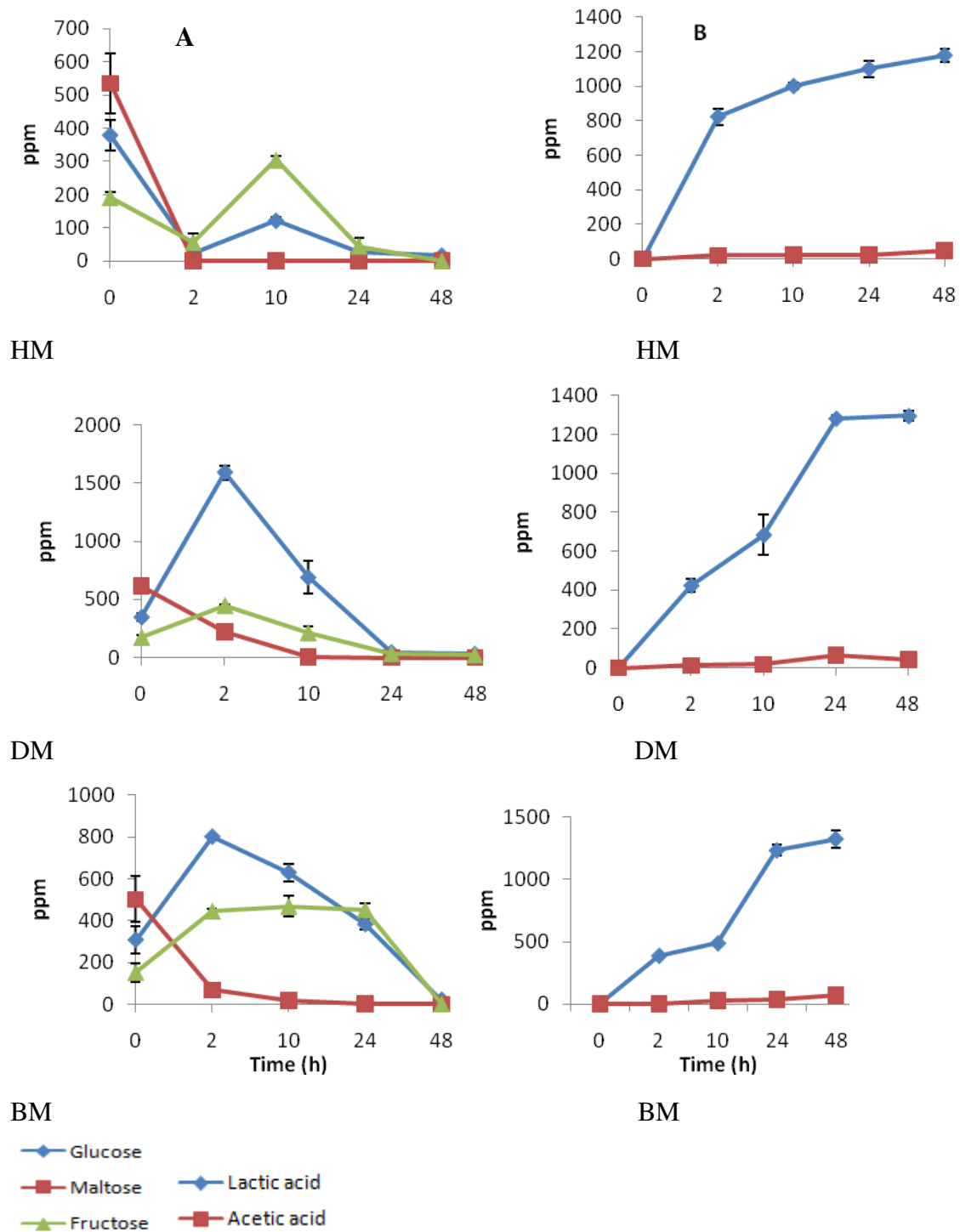


Figure 7.3 Change in mono and disaccharide concentration (A) and lactate, acetate and ethanol (B) during fermentation of injera sourdough. HM, DM and BM stand for dough from hammer, disc and blade mill. Error bars represent the SD of means.

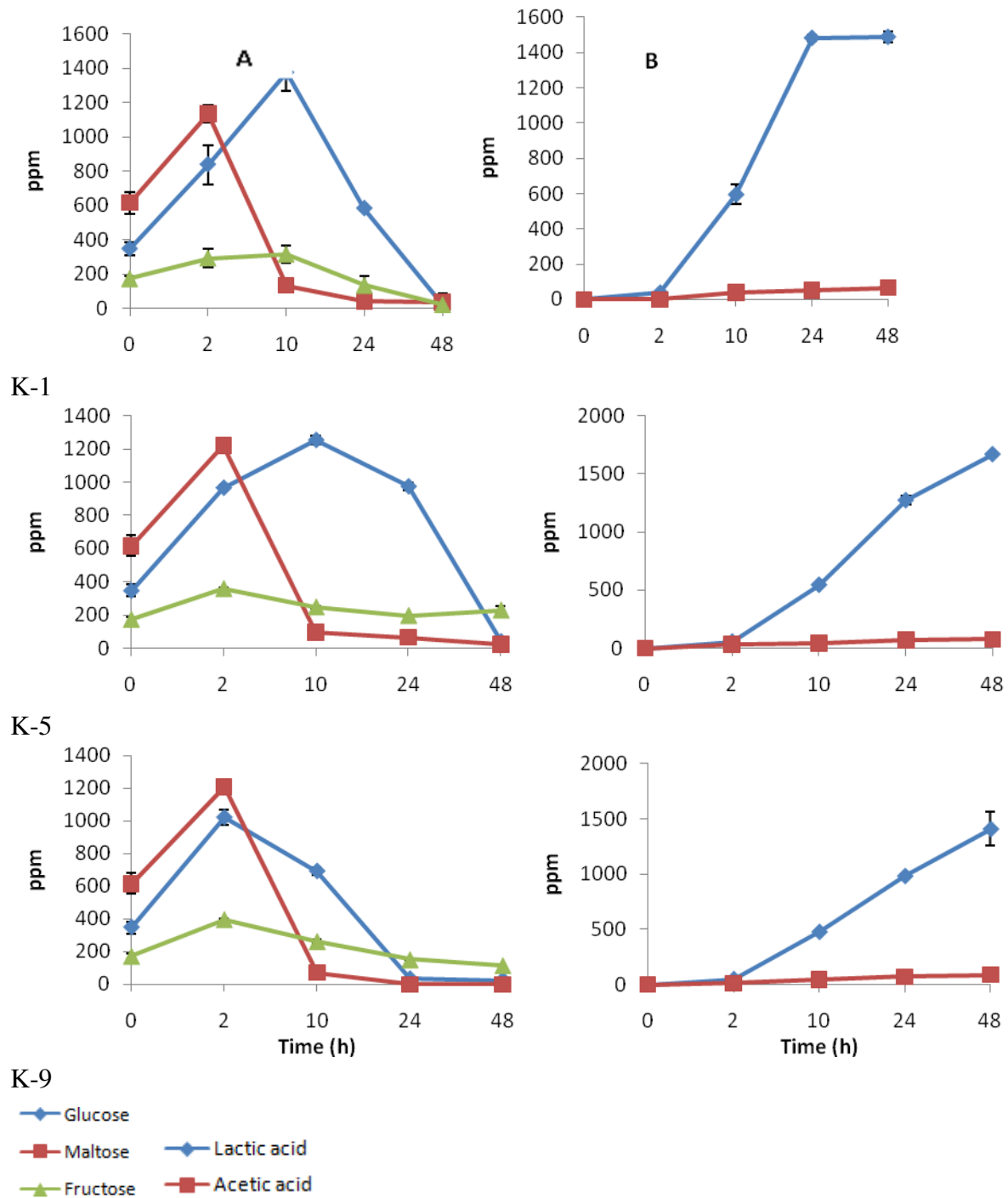


Figure 7.4 Change in mono and disaccharide concentration (A) and lactate, acetate and ethanol (B) during fermentation of injera sourdough. K1, K2, and K3 stand for dough with three-time/rpm kneading combinations. Error bars represent the SD of means.

Table 7.1 Phytate, mineral and phytate/mineral molar ratio of injera

Injera	Phytate	Minerals (mg/100g)			Molar ratio		
		Fe	Zn	Ca	Phy/Fe	Phy/Zn	Phy/Ca
Milling							
HMI	130.5±0.7 ^a	16.3±0.0 ^a	1.97±0.0 ^a	6.55±0.1 ^a	0.68 ^a	6.57 ^a	1.21 ^a
DMI	134.5±0.5 ^b	16.8±0.1 ^a	2.03±0.0 ^a	6.61±0.0 ^a	0.68 ^a	6.55 ^a	1.23 ^{ab}
BMI	135.8±0.5 ^b	16.3±0.1 ^a	1.97±0.0 ^a	6.50±0.1 ^a	0.71 ^b	6.85 ^b	1.27 ^b
Kneading							
K1	188.3±0.1 ^c	15.9±0.1 ^a	1.97±0.0 ^a	6.41±0.1 ^a	1.00 ^c	9.49 ^c	1.78 ^c
K5	153.8±0.3 ^b	16.1±0.1 ^a	1.99±0.0 ^a	6.44±0.0 ^a	0.81 ^b	7.65 ^b	1.45 ^b
K9	123.4±0.5 ^a	16.6±0.1 ^a	2.05±0.0 ^a	6.64±0.0 ^a	0.63 ^a	5.98 ^a	1.13 ^a

HMI, DMI, and BMI stand for injera from hammer, disk and blade mill respectively. K1, K5, and K9 stand for injera with three time-rpm kneading combinations. Data are expressed as mean ± standard deviations; Different superscripts in the same column within milling and kneading indicate statistically significant differences ($P < 0.05$).

Table 7.2 Kinetic parameters of pH change during injera sourdough fermentation

HM, DM and BM stand for dough from the hammer, disc, and blade milled flour; K1, K5, and K9 stand for dough with three-time/rpm kneading combinations. Data are expressed as mean ± standard deviations

Sample dough	Initial (flour) pH	($-\text{dpH}/\text{dt}$) _{max}	Time (h) to reach ($-\text{dpH}/\text{dt}$) _{max}	pH at 48h
Milling				
HM	6.23±0.03	0.2±0.00	10	3.47±0.01
DM	6.32±0.00	0.22±0.01	10	3.22±0.00
BM	6.41±0.01	0.21±0.01	10	3.32±0.00
Kneading				
K1	6.34±0.01	0.22±0.01	10	3.53±0.00
K5	6.34±0.01	0.24±0.01	2	3.51±0.00
K9	6.34±0.01	0.21±0.01	10	3.80±0.01

7.3.2 Phytate/mineral molar ratio

The effects of mill type and kneading speed and time combinations on phytate and mineral (Fe, Zn, Ca) content of tef injeras and their molar ratio was investigated (Table 7.1). There was no significant difference ($p < 0.05$) in phytate content between injera samples which made from disc mill (DMI) (134.5) and blade mill (BMI) (135.8). However, injera made from hammer mill (HMI) had significantly lower phytate content than that of DMI and BMI. This may be as result of using different types of mills which have different milling principles and lead to varied

particle size distributions. This agreed with Majzoobi, et al. (2014), as phytic acid content of wheat bran decreased from 50.1 mg/g to 21.6 mg/g due to particle size reduction.

There was a significant difference ($p < 0.05$) in phytate content between injera samples obtained from different kneading conditions (K1, K5, and K9). K9 which combined longer kneading time with a faster speed had lower phytate content (123.4) than that of K1 (188.3) which combined shorter kneading time with slower rpm. K5 which had moderate kneading time and speed combination showed the higher content of phytate than that of K9 and lower content than K1. Although there is lack of study in effects of kneading speed and time combinations on phytate content, the reason for having different content of phytate may come from the influence of kneading which can affect dough fermentation as many studies reported (Urga and Narasimha, 2017; Baye, 2014; Rasane et al., 2015a).

There was no significant difference in mineral content between HMI, DMI, and BMI. Similarly, the mineral content among different kneading conditions (K1, K5, and K9) was not varied significantly. However, its phytate/mineral molar ratio was affected significantly between samples. The absorption of minerals (Fe, Zn, and Ca) from a meal corresponds directly to its phytate content (Brune et al., 1992; Barbro et al., 1985; Morris and Ellis, 1985). The phytate/minerals molar ratios are used to predict its inhibitory effect on the bioavailability of minerals (Ma et al., 2005). Phytate/iron, phytate/zinc and phytate/calcium molar ratio between HMI and DMI were not varied (Table 7.1). However, BMI had a higher molar ratio (Phytate/iron, phytate/zinc, and phytate/calcium) than that of HMI and DMI. Although the samples had similar amount of minerals, the difference in phytate content which was as result of using different mill type justified the reason for having a different molar ratio. A similar trend was seen again between K1, K5, and K9 with a significant difference in phytate content which

caused by mechanical kneading lead to have different molar ratio between samples. Phytate/iron molar ratio of samples varied significantly in the order of K9 (0.63) < K5 (0.81) < K1 (1.00). The result showed that it has less risk of bioavailability. Hallberg et al., (1989) also stated, phytate/iron molar ratio >1 is regarded as indicative of poor iron bioavailability. Similarly phytate/zinc and phytate/calcium molar ratio of samples were significantly varied in the order of K9 (5.98) < K5 (7.65) < K1 (9.49) and K9 (1.13) < K5 (1.45) < K1 (1.78), respectively. The bioavailability of calcium in HMI, DMI, and BMI and K1, K5 and K9 were less. According to Morris and Ellis (1985), phytate/calcium molar ratio >0.24 will impair calcium bioavailability. On the other hand, Turnlund et al. (1984) reported that, zinc absorption is greatly reduced and results in a negative zinc balance when the phytate/ zinc molar ratio is 15.

7.4. Conclusions

The effect of mill type during injera making process varied fermentation kinetics and dough composition significantly. Fermentation pattern and dough compositions are also influenced by using different mechanical kneading time and speed combinations. Both tef milling and mechanical kneading methods play important role in the degradation of phytate and increase mineral bioavailability. BMI which was made from tef flour with larger particle size had low minerals (Fe, Zn and Ca) bioavailability than DMI and HMI. Increasing of mechanical kneading speed for a longer period of time, can decrease the phytate content of injera and lead to have better mineral bioavailability. However, although phytate/calcium molar ratio decreased with increasing of kneading time and speed (K9), the calcium bioavailability still needs to be improved.

Chapter 8-*General Conclusion*

Recommendation and perspectives

Chapter 8

Conclusion, Recommendation and perspectives

8.1 General conclusion

Based on the results found in the studies undertaken on tef sample (DZ-Cr-387), the following conclusions can be drawn:

- Mill type has important effect on flour particle size distribution, starch damage and bulk density, affecting the processing performance of the flours and determining the hydration and pasting properties. Difference in particle size distributions and starch damage levels of tef flour induced different *in vitro* starch digestibility, and result in having different sensorial acceptability of injera. The stone-disc attrition mill led to higher acceptability of injera texture and eye size which result in having higher injera overall acceptability.
- The application of software based injera quality evaluation which includes injera number of eyes, eye size and eye distribution examination gives similar result to visual human evaluation.
- The difference in flour particle size distribution and damage starch levels which resulted from using different mill type, significantly influence rheological, textural and functional properties of tef flours. Tef flour produced from Stone-disk attrition mill lead to highest percentage of starch damage and WSI that showed its suitability to produce higher quality tef injera than hammer and blade mills.
- The difference in kneading time-speed combinations has important impact on tef injera sensorial quality. Polyphenol content of injera was also affected by kneading conditions. Tef

dough kneading method was optimized at kneading speed 6 with 3 minutes of kneading time which has higher desirability to give 8-9 point hedonic scale of injera overall acceptability.

- Absit preparation method difference during injera making process was significantly affecting the sensorial quality of injera absit preparation method was optimized with a combination of 100 ml of batter to 900 ml of water to gives the higher desirability in terms of injera overall acceptability for qouncho tef variety (DZ-Cr-387).
- Fermentation patterns of *injera* dough during injera making process were also shown to be different, mainly due to difference in milling type and kneading time-speed combinations. Both milling and mechanical kneading difference, in injera making process, play important role in the degradation of phytate and increase mineral bioavailability.

In general, prerequisite factors which are mandatory in the transformation of traditional injera making process to industry levels was studied. Milling type, kneading time-speed combinations and absit preparation difference in industrial injera manufacturing process have significant contribution to give high product quality and uniformity. The application of software to evaluate injera quality during industrial injera manufacturing process can increase productivity and efficiency.

8.2 Recommendations

Based on the findings of this thesis several recommendations which help in transforming the traditional injera making process to industry level can be made.

- To maintain high injera quality and uniformity, the effects of mill type on injera quality should be considered during the transformation of traditional tef injera making to industry level. Stone-disc attrition mill can be used to maintain high quality injera than hammer and blade mill.

- Mass production of injera in industry may require fast, accurate and continues quality evaluation technique. Due to this, human visual quality evaluation should be replaced by software based quality evaluation method. ImageJ evaluation of injera number of eyes, eye size and eye distribution can be used as quick injera quality indicator beside the sensorial evaluation.
- Results from rheological analysis of this study can be used as input in industrial setup. Result from forward extrusion analysis can be used for setting pump capacity which required pumping tef batter through defined pipe size. The minimum force required to push the tef batter through 5mm opening is 1.35N.
- Due to the mass production of injera, industrial injera making process should include mechanical kneading mechanism to give high volume of output. The result from the effect of mechanical kneading on injera quality can be used for setting time/speed of kneading machine to give injera with acceptable quality and uniformity.
- One to nine dough to water ratio can be used during absit preparation to give acceptable injera quality for qouncho tef variety (DZ-Cr-387).
- Drop in pH during tef dough fermentation can be used in the industry as indicator of fermentation stage or status.

8.3 Perspectives

- Further studies are required on the effect of other tef varieties and milling type on injera quality. It is also advised to link starch digestibility with glycemic index on future studies. Mineral bioavailability also requires further investigation with more advanced method.

- Further studies are required on fermentation and its kinetics with regard to ethanol production, raffinose and stachyose content as affected by tef varieties, milling type and kneading conditions.

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Annexes

Annex A- Injera sensorial quality evaluation form

Health status: ok <input type="checkbox"/> not ok <input type="checkbox"/>	Age: Sex: Male <input type="checkbox"/> Female <input type="checkbox"/>
Panelist No:- Sample Code:	Date:

Instruction:-Please mark on the scale below to describe the given sample.

1. Color

Much too dark |—————| Much too light

2. Taste

Not sour enough |—————| Much too sour

3. Texture

Much too brittle |—————| Much too soft enough

4. Appearance

4.1. Number of eyes

Many too few |—————| Many too many

4.2. Eye size

Much too small |—————| Much too big

4.3. Eye distribution

Much too irregular |—————| Much too regular

4.4. Top and bottom surfaces

Much too powdery, sticky |—————| Much too non powdery, non-sticky

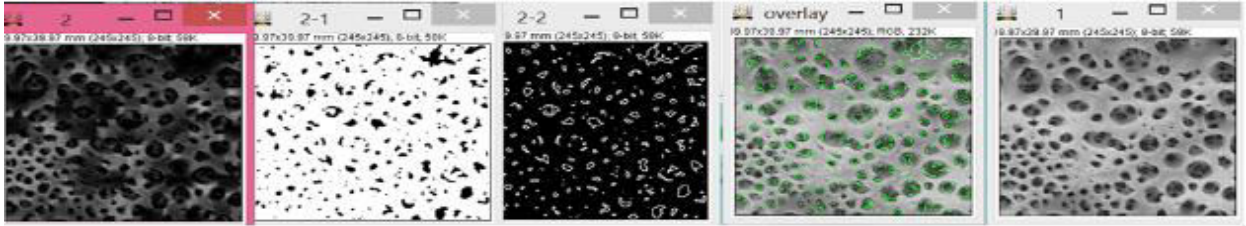
5. Smell

Much too un-normal |—————| Much too Normal

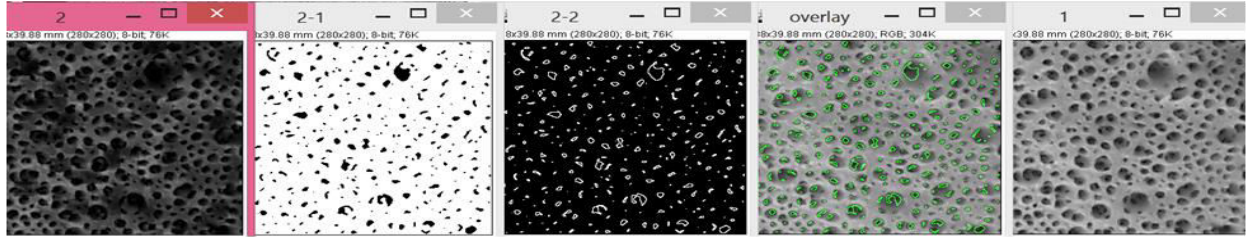
6. Overall acceptability

Dislike extremely |—————| Like extremely

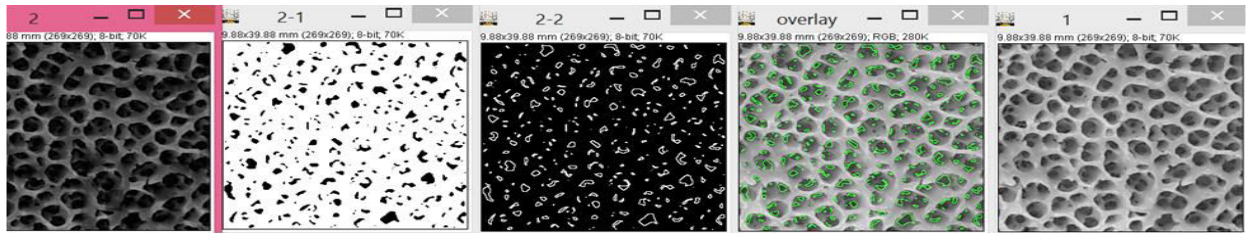
Annex B



HMI



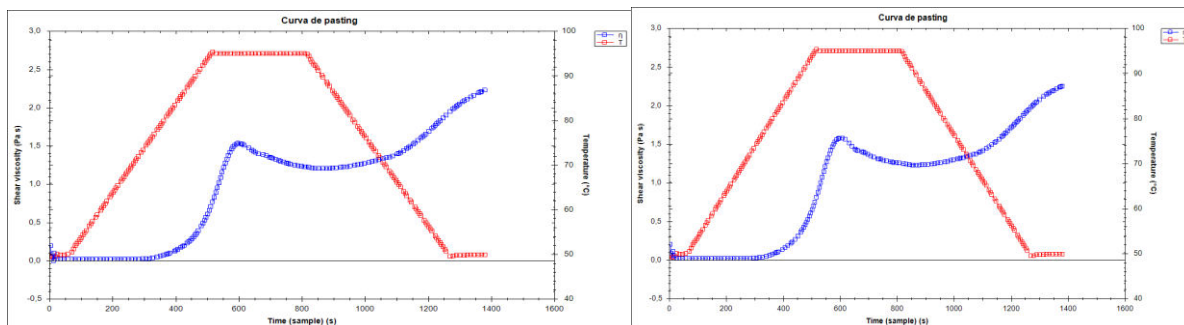
DMI



BMI

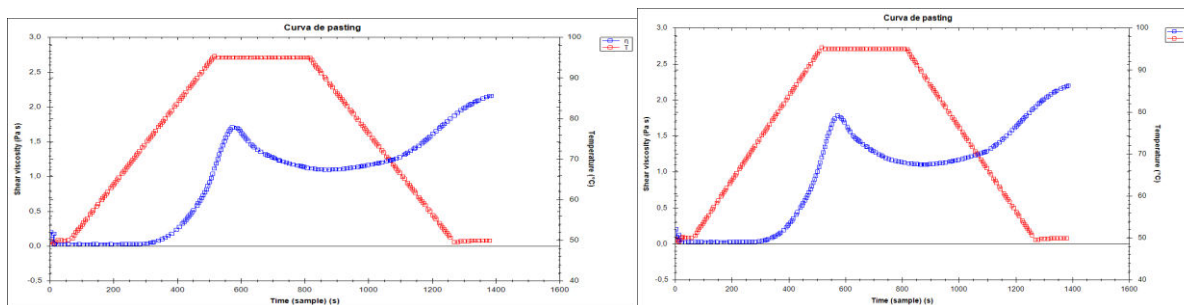
Annex B fig. S. Software based injera quality evaluation. HMI, DMI and BMI stand for injera from hammer mill, injera from disk mill and injera from blade mill respectively.

Annex C



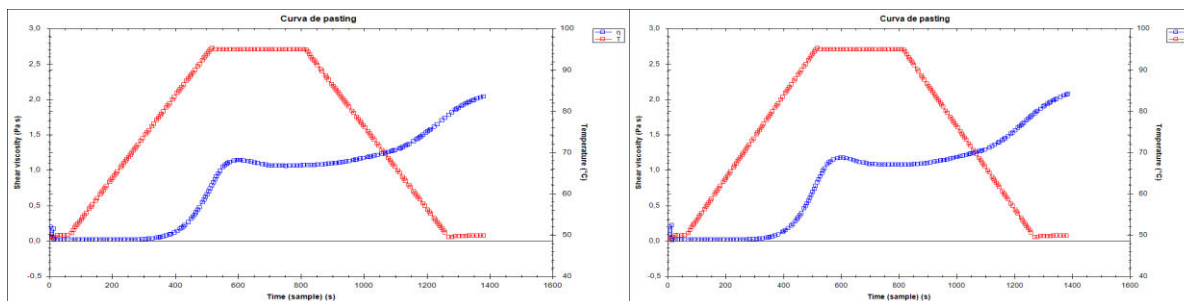
HM1

HM2



DM1

DM2



BM1

BM2

Annex C. Duplicate pasting curves for hammer (HM), disc (DM) and blade (BM) flour.

Annex D



K4



K5



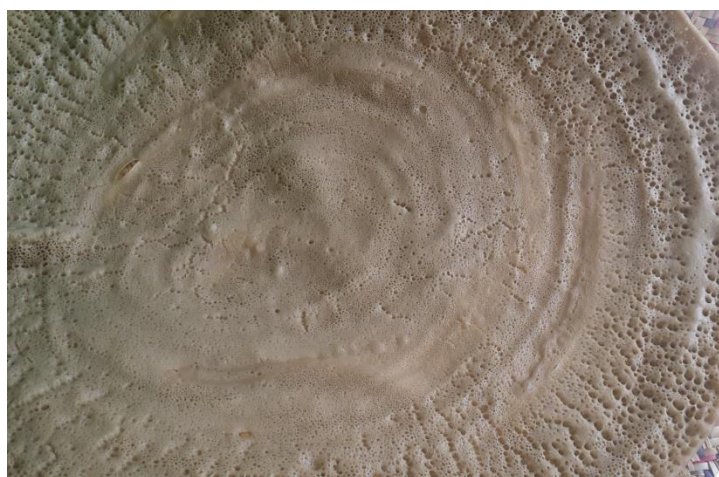
K9

Annex D. Injera fig. for kneading #4 (K4), #5 (K5) and #9 (K9)

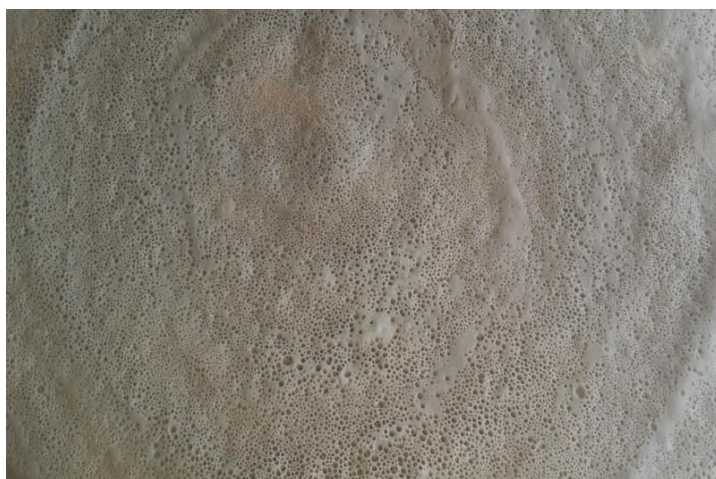
Annex E



A3



A4



A9

Annex E. Injera fig. for Absit #3 (A3), #4 (A4) and #9 (A9)

