

## Research Article

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## Antioxidant Properties of Ethiopian Traditional Bread (*Injera*) as Affected by Processing Techniques and Tef Grain (*Eragrostis tef* (Zucc.)) Varieties

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**Abstract:** The purpose of this study was to compare antioxidative potential of methanolic extracts of three tef grain (*Eragrostis tef*) varieties (white, brown and red tef) and their respective injera (pancake-like, soft, sour, circular flatbread and a staple food for majority of Ethiopians) and enriched one with fenugreek. Injera was prepared from dough fermented for 18 hours and 72 hours. The level of IC<sub>50</sub> values of free radical and reducing power of various tef varieties varied from 0.6 to 0.88 mg/ml and 2.25 to 2.5 mg/ml respectively. The highest free radical scavenging activities (0.6 mg/ml) and reducing power (2.25 mg/ml) was observed in red tef while the lowest scavenging activities and reductive potential were shown in white tef (0.86 mg/ml) and (2.5g/ml), respectively. Total phenol content was higher in red tef (11.47 mg GAE/g) as compared to brown tef (9.72 mg GAE/g) and white tef (8.28 mg/ GAE/g). Total flavonoids for white, brown and red tef were 1.03 mg/CE/g, 1.78 mg CE/g and 2.13 mg CE/g, respectively. The phenol and flavonoid levels of three tef varieties were significantly affected (P<0.05) by processing. In all the four total antioxidants parameters, antioxidant activities of injeras decreased in the order of red tef injera > brown tef injera > white tef injera in the same processing conditions. The study showed that partly fermented tef injera (18 hrs of fermentation) had high antioxidant capacity than fully fermented tef injera (72 hrs of fermentation) among the same tef varieties. However, partly fermented tef injera extracts showed lower antioxidant activities than raw tef flour.

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Keywords: *Injera*, Tef, Antioxidant Activity, Free Radical Scavenging Activity, Reducing Power, Phenol, Flavonoid

## 1. INTRODUCTION

Africa is the centre of origin and still today the major producing area for several cereal crops, notably sorghum, pearl millet, finger millet, tef and African rice. These traditional African cereals are sometimes called “Orphan Crops”, or even “Lost Crops” [1]. This is despite the fact that they are staple foods for millions of people in the semi-arid regions of the world, and particularly those who live by subsistence farming.

Tef (*Eragrostis tef* (Zucc.) Trotter) is a self-pollinated, annual, warm season grass that is used throughout the world as grain for human consumption and as forage for livestock. The amount of tef produced in the world is increasing rapidly due to the plant's popularity as an especial nutritious grain. Tef grain does not contain gluten and is an increasingly important dietary component for individuals who suffer from gluten intolerance. When grown as a grain it is normally ground into flour, which is used to make *injera*, flat bread eaten with every meal. It is also used as porridge, similar to cream of wheat or fermented and used to make an alcoholic beverage [2].

There are few different varieties of tef that vary in color from light to dark. The color can be ivory, light tan to deep brown or dark reddish brown purple, depending on the varieties. According to EHNRI [3] the various types of *injera* produced from the different varieties of tef do not have significant variation in their calorie, moisture, protein, carbohydrate, or phosphorus nutrients.

The biological activities of many phytochemicals are attributed to their antioxidant properties [4]. Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. The free radicals produced by oxidation reactions start chain reactions that damage cells. Antioxidant terminates these chain reactions by removing free radical intermediates and inhibits other oxidation reactions by being oxidized themselves [5]. Antioxidants play a major role in the prevention and treatment of a variety of diseases. The proposed mechanism by which antioxidants protects cells from oxidative stress is by scavenging free radicals, chelating catalytic metals and halting lipid peroxidation chain reactions. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases [6].

In recent years, nutritionists and the general public have come to regard cereals as more than sources of energy and essential nutrients. Certain minor components of foods are now recognized for their health-promoting properties, in particular for their roles in preventing or alleviating the effects of some of the chronic diseases such as cardiovascular disease and certain cancers.

Vegetables and fruits are the most important sources of these antioxidants. However, grains have largely been ignored as important contributors of dietary antioxidants, despite the fact that they are a staple dietary component for most of the world's population. Antioxidants found in whole grain foods are polyphenols including phenolic acids and flavonoids, which are responsible for the high antioxidant activity [7]. Although, many studies have been done for cereals antioxidant activities in other parts of the world, very little information is available about the antioxidant properties of tef. This study was undertaken to determine the antioxidant content of three tef varieties (white tef, red tef, and brown tef) and their processed product (*Injera*).

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

Standards BHT (2-tetra-butyl-4-hydroxyphenol), Gallic acid and reagents, 2, 2-diphenyl-1-picrylhydrazyl

(DPPH), Folin-Ciocalteu's reagent (FCR), potassium hexacyanoferrate, and methanol were purchased from Sigma-Aldrich. Ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), ferrous chloride ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ), trichloroacetic acid (TCA), aluminum chloride ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), sodium nitrite ( $\text{NaNO}_2$ ), phosphate buffer ( $\text{K}_2\text{HPO}_4 / \text{KH}_2\text{PO}_4$ ) are from Fluka, while sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) are from HiMedia.

### 2.1 Samples

The samples (white, red, and brown Quncho tef (DZ.Cr-387)) were brought from Bishoftu Agricultural Research Institute, Ethiopia with purpose. This sampling site was chosen because of occurrence of diverse varieties of tef and to get pure breed (Figure 1).



**Figure 1.** White, brown and red tef varieties

### 2.3 Samples Preparation

All tef samples were taken at one lot, cleaned, and stored in closed polyethene bags and used for the entire study. The samples were divided in to two flour portions. Half of the portion had three tef flour varieties (white, red and brown) and the rest flour portions were fermented either for 18 hours or 72 hours and were baked to obtain *injera*. Tef flour and *injera* prepared were used for further analysis of antioxidant capacity determination.

### 2.4 Preparation of Raw tef Flour

The samples which were collected from Bishoftu Agricultural Research Institute were grinded by using *lika-weke* grinding mill of model M2059. The grinded powders of tef samples were sieved through 1mm sieve. These sieved powders were collected and packed in dry polyethylene bags

### 2.5 Preparation of *injera*

*Injera* baking consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures. The only required ingredients are the tef flour and water. Tef flour was mixed with twice its weight of water. Inoculation was accomplished by consistently using partially cleaned fermentation container and by adding some *ersho* (a clear, yellow liquid that accumulates on the surface of the batter towards the final stage of fermentation). About 10% of the fermenting dough was mixed with three parts of water and boiled for 2 to 5 minutes. This is called '*absit*'. *Absit* ensures that *injera* had the proper texture and consistency. The tef dough was baked in an electric heated oven to obtain *Injera*. *Injera* was dried under electric heated oven at 40 °C over night, ground to a fine powder (to pass through a 40 mesh sieve) and stored in polyethylene bags for further extractions.

### 2.6 Preparation of Partially Fermented *injera*

Partially fermented *injera* was baked at 18 hours of fermentation and has sweet taste and characterized by

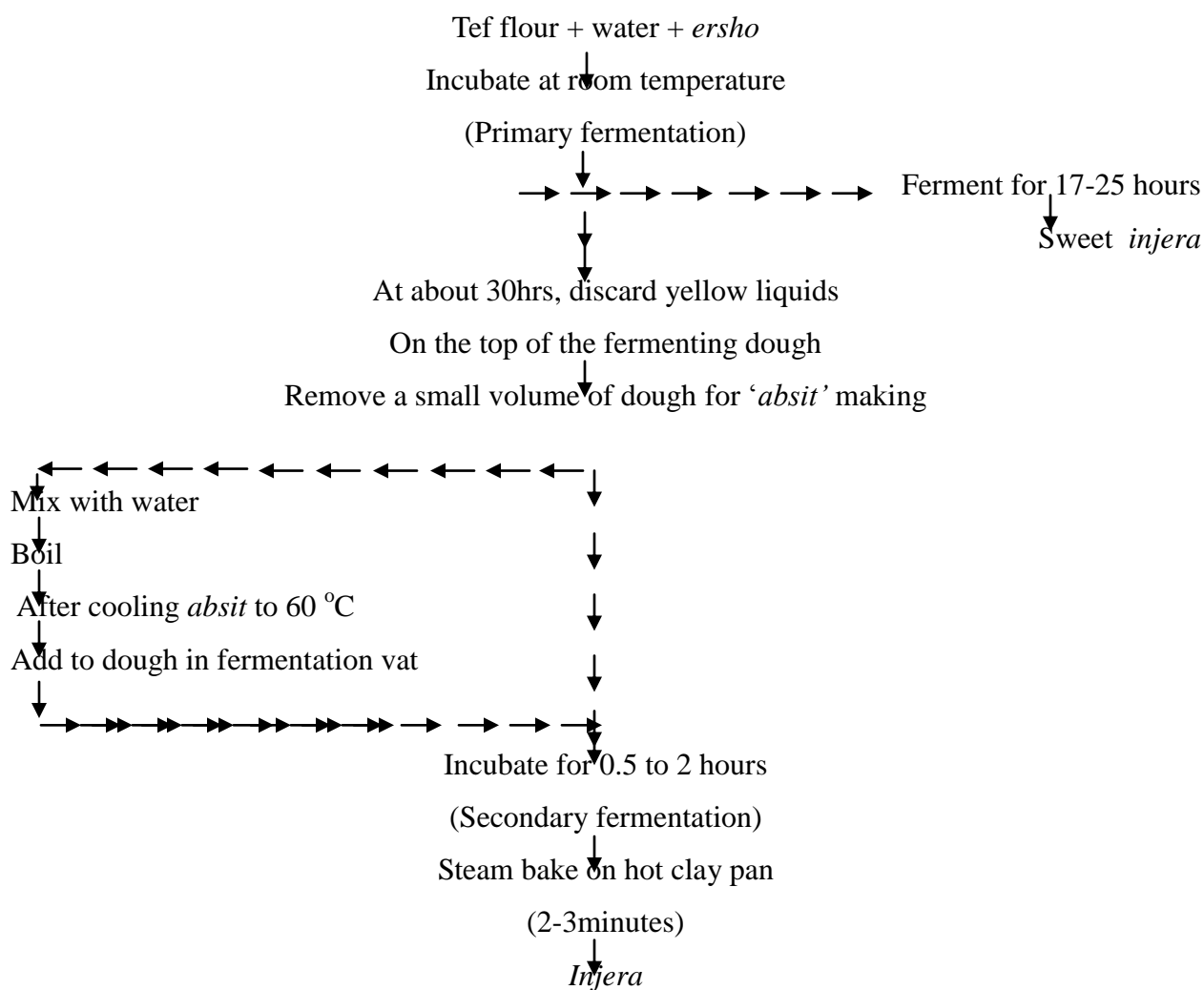
vigorous evolution of gas and maximum dough-rising. It is recommended for people suffering from gastritis and, thus, do not tolerate acidic foods.

### 2.7 Preparation of Fully Fermented *injera*

The fermentation process of fully fermented *injera* lasts for three days. The appearance of an acidic yellowish liquid on the surface of the dough at about 30 hours of fermentation was discarded. As soon as the liquid layer was poured off *absit* was mixed with the rest in fermentation vat after being cooled to 60°C. This process signals the initiation of the second stage of fermentation. By mixing the boiled dough with the rest in the vat, the dough-rising and gas formation process was enhanced. The fermenting dough was thin enough to pour on to the hot flat pan, locally known as '*mitad*' for steam-baked in to fully fermented *injera*. The preparation of *injera* is shown in Figure 2.

### 2.8 Enriching tef Flour with Fenugreek

In the study a 5% fenugreek flour was incorporated to a 95% of tef flour.



**Figure 2.** Flow chart for injera preparation [8] *Antioxidant Capacity Determination*

## 2.9 Sample Extraction

Samples were extracted based on the procedures outlined by Barros and Babbista *et al.* [9] and Ferreira *et al.* [10]. The tef grain varieties processed into flour and *injera*, were homogenized, weighed in to ten gram of dried powder. The powder was extracted by stirring with 100 ml of methanol at 25°C at 150 rpm for 24 hrs using an incubator shaker (ZHWHY-103B) and then filtered through Whatman No.1 filter paper. The residue was then extracted with two additional 100 ml portions of methanol as described above. The combined methanolic extracts were evaporated at 40 °C to dryness using a rotary evaporator (Stuart R3300) and re-dissolved in methanol at a concentration of 50 mg/ ml and stored at 4°C for further use.

### 2.9.1 Determination of Free Radical Scavenging Activity

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH [11]. The effect of methanolic extracts on DPPH radical was estimated according to Kirby and Schmidt [12]. A 0.004% solution of DPPH radical solution in methanol was prepared and then 2 ml of DPPH solution was mixed with 1 ml of various concentrations (0.1- 4 mg/ml) of the extracts in methanol. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read spectrophotometrically (Perkin Elmer Lambda 950 UV/VIS/NIR) by monitoring the decrease in absorbance at 517 nm. This absorption maximum was first verified by scanning freshly prepared DPPH from 200-800 nm using the scan mode of the spectrophotometer. Butyl hydroxytoluene (BHT) was used as a standard and mixture without extract was used as the control. Inhibition of free radical DPPH in percent (I %) was then calculated:

$$\text{Radical Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100\%$$

Where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the sample. The extract concentration providing 50% of radicals scavenging activity ( $IC_{50}$ ) was calculated from the graph of RSA percentage against extract concentration [9, 13].

### 2.9.2 Determination of Total Reducing Power

Total reducing power was carried out according to the method established by Oyaizu [14]. One millilitre of the extract at different concentrations (1- 6 mg/ml), phosphate buffer (0.2 M, pH 6.6, 2.5 ml) and potassium hexacyanoferrate solution (1% v/ m, 2.5 ml) were mixed in a test tube and incubated for 20 min at 50°C. Then 2.5 ml trichloroacetic acid (10%) was added, and the mixture was centrifuged at 2000 x g for 10 min. The upper layer (2.5 ml) was transferred into another tube and mixed with 2.5 ml deionized water and 0.5 ml ferric chloride( 0.1% ) and left to react for 10 min. Finally, the absorbance of the reaction mixture was measured at 700 nm. Stronger absorbance at this wavelength indicates higher reducing power of the antioxidant. The extract concentration providing 0.5 of absorbance ( $IC_{50}$ ) was calculated from the graph of RPA absorbance at  $\lambda = 700$  nm against extract concentration. BHT was used as standard [9, 13].



### 2.9.3 Total Phenolics Determination

Phenolic compounds concentration in the tef and methanolic extracts of *injeras* were estimated based on procedures described by Ferreira *et al.* [10]. One millilitre of sample (2000 µg) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to construct the standard curve (7.5–50 µg/ml). The results were mean values ± standard error of mean and expressed as mg of gallic acid equivalents/g of extract (GAEs). Total content of phenolic in teff and *injera* extracts in 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; c the concentration of gallic acid established from the calibration curve ( $y=17.00x + 0.114$ ;  $R^2= 0.997$ ); V the volume of extract, L; m is the weight of extract, g.

### 2.9.4 Assay for Total Flavonoids

Total flavonoid was determined by a colorimetric method as described in Xu and Chang [15]. Briefly, 0.25 ml of sample (50 mg) was mixed with 1.25 ml of deionized water and 75 µl of a 5% NaNO<sub>2</sub> solution. After 6 min, 150 µl of a 10% AlCl<sub>3</sub>.6H<sub>2</sub>O solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the pink colour was measured at 510 nm against the blank. For calibration curve (+) - Catechin was used with a concentration range of 10–250 µg/ml. Results were expressed as mg (+)-catechin equivalent (CE)/g of extract. The standard curve equation  $y = 2.657x + 0.049$ ,  $R^2 = 0.997$  was constructed from catechin to establish the actual concentration of the extracts.

### 2.10 Data Entry and Analysis

The experimental results were expressed in mean ± standard error (SE) of three parallel measurements. Data were evaluated by using two way variance analysis (ANOVA) and means were separated by Duncan's multiple range test ( $p<0.05$ ) by using SPSS version 15.0. For the construction of graphs and interpolating IC<sub>50</sub> of the respective antioxidant activities Microsoft Excel was used.

## 3. RESULTS AND DISCUSSIONS

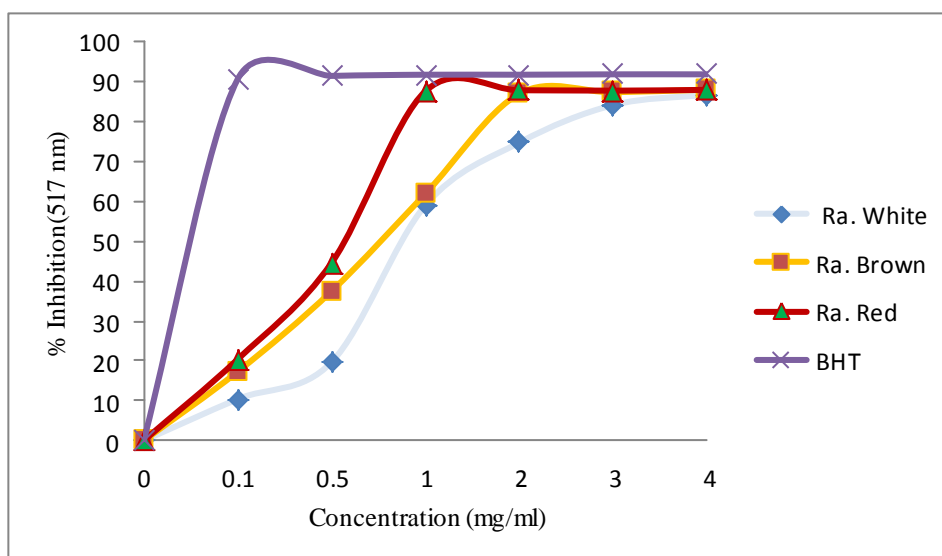
### 3.1 Free Radical Scavenging Activity

In this study, optimization of the concentration range required to scavenge DPPH was evaluated with pre-tests by tracking the purple to yellow color change of DPPH with increased concentration for each sample. This had been crucial part of the study since setting a much lower or much higher concentration range might not indicate the difference in scavenging power between samples clearly and more so, the IC<sub>50</sub> (the concentration required to scavenging 50 % of the radical) might be skipped. It is after this optimization process that 0.1 - 4 mg/ml was chosen and prepared by diluting the stock solution (50

mg/ml) with methanol. This concentration optimization process was applied in similar way for reducing, total phenolics and total flavonoids assays.

### 3.2 Free Radical Scavenging Activity of tef Varieties

The  $IC_{50}$  of white, brown, and red tef is 0.875, 0.75 and 0.6 mg/ml, respectively as compared to standard (BHT ( $IC_{50}$  =0.056)). Red tef was the most potent of all that could scavenge most free radicals and had the lowest  $IC_{50}$  value while white tef with the highest  $IC_{50}$ , is the least potent (Figure 3). The results obtained were in agreement with those of Brunswick laboratories; Norton, MA [16]. The studies show that ivory tef (3600  $\mu$ mol TE/100) had strong antioxidant activity as compared to brown tef (3400  $\mu$ mol TE/100) by oxygen radical absorbance capacity (ORAC).



**Figure 3.** The DPPH scavenging activity of raw tef extracts

### 3.3 Free Radical Scavenging Activities of Partly Fermented, Fully Fermented and Enriched *injera*

Methanol extracts from white tef *injera* were evaluated for free radical scavenging activity by DPPH method (Figure 4). Among the *injer*as extracts the  $IC_{50}$  of the enriched partly fermented white tef showed higher scavenger property (2.63 mg/ml). Fully fermented white tef *injera* extract had the highest  $IC_{50}$  value (3.25 mg/ml). The methanolic extracts of 50% inhibition value of white partly fermented, and enriched fully fermented white tef *injera* was 2.80 mg/ml and 3.00 mg/ml, respectively. The enrichment of white raw tef with fenugreek reduced from 0.875 mg/ml to 0.81 mg/ml ( $IC_{50}$ ).

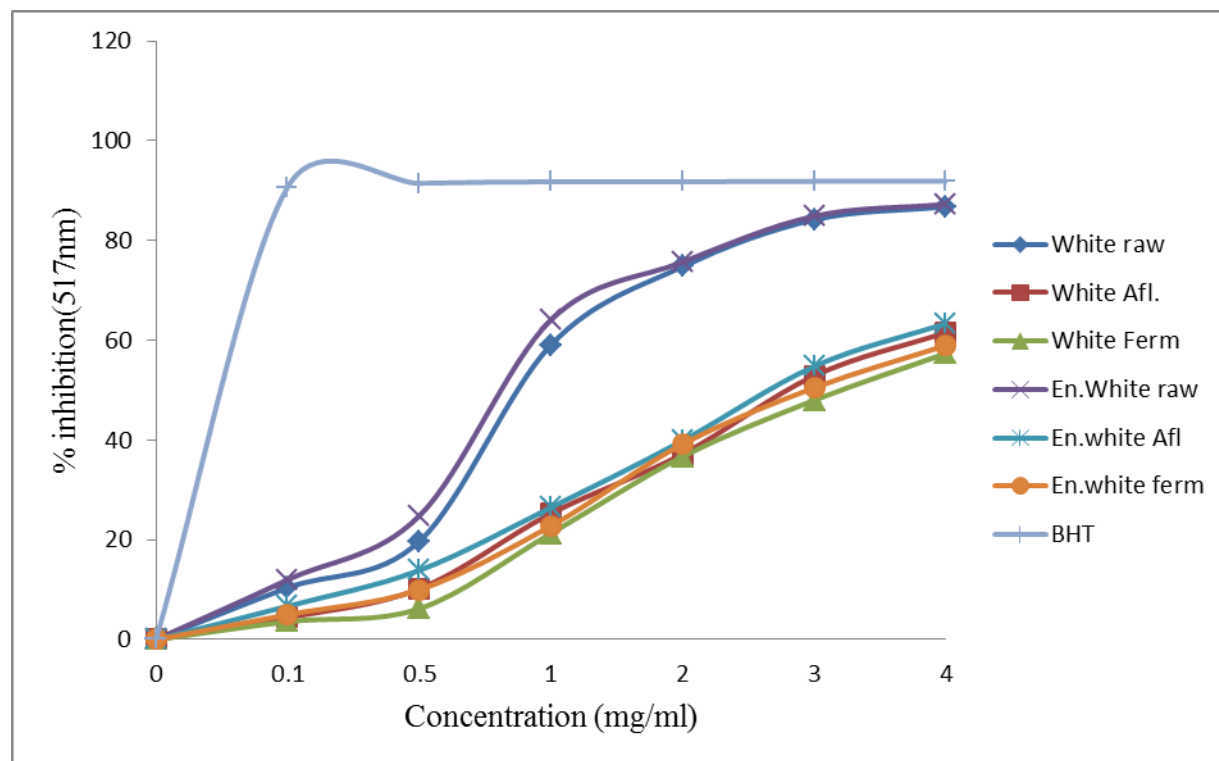
Processing of brown tef in to partly or fully fermented *injera* affected scavenging activities (Figure 5). The  $IC_{50}$ - values for partly fermented brown tef *injera*, fully fermented brown tef, enriched partly fermented brown tef *injera* and enriched brown fully fermented tef *injera* were 2.30, 2.75 2.25 and 2.50 mg/ml, respectively.

Scavenging activities of enriched red tef *injera* were stated in Figure 6. From the figure, it is observed that the  $IC_{50}$  ranges from 1.15 – 1.6 mg/ml. The lowest scavenging activities were found in red fully fermented *injera* (1.6 mg/ml) while the highest was observed in enriched partly fermented *injera* (1.15

mg/ml). The  $IC_{50}$ -value for partly fermented red tef *injera* and enriched fully fermented red tef *injera* were 1.25 mg/ml.

Results of this study indicated that the antioxidant contents of tef varieties had been affected by processing into partly and fully fermented *injera* and enrichment with fenugreek. There were slight decreases in the antioxidant levels of *injer*as (both partly and fully fermented) compared to raw tef flour. The decrease in the free radical scavenging activities between raw flour varieties and their processed products, may be attributed to the thermal effect (90 °C) during baking and it might be some of the antioxidants in tef were relatively not heat stable. This observation is however similar to that obtained by other studies [17, 18, 19]. When cereals are exposed to various conventional processing methods including thermal loss of antioxidant contents could occur.

The free radical retention capacity of partly fermented *injera* was higher than fully fermented *injer*as in all tef varieties in the same processing conditions. However, sweet *injera* extracts showed lower activity than raw tef flour at the same concentration (protocol) in the same varieties. The order of free radical scavenging capacity was: raw tef extract > sweet *injera* > fully fermented *injera* among the same varieties of tef.



**Figure 4.** The DPPH scavenging activity of white tef flour and its *injera*

### 3.4 Total Reducing Power

Reducing power measures the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts [20]. The reducing power of methanolic extracts of tef and tef *injera* were summarized in Figure 7. From the Figure, reducing power increased with increased extract concentrations. The ability to reduce Fe (III) may be attributed from hydrogen donation



from phenolic compounds [21] which is also related to the presence of a reductant agent [22]. In addition, the number and position of hydroxyl group of antioxidants compounds also rule their antioxidant activity [23].

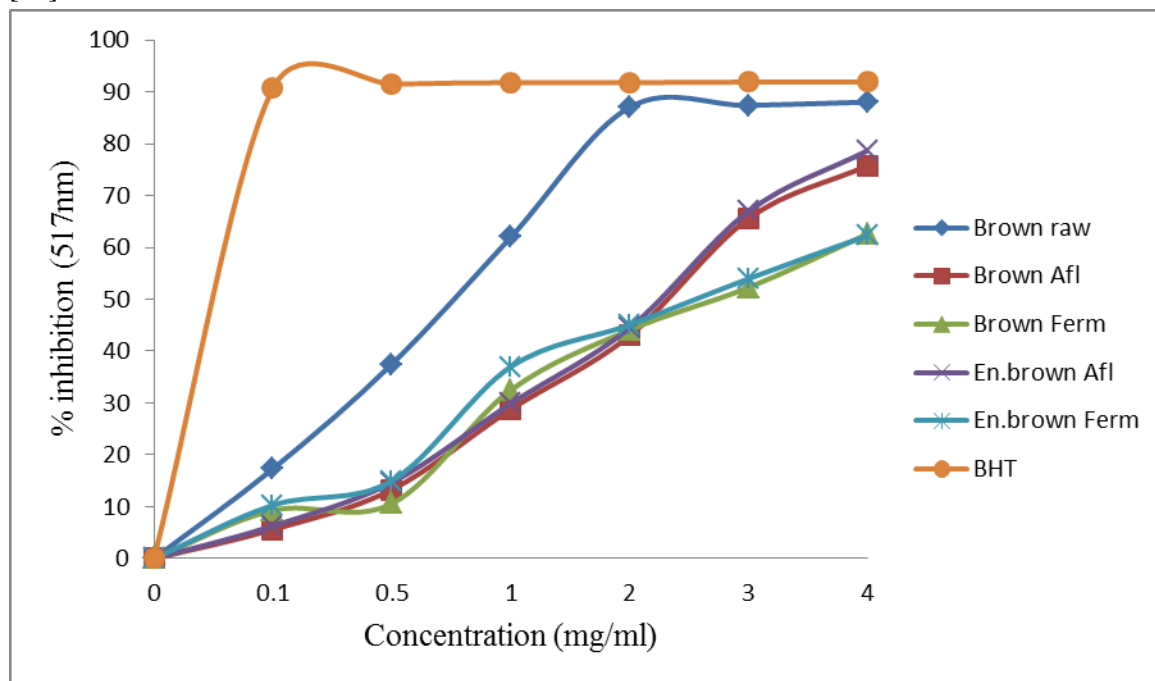


Figure 5. The DPPH scavenging activity brown tef flour and its injera

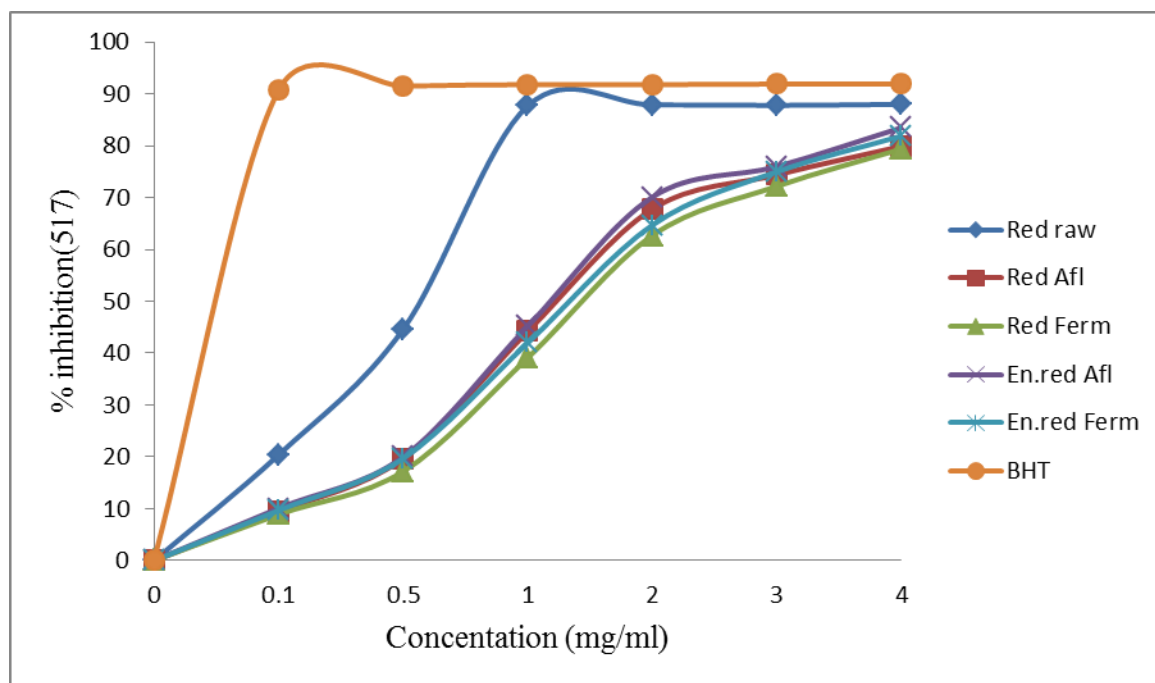
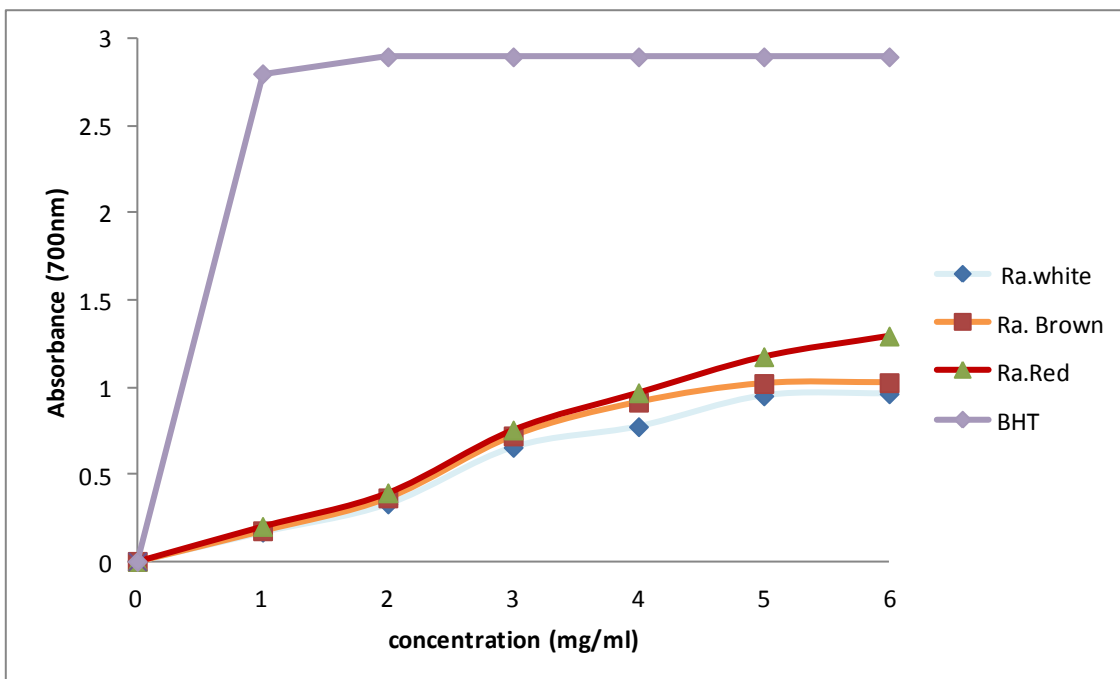


Figure 6. The DPPH scavenging activity red tef flour and its injera

### 3.4.1 Total Reducing Power of tef Varieties

From Figure 7, it is observed that the highest reducing ability was seen in red tef (2.25 mg/ml), followed by brown tef (2.3 mg/ml) and the lowest reduction observed in white tef (2.5 mg/ml). High reductive potential in red tef may be due to the presence of high tannins and pigment-contributing compounds such as the anthocyanins or the existence of phytochemicals that are responsible to the variable distribution of structural parts such as bran, endosperm, and germ [24].



**Figure 7.** Total reducing power of tef varieties

### 3.4.2 Reducing Power of Partly Fermented, Fully Fermented and Enriched tef injera

As indicated in Figure 8, the  $IC_{50}$  - values for total reducing power of white tef *injera* extracts were presented. Enriched partly fermented white tef *injera* had the minimum  $IC_{50}$  (2.7 mg/ml) while fully fermented white tef *injera* had the lowest reducing capability; it had 3.30 mg/ml. The  $IC_{50}$  value for partly fermented white tef *injera* was 2.75 mg/ml.

There were changes in the total reducing power; the extractions of enriched fenugreek brown tef *injera* had higher reductive potential with respect to the unenriched *injera* (Figure 9). The  $IC_{50}$  values for partly fermented brown tef *injera* (2.75 mg/ml), fully fermented brown tef *injera* (2.75 mg/ml), enriched partly fermented brown tef *injera* (2.50 mg/ml) and enriched fully fermented brown tef *injera* was 2.60 mg/ml.

As shown in Figure 10, the  $IC_{50}$ - values for methanolic extracts of partly fermented red tef *injera*, fully fermented red tef *injera*, enriched partly fermented red *injera* and enriched fully fermented red tef *injera* were 2.5 mg/ml, 2.75 mg/ml, 2.20 mg/ml and 2.4 mg/ml respectively.

There were overlap in the values of reductive potential for partly fermented white tef *injera*, partly fermented brown tef *injera* and fully fermented brown tef *injera* and red fermented *injera* (2.75 mg/ml)

this might be due to the reducing power assay is considered to be a sensitive method for the quantitative determination of dilute concentrations of total antioxidants [25].

There were decreases in reducing power of *injera* in contrast to the flours. As Friedman and Dao [26] observed in grains like sorghum, cooking and baking results in the partial loss of reductive potential. These observations however, are in contrast to the observation of Hye-Min and Bong-Kyung [27], which observed that grains (wheat flour) with longer baking times or higher temperatures generally corresponded to higher levels of antioxidants in comparison to less intense baking conditions. This is because most of the antioxidants found in the inside of bran and endosperm of the grain had been obtained in high temperature.

Extracts of *aflegna injera* (partly fermented) had higher scavenging activities and reductive potential than fully fermented *injer*as because in fully fermented *injera* at about 30 hours, the yellow liquid on the top of the fermented dough would be discarded this result in the loss of water-soluble antioxidants (Table 1). This result similar with the finding of Rovio *et al.* [28], he suggested that water may be used as an extraction solvent for antioxidants and has gained an increasing attention due to its unique antioxidants solvation properties.

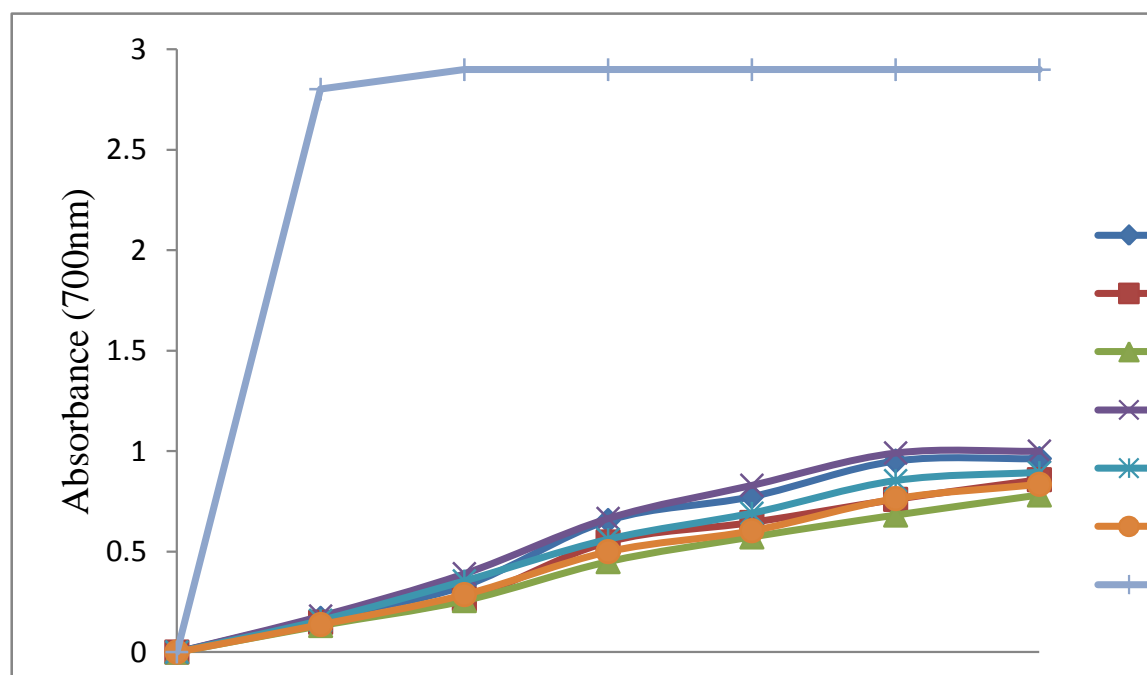


Figure 8. Reducing power of white tef flour and *injera*

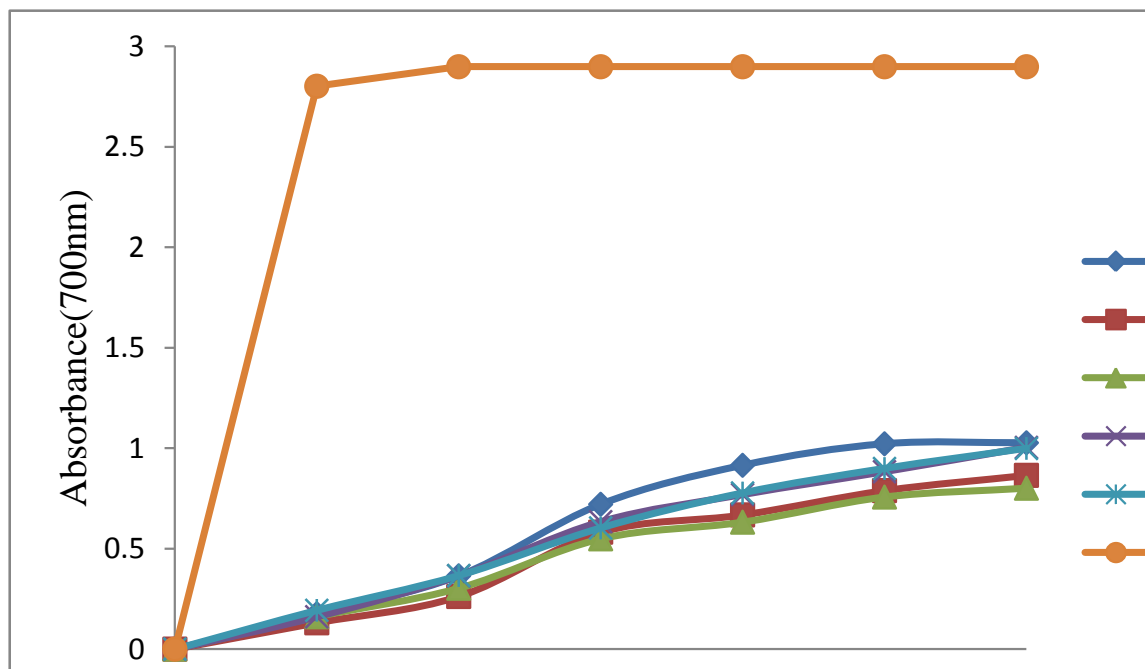


Figure 9. Reductive potential of brown tef flour and injera

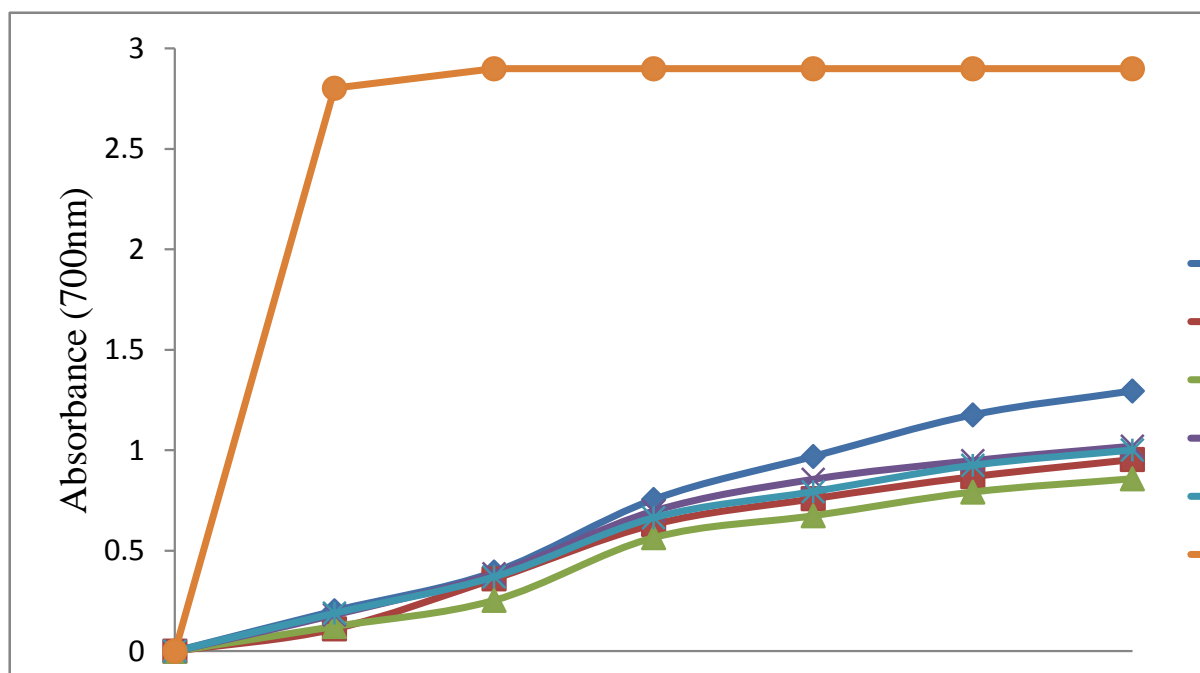


Figure 10. Reductive potential of red tef flour and injera.

A number of studies have documented significant reduction in antioxidant capacity of cereals in fermented foods. In the study of millet fermented foods, the fermentation reduced antioxidants levels

[29]; this is because antinutritional factors which sometimes act as antioxidants show a decline during fermentation. In contrast, in majority of cereals, fermentation often increases the content of soluble vitamins and antioxidants.

The finding of Iuliana *et al.* [30] indicates that antioxidant activity of the rye sour dough highly depends on the fermentation time. Long fermentation process is tools to increase the bioactive compounds and antioxidants activities. However, the finding of Iuliana *et al.*[30] contrasts with this finding since the fully fermented tef *injera* fermented for 72 hours had low antioxidant activity than partly fermented tef *injera* which had been fermented only for 18 hours. This might be due to loss of much water soluble antioxidants with the liquid on the top of the fermented tef sour dough before fully fermented *injera* was baked in respect to enhancement antioxidants during fermentation.

The extracts from the enriched *injera* appear to possess greater antioxidant activity when compared to unenriched *injera*. The reasons for the higher free-radical scavenging and total reducing ability of the enriched *injera* were due to the presence of fenugreek. This is in agreement with the result reported by park *et al.* [31], when wheat fortified with fenugreek; the fortified product increased its antioxidant levels.

**Table 1.** Free radical scavenging activities and reducing power of three tef varieties and their *injer*as

Extracts	IC <sub>50</sub> (mg /ml)	IC <sub>50</sub> (mg /ml)
	for RSA	for TRP
White tef	0.875	2.50
Brown tef	0.75	2.30
Red raw tef	0.60	2.25
Partly fermented white tef <i>injera</i>	2.80	2.75
Fully fermented white tef <i>injera</i>	3.25	3.30
En. Raw white tef	0.81	2.3
En. partly fermented white tef <i>injera</i>	2.63	2.70
En. fully fermented white tef <i>injera</i>	3.00	3.00
Partly fermented brown tef <i>injera</i>	2.30	2.75
Fully fermented brown tef <i>injera</i>	2.75	2.75
En. partly fermented brown tef <i>injera</i>	2.25	2.50
En. fully fermented brown tef <i>injera</i>	2.50	2.60
Partly fermented red tef <i>injera</i>	1.25	2.50
Fully fermented red tef <i>injera</i>	1.60	2.75
En. partly fermented red tef <i>injera</i>	1.15	2.20
En. fully fermented red tef <i>injera</i>	1.25	2.40

### 3. 5 Total Phenols

Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, antiatherosclerotic, and anti-carcinogenic activities. These activities may be related to their antioxidant activity [32]. Moreover, it had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds. Therefore, it is important to consider the effect of the total phenolic content on the antioxidant activity of tef extracts. The Folin-ciocalteu reagent is used to obtain a crude estimate of the amount of phenolic compounds present in the extracts. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the reagent. However, the assay has been shown not specific to just phenolic compounds but to any other substance that could be oxidised by the folin reagent. In addition, phenolic compounds depend on the number of phenolic groups they have, responded differently to the Folin-ciocalteu reagent.

#### 3.5.1 Phenol Contents of tef Varieties

Total phenolic compound contents of each tef varieties and *injera* were expressed as g gallic acid equivalent (GAE); the result showed that total phenolic compound content in red tef was highest (11.47 mg GAE/g). Brown tef extract was ranked second (9.87 mg GAE/g) possessing higher antioxidant properties than white tef (8.248 mg GAE/g). The results showed that the phenolic content of raw tef varieties were significantly different ( $P < 0.05$ ). This is in agreement with the results reported by Toyokuni *et al.* [33]; in which pigmented grains have been shown to have higher phenolic compounds than the regular non-pigmented grains.

#### 3.5.2 Phenol Contents of Partly Fermented, Fully Fermented, and Enriched tef *injer*as

The total phenolic content results of white tef *injera* were reported in Table 2, the average phenol values of partly (*aflegna*) and fully fermented white tef *injera* were 5.81 and 4.03 mg GAE/g respectively whereas the total phenolic content for enriched partly fermented white tef *injera* and enriched fully fermented white tef *injer*as were found to be 6.77 mg GAE/g and 5.59 mg GAE/g, respectively.

Phenol content of partly and fully fermented as well as enriched brown tef *injer*as compared to unenriched brown tef *injera* was given in Table 6. From Table 2, it is observed that there is enhancement in phenol content among enriched *injera*. The total phenol of brown tef *injera* ranged between 5.81 mg GAE to 8.53 mg GAE/g. The highest increase in phenol content was observed in enriched partly fermented brown tef *injera* (8.53 mg GAE/g) and the lowest increase was in fully fermented white tef *injer*as (5.81 mg GAE/g). Total phenol values of partly fermented and enriched fermented brown tef *injera* were found to be 7.19 and 7.94 mg GAE/g, respectively.

Processing of red tef in to partly or fully fermented *injera* affected the amount of total phenol content. The total phenol contents for partly fermented, fully fermented, enriched partly fermented and enriched fully fermented red tef *injera* were found to be 8.75, 7.36, 9.12 and 8.24 mg GAE/g respectively.

The raw tef flour showed the highest total phenol content with respect to both partly and fully fermented *injera* in all three varieties. This may be due to treatment of foods with high thermal processing. According to Julkunen [34], increasing the temperature above 60 °C, lowered the phenolic compounds considerably in the majority of cereals. The study also found that the total phenolic compounds of partly fermented *injera* was greater than fully fermented *injera* this is might be due to solubility of phenolics in water discarded before *injera* was baked. However, there were no large phenolic differences between



partly and fully fermented in the same varieties since the predominant phenolic compounds (Ferulic) in cereals were identified as lipid acid soluble derivatives. Similarly, Collins [35], he suggested that some phenolic acids and flavonoids are water soluble compounds, but lipid soluble derivatives are common to grains such as ferulic acid. Enhancement of phenol content in enriched *injera* as compared to unenriched is attributed to the addition of antioxidant rich food ingredient, fenugreek.

### 3.6. Total Flavonoids

Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities [36]. In food systems, flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides. Therefore, they present antioxidative efficiency in oils, fats and emulsions.

#### 3.6.1 Flavonoid Content of tef Varieties

Results presented in the Table 2 show significant variation ( $P < 0.005$ ) in flavonoid contents of tef varieties. These values ranged between 1.03 mg CE/ g and 2.13 mg CE/ g. The flavonoid contents for white tef, brown tef and red tef were: 1.03, 1.78 and 2.12 mg CE/ g respectively. The results also illustrated that red tef had highest flavonoids as compared to white and brown tef. This may due to the seed colour. Seed colours affect flavonoid contents. Cereal seeds grown on plants with red secondary color had higher levels of flavonoids than those less coloured seed is present. The results were similar to the values obtained from different colours of sorghum [37].

#### 3.6.2 Flavonoids of Partly Fermented, Fully Fermented and Enriched tef *injer*as

Among white tef *injera* (Table 2), the flavonoids content ranged between 0.88 mg CE/g to 1.09 mg CE/g. The flavonoid contents for partly fermented, fully fermented, enriched partly fermented and enriched fully fermented white tef *injera* were 0.89, 0.88, 1.09 and 1.06 mg CE/g respectively.

In brown tef *injera*, the highest flavonoid content was noticed in enriched partly fermented *injera* (1.9 mg CE/ g). The lowest content was in fully fermented brown tef *injera* (1.18 mg CE/ g). The flavonoid content of partly fermented brown tef *injera* and enriched fermented brown tef *injera* were 1.33 mg CE/ g and 1.264 mg CE/g, respectively.

Results in Table 2 shows that flavonoid contents of red tef *injera*. The values ranged between 1.64 mg CE/ g and 2.03 mg CE/ g. The results of partly fermented, fully fermented, enriched partly and enriched fully fermented red tef *injera* were: 1.77, 1.64, 2.03, and 1.87 mg CE/ g, respectively.

From Table 2 it can also be observed that there was reduction of flavonoids in *injera*. Processing significantly affected the flavonoid distribution of tef flour varieties ( $P < 0.05$ ). The relative difference of flavonoids content may be attributed to flavonoids which are present in the tef flour can be destroyed or transformed into other phytochemicals during heat treatment and processing. Kikuzaki and Nakatani [38] reported the reduction in the value of flavonoids during heat treatment and processing because transformation of existing flavonoid structure happened in oxidation or may interact with the other compounds. Between un enriched and enriched tef *injera* with fenugreek, enriched *injer*as showed the highest flavonoid value.

*Injer*as prepared from the three tef varieties showed the same trend in their phenol and flavonoid values as the raw tef varieties in the order: red tef *injer*as > brown tef *injer*as > white tef *injer*as in the same processing parameters. The phenol and flavonoids contents of the three tef varieties

were significantly affected ( $P < 0.05$ ) by processing. However, variances of analysis of variety \* processing was found to be insignificant ( $P > 0.005$ ).

**Table 2.** Effects of processing on the phenol and flavonoid contents of tef varieties (white, brown and red) and *injeras*

Variety	Treatment	Phenol ( mg GAE/gm)	Flavonoid ( mg CE/gm)
White tef	WR	8.28 ± 0.03 <sup>ab</sup>	1.03 ± 0.06 <sup>ab</sup>
	WA	5.81 ± 0.23 <sup>bcd</sup>	0.89 ± 0.02 <sup>a</sup>
	WF	4.03 ± 0.31 <sup>d</sup>	0.87 ± 0.09 <sup>a</sup>
	WEA	6.77 ± 0.77 <sup>bc</sup>	1.09 ± 0.08 <sup>ab</sup>
	WEF	5.59 ± 0.84 <sup>cd</sup>	1.06 ± 0.06 <sup>ab</sup>
	WER	9.41 ± 0.86 <sup>a</sup>	1.24 ± 0.09 <sup>a</sup>
Brown tef	BR	9.73 ± 0.26 <sup>e</sup>	1.78 ± 0.09 <sup>ef</sup>
	BA	7.19 ± 0.29 <sup>fg</sup>	1.32 ± 0.05 <sup>f</sup>
	BF	5.81 ± 0.976 <sup>g</sup>	1.18 ± 0.03 <sup>f</sup>
	BEA	8.53 ± 0.64 <sup>ef</sup>	1.90 ± 0.44 <sup>e</sup>
	BEF	7.94 ± 0.92 <sup>efg</sup>	1.26 ± 0.14 <sup>f</sup>
Red tef	RR	11.47 ± 0.84 <sup>x</sup>	2.13 ± 0.01 <sup>x</sup>
	RA	8.75 ± 0.06 <sup>xy</sup>	1.77 ± 0.03 <sup>y</sup>
	RF	7.36 ± 0.89 <sup>y</sup>	1.64 ± 0.06 <sup>z</sup>
	REA	9.12 ± 0.98 <sup>xy</sup>	2.03 ± 0.01 <sup>x</sup>
	REF	8.24 ± 0.97 <sup>xy</sup>	1.872 ± 0.07 <sup>y</sup>

Reported values were mean ±SE (n=3). Means with different letters in the same column are significantly different ( $P < 0.05$ ). NB: WR, WA, WF, WEA & WER were white raw tef, white *aflegna*, white fermented, white enriched *aflegna* and enriched white tef *injeras* respectively) and BR, BA, BF, BEA & BEF represent brown raw tef, *aflegna*, brown enriched *aflegna* and enriched brown fermented *injeras* respectively while RR ( raw red tef), RA (*aflegna* red tef *injeras*), RF (fully fermented red tef *injeras*), REA ( enriched *aflegna* red tef *injeras*) & REF (red enriched fully fermented *injeras*).

#### 4. CONCLUSIONS

The research was carried out to evaluate the antioxidant content and activity of three tef varieties. Red tef had the most potent of all that could scavenge most free radical and highest reductive potential and the lowest IC<sub>50</sub> value accompanied by having the highest phenolic and flavonoids contents. White tef with the highest IC<sub>50</sub> is the least potent. The study also showed that processing of tef flour into partly or fully fermented *injera* had reduced the total antioxidant contents with respect to raw tef flour. The total antioxidants retention capacity of partly fermented *injera* was higher than fully fermented *injeras*. From the study, it was also observed that there was enhancement in antioxidant activities of enriched *injera* with

fenugreek at 95:5 ratio of tef flour to fenugreek, respectively.

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