Effect of Mill Type and Mechanical Kneading Conditions on Fermentation Kinetics of Tef dough During Injera making and Phytate to Mineral Molar Ratio of Injera

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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.

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, e*rsho*, 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 is 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 &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 (Angioloniand Rosa, 2005), dough rheological properties (Angioloniand 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.

2. Materials and methods

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.

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 min in case of BM.

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's 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 1 min 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 ºC).

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.

2.4 Flour characterization

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.

2.4.2 Mineral determination

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

2.5 Fermentation kinetics

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 (_dpH/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).

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 $HClO_4$ solution was added to a 10 mL aliquot of the homogenate. The mixture was centrifuged for 15 min at 4000 g at 15 1C, 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).

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 Shimatzu 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 H_2SO_4, flow rate 0.6 mL/min, column temperature 35 ˚C.

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.

3 Results and discussion

3.1 Fermentation kinetics

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. 1 & 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 1). This difference might be due to the difference in kneading speed/time combination which may affect the fermentation kinetics. In all doughs, 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 and3.32, 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.

Table 1 Kinetic parameters of pH change during injera sourdough fermentation

Sample dough	Initial (flour) pH	$(\_dpH/dt)_{max}$	Time (h) to reach $(\_dpH/dt)_{max}$	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

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

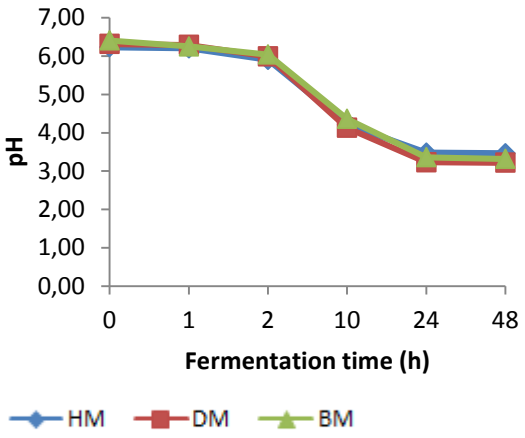


Figure 1 Changes in pH during injera sourdough fermentation. HM, DM and BM stand for dough from hammer, disc and blade mill. Error bars represent the standard deviation of means.

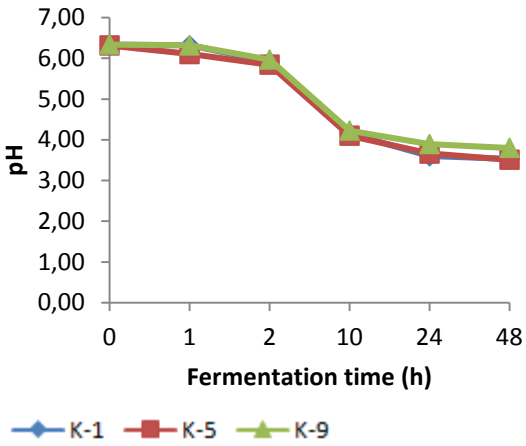


Figure 2 Changes in pH during injera sourdough fermentation. K1, K5, and K9 stand for dough with three-time/rpm kneading combinations.. Error bars represent the standard deviation of means.

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 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 that 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).

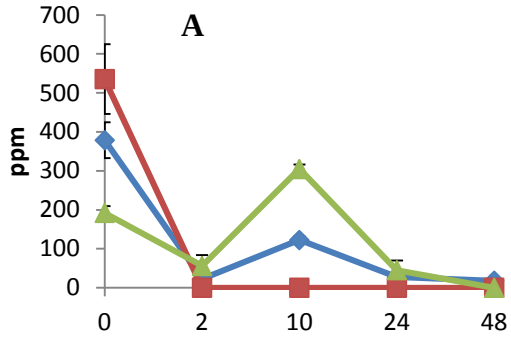
On the other hand, at 2 h of fermentation, DM and BM were seen with the increased value of glucose and fructose and then start decreasing to the final concentration of 32.44 ppm and 21.15 ppm of glucose and fructose consecutively in DM and 19.83 ppm and zero in BM. Similar to Kulp & Lorenz (2003), This 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. 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

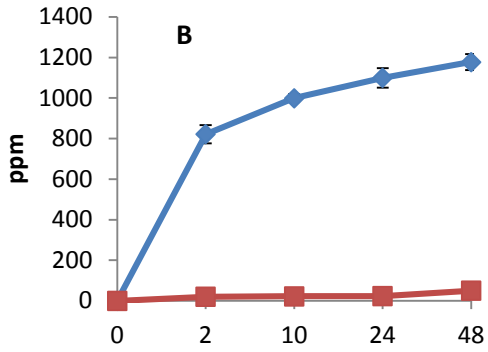
concentration was noted until it reached to the final value (20.79 ppm in K1 and 42.98 ppm in K5) at 48h of fermentation. K9 had different trends of glucose break down than that of K1 and K5. For K1 and K9, the lower initial concentration of fructose (173.03 ppm) showed increment during 2hr of fermentation 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 relate to 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 & Ahrné (2000) who stated, lactic acid bacteria convert starch to lactic acid. Kulp & 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.

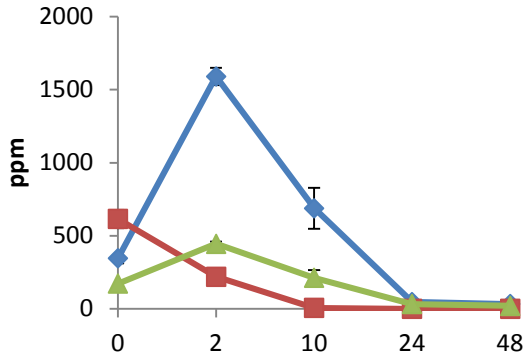
Acetic acid concentration for K1, K5, and K9 showed similar trends like HM, DM, and BM. However, K9 had different trends of lactic acid concentration as it had a different pattern of glucose breakdown than that of K1 and K5. The highest concentration rate was seen between 2hrs and 24hrs of fermentation. 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.



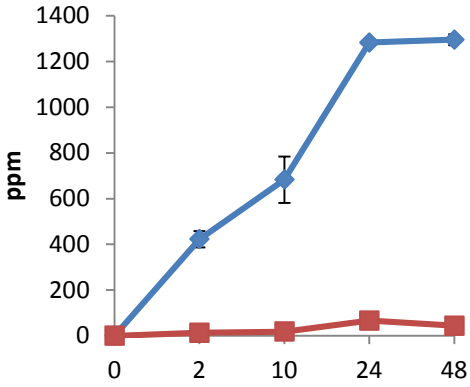
HM



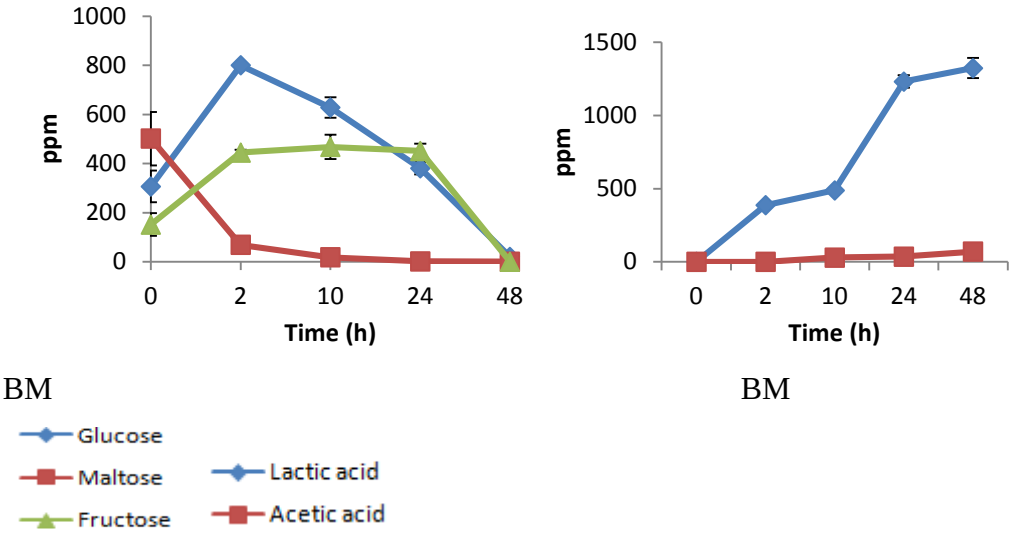
HM



DM

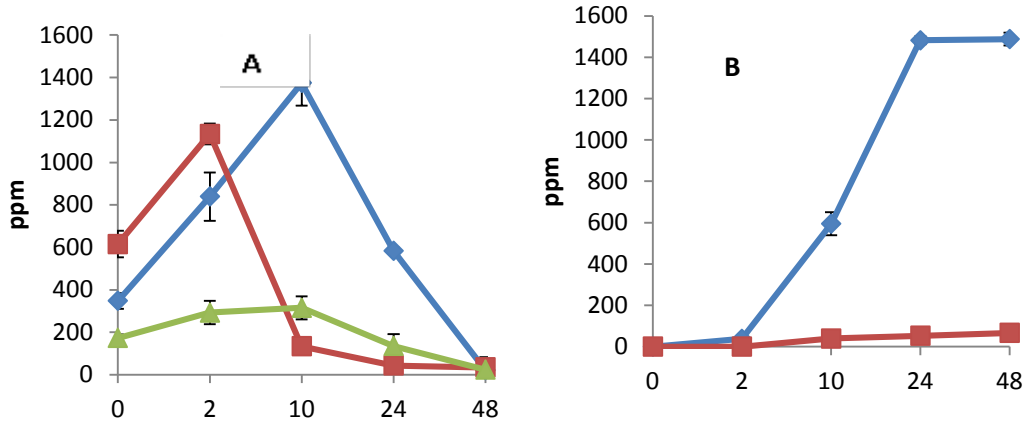


DM

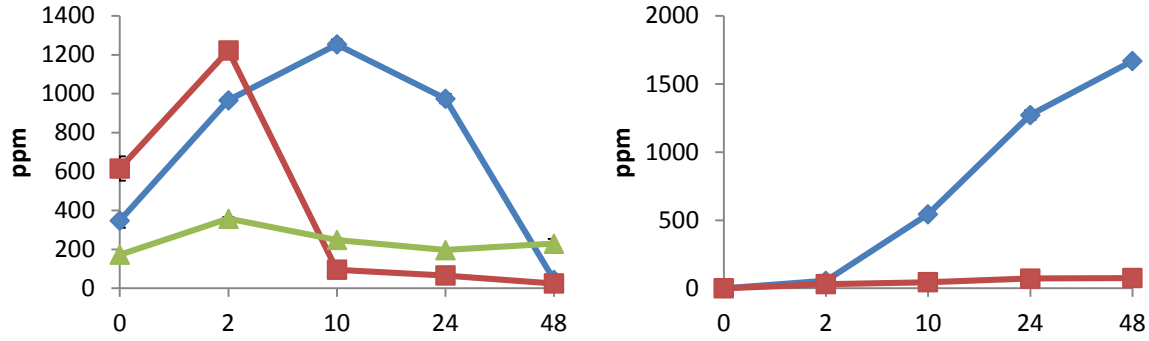


BM BM

Figure 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.



K-1



K-5

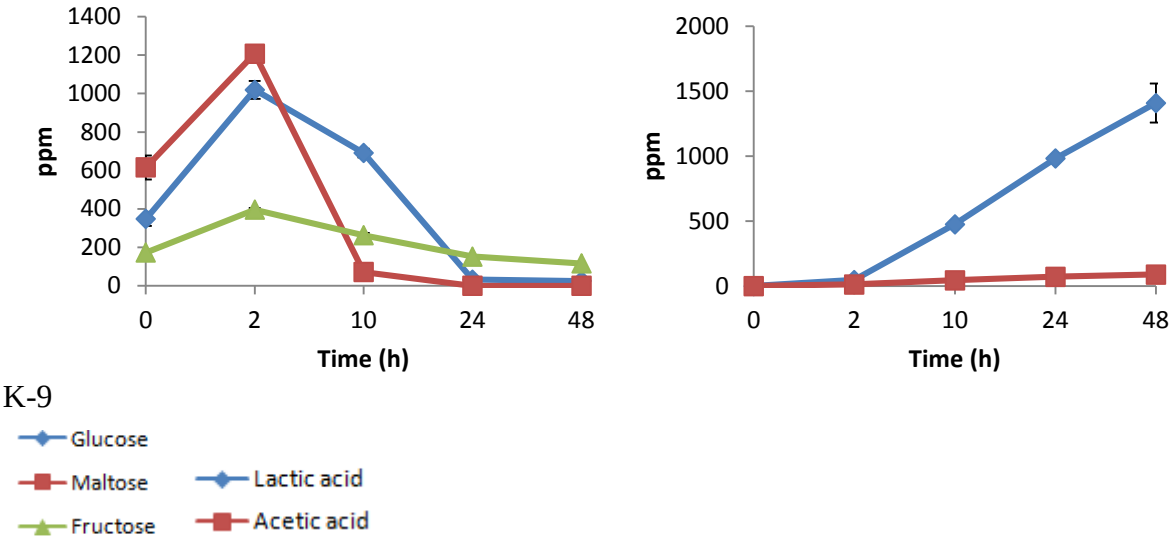


Figure 4 Change in mono and disaccharide concentration (A) and lactate, acetate and ethanol (B) during fermentation of injera sourdough. K1, K2, and K3 stand for dough with three-time/rpm kneading combinations. Error bars represent the SD of means.

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 2). 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 variedparticle 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 K1 and lower content than K9. 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).

Table 2 Phytate, mineral and phytate/mineral molar ratio of injera

		Minerals (mg/100g)			Molar ratio		
Injera	Phytate	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 ($P < 0.05$).

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 2). 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 ithas 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.

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 increase in kneading time and speed (K9), the calcium bioavailability still needs to be improved.

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References

Abebe, W., & Ronda, F. (2014).Rheological and textural properties of tef [Eragrostis tef (Zucc.) Trotter] grain flour gels. Journal of Cereal Science, 60(1), 122-130.

Abebe, W., Collar, C., & Ronda, F. (2015).Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours. Carbohydrate polymers, 115, 260-268.

Abebe, Y., Bogale, A., Hambidge, K. M., Stoecker, B. J., Bailey, K., & Gibson, R. S. (2007). Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability. Journal of Food Composition and Analysis, 20(3), 161-168.

Angioloni, A., & Dalla Rosa, M. (2005). Dough thermo-mechanical properties: influence of sodium chloride, mixing time and equipment. Journal of Cereal Science, 41(3), 327-331.

Angioloni, A., & Dalla Rosa, M. (2007). Effects of cysteine and mixing conditions on white/whole dough rheological properties. Journal of food engineering, 80(1), 18-23.

Axelsson, L., & Ahrné, S. (2000). Lactic acid bacteria. In Applied microbial systematics (pp. 367-388). Springer, Dordrecht.

Barbro, N., Brittmarie, S., & Cederblad, Å. K. E. (1985). Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. British Journal of Nutrition, 53(1), 47-53.

Baye, K. (2014). Teff: nutrient composition and health benefits (Vol. 67). Intl Food Policy Res Inst.

Baye, K., Mouquet-Rivier, C., Icard-Vernière, C., Rochette, I., & Guyot, J. P. (2013). Influence of flour blend composition on fermentation kinetics and phytate hydrolysis of sourdough used to make injera. Food chemistry, 138(1), 430-436.

Bervas, E. (1991). Mise au point de levains bactériens pour la panification (Doctoral dissertation, Clermont-Ferrand 2).

Brune, M., Rossander-Hultén, L., Hallberg, L., Gleerup, A., & Sandberg, A. S. (1992). Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. The Journal of nutrition, 122(3), 442-449.

Bultosa, G., & Taylor, J. (2004).Paste and gel properties and in vitro digestibility of tef [Eragrostis tef (Zucc.)Trotter] starch. Starch-Stärke, 56(1), 20-28.

D'Amore, T., Russell, I., & Stewart, G. G. (1989). Sugar utilization by yeast during fermentation. Journal of industrial microbiology, 4(4), 315-323.

De la Hera, E., Rosell, C. M., & Gomez, M. (2014). Effect of water content and flour particle size on gluten-free bread quality and digestibility. Food chemistry, 151, 526-531.

Demissie, A. (2001). Tef genetic resources in Ethiopia. Narrowing The Rift, 27-31.

Esselink, E., van Aalst, H., Maliepaard, M., Henderson, T. M., Hoekstra, N. L., & van Duynhoven, J. (2003). Impact of industrial dough processing on structure: a rheology, nuclear magnetic resonance, and electron microscopy study. Cereal Chemistry, 80(4), 419-423.

Gashe, B. A. (1985). Involvement of Lactic Acid Bacteria in the Fermentation of TEF (Eragrosfis tef), an Ethiopian Fermented Food.Journal of food science, 50(3), 800-801.

Gifawesen, C., & Besrat, A. (1982). Yeast flora of fermenting tef (Eragrostis Tef) dough. SINET: Ethiop. J. Sci., 5(1)), 21-25.

Gobbetti, M., Corsetti, A., & Rossi, J. (1994). The sourdough microflora. Evolution of carbohydrates during the sourdough fermentation. Microbiologie, Aliments, Nutrition, 12, 9-15.

Gobbetti, M., Simonetti, M. S., Corsetti, A., Santinelli, F., Rossi, J., & Damiani, P. (1995). Volatile compound and organic acid productions by mixed wheat sour dough starters: influence of fermentation parameters and dynamics during baking. Food Microbiology, 12, 497-507.

Greiner, R., & Konietzny, U. (2006). Phytase for food application. Food Technology & Biotechnology, 44(2).

Hallberg, L., Brune, M., & Rossander, L. (1989). Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. The American journal of clinical nutrition, 49(1), 140-144.

Hammes, W. P., Brandt, M. J., Francis, K. L., Rosenheim, J., Seitter, M. F., & Vogelmann, S. A. (2005). Microbial ecology of cereal fermentations. Trends in Food Science & Technology, 16(1-3), 4-11.

Hang, W., & Lantzsch, H. J. (1983). Comparative methods for the rapid determination of oxalate and phytate in cereal products. J. Sci. Food Agric, 34, 1423-1426.

Kadan, R. S., Bryant, R. J., & Miller, J. A. (2008). Effects of milling on functional properties of rice flour. Journal of food science, 73(4).

Kim, J. C., & De Ruiter, D. (1968). Bread from non-wheat flours. Food Technol, 22(7), 867-878.

Kingamkono, R., Sjögren, E., Svanberg, U., & Kaijser, B. (1994). pH and acidity in lactic-fermenting cereal gruels: effects on viability of enteropathogenic microorganisms. World Journal of Microbiology and Biotechnology, 10(6), 664-669.

Kulp, K., & Lorenz, K. (Eds.). (2003). Handbook of dough fermentations (Vol. 127). Crc Press.

Latimer, G. W. (2016). Official methods of analysis of AOAC International. Rockville, MD: AOAC International, ISBN: 978-0-935584-87-5.

Lefebvre, D., Gabriel, V., Vayssier, Y., & Fontagne-Faucher, C. (2002). Simultaneous HPLC determination of sugars, organic acids and ethanol in sourdough process. LWT-Food Science and Technology, 35(5), 407-414.

Li, E., Dhital, S., & Hasjim, J. (2014). Effects of grain milling on starch structures and flour/starch properties. Starch-Stärke, 66(1-2), 15-27.

López, A. C. B., Pereira, A. J. G., & Junqueira, R. G. (2004). Flour mixture of rice flour, corn and cassava starch in the production of gluten-free white bread. Brazilian Archives of Biology and Technology, 47(1), 63-70.

Ma, G., Jin, Y., Piao, J., Kok, F., Guusje, B., & Jacobsen, E. (2005). Phytate, calcium, iron, and zinc contents and their molar ratios in foods commonly consumed in China. Journal of Agricultural and Food Chemistry, 53(26), 10285-10290.

Majzoobi, M., Pashangeh, S., Farahnaky, A., Eskandari, M. H., & Jamalian, J. (2014). Effect of particle size reduction, hydrothermal and fermentation treatments on phytic acid content and some physicochemical properties of wheat bran. Journal of food science and technology, 51(10), 2755-2761.

Mathewson, P. R. (2000). Enzymatic activity during bread baking. Cereal Foods World.

MORRIS, E. R., & ELLIS, R. (1985). Bioavailability of dietary calcium: effect of phytate on adult men consuming nonvegetarian diets.

Nout, M. J. R., & Motarjemi, Y. (1997). Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. Food Control, 8(5-6), 221-226.

Nout, M. R. (2009). Rich nutrition from the poorest–Cereal fermentations in Africa and Asia. Food Microbiology, 26(7), 685-692.

Nout, M. R., Rombouts, F. M., & Havelaar, A. (1989). Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. International Journal of Food Microbiology, 8(4), 351-361.

Orlowski, J. H., & Barford, J. P. (1987). The mechanism of uptake of multiple sugars by Saccharomyces cerevisiae in batch culture under fully aerobic conditions. Applied microbiology and biotechnology, 25(5), 459-463.

Parker, M. L., Umeta, M., & Faulks, R. M. (1989). The contribution of flour components to the structure of injera, an Ethiopian fermented bread made from tef (Eragrostis tef). Journal of Cereal Science, 10(2), 93-104.

Rasane, P., Jha, A., Sabikhi, L., Kumar, A., & Unnikrishnan, V. S. (2015). Nutritional advantages of oats and opportunities for its processing as value added foods-a review. Journal of food science and technology, 52(2), 662-675.

Röcken, W., Rick, M., & Reinkemeier, M. (1992). Controlled production of acetic acid in wheat sour doughsKontrollierte Essigsäurebildung in Weizensauerteigen. Zeitschrift für Lebensmittel-Untersuchung und Forschung, 195(3), 259-263.

Rogers D, Langemeier J. (1995). Sugars in doughs and resultant breads and biscuits. Technical Bulletin, American Institute of Baking Research Department 17(5):1-5.

Serrano, R., & Delafuente, G. (1974). Regulatory properties of the constitutive hexose transport in Saccharomyces cerevisiae. Molecular and cellular biochemistry, 5(3), 161-171.

Spaenij-Dekking, L., Kooy-Winkelaar, Y., & Koning, F. (2005).The Ethiopian cereal tef in celiac disease. New England Journal of Medicine, 353(16), 1748-1749.

Svanberg, U., & Lorri, W. (1997). Fermentation and nutrient availability. Food Control, 8(5-6), 319-327.

Turnlund, J. R., King, J. C., Keyes, W. R., Gong, B., & Michel, M. C. (1984). A stable isotope study of zinc absorption in young men: effects of phytate and a-cellulose. The American journal of clinical nutrition, 40(5), 1071-1077.

Umeta, M., & Faulks, R. M. (1988).The effect of fermentation on the carbohydrates in tef (Eragrostis tef).Food chemistry, 27(3), 181-189.

Urga, K., & Narasimha, H. V. (2017). Traditional sour dough bread (Difo Dabbo) making: I. Effects on phytic acid destruction. The Ethiopian Journal of Health Development (EJHD), 12(3).

USDA, (2007).National Nutrient Database for Standard Reference.Release 20.USDA.

Viswanath, N. (2012). The Hierarchy of Poor: The Tension Between Favoring Smallholder Farmers or Domestic Consumers in Ethiopian Agricultural Development. Africa Policy Journal, 8, 30.

Waley, S. G. (1981). An easy method for the determination of initial rates. Biochemical Journal, 193(3), 1009-1012.

Zegeye, A. (1997). Acceptability of injera with stewed chicken.Food quality and preference, 8(4), 293-295.