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Variety and baking effects on injera making quality, polyphenols content and antioxidant activity of millet flours and injera

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Injera or Biddena commonly made from tef (*Eragrostis tef* (Zucc) Trotter) grain and a combination with other grains is a staple food of Ethiopian and neighboring countries. Millet and millet-based food products are rich in polyphenols and antioxidant activities. This study aimed to evaluate millet varieties for their injera making quality and polyphenol content and antioxidant activity. Five millet varieties and one tef variety called Quncho were collected, investigated and compared. A significant (p<0.05) variations were noticed in rollability, adhesiveness and overall acceptance of injera. Quncho was perceived differently and rated higher in its overall acceptance (8.04). Among millet injera's, Kola-1 rated higher whereas Padet was perceived lower in overall consumer acceptance. The result showed that the total phenols, flavonoids and anthocyanin contents of flour and injera ranged from 18.63 to 27.29 µg GAE/g, 11.99 to 15.43 µg catechin equivalent per g of sample, and 5.11 to 53.23 mg/l for flours and 22.99 to 27.25 µg GAE/g, 13.47 to 14.49 µg catechin/g and 5.53 to 24.27 mg/l for injera. Tesema had the highest total phenols (27.29 µg GAE/g), total flavonoid content (15.43 µg catechin/g) and antioxidant activity (41.07%) against the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Kola-1 showed the lowest L* (59.76) and a* (-0.11) and the highest b* (4.21) flour color characteristics. Baking caused a non-significant (p>0.05) reduction in total phenols, total flavonoids contents and DPPH free radical scavenging capacity. Hence, it is recommended that Tesema and Kola-1 varieties could be used for functional food development and injera making quality, respectively.

Key words: Millet varieties, tef, injera, baking, polyphenols, antioxidant effects.

INTRODUCTION

Injera or *biddena* is a staple food of Ethiopian which accounts for approximately 70% of dietary calories. It is made from quite a lot of cereal grains such as tef, sorghum, millet, maize, barley and wheat depending on the regions and availability. However, best *injera* is made from tef (*Eragrostis tef* (Zucc) Trotter) grain. Recently,

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because of its being a whole grain product and gluten free nature (the cause for celiac disease) tef *injera* is gaining popularity in the developed countries as well (Abiyu et al., 2013). *Injera* is also considered as good sources of energy, fiber, iron, calcium and vitamins although the fermentation process during preparation results in significant reduction of most of the nutrients found in the cereals flour (Mezemir, 2015). *Injera* from white tef is most preferred due to its softer texture, preferred taste and color, and can be rolled without cracking (Boka et al., 2013). Tef is commonly grouped as a small millet along with fonio, finger millet and proso millet and it belongs to the same subfamily and tribe (Eragrostideae). As the tef price goes up, even middle income households tend to mix tef flour with cheaper cereals such as millet, sorghum maize or rice in preparing *injera* (Di Marcantonio and Demeke, 2013). Reports have shown that millet is inexpensive and nutritionally comparable or even superior to major cereals (Pathak et al., 2000).

Nowadays, agricultural research institutes have been releasing new varieties with the aim of increasing crop yield. However, grain quality and flour functionality are the most important criteria for good dough handling properties and specific health benefits. Millets and millet based food products are rich in phytochemicals which exhibit antioxidant and free-radical scavenging activity. As these are also gluten-free it could be suitable for persons suffering from celiac disease. Free radicals and reactive oxygen species are fundamentally the core cause of several disorders in humans that are generated as an imbalance between formation and neutralization of pro-oxidants resulting in oxidative stress as they cause oxidative damage to lipids, proteins, and DNA. Numerous studies and epidemiological evidences had shown that whole-grain cereal based foods are rich in plant polyphenols which could protect the body against age-related diseases such as cancer, cardiovascular ailments, diabetes, metabolic syndrome, and Parkinson's disease (Chandrasekara and Shahidi, 2012; Fardet et al., 2008; Manach et al., 2005; Scalbert et al., 2005). Antioxidants are thought to be important in reducing oxidative damage (Halliwell, 1994).

Prior phytochemical profiling of millet indicated that it contained significant amounts of antioxidants such as carotenoids, phenolics, and tocopherols (Asharani et al., 2010). Several forms of phenolics which exist in the grain have been reported to render antioxidative and antiproliferative effects and are responsible for the control of cholesterol oxidation in vitro systems (Madhujith and Shahidi, 2007, 2009; Liyana-Pathirana and Shahidi, 2006). Health benefits imparted by cereal phenolics may be a result of additive and synergistic effects of multiple compounds present in the grains. Phenolic compounds are secondary plant metabolites and their type and content in the grains may depend on a number of factors such as the type of cereal, variety, and part of the grain, climatic conditions, and cultivation practices (Naczk and Shahidi, 2004). In addition, thermal treatments may reduce or increase the phenolic content and the antioxidant activities of cereals (Zielinski et al., 2006). Siroha and Sandhu (2017) revealed that toasting significantly increased the polyphenols content and antioxidant activity of pearl millet than cooking. Gull et al. (2018) noticed a significant reduction of antioxidant activity in cooked millet-pomace based pasta due to thermal degradation. Therefore, as *injera* is a staple food of Ethiopian, the investigation of millet varieties grown in several agro-ecological parts of the country and the effect of processing on the product making quality, and phenolic contents and antioxidant potential is unquestionable. Admassu et al. (2009) studied the chemical composition of local and improved finger millet varieties grown in Ethiopia. However, information on the polyphenol contents and antioxidant activity, and product making quality of Ethiopian millets are very limited. Thus, the objective of this study was to evaluate the polyphenol profiles, antioxidant activity and *injera* making quality of improved millet varieties grown in Ethiopia.

MATERIALS AND METHODS

In this study five samples of released millet varieties (four finger millet and one pearl millet) namely *Padet, Tessema, Tadesse, Aksum* and *Kola-1* grown in 2018/2019 season in moisture stress areas under similar but not the same agroecologies of Ethiopia were collected from Melkassa Agricultural Research Center, Ethiopia. One *tef* variety called *Quncho* was obtained from Debrezeit Agricultural Research Center and was used as a control. All millet varieties were grown in dry land areas and the tef grain was grown in moist and humid area. The grains were sorted, cleaned, washed, drained, sun dried and ground into flour. All the required chemicals were purchased from a company found in Can Tho City, Vietnam. The experiment was conducted at Food Science and Nutrition laboratory, Melkassa Agricultural Research center, Ethiopia and Food Technology laboratory of Can Tho University, Vietnam.

Injera processing and sensory evaluation

Injera was prepared using a standardized *injera* making procedure (Yetneberk et al., 2004). The procedure involved milling whole millet grain into a flour, preparation of a dough, and fermentation of the dough after adding yeast (a batter from a previous batch) and fermenting at room temperature for 48 h. After fermentation, 80 g of the fermented dough was thinned with 30 mL of water and cooked in 200 ml of boiling water for 1 min. The gelatinized batter was cooled to 45°C at room temperature and added back to the fermenting dough. After thorough mixing, 100 ml of water was added and the batter was fermented at room temperature for 3 h. Additional water (20 ml) was added to the fermented dough to bring to batter consistency. About 500 g of the fermented batter was poured in a circular manner on a 50 cm diameter hot clay griddle, covered, and baked for 2 min. The overall image of *injera* processing technique is shown in Figure 1.

A semi-trained panel (a panel briefed about the scoring of sensory attribute) of 40 people, who are consuming that particular product evaluated the samples. Accordingly, 20 consumer panelists

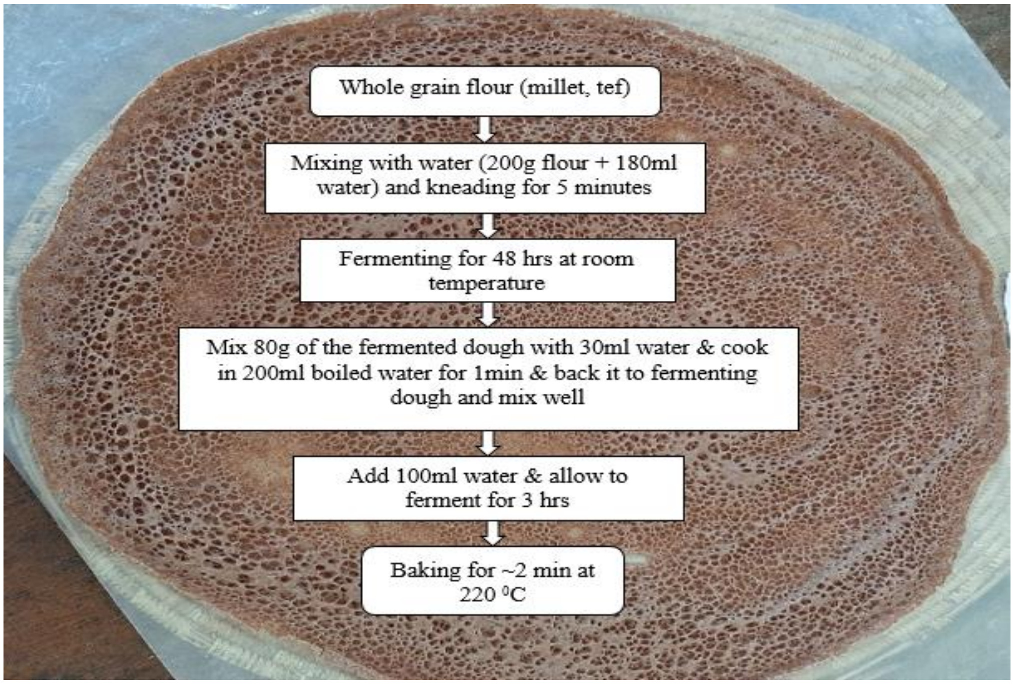

Figure 1. Injera processing flowchart (pictured: *injera* from *Tesema* variety).

were considered as replication one and the other 20 panelists as second replication. A rolled piece of *injera* was presented for panelist on a tray at room temperature within 2 h after baking. Panelists were served with a three digit coded samples on white plates in fluorescent-lighted rooms and was asked to evaluate some basic *injera* attributes (eye distribution, color, rollability, taste, adhesiveness, bitterness after taste and overall acceptability) using a nine point hedonic scale (1= extremely dislike, 9= extremely like).

Color characteristics

Color measurements of flour and *injera* samples were carried out using a Hunter colorimeter (Model, NR60CP 3NH Technology Co., LTD) optical Sensor on the basis of L*, a*, and b* values as described by Kaur and Singh (2005). A glass cell containing flour was placed above the light source, covered with a white plate and L*, a*, and b* color values were recorded. The instrument was calibrated against a standard red-colored reference tile (Ls = 25.54, as = 28.89, bs = 12.03). Total color difference (ΔE) was calculated by applying the equation:

$$\Delta E = [(L_s - L)^2 + (a_s - a)^2 + (b_s - b)^2]^{1/2}$$

where, the L* value indicates the lightness, 0–100 representing dark to light. The a* value gives the degree of the red-green color, with a higher positive a* value indicating redder. The b* value indicates the degree of the yellow-blue color, with a higher positive b* value indicating more yellow.

Polyphenol compounds and antioxidant activity

Sample preparation

Because of its high crude fat content, the flour from *Kola-1* was defatted first by dissolving in hexane (1:5 w/v, 5 min for 3 times) to remove interfering lipids. A flour sample (2.5 g) was extracted with 25 ml acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) for 2 h while shaking. The mixture was centrifuged at 3000×g for 10 min. The supernatant was used for determination of total phenols, total flavonoids and DPPH radical scavenging activity. The absorbance of the extracts was measured using Visible Spectrophotometer (Model 722, China). Note that both flour and injera were analyzed for the aforementioned parameters.

Anthocyanin content

The anthocyanin content (ACC) was determined by the pH differential method (Li et al., 2012). The diluted sample extracts (100 µL) in 25 mmol L^{-1} potassium chloride solution (pH 1.0, 5.0 mL) and 0.4 mol L^{-1} sodium acetate buffer (pH 4.5, 5.0 mL) were measured at 510 and 700 nm, respectively, after 15 min of incubation at 23°C. Finally, absorbance (A) variation was calculated as:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$$

Total anthocyanin content of samples (mg cyanidin3- glucoside L^{-1} of sample extract) was calculated from the following equation:

$$ACC = \frac{A\ x\ M\ x\ DF\ x\ 1000}{(\varepsilon\ x\ 1)}$$

Where A is absorbance value, M is molecular weight (449.2 g mol^{-1}), DF is dilution factor (51), and e is the molar absorptivity of cyanidin3-glucoside (26,900 L mol^{-1} cm^{-1}). The result was calculated in g cyanidin3-glucoside equivalents (CGE)/kg of sample wet weight.

Total phenolic content

The total phenolic content (TPC) was determined by following the Folin–Ciocalteu spectrophotometric method described by Gao et al. (2002). Aliquot of extract (250 µl) was added to 1.5 ml freshly diluted Folin–Ciocalteu reagent. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 ml of sodium carbonate solution (60 g/L). Then after incubation at 25ºC for 90 min, the absorbance of the mixture was read at 725 nm. Acidified methanol was used as a blank and the result was expressed as a µg of gallic acid equivalents (GAE)/g of flour.

Total flavonoids content

The total flavonoids content (TFC) was determined following the method as described by Zhishen et al. (1999). Extract (250 µl) was diluted with 1.25 ml distilled water. Sodium nitrite (750 µl of 5% solution) was added and the mixture was allowed to stand for 6 min. Then, 150 µl of a 10% aluminum chloride was added and the mixture was allowed to stand for 5 min. Finally, 0.5 ml of 1 M sodium hydroxide was added and solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Catechin was used as standard and the result was reported as µg of catechin equivalents (CE)/g of flour.

DPPH free radical scavenging activity

Antioxidant activity by DPPH free radical scavenging capacity was measured following a modified version of the method described by Brand-Williams et al. (1995) with slight modification. The supernatant (250 µl) was reacted with 4 ml of a 6×10^{-5} mol/L of DPPH solution. Absorbance (A) at 515 nm was read at 0 and 30 min taking a methanol as a blank. DPPH free radical scavenging activity was calculated as % discoloration.

$$\% \text{ DPPH Activity} = (1 - (\text{A of sample}_{t=30}/\text{A of control}_{t=0})) \times 100$$

Statistical analysis

A duplicate data was used and analyzed using Minitab 16 statistical software package and Tukey's multiple comparison tests was used to determine the significance of variation between treatments at 95% confidence level. Results were given as mean ± standard deviation.

RESULTS AND DISCUSSION

Sensory properties of injera

In this study, a nine point hedonic scale (1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8= like very much, 9= like extremely) was used. Obviously *injera* with characteristics of white color, even eye distribution, less sourness and bitterness, rollable and less stick is preferred by consumers. The results of sensory characteristics of *injera* from five millet varieties and tef *injera* which was used as a control are shown in Table 1. In terms of aroma, taste and bitter aftertaste, no significant difference (p>0.05) were observed between and within millet varieties and control sample. *Padet* significantly (p<0.05) differed from *Axum* and rated lower in its *injera* eye evenness (6.25) and color (5.71). *Injera* eye is a honeycomb like structure of the top surface of the product and it is formed during baking.

A significant (p<0.05) variations were noticed in rollability, adhesiveness and overall acceptance of *injera*. *Quncho* was perceived differently and rated higher in its overall acceptance (8.04). Conversely, injera from *Padet* was rated lower in overall acceptance (6.21). In addition, among finger millet cultivars, *Padet* was perceived lower in its *injera* eye distribution and color; and a highest score of bitter aftertaste sensory attribute was for *injera* made from *Axum* (7.31) and *Tesema* (7.24) varieties with non-significant (p>0.05) variation in between. Bitter aftertaste could be due to the presence of polyphenols in the grain. Rollability is one the most important *injera* sensory attribute as it describes the ability of *injera* being rolled. The result showed that *Quncho* (7.99) and *Tesema* (7.04) had the highest and the lowest rollability with a significant difference among them and more preferred from others. This difference might be due to retro gradation of starch components.

Yetneberk et al. (2004) revealed that sorghum cultivar with floury endosperm were characterized by soft and rollable *injera*. The degree of *adhesiveness* of *injera* during consumption is desirable to consumer acceptance and it is a quality of being stick to human sense organs while eating. *Tesema* showed the lowest degree of stickiness among finger millet cultivars.

Polyphenols content and antioxidant activity of flours and injera

Anthocyanin content (ACC), total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging capacity of whole grain flours and *injera* from different millet varieties are shown in Table 2. Importantly, antioxidants are known to limit the amount of free radicals produced in human bodies. Phenolics are one of the major antioxidants found in millets, which chemically act by donating hydrogen atoms via hydroxyl groups on benzene rings to electron-deficient free radicals, and form a resonance-stabilized and less-reactive phenoxyl radical. Phenolics from millets have also shown their ability as reducing agents, singlet oxygen quenchers, and metal chelators (Devi et al., 2014).

Polyphenols content and antioxidant activity of flours

Anthocyanin content: The anthocyanin content of millet flours varied significantly (p<0.05) among millet varieties and ranged from 5.11 to 53.23 mg/L, the lowest for

Table 1. Sensory characteristics of millet and tef injera.

Variety	Sensory attributes							
	Eye distribution	Color	Aroma	Taste	Bitterness after taste	Rollability	Adhesiveness	Overall acceptance
Axum	7.49±0.38[a]	8.01±0.28[a]	7.38±0.38[a]	7.49±0.38[a]	7.31±0.35[a]	7.35±0.42[ab]	6.91±0.45[ab]	7.05±0.08[bc]
Kola-1	7.28±0.09[ab]	8.26±0.38[a]	7.78±0.17[a]	7.14±0.20[a]	6.97±0.04[a]	7.14±0.21[ab]	6.79±0.21[ab]	7.51±0.11[ab]
Padet	6.25±0.05[b]	5.71±1.07[b]	7.33±0.11[a]	7.09±0.28[a]	6.83±0.09[a]	7.42±0.18[ab]	6.84±0.18[ab]	6.21±0.11[d]
Tadese	6.65±0.15[ab]	7.40±0.09[ab]	7.26±0.37[a]	7.08±0.03[a]	7.21±0.28[a]	7.11±0.17[ab]	6.495±0.12[ab]	6.44±0.12[cd]
Tesema	6.57±0.34[ab]	7.41±0.57[ab]	7.48±0.23[a]	7.11±0.16[a]	7.24±0.36[a]	7.04±0.06[b]	6.48±0.09[b]	7.14±0.29[b]
Quncho	7.35±0.41[ab]	8.47±0.08[a]	8.09±0.21[a]	7.96±0.05[a]	7.06±0.57[a]	7.99±0.16[a]	7.43±0.18[a]	8.04±0.22[a]

Mean ± SD with different superscripts in a column varied significantly ($p < 0.05$) within and between different millet cultivars and control, n=2.

Table 2. Polyphenols content and antioxidant activity of flour and injera.

Variety	Flour				Injera			
	TPC (µgGAE/g)	TFC, µgCE/g	ACC (gCGE/kg)	DPPH scavenging activity (%)	TPC (µgGAE/g)	TFC (µgCE/g)	ACC (gCGE/kg)	DPPH scavenging activity (%)
Axum	26.99±0.008[a]	15.08±0.084[b]	25.97±3.011[bc]	39.31±1.05[ab]	26.22±0.045[b]	14.09±0.01[b]	24.27±1.81[a]	40.55±0.18[a]
Kola-1	22.96±0.187[d]	13.49±0.009[d]	5.11±1.204[c]	31.81±0.60[c]	24.77±0.018[c]	13.47±0.01[c]	5.53±0.60[d]	31.32±1.41[c]
Padet	25.15±0.062[c]	14.08±0.122[c]	46.84±13.248[ab]	38.17±0.66[b]	23.61±0.035[d]	13.56±0.04[c]	16.39±0.60[d]	36.37±0.38[b]
Tadese	25.76±0.036[b]	13.91±0.056[c]	35.34±1.807[ab]	38.15±0.00[b]	24.89±0.045[c]	13.57±0.00[c]	9.79±1.81[cd]	39.43±0.71[a]
Tesema	27.29±0.045[a]	15.43±0.028[a]	53.23±1.807[a]	41.07±0.60[a]	27.25±0.018[a]	14.49±0.13[a]	20.01±1.81[ab]	41.21±0.10[a]
Quncho	18.63±0.098[e]	11.99±0.075[e]	29.81±4.818[ab]	27.54±0.69[d]	22.99±0.027[e]	13.50±0.05[c]	15.33±3.61[bc]	32.19±1.41[c]

Mean ± SD with different superscripts in a column varied significantly ($p < 0.05$) within and between different millet cultivars and control, n=2. TPC, Total phenols content; TFC, Total flavonoids content; ACC, Anthocyanin content; GAE, Gallic acid equivalent; CE, Catechin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; CGE, Cyanidin3-glucoside equivalent.

Kola-1 and the highest for *Tesema* was noticed. Finger millet cultivars, *Padet*, *Tadese*, and *Tesema* were statistically similar in their anthocyanin content. Except from *Axum*, all finger millets varieties had the highest ACC than *Kola-1* and showed significant variation. *Tesema* and *Padet* was found to possess the highest ACC among all the studied varieties which had nine times higher than the ACC of *Kola-1*. *Axum* had the lowest ACC (25.97 mg/g) among finger millet varieties. *Quncho*, which is under the same family of millets showed a significant difference with *Axum* and *Kola-1*. Anthocyanins are a group of naturally occurring and water soluble pigments that are responsible for the red-blue color of many grains, and can be found in glycosylated forms linked with sugars such as glucose, galactose, arabinose, xylose, rutinose. These compounds become increasingly popular due to their attractive colors and suggested benefits for human health (Pojer et al., 2013) and in addition, protect plants against various biotic and abiotic stresses (Ahmed et al., 2014).

Total phenols and total flavonoids contents: The result showed that contents of total phenols and flavonoids differed significantly ($p<0.05$) between and within millet varieties and control sample as well. The total phenols and total flavonoids contents of flours ranged from 18.63 to 27.29 µg of GAE/g and 11.99 to 15.43 µg of CE/g, with the highest value for *Tesema* and the lowest content for *Quncho*, respectively. *Kola-1* was significantly (p<0.05) different and had lower contents of total phenols (22.96 µgGAE/g) and total flavonoids (13.49 µgCE/g) compared to finger millet varieties. Among finger millet cultivars, *Padet and Tadese* showed the lowest TPC (25.15 µgCE/g) and TFC (13.91 µgGAE/g), respectivley. The lowest TPC and TFC were observed with *Quncho*. Chandrasekara and Shahidi (2011) revealed that the phenolic content of whole pearl and kodo millet varieties was 8.63 and 32.4 ferulic acid equiv µmol/g defatted meal, respectively. Contrarily, Ragaee et al. (2006) reported the highest total phenols content (1387 µg GAE/g) for pearl millet variety. This variation could be due to agronomic, environmental and varietal differences, and forms and level of sample processing.

DPPH free radical scavenging activity: The antioxidant activity assay by DPPH was significantly (p<0.05) different among millet varieties. The highest DPPH free radical scavenging activity was observed for *Tesema* (41.07%) and the lowest for *Quncho* (27.54%). From the studied millet varieties, *Kola-1* was found to have the lowest (31.81%) antioxidant activity than finger millets and the greater antioxidant than *Quncho* with statistical difference. *Tadese* showed the lowest DPPH free radical scavenging activity (38.15%) with non-significant variation among finger millet cultivars. With the exception of *Tesema*, finger millet cultivars was not significantly (p>0.05) different. The DPPH free radical scavenging ability of all finger millet varieties was nearly 1.5 times the extracts from *Quncho*. An order of a free radical scavenging capacity, *Tesema* > *Axum* > *Padet* > *Tadese* > *Kola-1* > *Quncho* was observed. DPPH radical is a synthetic organic radical that is widely used to evaluate free radical scavenging possessions of antioxidative compounds. Similar study by Siroha et al. (2016) noticed that the antioxidant activity of pearl millet flours in the range of 31.8 to 46.7%. In addition, Ragaee et al. (2006) revealed that pearl millet had DPPH scavenging capacity of 23.83 µmol/g. Conversely, Chandrasekara et al. (2012) reported DPPH scavenging activities of 13.8 µmol ferulic acid equivalent/g for pearl millet flour. The antioxidant activity of grains could be influenced by genetic and environmental factors, and the level and method of grain processing.

Injera polyphenols content and antioxidant activity: After fermentation and baking, the polyphenol contents and antioxidant activity were ranged from 22.99 to 27.25 µgGAE/g for TPC, 13.47 to 14.49 µgCE/g for TFC, 5.53 to 24.27 mg/g for ACC and 31.32 to 41.21% for DPPH radical scavenging activity with a significant (p<0.05) difference among all varieties (Table 2). *Tesema* had the highest TPC, TFC and DPPH free radical scavenging capacity with a values 27.25 µgGAE/g, 14.49 µgCE/g and 41.21%, respectively. The lowest (even lower than *injera* made from *Quncho*) contents of total flavonoid, anthocyanin and antioxidant capacity was observed with *Kola-1*. Among finger millet varieties, *Padet* showed the lowest content in TPC (23.61 µgGAE/g) and TFC (13.56 µgCE/g). Except for the anthocyanin content, a non-significant (p>0.05) reduction or increment in total phenols, total flavononids and DPPH free radical scavenging activity were observed in *injera* compared to flours. This could be attributed to the partial degradation of phenols by microorganisms during fermentation (Bravo, 2009) and loss of some anthocyanin, which have been reported as labile to heat. An increment in DPPH free radical scavenging activity was noticed in finger millet cultivars and tef which is likely due to the amount reducing sugars that contribute to maillard reaction. Gélinas and McKinnon (2006) also observed that the crust of white bread contained more phenolic compounds than the crumb because of the Maillard reaction.

On the other hand, Salar et al. (2017) revealed that fermentation resulted in an increased TPC with fermented pearl millet grains showing higher values than unfermented pearl millet grains. According to these authors, as fermentation increased from 0 to 6 days, the TPC increased from 6.4 to 34.1 mg GAE/g dwb. However, beyond these fermentation days a reduction in TPC was noticed. In the present study, fermentation and then baking did not result in a significant (p>0.05) reduction and/or increment of DPPH radical scavenging activity of the millets extracts, except for *Quncho* which showed an increment (Figure 2). A strong correlation (r=0.98) was noticed between total phenolic content and DPPH radical scavenging activity. In addition, total flavonoids content and DPPH activity were highly correlated (r=0.94).

Color characteristics

Table 3 shows color characteristics (L*, a*, b* and ΔE) of flours and *injera* which were evaluated using hunter color lab. The L* value of flours ranged from 59.73 to 64.77, the more darken and light colors for *Kola-1* and *Tesema*, respectively. After baking, the L* value of *injera* ranged from 48.04 to 57.92 and except for *Kola-1* (which showed an increment) was significantly reduced in comparison with flours. *Kola-1* and *Quncho,* and among finger millet varieties, *Axum* and *Padet* in both flours and *injera* did not show significant (p>0.05) variation in their L* value. The variation observed might be due to the chemical compositions of the grain mainly because of protein, starch and reducing sugar contents that affects Maillard reaction. The degree of a red-green color (a*) ranged from -0.12 to 1.68 for flours and 2.14 to 4.47 for injera, and baking significantly increased a* value compared to flours. The highest and the lowest a* value with a significant difference was noticed with *Quncho* and *Kola-1* in flours, but had the lowest and exhibited insignificant variation in injera. *Kola-1* was statistically different and showed the minimum redness and maximum greenness

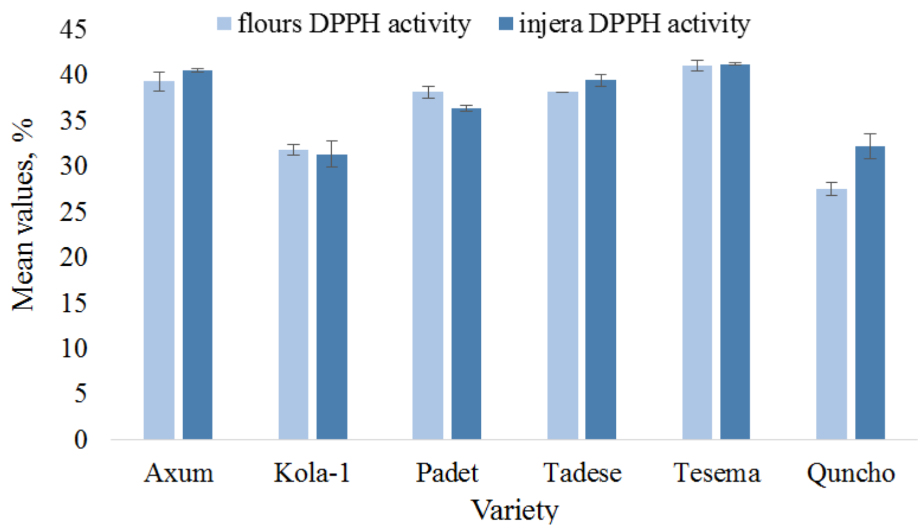

Figure 2. DPPH radical scavenging activity of flours and injera.

Table 3. Color properties of flours and injera.

Variety	Flour				Injera			
	L*	a*	b*	ΔE	L*	a*	b*	ΔE
Axum	61.93±0.28^{c}	1.13±0.25^{b}	2.81±0.31^{c}	46.85±0.06^{c}	49.73±0.35^{c}	3.88±0.07^{b}	4.91±0.11^{d}	35.52±0.25^{cd}
Kola-1	59.73±0.20^{d}	-0.12±0.03^{c}	4.21±0.11^{b}	45.59±0.04^{d}	57.92±0.15^{a}	2.14±0.01^{d}	8.39±0.03^{a}	42.08±0.36^{a}
Padet	62.16±0.41^{c}	1.05±0.06^{b}	2.53±0.46^{cd}	46.99±0.42^{c}	48.04±0.35^{d}	3.16±0.02^{c}	3.69±0.04^{e}	35.17±0.23^{d}
Tadese	63.61±0.27^{b}	0.78±0.17^{b}	2.18±0.10^{d}	48.34±0.31^{b}	50.25±0.42^{c}	3.78±0.13^{b}	4.77±0.15^{d}	35.99±0.15^{c}
Tesema	64.77±0.44^{a}	1.14±0.19^{b}	2.78±0.12^{c}	49.08±0.26^{a}	49.54±0.64^{c}	4.47±0.09^{a}	5.32±0.11^{c}	34.89±0.15^{d}
Quncho	60.05±0.13^{d}	1.68±0.03^{a}	6.85±0.12^{a}	44.25±0.09^{e}	53.14±0.35^{b}	2.08±0.02^{d}	5.88±0.07^{b}	38.88±0.22^{b}

Mean ± SD with different superscripts in a column varied significantly ($p < 0.05$) within and between different millet cultivars and control, n=3. L*, a*, b* and ΔE represents degree of lightness, red-green, yellow-blue and total color difference, respectively.

color in flours and injera, respectively, whereas *Tesema* exhibited the maximum a* value in injera indicating more redness. In comparison with the flour from pearl millet variety, flours from finger millet varieties showed the maximum redness with non-significant ($p<0.05$) difference among them. A significant difference was observed among flours of millet varieties in their b* value (yellow-blue color) which ranged from 2.18 to 4.21, the highest for *Kola-1* (which exhibited maximum blueness) and the lowest for *Tadese* (attained maximum yellowness). *Quncho* showed the highest b* value, which indicated a less yellowness and more blueness. Baking increased the b* value in injera and ranged from 3.69 to 8.39 with the highest and lowest for *Kola-1* and *Padet*, respectively. The total color difference (∆E) ranged from 44.25 to 49.08 for flours and from 34.89 to 42.08 for injera, with *Tesema* having thehighest and the lowest in flour and injera, respectively. Significant reduction of ∆E was noticed after baking. Among finger millet cultivars, the lowest ∆E was noticed in *Axum* and *Padet* varieties with statistically non-significant difference in between. The total color difference of flours and *injera* was negatively correlated (r=-0.74), whereas a* value was positively correlated with the anthocyanin content in both flours (r=0.62) and *injera's* (r=0.59) and were statistically different. Siroha and Sandhu, (2017) revealed that toasting significantly reduced the L* value and increased a* and b* values in flours from pearl millet cultivars.

Conclusion

Even though tef *injera* is more popular than millet, it contains less health-promoting compounds in relation to its phytochemicals content and less effective on oxidative stress and probably associated health disorders. The present study revealed that among millet varieties, *injera* made from *Kola-1* and *Tesema* varieties were more preferred in its overall consumer acceptance and the result was comparable to the most preferred tef *injera*. Finger millet varieties had the highest contents of total phenols, total flavonoids and DPPH radical scavenging activity than pearl millet variety. Among finger millet variety, *Tesema* showed the highest in polyphenols content and antioxidant activity. A lighter flour color was observed with *Tesema*. *Kola-1* had the highest total color difference both in flour and *injera*.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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