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The effect of mechanical kneading and absit preparation on tef injera quality

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The aim of this study was to investigate the effect of mechanical kneading and 'absit' preparation difference on the quality of tef injera, the staple food of Ethiopians. Standard methods were adopted to determine the starch fraction, total phenol, flavonoid, phytate and tannins of injera. Sensory injera quality was assessed using 9-point-hedonic scale. Change in kneading conditions (time/speed) did not significantly affect the free sugar (FSG), slowly digestible starch (SDS), resistant starch (RS), total starch(TS) and starch digestion rate index (SDRI). On the other hand, significant variation was observed in rapidly available glucose and rapidly digestible starch (RAG and RDS). Flavonoids, total phenolics and phytate contents varied significantly at different kneading time- speed combinations. Injera sensory quality was also significantly affected due to change in kneading conditions. Kneading condition 5 (3 min at speed 6) has the highest injera overall acceptability while kneading condition 9 (7 min at speed 12) had the lowest. In addition to kneading conditions, absit preparation (water to fermented dough ratio) was also found to affect the quality of tef injera. Absit # 3 made from 100 ml of fermented dough and 900 ml of water had the highest injera overall acceptability while, the lowest was observed on Absit # 4 made from 300 ml of fermented dough and 100 ml of water. In conclusion, both kneading and absit preparation significantly influenced starch hydrolysis, flavonoids, total phenolics and phytate contents as well as sensory quality of injera.

Key words: Kneading, sensory quality, absit preparation, starch fractions.

INTRODUCTION

More than 70% of Ethiopian populations rely on injera for their diet, which is a traditional Ethiopian sourdough flatbread (Dijkstra et al., 2008). 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). Quality characteristics of injera are directly related to its

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Table 1. Kneading and absit variables.

Kneading variables			Absit variables		
Kneading treatment	Time (min)	Speed (speed)	Absit treatment	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

appearance, texture and taste. According to Assefa et al. (2018), 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 starts by 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 on these changes at 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, 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 optimum kneading 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.

Dough processing is also an important factor determining the quality of baked goods (Amjid et al., 2013). 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, Li et al. (2015) also described as food processing including: mixing, kneading, and heating affect the antioxidant properties of foods for which polyphenols are responsible.

Once fermentation 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’ (Assefa et al., 2018). Zannini et al. (2012) stated that the functionality of absit in the injera 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 absit is a dough enhancer (improves the texture of the dough) and Girma et al. (2013) also mentioned that absit is a dough binder, but does not define these terms or suggest a mechanism for the effect. It is believed that the main function of a dough enhancer and binder is to enhance the viscosity of batters. Yetneberk et al. (2004) stated that the objective of gelatinization is primarily to bring about cohesiveness of the batter and secondly to provide easily fermentable carbohydrate to leaven the injera. Yetneberk et al. (2004) also 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. However, this study is lacking on the influence of absit preparation on injera quality. Therefore, the present study was done to investigate the effect of mechanical kneading and ‘absit’ preparation difference on tef injera quality.

MATERIALS AND METHODS

Among the different varieties released by Debre Zeit 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 Debre Zeit 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 1) were used based on preliminary assessment of injera exporters, kneading machine capacity, and other works on bread dough kneading; similarly the ratio of water to fermented dough for absit preparation was based on previous work (Assefa et al., 2018) and traditional practices. Based on the sensory results, Kneading # 1, with shorter kneading time and slower kneading speed, had moderate overall injera acceptability; #5, with moderate kneading time and moderate speed, had the highest injera acceptability; and

#9, with longer kneading time and fastest kneading speed, had the lowest injera acceptability; they were selected for starch hydrolysis, flavonoids, total phenolics, phytate and tannins analysis.

Tef dough kneading and Injera preparation

Tef dough samples were prepared according to Parker et al. (1989) and Zegeye (1997) with little modification. Amount equal to 60 ml of starter (ersho) was initially added to each kg of flour. Accordingly, the tef flour (from stone-disc mill) was mixed at 2:3 (w/w) with potable water and kneaded by kitchen aid (Moulinex Masterchef 720, France). The dough was allowed to ferment for 60 h at room temperature $30 \pm 5^\circ\text{C}$. After this primary fermentation, different dough-water combinations (Table 1) were used to prepare absit that was 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 h 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 to 3 min of cooking using electric injera baking equipment, injera was removed and placed in a basket.

Starch fractions analysis

Englyst et al. (1992) methods were used to measure *in vitro* starch digestibility of tef injera with modifications by Englyst et al. (1999 and 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 (Englyst et al., 2000). The free sugar glucose (FGS) content was measured by a separate test according to 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.

Sample extraction for further analysis

Samples were extracted based on the procedures outlined by Barros et al. (2007) and Ferreira et al. (2007). Five gram of injera sample was extracted by stirring with 100 ml of methanol at 25°C and 150 rpm for 24 h using temperature shaker incubator (ZHWY-103B, China) and then filtered through Whatman No. 4. 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, UK) and re-dissolved in methanol at the concentration of 50 mg/ml and stored at 4°C for further use.

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-Ciocalteus 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 $\mu\text{g}/\text{ml}$). Total content of phenolic in injera extracts and gallic acid equivalent (GAE) was calculated by the following formula:

$$C = \frac{c \times V}{m}$$

Where, C is the total content of phenolic compounds (mg/g fresh material in GAE) and c the concentration of gallic acid established from the calibration curve.

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% NaNO_2 solution. After 6 min, 150 μl of a 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1 M 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 $\mu\text{g}/\text{ml}$. Results were expressed as mg (+)- catechin equivalent (CE)/g of extract.

Determination of phytate

The phytate content in the sample was determined according to Oyaizu, (1986). About 0.1 g of fresh samples was extracted with 10 ml 2.4% HCl in a mechanical shaker for 1 h at a room temperature. The extract was centrifuged at 3000 rpm for 30 min. The clear supernatant was used for phytate estimation. One ml of Wade reagent (containing 0.03% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 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 s. 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.

Determination of 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 h at room temperature on a mechanical shaker (ZHWY-103B, China). The solution was centrifuged at 1000 rpm for 5 min. 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 min, the absorbance of the solutions and the standard solution were measured at 500 nm. Blank sample consisted of 1 ml of extract solution with 5 ml of 1% HCl without vanillin-HCl reagent. (+) catechin (0.5–12 mg /100 ml) was used as standard for construction of calibration curve.

Sensory evaluation

The sensory evaluation was carried out by a panel trained according to Einstein (1991). The selected panelists were tested for their ability to detect basic tastes (Jellinek, 1985). The panel comprised 10 people as recommended by Stone and Sidel (1985). They were female and male, who were students in Addis Ababa University, Ethiopia. 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

Table 2. Effect of kneading conditions on starch fraction of tef injera.

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

Data are expressed as mean ± standard deviations (SD); n=10; 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. Different superscripts in the same column indicate statistically significant differences (P < 0.05).

Table 3. Effect of kneading conditions on total phenolics, flavonoids, phytate and tannins contents (mg /100 g).

Kneading	Flavonoids	Total Phenols	Phytate	Tannins
1	0.12 ^b ±0.00	0.13 ^c ±0.00	94.1 ^a ±0.16	198.5 ^a ±6.8
5	0.15 ^a ±0.00	0.32 ^b ±0.00	70.0 ^b ±0.31	177.6 ^a ±0.00
9	0.15 ^a ±0.02	0.38 ^a ±0.00	46.5 ^c ±0.31	172.4 ^a ±7.4

Data are expressed as mean ±SD, n=10 ; means with different superscripts in the same column are statistically different (α <0.05).

acceptability was also evaluated. A score sheet was prepared using the selected descriptors. Each one of attribute was evaluated using a 9-point numerical scale (0–9) anchored on both sides with verbal descriptions (that is 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 3–4 h after baking. A glass of drinking water was used for rinsing between samples.

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) (SPSS Inc., USA) and means of duplicate results were compared by Tukey's Honestly Significant Difference Test.

RESULTS AND DISCUSSION

Effect of kneading (time/speed) on injera quality

Starch fractions at different kneading conditions

Table 2 presents the effect of kneading at different time/speed combinations on starch fractions. The changes of kneading conditions (time/speed) did not significantly affect the FSG, SDS, RS, TS and SDRI. On the other hand, significant variation was observed on RAG and RDS which were kneaded at different time/speed combinations. 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 (Singh et al., 2010). Though insignificant difference was observed with increasing time and speed of kneading, the SDS value increased with increasing time and speed. 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 to enzymatic attacks; increased hydrolysis; faster digestion. Kneading #5 (3 min at speed 6) had higher RAG (70.6) and RDS (63.4) while the lower RAG (68.0) and RDS (61.0) were observed in 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 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).

Effect of kneading conditions on total phenolics, flavonoids, phytate and tannin contents

Table 3 presents the effect of kneading conditions on flavonoids, total phenolics, phytate and tannins contents. The finding showed that changing kneading conditions

Table 4. Effect of kneading conditions on the sensory quality of injera.

Kneading	Color	Taste	Texture	Number of eyes	Eye size	Eye distribution	Top and bottom surfaces	Odor	Overall acceptability
1	5.3 ^a ±1.3	5.5 ^a ±0.1	4.1 ^{ab} ±0.7	6.7 ^a ±1.4	1.4 ^a ±0.4	5.6 ^{ab} ±1.1	5.6 ^b ±0.9	5.5 ^a ±0.4	5.1 ^{bc} ±0.3
2	6.0 ^a ±0.0	5.4 ^a ±0.6	6.5 ^{bc} ±1.0	6.1 ^a ±0.1	3.6 ^b ±0.1	3.7 ^{ab} ±0.1	6.2 ^b ±0.2	6.3 ^a ±0.3	7.3 ^{cde} ±0.3
3	7.4 ^a ±0.1	6.2 ^a ±0.3	6.3 ^{bc} ±0.4	5.2 ^a ±0.2	6.3 ^c ±0.4	4.4 ^{ab} ±0.8	5.8 ^b ±0.6	5.4 ^a ±2.1	8.2 ^{de} ±0.0
4	6.0 ^a ±0.5	5.1 ^a ±1.5	2.8 ^a ±1.8	4.2 ^a ±1.1	4.7 ^{bc} ±0.9	3.1 ^{ab} ±1.8	3.9 ^{ab} ±0.1	4.2 ^a ±2.1	3.1 ^{ab} ±2.2
5	6.3 ^a ±1.6	5.3 ^a ±1.8	6.7 ^{bc} ±0.1	5.3 ^a ±0.8	5.2 ^{bc} ±0.1	6.9 ^b ±0.4	6.7 ^b ±1.4	6.1 ^a ±1.1	8.6 ^e ±0.1
6	4.8 ^a ±0.3	4.9 ^a ±0.8	3.1 ^a ±0.4	5.5 ^a ±0.4	5.9 ^c ±0.3	5.9 ^{ab} ±0.3	6.2 ^b ±0.7	5.1 ^a ±0.5	3.5 ^{ab} ±0.6
7	5.4 ^a ±0.8	5.2 ^a ±0.8	6.8 ^{bc} ±0.6	6.0 ^a ±0.4	6.0 ^c ±1.0	3.3 ^{ab} ±0.7	5.2 ^b ±1.1	6.2 ^a ±0.1	5.3 ^{bcd} ±0.2
8	4.6 ^a ±1.2	4.5 ^a ±0.2	4.3 ^{ab} ±0.1	6.0 ^a ±0.1	3.5 ^{ab} ±0.6	4.8 ^{ab} ±1.3	1.8 ^a ±0.2	6.1 ^a ±0.4	5.1 ^{bc} ±0.2
9	6.3 ^a ±0.1	6.3 ^a ±0.1	8.7 ^c ±0.2	5.4 ^a ±0.6	4.5 ^{bc} ±0.3	2.0 ^a ±0.7	1.3 ^a ±0.2	6.4 ^a ±0.5	1.9 ^a ±0.1

Data are expressed as mean ±SD, n=10; means with different superscripts in the same column are statistically different ($\alpha < 0.05$).

have non-significant effect on the tannins content of the final product. Unlike tannins, flavonoids, total phenolics and phytate contents 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 occurred during the initial dough mixing and the remolding into smaller pieces. Contrary to the observation of Chlopicka et al. (2012), the total phenolic content increased with increasing 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 flavonoids and total phenol contents of injera. Phytate content degraded significantly as kneading time and speed increased. It decreased in the order: Kneading #1 (94.1 mg/100 g)>

Kneading #5 (70.0 mg/100 g)> Kneading #9 (46.5 mg/100 g). 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) 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 et al., 2003) and glucose lowering effects (Lee et al., 2005, 2006).

Effect of kneading conditions on sensory quality of injera

The effect of kneading conditions on the sensory

quality of injera is presented in Table 4. There was non-significant difference in 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, top and bottom surfaces and over all acceptability 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 to the quality of the product (Rosell, 2011). The sensory qualities texture, eye size and distribution, top and bottom surfaces and overall acceptability rated more with kneading time of 3 min and speed 6 (Kneading #5). On the other hand, Kneading #9 (7 min at speed 12) had the lower injera overall acceptability. Banu (2000) described that kneading is one of the most important operations 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

Table 5. Effect of different water to fermented dough proportions on the sensory quality of injera (mean \pm SD).

Absit	Color	Taste	Texture	Number of eyes	Eye size	Eye distribution	Top and bottom surfaces	Odor	Overall acceptability
1	5.4 ^a \pm 0.0	4.1 ^a \pm 0.9	4.9 ^{ab} \pm 0.8	5.8 ^a \pm 0.4	3.9 ^{bc} \pm 1.3	4.8 ^{ab} \pm 0.8	5.3 ^b \pm 0.6	5.7 ^a \pm 0.2	4.4 ^{bc} \pm 0.3
2	6.0 ^a \pm 0.2	4.3 ^a \pm 0.1	6.0 ^{ab} \pm 0.2	6.0 ^a \pm 0.2	3.2 ^{bc} \pm 1.3	6.1 ^{ab} \pm 1.9	5.4 ^b \pm 0.0	6.8 ^{ab} \pm 0.1	5.0 ^c \pm 0.1
3	6.6 ^a \pm 0.7	4.8 ^a \pm 0.0	7.5 ^b \pm 0.4	6.2 ^a \pm 0.0	5.2 ^b \pm 0.4	6.9 ^{ab} \pm 0.0	6.3 ^b \pm 0.9	7.3 ^b \pm 0.2	7.95 ^d \pm 0.1
4	5.9 ^a \pm 0.4	5.1 ^a \pm 0.1	3.9 ^a \pm 1.1	4.8 ^a \pm 0.4	2.9 ^{bc} \pm 0.6	2.4 ^a \pm 0.4	5.5 ^b \pm 0.5	5.7 ^a \pm 0.1	2.2 ^a \pm 0.0
5	6.9 ^a \pm 0.7	4.3 ^a \pm 0.2	4.1 ^a \pm 0.5	5.2 ^a \pm 1.5	1.9 ^a \pm 1.0	4.5 ^{ab} \pm 2.1	5.7 ^b \pm 0.0	6.3 ^{ab} \pm 0.8	3.2 ^{ab} \pm 0.8
6	6.9 ^a \pm 0.8	4.1 ^a \pm 0.0	7.5 ^b \pm 0.3	5.5 ^a \pm 0.2	8.7 ^c \pm 0.1	8.4 ^b \pm 0.3	6.3 ^b \pm 0.2	6.6 ^{ab} \pm 0.2	4.4 ^{ab} \pm 0.0
7	5.5 ^a \pm 0.1	4.8 ^a \pm 1.3	5.1 ^{ab} \pm 1.4	6.0 ^a \pm 0.6	1.5 ^a \pm 0.0	5.1 ^{ab} \pm 0.4	5.1 ^b \pm 0.3	6.3 ^{ab} \pm 0.4	3.1 ^{ab} \pm 0.4
8	5.7 ^a \pm 0.4	4.1 ^a \pm 0.2	4.4 ^a \pm 0.1	5.9 ^a \pm 0.5	1.2 ^a \pm 0.1	5.3 ^{ab} \pm 1	5.3 ^b \pm 0.6	5.8 ^a \pm 0.2	3.5 ^{ab} \pm 0.4
9	6.3 ^a \pm 0.3	4.3 ^a \pm 0.3	4.9 ^{ab} \pm 0.6	4.7 ^a \pm 0.3	2.2 ^a \pm 0.3	3.0 ^a \pm 1.5	3.2 ^a \pm 0.5	5.7 ^a \pm 0.1	2.3 ^a \pm 0.2

Data are expressed as mean \pm SD, n=10, Means with different superscripts in the same column are statistically different (α <0.05).

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 makes the dough temperature to rise. 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 is being held by the physical and colloidal processes (Bordei, 2004).

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).

Effects of water to fermented dough ratio during absit preparation on the quality of injera

The impacts of water to fermented dough ratio during absit preparation on the sensory quality of injera are presented in Table 5. The ratio of water to fermented dough did significantly affect the quality of injera. Texture, number of eyes, top and bottom surface, odor and overall acceptability of injera obtained from absit #3 prepared from a ratio of 900:100 ml of water to fermented dough with higher sensory score. Injera overall acceptability is the result of other quality attributes like texture, number of eyes, top and bottom surface, odor and others. Absit #6 had less overall acceptability than that of Absit # 3, but the individual sensory attribute gained 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 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 activate yeasts is 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". 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% (Zanniniet 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 significantly depend on tef varieties.

Conclusion

Kneading conditions considerably affect the starch fractions (RAG and RDS) and sensory quality of injera. Kneading time of 3 min and speed of 6 rpm had the highest injera overall acceptability. Flavonoid, total phenolics and phytate contents 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 which is made from 100 ml of fermented dough and 900 ml of water had the highest injera overall acceptability and further studies are required on different tef varieties.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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REFERENCES

- 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.
- Abiyu HT, Zewdu AW, Haki GD (2013). Preparation of injera from pre-fermented flour: nutritional and sensory quality. *International Journal of Science Innovations and Discoveries* 3:165-175.
- Alonso R, Aguirre A, Marzo F (2000). Effects of extrusion and traditional processing methods on anti-nutrients and *in vitro* digestibility of protein and starch in faba and kidney beans. *Food Chemistry* 68: 159-165.
- Altan A, McCarthy K, Maskan M (2009). Effect of extrusion cooking on functional properties and *in vitro* starch digestibility of barley-based extrudates from fruit and vegetable by-products. *Journal of Food Science* 74: 77-86.
- Amjid MR, Shehzad A, Hussain S, Shabbir MA, Khan MR, Shoab M (2013). A comprehensive review on wheat flour dough rheology. *Pakistan Journal of Food Sciences* 23(2):105-123.
- Ashagrie Z, Abate D (2012). Improvement of injera shelf life through the use of chemical preservatives. *African Journal of Food, Agriculture, Nutrition and Development* 12:6409-6423.
- Ashenafi M (2006). A review article on the microbiology of indigenous fermented foods. *Ethiopian Journal of Biological Sciences* 5:189-245.
- Assefa Y, Emire S, Villanueva M, Ababe W, Ronda F (2018). Influence of milling type on tef injera quality. *Food Chemistry* 266:155-160.
- Attuquayefio WD (2014). Influence of processing parameters on eye size and elasticity of tef-based injera. Available at: <https://etda.libraries.psu.edu/catalog/23432>
- Banu C (2000). Handbook engineer food industry. Vol. II. (in Romanian). Technical Publishing House, Bucharest.
- Barros L, Ferreira MJ, Queirós B, Ferreira IC, Baptista P (2007). Total phenols, ascorbic acid, β -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry* 103:413-419.
- Baye K (2014). Tef: nutrient composition and health benefits. *International Food Policy Research Institute* 67:10.
- Bordei D (2004). Modern technology of bakery. (in Romanian). Bucharest AGIR P 447.
- Burns RE (1971). Method for estimation of tannin in grain sorghum. *Agronomy Journal* 63(3):511-512.
- Canja CM, Lupu M, Taulea G (2014). The influence of kneading time on bread dough quality. *Bulletin of the Transilvania University of Brasov. Forestry, Wood Industry, Agricultural Food Engineering, Series II* 7(1):79.
- Cauvain S (2003). *Breadmaking: Improving quality*. Cambridge, UK: Woodhead. Publishing Limited, Cambridge, Anglia.
- Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereals breads. *LWT-Food Science and Technology* 46(2):548-555.
- Curhan GC, Willett WC, Knight EL, Stampfer MJ (2004). Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II. *Archives of Internal Medicine* 164(8):885-891.
- Dijkstra A, Polman J, van Wulfften-Palthe A, Gamboa PA, van Ekris L (2008). Survey on the nutritional and health aspects of tef (*Eragrostis tef*). Available at: https://www.doc-developpement-durable.org/file/Culture-plantes-alimentaires/FICHES_PLANTES/teff/Survey%20o n%20the%20nutritional%20and%20health%20as pects%20of%20teff.pdf
- Dobraszczyk B, Morgenstern M (2003). Rheology and the breadmaking process. *Journal of Cereal Science* 38: 229-245.
- Einstein MA (1991). Descriptive techniques and their hybridization. In: *Sensory Science Theory and Applications in Foods* pp. 317-338.
- Englyst HN, Kingman SM, Cummings JH (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* 46:S33-50.
- Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH (1999). Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. *The American Journal of Clinical Nutrition* 69(3):448-454.
- Englyst K, Hudson G, Englyst H (2000). Starch analysis in food. RA Meyers (Ed.), *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*.
- Ferreira IC, Baptista P, Vilas-Boas M, Barros L (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from Northeast Portugal: Individual cap and stipe activity. *Food Chemistry* 100: 1511-1516.
- Girma T, Bultosa G, Bussa N (2013). Effect of grain tef [*Eragrostis tef* (Zucc.) Trotter] flour substitution with flaxseed on quality and functionality of injera. *International Journal of Food Science & Technology* 48:350-356.
- Gómez M, Talegón M, Hera E (2013). Influence of mixing on quality of gluten-free bread. *Journal of Food Quality* 36(2):139-145.
- Hurrell R, Egli I (2010). Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition* 91(5):1461S-1467S.
- Jellinek G (1985). *Sensory evaluation of food. Theory and practice*. Ellis Horwood Ltd.
- Keiffer R (2006). The role of gluten elasticity in the baking quality of wheat. In: Popper L, Schäfer W, Freund W (Eds.), *Future of flour-A Compendium of flour improvement. Verlag Agrimedia* pp. 169-178.
- Lawless HT, Heymann H (1999). *Sensory evaluation of food. Principles and practices*; Aspen Publishers. Inc. Gaithersburg, MD, USA.
- Lee SH, Park HJ, Cho SY, Jung HJ, Cho SM, Cho YS, Lillehoj HS (2005). Effects of dietary phytic acid on serum and hepatic lipid levels in diabetic KK mice. *Nutrition Research* 25(9):869-876.
- Lee SH, Park HJ, Chun HK, Cho SY, Cho SM, Lillehoj HS (2006). Dietary phytic acid lowers the blood glucose level in diabetic KK mice. *Nutrition Research* 26(9):474-479.
- Li Y, Ma D, Sun D, Wang C, Zhang J, Xie Y, Guo T (2015). Total phenolic, flavonoid content, and antioxidant activity of flour, noodles, and steamed bread made from different colored wheat grains by three milling methods. *The Crop Journal* 3(4):328-334.
- Maloney D, Foy J (2003). Yeast fermentations. In: Kulp K, Lorenz K. (Eds.). *Handbook of Dough Fermentations*. Marcel Dekker, Inc., New York pp. 43-60.
- Manary MJ, Hotz C, Krebs NF, Gibson RS, Westcott JE, Broadhead RL, Hambidge KM (2002). Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet. *The American Journal of Clinical Nutrition* 75(6):1057-1061.
- Maxon ED, Rooney LW (1972). Evaluation of method for tannin analysis in sorghum grain. *Cereal Chemistry* 49:719-729.
- Morris ER, Ellis R (1985). Bioavailability of dietary calcium: effect of phytate on adult men consuming non vegetarian diets. In ACS Symposium series-American Chemical Society (USA).
- Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* 44:307-315.
- Parker ML, Umata M, Faulks RM (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.

- Rosell CM (Ed.) (2011). The science of doughs and bread quality. In: Flour and breads and their fortification in health and disease prevention pp. 3-14.
- Rus FI, Bică (Canja) CM, Șerban E (2008). Unit operations, mixing and crowding. (in Romanian). Transilvania University Press, Brasov.
- Singh J, Dartois A, Kaur L (2010). Starch digestibility in food matrix: A review. *Trends in Journal of Food Science and Technology* 21:168-180.
- Singh RP, Agarwal C, Agarwal R (2003). Inositol hexaphosphate inhibits growth, and induces G1 arrest and apoptotic death of prostate carcinoma DU145 cells: modulation of CDK1-CDK-cyclin and pRb-related protein-E2F complexes. *Carcinogenesis* 24(3):555-563.
- Stewart RB, Getachew A (1962). Investigations of the nature of injera. *Economic Botany* 16:127-130.
- Stone H, Sidel JL (Eds.) (1985). *Sensory evaluation practices*. 2nd. Academic Press. *San Diego*.
- Sujka M, Jamroz J (2013). Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour. *Food Hydrocolloids* 31:413-419.
- Xu BJ, Chang SK (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science* 72:S159-S166.
- Yetneberk S, de Kock HL, Rooney LW, Taylor JRN (2004). Effects of sorghum cultivar on injera quality. *Cereal Chemistry* 81:314-321.
- Zannini E, Jones JM, Renzetti S, Arendt EK (2012). Functional replacements for gluten. *Annual Review of Food Science and Technology* 3:227-245.
- Zegeye A (1997). Acceptability of injera with stewed chicken. *Food Quality and Preference* 8(4):293-295.