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Effect of fermentation on nutrient composition, antinutrients, and mineral bioaccessibility of finger millet based *Injera*: A traditional Ethiopian food

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ABSTRACT

Finger millet, like other cereals, contains high amounts of antinutrients that bind minerals, making them unavailable for absorption. This study explores the effect of traditional fermentation on nutritional, antinutritional, and subsequent mineral bioaccessibility (specifically iron, zinc, and calcium) of finger millet based *Injera*. Samples of fermented dough and *Injera* prepared from light brown and white finger millet varieties were analyzed for nutritional composition, antinutritional content, and mineral bioaccessibility following standard procedures. With some exceptions, the proximate composition of fermented dough was significantly affected by fermentation time. Compared to unfermented flour, the phytate and condensed tannin content significantly ($p < 0.05$) decreased for fermented dough and *Injera* samples. A strong decline in phytate and condensed tannin content was observed in white finger millet *Injera* as fermentation time increased, compared to light brown finger millet based *Injera*. The mineral bioaccessibility of *Injera* prepared from finger millet and maize composite flour increased with fermentation time, leading to a significant increase in bioaccessible iron, zinc, and calcium, ranging from 15.4–40.0 %, 26.8–50.8 %, and 60.9–88.5 %, respectively. The results suggest that traditional fermentation can be an effective method to reduce phytate and condensed tannin content, simultaneously increasing the bioaccessibility of minerals in the preparation of finger millet based *Injera*.

1. Introduction

Finger millet (*Eleusine coracana*) is a small-seeded cereal of Ethiopian origin, grown widely in the semi-arid tropics and subtropics of the world (Ramashia et al., 2018; Sood et al., 2016). It is the sixth major crop in Ethiopia following Teff, wheat, maize, sorghum, and barley (CSA, 2021). Specifically, the country produces more than 12 million quintals of finger millet, out of which 55 % is produced in the Amhara region. Finger millet is a resilient crop, offering a good grain yield even in conditions where other crops fail to produce a reasonable yield. It represents hence a food crop included in food security plans for drought-prone areas (Adekunle et al., 2012). However, the crop remains underutilized in Ethiopia. Nutritionally, finger millet is a good source of carbohydrates, proteins, sugars, starch, and dietary fiber, being particularly rich in essential minerals, calcium, and iron (Chandra et al., 2016; Ramashia et al., 2019). Hence, in developing countries like Ethiopia,

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where severe iron deficiency is reported, incorporating finger millet in the common food basket may help alleviate health issues such as anemia (Gebre, 2019). Although finger millet has a significant potential of being an important source of minerals (i.e. iron, zinc, calcium, etc.), like other cereals, it contains a high amount of antinutritional components such as phytic acid, phenolic compounds, and condensed tannins. Antinutrients are among the major causes of iron and zinc deficiency (Baye et al., 2015) by reducing mineral bioavailability. They also impair protein and starch digestibility (Pragya, 2012). The utilization of conventional food-processing techniques such as fermentation can enhance the bioavailability of micronutrients and nutrient digestibility (Devisetti et al., 2014; Gabaza et al., 2018b; Saleh et al., 2013; Séye et al., 2018; Singh & Sarita, 2018). *Injera,* a traditional fermented flatbread, is the most widely consumed foodstuff in Ethiopia. While *Injera* is widely produced from Teff (*Eragrostis teff*), finger millet based *Injera* is also a staple in several parts of Ethiopia. However, literature on the effect of fermentation on finger millet based *Injera* is scarce. This study therefore aimed to evaluate the effect of traditional fermentation on the nutritional, antinutritional, and mineral bioaccessibility of finger millet based *Injera.*

2. Materials and methods

2.1. Finger millet Injera preparation

Sample areas for *Injera* recipe identification were selected based on the communities' wide experience in consuming finger millet-based Injera. To assess if there are differences in preferences for finger millet varieties and recipes between producers and buyers, two nearby areas from Bahir Dar city, Dangila and Zege districts were included in the study. While communities in Dangishita Kebele of Dangila district are finger millet producers, communities from Zege are generally characterized as buyers. Dangila district, Dangishita sub-district (Kebele) is located approximately 90 km away from Bahir Dar, the capital city of the Amhara region, Ethiopia, and Zege is located 30 km away from Bahir Dar to the west.

The identification of finger millet *Injera* recipes involved significant contributions from women in the two different communities during focus group discussions. The entire process of *Injera* preparation from finger millet was also observed in local households (Fig. 1). A total of 12 women (randomly selected) from each community participated in the data collection process, providing information on the type of finger millet preferred for *Injera* preparation, the practice of mixing finger millet with other cereals, the fermentation period, and the practice of back slopping.

Producers prefer light brown (brown) finger millet for *Injera*, while buyers prefer the white-colored varieties (Fig. 2). The preference for light brown finger millet for *Injera* preparation stems from the fact that *Injera* prepared from this type of finger millet is soft and has a better appearance compared to the other types of finger millet. On the contrary, the preference for the white-colored variety by the buyers or Zege community relates to the fact that *Injera* from this variety is comparable in texture and appearance with *Injera* prepared from Teff (the cereal most widely used for *Injera* preparation). Unlike buyers, mixing finger millet with other cereals, especially maize (*Zae Mays)*, is a common practice by finger millet producers as maize is also one of the major cereals produced by this community. Women from the producer community reported *Injera* preparation exclusively from finger millet, as well as its mixing with maize, which is readily available and relatively cheap. The differences in the amount of maize used for *Injera* preparation among producer communities correspond to differences in wealth, as relatively poor households tend to increase the ratio of maize added.

Two recipes from finger millet producing communities and one from buyers were identified. The major difference between the two recipes from producer communities is the proportion of maize mixed with finger millet for *Injera* preparation; some use a 1:1 ratio for *Injera* preparation, while others use a 2:1 ratio. An additional difference is associated with the stage at which the two cereals are mixed. When women use a 1:1 ratio, the cereals are milled separately. Maize dough is prepared initially, and finger millet is added on the third fermentation day. On the other hand, in the 2:1 ratio, flours are milled together and the dough is

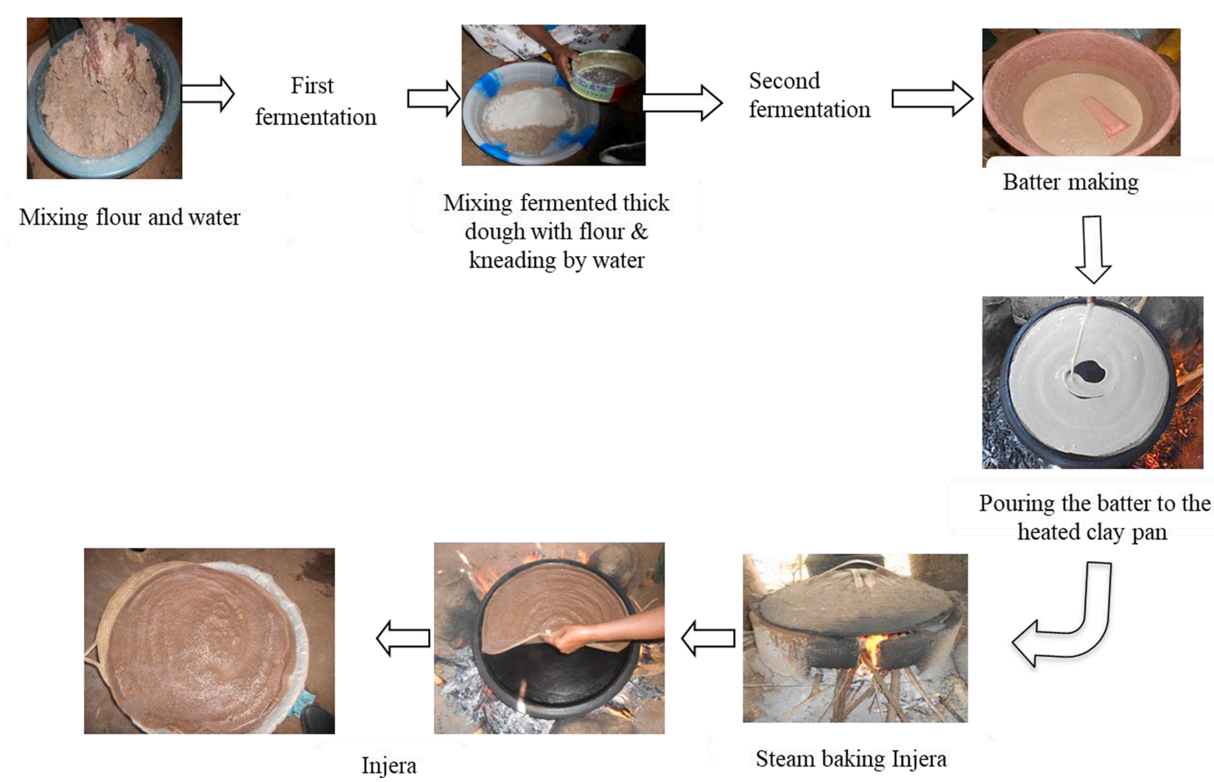

Fig. 1. Flow diagram of the preparation of light brown finger millet based *Injera*.

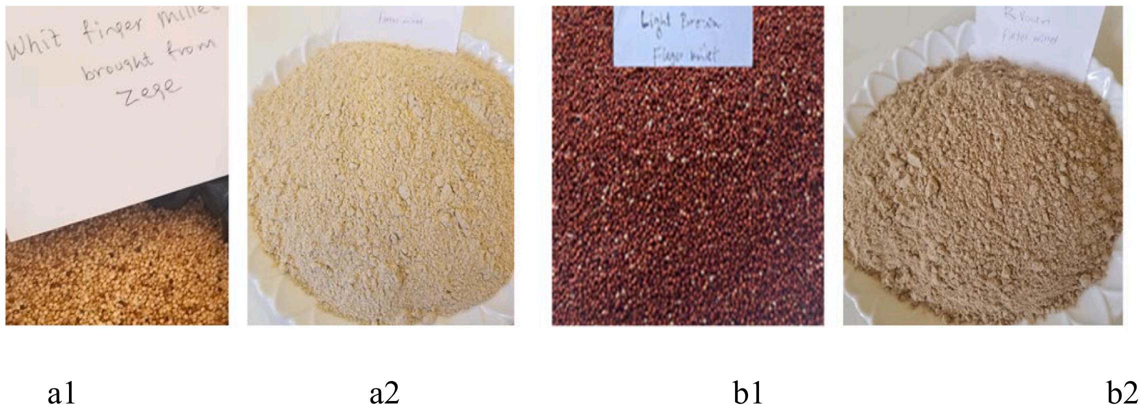

Fig. 2. Finger millet grain (1) and flour (2): white (a1, a2); light brown (b1, b2).

prepared from the blended flour, and another batch of blended flour is added on the third fermentation day. In both cases, the dough is ready for baking after the fourth day of fermentation and can continue to be used up to the seventh day. Back-slopping is not a common practice among producers.

Unlike producers, finger millet buyers prepare finger millet dough and use back slopping (locally called '*Ersho*') to hasten the fermentation process. On the fourth day, the dough is converted to batter by adding water and is ready for baking. It was also reported that the dough can be left fermented for up to 7 days and *Injera* is subsequently baked as needed.

2.2. Injera preparation in the laboratory and sample preparation

To reproduce the traditional *Injera* preparation process used by the the local women in the laboratory, finger millet and maize seeds were purchased and thoroughly cleaned to remove foreign matter such as stones, dust, and straw. The maize was manually decorticated and winnowed. Then, the white finger millet, light brown finger millet, maize, and the combined grain of light brown finger millet and maize (1:2 ratio) were milled in local community milling units that use mechanical millers to obtain flour to the fineness level traditionally used for *Injera* preparation. Finally, the flour was sieved using a 500-µm sieve and used for *Injera* preparation.

The actual procedure of *Injera* preparation was fully performed in the Food Processing Laboratory of Chemical and Food Engineering Faculty, Bahir Dar Institute of Technology, Bahir Dar University following procedures of producer communities from Dangishita subdistrict (Fig. 3) and of buyers from Zege (Fig. 4).

From each recipe, fermented dough samples were taken at 24-hour fermentation time intervals and freeze-dried (Coolsafe 55–9, Scanvac, Lynge, Denmark). Similarly, *Injera* samples were also freeze-dried and ground in a grain miller to a fine powder and stored in sealed bags at −20 °C until needed for analysis. Samples for mineral content analysis and mineral bioaccessibility determination were transported to Greece under cooled conditions and immediately frozen at −20 °C at the Laboratory of Environmental Chemistry of the Department of Chemistry of the National and Kapodistrian University of Athens.

2.3. Nutrient analysis

2.3.1. Proximate composition

The proximate composition of raw flour, fermented dough flour, and *Injera* samples was determined. Moisture, crude protein, crude fiber, crude fat, and total ash contents of the samples were determined following the AOAC method (2005) and the total carbohydrate was estimated by the weight difference method (Monro & Burlingame, 1996). Gross energy was determined by multiplying the percentage of crude protein, crude fat, and carbohydrate by factors 4, 9, and 4, respectively, and the estimation was recorded as kcal/100 g (Nguyen et al., 2007).

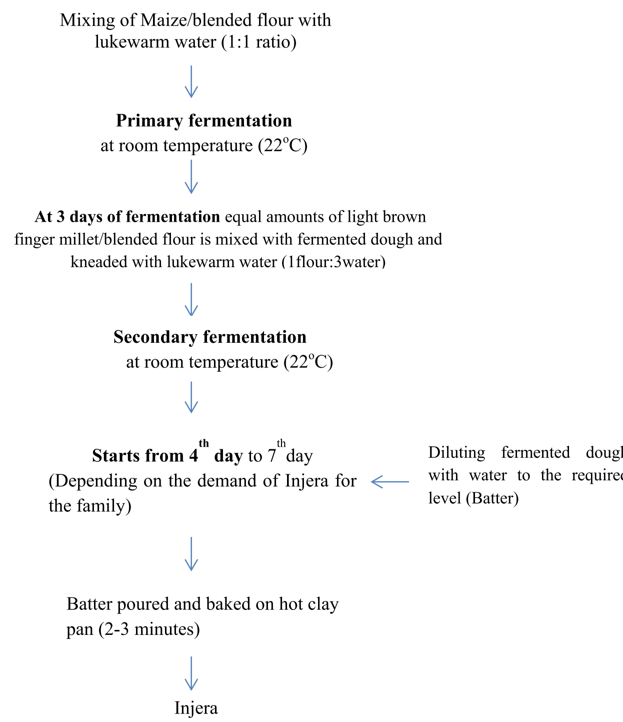

Fig. 3. Flow diagram of *Injera* preparation from light brown finger millet and maize in Dangishita Kebele, Dangila, Ethiopia.

2.3.2. Mineral composition analysis using ICP-OES

The mineral content of the grain flour and *Injera* samples was determined according to the method of Grigoriou et al. (2022), slightly modified. The samples (0.1 g) were wet digested with the addition of HNO_3 acid 65 % supra pure, employing a microwave digester (Multiwave Go Plus, Anton Paar, Graz, Austria) and subsequently diluted to a final volume of 25 mL with Milli-Q water of 18.2 MΩ.cm (Millipore, Bedford, MA, USA).

The determination of Ca, Fe, and Zn was performed by ICP-OES, with a Perkin Elmer Optima 2100DV (Perkin Elmer, USA) instrument. Limits of detection (LODs) were calculated by multiplying the standard deviation of seven replicate samples prepared at an approximately low concentration by 3.14 (USEPA, 1997). LODs in µg/g of dry weight were

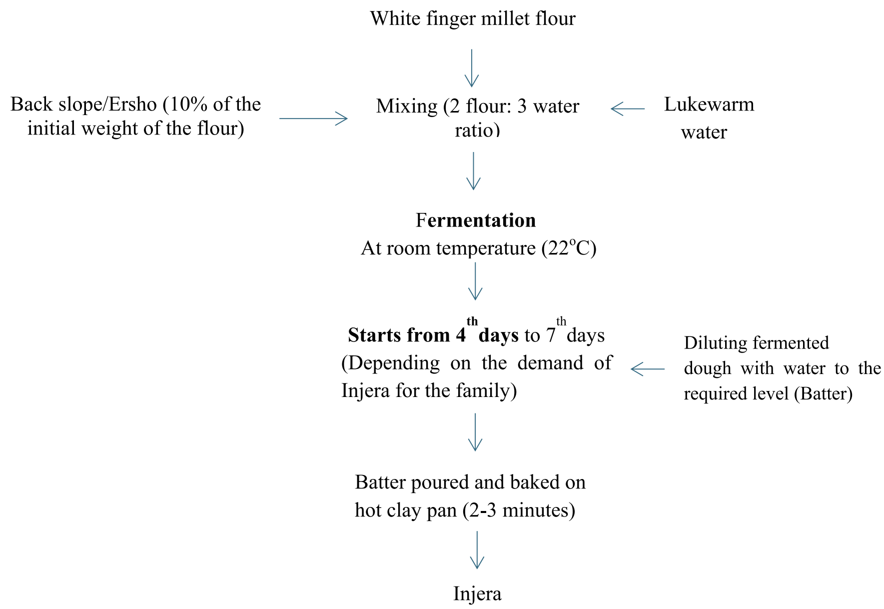

Fig. 4. Flow diagram of white finger millet *Injera* preparation in Zege.

calculated equal to 0.140 for Ca, Fe, and Zn.

For quality assurance purposes, within each batch of samples, at least one procedural blank was included. For the accuracy and precision of the analysis, a certified reference material (CRM) BCR 191 (brown bread) was analyzed and recoveries for Ca, Fe, and Zn were calculated at 100 ± 10 %.

2.3.3. Determination of bioaccessible calcium (Ca), iron (Fe) and zinc (Zn)

The Ca, Fe, and Zn bioaccessibility of *Injera* samples was determined according to the method presented by Khoja et al. (2020) and Glahn et al. (1998), simulating the gastrointestinal digestion system with some modifications. In a 15 mL tube, 1 g of sample was mixed with 10 mL of saline solution (140 mmol/L NaCl and 5 mmol/L KCl), vortexed, and left for 15 min. Then, the pH was adjusted to 2.0, using 1 M HCl. Subsequently, 1 mL of pepsin (Sigma-P7012) (9.6 mg mL^{-1}) was added and vortexed. The sample was incubated at 37 °C in a shaking water bath (150 rpm) for 90 min. The pH of the samples was then adjusted to 7 using 1 M NaOH. A 2.5 mL mixture of Bile extract (Sigma-B8631) and pancreatin (Sigma-P7545) (8.5 mg mL^{-1} bile extract and 1.4 mg mL^{-1} pancreatin) were added. The solution was made up to 18 mL with saline solution and the samples were incubated at 37 °C for 90 min. At the end of the incubation period, samples were centrifuged at 1000 rpm for 10 min, the supernatants were decanted, and 1 mL of the supernatant was taken into a 15 mL tube. The supernatant (l mL) was digested with HNO_3 acid 65 % supra pure in an oven at 60 °C overnight. The digested sample was diluted with distilled water and Ca, Fe, and Zn were analyzed by ICP-OES.

2.4. Antinutrient analysis

2.4.1. Phytic acid content

The phytic acid content of the grain, fermented dough, and *Injera* samples was determined by using the Megazyme (Megazyme- KPHYT, Bray, Ireland) kit, following the protocol described by the manufacturer (McKie & McCleary, 2016). Flour samples were digested by hydrochloric acid. The extracted phytate that was digested with phytase and alkaline phosphatase suspension was used to release phosphate from all the myoinositol phosphate forms. The phosphate released was measured using a modified colorimetric method at 655 nm. The phytic acid content was calculated from the standard calibration curve (Supplementary Fig. 1) and expressed as milligrams of phytic acid per 100 g of sample.

2.4.2. Condensed tannin content

The condensed tannin content of the samples (finger millet and maize flour, fermented dough flour, and *Injera*) was estimated following the method reported by Dykes (2019) and Price et al. (1978) using a modified vanillin-HCl assay. The extract was prepared by mixing 0.3 g of sample flour with acidified methanol (1 % HCl in methanol) in a centrifuge tube. The mixture was subsequently vortexed and placed in a water bath for 20 min. The extract was then vortexed again and centrifuged at 4,000 g for 10 min. From the supernatant, 2 mL was taken and placed into two separate test tubes, 1 mL in each of the tubes. Then 5 mL of Vanillin reagent was added to the fraction of 1 mL containing supernatant (labeled as "sample") and 4 % HCl in methanol was added to the other fraction of 1 mL of the supernatant (labeled as "blank"). The mixtures were allowed to stand for 20 min before their absorbance reading at 500 nm using a spectrophotometer (G6860A, Agilent, Malaysia). A calibration curve was prepared using a standard solution of catechin (Supplementary Fig. 2). The tannin content was expressed as mg CE/100 g.

2.5. Statistical analyses

All the experiments were performed in triplicate, and data is shown as mean ± standard deviation (SD). Data were analyzed using analysis of variance (ANOVA), following the general model procedure of SPSS statistical software version 20, followed by Tukey's post hoc test. The significance level was at $p < 0.05$.

3. Results and discussion

3.1. Effect of fermentation time on the proximate composition of finger millet based Injera

The proximate composition of finger millet based *Injera* is presented in Table 1. The proximate composition of unfermented finger millet flour, maize flour, and finger millet-maize composite flour shows a significant difference with *Injera* samples.

In the case of composite flour, the unfermented flour is significantly different in ash and crude fat content, with *Injera* prepared from 1:1

Table 1
Effect of fermentation time on the proximate composition (g/100 g d.w.) of *Injera* prepared from finger millet and composite flour of finger millet-maize.

Fermentation time (h)	Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fibre (%)	Total CHOs (%)	Energy (Kcal/100 g)
Brown Finger millet (BF) Injera						
0	3.31 ± 0.07^{a}	1.84 ± 0.01^{b}	7.61 ± 0.22^{bc}	3.84 ± 0.05^{a}	87.2 ± 0.24^{b}	396.0 ± 0.28^{b}
96	3.21 ± 0.04^{ab}	2.17 ± 0.08^{a}	8.05 ± 0.05^{a}	3.20 ± 0.02^{b}	86.6 ± 0.15^{c}	398.0 ± 0.30^{a}
120	3.18 ± 0.04^{ab}	2.03 ± 0.09^{a}	7.80 ± 0.03^{ab}	2.84 ± 0.10^{c}	87.0 ± 0.09^{b}	397.4 ± 0.30^{a}
144	3.14 ± 0.05^{bc}	1.58 ± 0.07^{c}	7.38 ± 0.03^{c}	2.77 ± 0.02^{c}	87.9 ± 0.14^{a}	395.4 ± 0.16^{b}
168	3.01 ± 0.07^{c}	1.57 ± 0.03^{c}	7.58 ± 0.08^{bc}	2.60 ± 0.09^{c}	87.8 ± 0.02^{a}	395.8 ± 0.18^{b}
Maize (M) Injera						
0	1.22 ± 0.03^{b}	3.72 ± 0.11^{a}	8.86 ± 0.23^{a}	2.81 ± 0.06^{a}	86.2 ± 1.3^{a}	413.7 ± 7.1^{a}
96	1.34 ± 0.04^{ab}	2.71 ± 0.21^{c}	8.96 ± 0.00 ^{a}	2.18 ± 0.17^{b}	87.0 ± 0.41^{a}	408.1 ± 2.7^{a}
120	1.37 ± 0.02^{ab}	2.86 ± 0.22^{c}	8.94 ± 0.11^{a}	1.93 ± 0.15^{bc}	86.9 ± 0.20^{a}	408.8 ± 0.77^{a}
144	1.37 ± 0.10 ^{a}	3.12 ± 0.20^{bc}	8.86 ± 0.22^{a}	1.63 ± 0.06^{c}	87.0 ± 0.40^{a}	410.2 ± 1.0^{a}
168	1.45 ± 0.03^{a}	3.37 ± 0.17^{ab}	8.49 ± 0.22^{a}	1.59 ± 0.03^{c}	86.2 ± 0.14^{a}	411.1 ± 1.0^{a}
1 Brown Finger millet: 1 Maize (BFM) Injera						
0	1.22 ± 0.03^{c}	3.72 ± 0.31^{a}	8.86 ± 0.23^{a}	2.79 ± 0.09^{a}	86.2 ± 1.3^{a}	413.7 ± 7.1^{a}
96	2.31 ± 0.01^{a}	3.17 ± 0.05^{b}	8.49 ± 0.25^{a}	2.77 ± 0.08^{a}	85.9 ± 0.30^{a}	407.3 ± 1.1^{ab}
120	2.22 ± 0.11^{a}	3.05 ± 0.09^{b}	8.67 ± 0.43^{a}	2.66 ± 0.02^{a}	87.4 ± 0.83^{a}	399.7 ± 2.2^{b}
144	2.21 ± 0.06^{a}	3.01 ± 0.19^{b}	8.69 ± 0.49^{a}	2.63 ± 0.01^{a}	86.3 ± 1.0^{a}	406.9 ± 2.3^{ab}
168	2.03 ± 0.05^{b}	2.77 ± 0.04^{b}	8.23 ± 0.21^{a}	2.54 ± 0.08^{a}	87.2 ± 0.34^{a}	402.7 ± 0.76^{b}
1 Brown Finger millet: 2 Maize (BF2M) Injera						
0	1.82 ± 0.07^{b}	3.20 ± 0.14^{b}	7.39 ± 0.19^{b}	2.98 ± 0.06^{a}	86.3 ± 3.1^{a}	415.3 ± 15^{a}
96	2.07 ± 0.06^{a}	3.72 ± 0.06^{b}	9.09 ± 0.35^{a}	2.21 ± 0.03^{b}	85.6 ± 2.2^{a}	407.7 ± 10^{a}
120	2.10 ± 0.04^{a}	3.84 ± 0.13^{b}	9.41 ± 0.02^{a}	2.13 ± 0.07^{b}	84.5 ± 2.5^{a}	410.9 ± 13^{a}
144	2.15 ± 0.06^{a}	3.90 ± 0.10^{b}	9.25 ± 0.30^{a}	2.07 ± 0.06^{b}	86.0 ± 0.38^{a}	402.9 ± 0.42^{a}
168	2.25 ± 0.10^{a}	3.69 ± 0.16^{b}	9.22 ± 0.44^{a}	2.10 ± 0.10^{b}	86.1 ± 0.52^{a}	403.0 ± 0.46^{a}
White Finger millet (WF) Injera						
0	2.47 ± 0.04^{a}	1.79 ± 0.05^{a}	7.68 ± 0.02^{a}	3.46 ± 0.01^{a}	88.1 ± 0.23^{a}	399.1 ± 0.09^{a}
96	3.15 ± 0.04^{b}	0.81 ± 0.04^{c}	7.76 ± 0.11^{a}	2.67 ± 0.02^{b}	88.3 ± 0.18^{a}	391.4 ± 0.05^{c}
120	3.16 ± 0.03^{b}	0.93 ± 0.04^{b}	7.80 ± 0.11^{a}	2.59 ± 0.10^{bc}	88.1 ± 0.07^{a}	392.0 ± 0.28^{b}
144	3.22 ± 0.02^{b}	0.89 ± 0.05^{bc}	7.70 ± 0.00^{a}	2.48 ± 0.05^{bc}	88.2 ± 0.07^{a}	391.6 ± 0.19^{bc}
168	3.26 ± 0.02^{b}	0.88 ± 0.01^{bc}	7.66 ± 0.11^{a}	2.43 ± 0.04^{c}	88.2 ± 0.07^{a}	391.4 ± 0.06^{c}

Mean values and standard deviation of triplicate replications. Means with no common letters within a row significantly differed ($p \leq 0.05$).

finger millet and maize composite flour (BFM). Likewise, the unfermented flour significantly increases in ash and crude protein, while it decreases in crude fiber compared with *Injera* prepared from 1:2 finger millet and maize composite flour (BF2M). The *Injera* prepared from BF2M flour has a significant increase, by 27.3 % protein content, compared to that of 7.4 g/100 g of the unfermented flour. However, the difference among different *Injera* samples is not significant. The increased protein concentration in the composite finger millet and maize *Injera* samples could be attributed to the synthesis of enzymes (such as amylases and proteases for the break down of starch into simple sugars and protein into amino acids respectively) by microorganisms and other newly formed proteins by fermentation, and the release of proteins from the degradation of other constituents such as antinutritional factors (Amankwah et al., 2009; Azeez et al., 2022; Ilowefah et al., 2017; Nkhata et al., 2018). The results show a slight increment in the protein content of other *Injera* samples when compared to their respective unfermented flour. Mutshinyani et al. (2020) recorded a significant increment in crude protein value (7.1–9.4 % for light brown and 7.8–9.5 % for dark brown) of finger millet after 96 h of fermentation. In contrast, it has also been noted that the protein content of pearl millet decreased by 4.5 % after undergoing spontaneous fermentation for 20 h during the preparation of Lohoh, a traditional Saudi Arabia leavened bread (Osman, 2011).

The crude fiber content decreased by 25.0 %, 22.8 %, and 16.7 % in 1:2 finger millet and maize composite flour (BF2M), white finger millet (WF), and brown finger millet (BF) *Injera*, respectively, compared to unfermented flour ($P < 0.05$). This is consistent with the result of previous studies (Adegbehingbe, 2013; Mutshinyani et al., 2020) reporting a significant decrease in the fiber contents of finger millet and maize flours with fermentation time. The observed reduction in crude fiber value after fermentation could be due to the enzyme activities, which degrade the cell wall matrix during fermentation (Chinma et al., 2020; Ilowefah et al., 2017; Ogodo et al., 2018; Ojokoh & Bello, 2014).

Except for brown finger millet (BF), the ash content of unfermented flour samples was significantly different across all *Injera* samples ($P < 0.05$). In the case of composite flour samples, the ash content increases with the increasing proportion of finger millet flour, possibly because of the high ash content of brown finger millet (Table 1). The increase in ash content could be attributed to the rearrangement of fiber composition or degradation of antinutritional substances (Ilowefah et al., 2017).

The fat content of unfermented flour was significantly ($p \leq 0.05$) different from that of all *Injera* samples except for 1:2 finger millet and maize composite flour (BF2M). However, no significant difference was recorded among *Injera* samples with fermentation time. As the proportion of brown finger millet flour in the flour composite increased, the crude fat content of the flour composite significantly decreased

(Table 1). Fermentation decreased the fat content of maize and composite flour of finger millet and maize *Injera* samples. Previous evidence also showed a decrease in the fat content of fermented millet flour (Afify et al., 2012; Ojokoh et al., 2015; Sade, 2009; Simwaka et al., 2017). This might be attributed to the use of lipids as an energy source or to lipolytic hydrolysis caused by lipase enzyme during fermentation (Adebiyi et al., 2019; Adebo et al., 2022).

The total carbohydrate content did not show any significant difference in all *Injera* recipes with fermentation time, except *Injera* prepared from brown finger millet *Injera*. However, significant differences were observed in the total energy content of *Injera* samples in all recipes. There were also inconsistencies in the total energy values at different fermentation times. The result has shown that the addition of maize flour into composite flour increased the energy content of *Injera*. This might derive from the higher fat and protein content of maize. The total carbohydrate and gross energy content of our *Injera* samples are higher than those reported for spontaneously fermented *Injera* prepared from other cereals (Agza et al., 2018; Anberbir et al., 2023; Mihret & Bultosa, 2017; Woldemariam et al., 2019; Yegrem et al., 2021).

3.2. Antinutritional content of finger millet based fermented dough and Injera

Antinutrients are chemical substances found in plants, referred to as "secondary metabolites", having distinct biological effects based on the structure of certain components (Parul, 2014). They have an adverse effect on nutrient values, by lowering nutritional digestibility and mineral absorption (Singh & Sarita, 2018). Tables 2 and 3 present the effect of fermentation time on the phytate and condensed tannin content of fermented dough (feremented at room temperature i.e. 22 °C) and *Injera*, prepared from finger millet and finger millet-maize composite flour, respectively. The phytate and condensed tannin content of both fermented dough and *Injera* reduced substantially with fermentation time.

The fermented dough flour of light brown finger millet (BF) in the first fermentation phase demonstrated a significant reduction of 46.8 % and 39.7 % of its phytate and condensed tannin content, respectively (Table 2). On the third day, the phytate and condensed tannin contents significantly increased ($p < 0.05$), possibly due to the newly added brown finger millet (BF) flour, while in the second fermentation phase, the phytate and condensed tannin contents decreased by 37.1 % and 23.3 %, respectively. A similar trend was observed for the fermented dough flour of maize and other finger millet-maize composites. Despite different processing conditions, fermentation resulted in a reduction in the content of antinutritional components of our samples.

Our findings are consistent with those of other studies. Elyas et al. (2002) found that natural fermentation of two varieties of pearl millet for 36 h reduced phytic acid by 50 % for both cultivars. Osman (2011) also reported that traditional fermentation of pearl millet for 24 h at 30 °C decreased the phytic acid content significantly (from 647 to 311 mg/100 g or 51.9 % reduction). However, Gabaza et al. (2018) stated that from four spontaneously fermented finger millet varieties, only white finger millet (WV2 variety) fermented for 36 h at 25–30 °C reduced its phytate content by 22 %, while other varieties increased it, suggesting that the trend differentiated among varieties.

The fermentation process could cause a decline in the phytate content, by increasing the activity of native or intrinsic phytase and microbial phytase, thereby hydrolyzing insoluble organic complexes with minerals (Azeez et al., 2022; Makokha et al., 2002; Olukomaiya et al., 2020; Osman, 2011; Sokrab et al., 2014). Phytate reduction in food is important, since it interferes with the availability of essential nutrients such as Fe, Zn, and Ca in the gastrointestinal tract of humans, reducing their bioavailability. Different studies (Fredlund et al., 2006; Frontela et al., 2011; Pires et al., 2023) also reported the adverse effect of phytate on calcium, iron, and zinc bioavailability.

The condensed tannin content of our dough samples decreased with fermentation time. A decrease in the condensed tannin content of fermented flour is in agreement with other studies (Adebiyi et al., 2017; Taylor & Duodu, 2015). Specifically, Gabaza et al. (2018) reported a decrement of 33–53 % of condensed tannin in finger millet slurries subjected to spontaneous fermentation. The decrease in condensed tannin can be attributed to the higher activity of phytases, polyphenol oxidase, and tannin acyl hydrolases during processing (Samtiya et al., 2020). Moreover, enzymes from microflora facilitate the breakdown of tannin-protein, tannic acid–starch, and tannin-iron complexes during fermentation, leading to the release of free nutrients which invariably enhance nutrient availability (Onweluzo & Nwabugwu, 2009).

As shown in Table 2, blending maize with light brown finger millet resulted in a decrease in the phytate and condensed tannin content of fermented dough flour. This might be attributed to the higher antinutrient content of finger millet compared to that of maize. Incorporation of maize flour to light brown finger millet flour in 2:1 ratio

Table 2
Effect of fermentation time on the antinutrient content (mg/100 g d.w.) of fermented dough.

Fermentation time (h)	Phytic acid (mg/100 g)	Condensed Tannin (mg/100 g)
Brown Finger millet (BF) dough		
0	824 ± 3.8[a]	248 ± 2.3[a]
24	709 ± 5.4[b]	211 ± 1.7[b]
48	520 ± 0.43[c]	150 ± 1.2[c]
72	573 ± 1.3[d]	162 ± 3.4[d]
96	440 ± 5.3[e]	112 ± 3.3[e]
120	322 ± 3.8[f]	90 ± 2.4[f]
144	266 ± 3.6[g]	73 ± 1.2[g]
168	232 ± 5.6[h]	69 ± 0.69[g]
Maize (M) dough		
0	432 ± 3.9[a]	38 ± 1.3[a]
24	345 ± 6.4[b]	28 ± 1.3[b]
48	259 ± 5.3[c]	19 ± 3.4[c]
72	332 ± 2.6[b]	23 ± 4.0[bc]
96	227 ± 2.0[d]	18 ± 2.6[ec]
120	176 ± 2.4[e]	13 ± 1.4[e]
144	116 ± 2.8[f]	11 ± 2.3[e]
168	81 ± 6.7[g]	11 ± 2.6[e]
1 Brown Finger millet: 1 Maize (BFM) dough		
0	419 ± 5.5 [a]	38 ± 1.3[a]
24	334 ± 3.8[b]	30 ± 1.3[b]
48	244 ± 1.7[c]	21 ± 2.3[c]
72	473 ± 5.6[d]	127 ± 5.6[d]
96	339 ± 1.8[b]	78 ± 2.8[e]
120	213 ± 4.4[e]	53 ± 2.3[f]
144	176 ± 3.5[f]	45 ± 1.3[a]
168	158 ± 4.0[g]	31 ± 1.3[b]
1 Brown Finger millet: 2 Maize (BF2M) dough		
0	597 ± 4.0[a]	122 ± 2.7[a]
24	482 ± 4.1[b]	99 ± 1.4[b]
48	328 ± 1.1[c]	62 ± 1.1[c]
72	402 ± 8.7[d]	80 ± 3.4[d]
96	261 ± 1.5[e]	53 ± 0.66[e]
120	150 ± 7.0[f]	33 ± 1.2[f]
144	131 ± 2.4[g]	22 ± 1.3[g]
168	118 ± 2.6[g]	21 ± 2.3[g]
White Finger millet (WF) dough		
0	634 ± 3.7[a]	233 ± 2.5[a]
24	418 ± 2.9[b]	166 ± 1.2[b]
48	279 ± 1.0[c]	83 ± 1.2[c]
72	159 ± 3.2[d]	46 ± 3.3[d]
96	127 ± 1.6[e]	38 ± 2.1[e]
120	108 ± 0.23[f]	37 ± 2.3[fe]
144	102 ± 2.5[f]	33 ± 1.3[fe]
168	98 ± 4.4[f]	32 ± 1.3[f]

Mean values and standard deviation of triplicate measurements. Means with no common letters within a row are significantly different ($p \leq 0.05$).

Table 3
Phytic acid and tannin content (mg/100 g d.w.) of finger millet based *Injera.*

Fermentation time (h)	Phytic acid (mg/100 g)	Condensed Tannin(mg/100 g)
Brown Finger millet (BF) Injera		
0	824 ± 3.8[a]	248 ± 2.3[a]
96	417 ± 2.7[b]	109 ± 3.9[b]
120	309 ± 4.0[c]	87 ± 1.3[c]
144	259 ± 0.85[d]	70 ± 2.4[d]
168	225 ± 0.43[e]	65 ± 2.5[d]
Maize (M)Injera		
0	432 ± 3.9[a]	38 ± 1.3[a]
96	215 ± 2.1[b]	17 ± 1.3[b]
120	172 ± 1.7[c]	13 ± 3.4[b]
144	114 ± 1.4[d]	11 ± 2.2[b]
168	80 ± 1.5[e]	10 ± 1.3[b]
1 Brown Finger millet: 1 Maize (BFM)Injera		
0	419 ± 4.6[a]	38 ± 1.3[a]
96	317 ± 7.4[b]	75 ± 2.2[b]
120	202 ± 1.8[c]	51 ± 1.3[c]
144	172 ± 7.8[d]	42 ± 3.3[a]
168	156 ± 4.3[d]	28 ± 2.2[d]
1 Brown Finger millet: 2 Maize (BF2M) Injera		
0	597 ± 4.0[a]	122 ± 2.7[a]
96	247 ± 3.4[b]	49 ± 2.2[b]
120	145 ± 2.9[c]	32 ± 1.3[c]
144	128 ± 0.42[d]	21 ± 3.4[d]
168	116 ± 1.5[d]	20 ± 2.2[d]
White Finger millet (WF) Injera		
0	634 ± 3.7[a]	233 ± 2.5[a]
96	120 ± 2.5[b]	37 ± 2.5[b]
120	104 ± 0.42[c]	33 ± 2.2[b]
144	99 ± 1.5[c]	32 ± 1.3[b]
168	95 ± 4.0[c]	32 ± 2.6[b]

Mean values and standard deviation of triplicate replications. Means with no common letters within a row significantly different ($p \leq 0.05$).

respectively (BF2M), leads to a further decrease in the antinutrient content of the fermented dough. Fermentation led to a significant decrease in the phytate and condensed tannin content by 23.2 % and 49.2 % respectively, in the first fermentation phase. However, the addition of blended flour on the third fermentation day resulted in a significant increment which decreased significantly in the second fermentation phase by 16.8 % and 60.5 %, respectively (Table 2). Likewise, the dough prepared from a 1:1 blending ratio of light brown finger millet to maize flour, BFM, also showed a significant reduction in phytate content with fermentation time. However, due to the incorporation of light finger millet into maize thick dough on the third fermentation day, the phytate content increased significantly and subsequently gradually decreased during the second fermentation phase. Overall, the trend in Table 2 shows that, except for white finger millet dough, the phytate and condensed tannin contents of all fermented dough samples significantly decreased until the fermentation time of 48 and 72 h, due to newly added flours, whereas the phytate content significantly increased and subsequently started to significantly degrade after 72 h fermentation time.

Our findings are in line with the study of Sharma et al. (2017), who reported that mixing red and white finger millet flour (GPU 28 and KMR 340, respectively) with refined wheat flour led to a significant increment in the phytate content of the blended flours by 12.5 % for wheat mixed with red finger millet and by 29.6 % for wheat mixed with white finger millet. According to Baye et al. (2014), the efficiency of phytate degradation varies, according to the type of cereals and fermentation conditions. Similarly, Herter-Aeberli et al. (2020) reported that the addition of 10 % whole wheat flour reduced the phytate content from 0.76 to 0.24 g/100 g, being almost completely degraded when the amount of wheat flour increased up to 25 %. The reduction of phytate content during fermentation might be attributed to endogenous phytase activities of raw materials, as well as to processing conditions such as pH, which is known to modulate the activity of both plant and microbial phytases (Greiner & Konietzny, 2006).

In the case of white finger millet (WF), the percentage of decline in the phytate content and condensed tannin following 7 days of fermentation was 73.77 % and 86.44 %, respectively, comparable to the values so far reported by several authors. These results were similar to those observed for the fermentation of finger millet (Azeez et al., 2022), pearl millet (Osman, 2011), sorghum and pearl millet (Onyango et al., 2013), sorghum and finger millet (Makokha et al., 2002) and corn (Sokrab et al., 2014). Our results showed that there is no significant decrement either of phytate content or of condensed tannin after a 96 h fermentation, possibly due to the maximum hydrolysis of the components occurring at this fermentation period.

Considering finger millet varieties, the reduction in the phytate and condensed tannin content of fermented dough varied between light brown and white finger millet. Specifically, as fermentation time increases, a stronger decline was observed in white finger millet fermented dough flour than in light brown one. This difference might derive from the addition of a backslope or starter (*Ersho*) in the preparation of white finger millet dough and the incorporation of new flour on the third day of fermentation while preparing brown finger millet dough. The addition of backslope (*Ersho*) during dough preparation is important in the hastening of the fermentation process, as it influences the microbial diversity of the fermented slurries, in addition to improving the flavor, structure, and stability of baked goods (Gänzle & Zheng, 2019; Karaman et al., 2018). Sharma and Sharma (2022) demonstrated that mixed strains of lactic acid bacteria fermentation of foxtail millet for 20 h at 38 °C reduced phytate and condensed tannin content by 30.0 % and 30.2 % respectively. Results in our study showed a higher percentage reduction (in the range of 62.2 %-84.6 % for phytate and 19.6 %-86.4 % for tannin) than those reported previously (Sakandar et al., 2019; Sharma & Sharma, 2022), possibly attributed to the relatively longer fermentation period. Azeez et al. (2022) also reported a 56.6 % reduction in phytic acid by using dry yeast after 16 h fermentation of brown finger millet at 27 °C. Overall, we found that the reduction in phytate and condensed tannin content varies depending on variety, processing, and fermentation conditions.

The phytate and condensed tannin contents of finger millet based *Injera* are shown in Table 3. There was a significant decrement ($P < 0.05$) in the phytate content of brown finger millet and maize *Injera*, as well as in the tannin content of the 1:1 finger millet and maize composite flour (BFM) *Injera* with an increase in fermentation time. Similarly, the phytate and condensed tannin content of *Injera* samples of the two blend types (1:1 finger millet and maize composite flour, BFM and 1:2 finger millet and maize composite flour, BF2M) showed a significant difference in all fermentation times except between 144 h and 168 h, while the tannin content of 1:1 finger millet and maize composite flour (BFM) significantly decreased with an increase in fermentation time. We observed that the combined effect of fermentation and cooking resulted in a decrease in the phytate and condensed tannin content of our *Injera* sample. This result is in line with a study by Gabaza et al. (2018), who reported a decrement of condensed tannin and total phenolic contents of finger millet porridge by 22.0–36.0 % and 0.6–40.7 %, respectively, following spontaneous and backslopped fermentation and cooking of finger millet varieties. Another study by Sharma et al. (2017) demonstrated that baking resulted in a significant reduction in the phytic acid content by 29.5 % for wheat flour, 20.9 % for red finger millet and wheat blend chapatti, and 19.9 % for white finger millet and wheat blend chapatti. Thus, apart from producing the desired flavor and taste, fermentation and cooking eliminate considerable amounts of phytic acid and condensed tannin in finger millet based *Injera*.

3.3. Proximate composition, phytate, and tannin content of fourth-day *Injera* samples

Finger millet *Injera* or finger millet based *Injera* is usually consumed after fermentation for four days. We therefore compared the proximate composition, phytate, and tannin content of *Injera* prepared according to the different recipes outlined above (Table 4). The protein and fat content of *Injera* samples prepared from 1:2 of light brown finger millet and maize were higher than those prepared according to other recipes. Maize seems to contribute to the higher fat and protein content observed in *Injera* prepared using this specific ratio. However, the ash and crude fiber content of *Injera* prepared from 1:1 ratio of finger millet to maize composite flour were higher than *Injera* prepared from 1:2 ratio of finger millet to maize composite flour. Brown finger millet is assumed to contribute to the higher fiber and ash content recorded for this *Injera* sample. The fat content of white finger millet *Injera* is lower compared to that of light brown finger millet *Injera*. Conversely, the light brown finger millet *Injera* has a higher protein content. The total carbohydrate and energy content of *Injera* samples showed insignificant variation, except for the energy content between white finger millet and maize based *Injera* samples. The mean value of total carbohydrate and energy values of all *Injera* samples were in the range of 85.6–88.2 % and 392–410 Kcal/100gm, respectively. A significant difference ($P < 0.05$) was observed in the phytate and tannin content of all *Injera* samples. High phytate and tannin content was observed in brown finger millet *Injera*.

3.4. Ca, Fe and Zn content

Ethiopia has significant problems with nutrient deficiencies, particularly with regard to micronutrients such as iron, calcium and zinc (EPHI and UNICEF, 2016; Teshome et al., 2019). Table 5 presents the total Ca, Fe, and Zn content of *Injera* samples at different fermentation time. The unfermented flour of light brown finger millet (BF) contains the highest Fe and Ca content (4.58 mg/100 g dw and 372.66 mg/100 g dw, respectively) followed by white finger millet (WF) with higher Zn content. Except for white finger millet *Injera*, the iron, zinc, and calcium contents of the other *Injera* samples significantly increased ($P < 0.05$) with fermentation time.

The iron content of *Injera* increased by 109.0 %, 102.3 %, 246.3 %, 100.6 %, and 72.6 % for brown finger millet (BF), maize (M), 1:1 finger millet and maize composite flour (BFM), 1:2 millet and maize composite flour (BF2M), and white finger millet (WF), respectively, at 168 h in comparison with unfermented flour. These results are consistent with those of previous studies (Azeez et al., 2022; Jan et al., 2022; and Mudau et al., 2022) which reported an increase in the iron content of fermented finger millet flours. When compared to unfermented flours, the iron content in brown finger millet (BF), maize (M), 1:1 finger millet and maize composite flour (BFM), and 1:2 finger millet and maize composite flour (BF2M) *Injera* was considerably higher at 168 h of fermentation, while iron reached its highest level at 96 h of fermentation for *Injera* prepared from White Finger Millet (WF). A significant difference ($P < 0.05$) was observed in the iron content of *Injera* samples during different fermentation times. Specifically, for brown finger millet (BF) and 1:2 finger millet and maize composite flour (BF2M), a significant difference was observed up to 144 h of fermentation. In the case of maize (M) and white finger millet (WF) *Injera*, a significant difference was observed up to 120 h of fermentation. Additionally, there was a significant difference in the iron content among all flour samples of 1:1 finger millet and maize composite flour (BFM).

A similar observation was recorded for zinc. Although brown finger millet (BF) and white finger millet (WF) *Injera* had higher zinc contents (3.2 mg/100 g) than other *Injera* samples prepared at 168 h and 96 h of

Table 5
Ca, Fe and Zn content (in mg/100 g of d.w.) of finger millet based *Injera* prepared at different fermentation times.

Fermentation time (h)	Fe	Zn	Ca
Brown Finger millet (BF) Injera			
0	4.58 ± 0.26[a]	2.13 ± 0.05[a]	373 ± 5[a]
96	6.67 ± 0.09[b]	2.67 ± 0.02[b]	400 ± 5[b]
120	8.06 ± 0.48[c]	2.99 ± 0.03[c]	435 ± 6[c]
144	9.50 ± 0.28[d]	3.18 ± 0.02[d]	444 ± 3[c]
168	9.57 ± 0.12[d]	3.20 ± 0.07[d]	448 ± 7[c]
Maize (M)Injera			
0	2.14 ± 0.01[a]	1.58 ± 0.02[a]	18.4 ± 0.5[a]
96	3.16 ± 0.05[b]	1.95 ± 0.07[b]	28.1 ± 0.2[b]
120	4.13 ± 0.26[c]	2.30 ± 0.07[c]	32.5 ± 0.8[c]
144	4.46 ± 0.18[c]	2.33 ± 0.02[c]	31.8 ± 0.2[c]
168	4.33 ± 0.14[c]	2.35 ± 0.09[c]	32.3 ± 0.7[c]
1 Brown Finger millet: 1 Maize (BFM)Injera			
0	2.14 ± 0.10[a]	1.61 ± 0.01[a]	18.5 ± 0.3[a]
96	4.53 ± 0.21[b]	2.16 ± 0.08[b]	244 ± 3[b]
120	5.67 ± 0.04[c]	2.52 ± 0.08[c]	265 ± 3[c]
144	6.36 ± 0.18[d]	2.87 ± 0.03[d]	287 ± 2[d]
168	7.41 ± 0.10[e]	2.94 ± 0.12[d]	291 ± 3[d]
1 Brown Finger millet: 2 Maize (BF2M) Injera			
0	3.17 ± 0.23[a]	1.73 ± 0.02[a]	129 ± 1[a]
96	4.08 ± 0.21[b]	2.24 ± 0.05[b]	173 ± 2[b]
120	5.00 ± 0.03[c]	2.50 ± 0.01[c]	184 ± 1[c]
144	5.96 ± 0.24[d]	2.69 ± 0.02[d]	189 ± 3[c]
168	6.36 ± 0.23[d]	2.73 ± 0.01[d]	193 ± 3[c]
White Finger millet (WF) Injera			
0	4.20 ± 0.15[a]	2.29 ± 0.06[a]	332 ± 4[a]
96	8.45 ± 0.12[b]	3.20 ± 0.04[b]	396 ± 4[b]
120	8.01 ± 0.02[bc]	2.91 ± 0.03[c]	392 ± 7[b]
144	7.64 ± 0.16[c]	2.71 ± 0.11[cd]	385 ± 7[b]
168	7.25 ± 0.14[c]	2.64 ± 0.05[d]	377 ± 8[b]

Mean values and standard deviation of duplicate replications. Means with no common letters within a row significantly differed ($p \leq 0.05$).

Table 4
Proximate and antinutrient content of fourth-day *Injera* samples.

Injera	Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fiber (%)	Total CHO (%)	Energy (Kcal/100 g)	Phytic acid (mg/100 g)	Condensed Tannin(mg/100 g)
Brown Finger millet (BF)	3.21 ± 0.04[a]	2.17 ± 0.08[a]	8.05 ± 0.05[ab]	3.20 ± 0.02[a]	86.6 ± 0.15[a]	398.0 ± 0.30[ab]	417 ± 2.7[a]	109 ± 3.9[a]
Maize (M)	1.34 ± 0.04[b]	2.71 ± 0.21[b]	8.96 ± 0.00[c]	2.18 ± 0.17[b]	87.0 ± 0.41[a]	408.1 ± 2.7[b]	215 ± 2.1[b]	17 ± 1.3[b]
1 Brown Finger millet: 1 Maize (BFM)	2.31 ± 0.01[c]	3.17 ± 0.05[c]	8.49 ± 0.25[bc]	2.77 ± 0.08[c]	85.9 ± 0.30[a]	407.3 ± 1.1[b]	317 ± 7.4[c]	76 ± 2.2[c]
1 Brown Finger millet: 2 Maize (BF2M)	2.07 ± 0.06[d]	3.72 ± 0.06[d]	9.09 ± 0.35[c]	2.21 ± 0.03[b]	85.6 ± 2.2[a]	407.7 ± 10[b]	247 ± 3.4[d]	49 ± 2.2[d]
White Finger millet (WF)	3.15 ± 0.04[a]	0.81 ± 0.04[e]	7.76 ± 0.11[a]	2.67 ± 0.02[c]	88.3 ± 0.18[a]	391.4 ± 0.05[a]	120 ± 2.5[e]	37 ± 2.5[e]

Mean values and standard deviation of triplicate measurements. Means with no common letters within a row are significantly different ($p \leq 0.05$).

fermentation time respectively, the content was within the range commonly found in cereals. The zinc content of brown finger millet (BF), 1:1 finger millet and maize composite flour (BFM), and 1:2 finger millet and maize composite flour (BF2M) *Injera* samples showed a statistically significant increase among *Injera* samples up to the fermentation time of 144 h, while in maize and white finger millet *Injera* samples, a statistically significant increase was observed up to the fermentation time of 120 h. The zinc content of samples in this study is in line with previous findings (Ahmed et al., 2020; Anberbir et al., 2023).

The highest calcium content was recorded for brown finger millet *Injera* (448 mg/100 g). The fact that relatively lower iron, zinc, and calcium contents were observed in maize *Injera* suggests that the addition of maize leads to a decrease in the mineral content of blended flour. Fermentation time affected the calcium content of *Injera* samples prepared at different fermentation times. This result is in line with previous research (Azeez et al., 2022; Mudau et al., 2022), reporting an increase in the calcium content of solid-state fermented finger millet flours. The results further show a significant difference in the calcium content among the samples of 144 h fermented *Injera* for 1:1 finger millet and maize composite flour (BFM) and of 120 h fermented *Injera* for brown finger millet (BF), maize (M), and 1:2 finger millet and maize composite flour (BF2M). However, in the case of white finger millet (WF), a significant difference is shown between the unfermented flour and the 96 h fermented *Injera*.

Generally, the mineral content of *Injera* samples prepared from light brown finger millet, maize, and blended flour showed an increment with fermentation time. This finding agrees with earlier studies of Azeez et al. (2022) and Mudau et al. (2022), who reported an increment of calcium, iron, and zinc content of fermented finger millet by 5.3–8.7 %, 57.4–117 %, and 12.1–76.9 % respectively. Adebiyi et al. (2017) also showed that the mineral content increases when pearl millet is fermented and malted to produce flour and the resultant biscuit. Another study by Balli et al. (2023) demonstrated that fermentation increased the iron, calcium, and phosphorus content of fermented pearl millet. The increase in the mineral content of *Injera* samples compared with those of unfermented flour might be due to the loss of dry matter during fermentation as microbes degrade carbohydrates, proteins, and antinutritional factors (Gabaza et al., 2018a; Kruger et al., 2013; Nkhata et al., 2018). Enzymatic activities occurring during fermentation, facilitate the decomposition of this insoluble complex (Srivastava et al., 2020).

3.5. Bioaccessibility of iron, zinc, and calcium from finger millet based *Injera*

The *in vitro* digestibility method was used to estimate the bioaccessibility of Fe, Zn, and Ca from our *Injera* samples. The bioaccessibility of iron, zinc, and calcium from unfermented flour and finger millet based *Injera* samples, as determined by the *in vitro* digestibility method, is presented in Fig. 5.

Fermentation significantly ($P < 0.05$) affected the bioaccessibility of iron, zinc, and calcium in all samples. Higher bioaccessible iron and zinc values were observed for unfermented maize flour (12.8 and 18.1 %, respectively), while higher bioaccessible calcium (48.5 %) was observed for unfermented white finger millet flour (WF). After fermentation, the bioaccessibile iron, zinc, and calcium increased significantly in the range 15.4–40 %, 26.8–50.8 %, and 60.9–88.5 %, respectively, for light brown finger millet, the blended, and maize *Injera*. Such increased bioaccessibility, however, was not evident in the case of white finger millet *Injera*; instead, there was a significant increment at 96 h fermentation time and a slight decrement at 168 h. The fact that fermentation affected the iron, zinc, and calcium bioaccessibility in this study may be due to a reduction in the phytate and tannin contents. Fermentation is one of the most effective methods used to degrade the antinutrient and mineral complexes by enzymes that are derived from naturally occurring microflora; making minerals free, easily assessable, and bioavailable (Nkhata et al., 2018; Pranoto et al., 2013). Some researchers also reported a significant increment of iron, zinc, and calcium bioaccessibility due to fermentation (Greffeuille et al., 2011; Hemalatha et al., 2007; Kruger et al., 2013; Mamiro et al., 2001; Proulx & Reddy, 2007), while others found no significant effect (Baye et al., 2014; Gabaza et al., 2018b) or a reduction (Hemalatha et al., 2007).

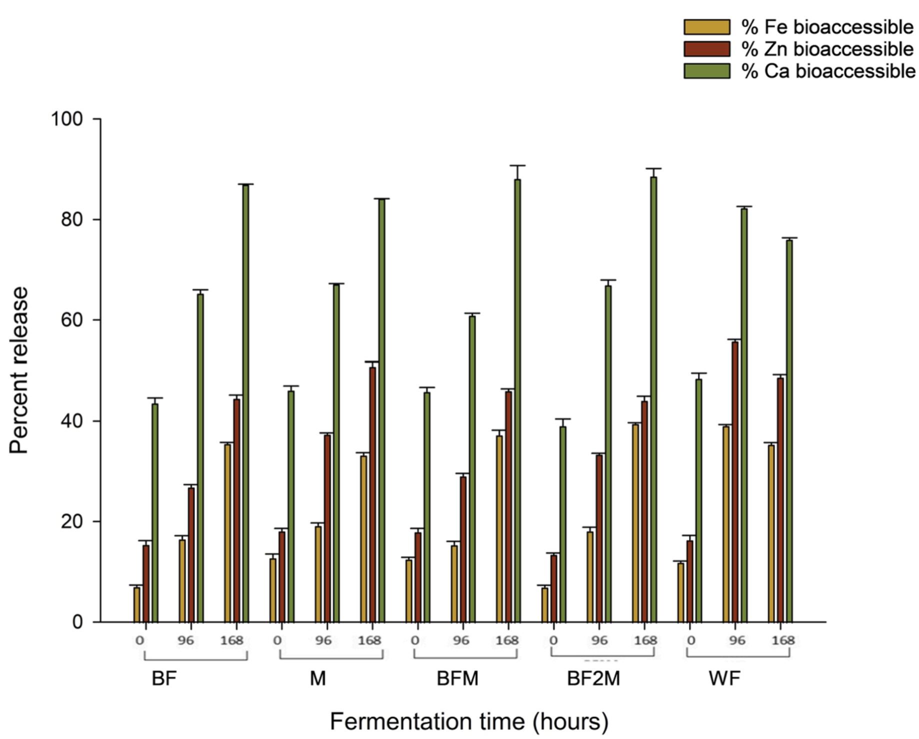

Fig. 5. Bioaccessibile iron, zinc, and calcium from finger millet based *Injera* at different fermentation times. Error bars represent mean ± SD.

4. Conclusion

This study showed that fermentation of finger millet in the preparation of finger millet based *Injera* effectively reduces antinutritional compounds. Consequently, this process leads to improvements in nutritional components and enhances mineral bioaccessibility. The differences in the composition of the flour blends used to prepare *Injera* influence fermentation patterns, ultimately impacting the final composition of the fermented dough and *Injera*. Notably, substantial changes are observed in protein, fat, mineral, phytate, and condensed tannin content. Furthermore, fermentation plays a pivotal role in reducing the phytate and condensed tannin content of both fermented flour and *Injera*. The most significant effects are observed when fermenting white finger millet flour with sourdough, particularly as fermentation time increases. This results in an enhanced proximate composition and mineral bioaccessibility of both fermented finger millet and *Injera* along with reduced phytate and condensed tannin content. The findings of this study suggest that fermenting finger millet flour shows promising results in improving its nutritional value to develop different food products. These insights provide valuable guidance for future investigations into various bioprocessing techniques, aiming to enhance the utilization of finger millet grains, whether for home or industry-scale *Injera* production.

CRediT authorship contribution statement

Helen Walle Endalew: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Minaleshewa Atlabachew:** Writing – review & editing, Supervision, Software, Project administration, Methodology, Investigation, Conceptualization. **Sotirios Karavoltsos:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Aikaterini Sakellari:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Mohamad Farshard Aslam:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Lara Allen:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Howard Griffiths:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Panagiotis Zoumpoulakis:** Writing – review & editing, Resources. **Anastasia Kanellou:** Writing – review & editing, Resources. **Tadesse Fenta Yehuala:** Investigation. **Metadel Kassahun Abera:** Writing – review & editing, Supervision. **Mesfin Wogahyehu Tenagashaw:** Writing – review & editing, Supervision. **Hirut Assaye Cherie:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114635.

References

Adebiyi, J. A., Kayitesi, E., Adebo, O. A., Changwa, R., & Njobeh, P. B. (2019). Food fermentation and mycotoxin detoxification: An African perspective. *Food Control, 106*, Article 106731. https://doi.org/10.1016/j.foodcont.2019.106731

Adebiyi, J. A., Obadina, A. O., Adebo, O. A., & Kayitesi, E. (2017). Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (Pennisetum glaucum) flour. *Food Chemistry, 232*, 210–217. 10.1016/j.foodchem.2017.04.020.

Adebo, J. A., Njobeh, P. B., Gbashi, S., Oyedeji, A. B., Ogundele, O. M., Oyeyinka, S. A., & Adebo, O. A. (2022). Fermentation of cereals and legumes: Impact on nutritional constituents and nutrient bioavailability. *Fermentation, 8*(2), Article 63. https://doi.org/10.3390/fermentation8020063

Adegbehingbe, K. T. (2013). Fermented sprouted and unsprouted maize for ogi production. *International Journal of Advanced Research, 1*(10), 428–434.

Adekunle, A. A., Ellis-Jones, J., Ajibefun, I., Nyikal, R., Bangali, S., Fatunbi, O., & Ange, A. (2012). Agricultural innovation in sub-Saharan Africa: Experiences from multiple-stakeholder approaches. *Forum for Agricultural Research in Africa (FARA), Accra, Ghana*.

Afify, A.-E.-M.-M., El-BELTAGI, H. S., El-Salam, S. M. A., & Omran, A. A. (2012). Effect of soaking, cooking, germination and fermentation processing on proximate analysis and mineral content of three white sorghum varieties (Sorghum bicolor L. Moench). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40*(2), 92–98.

Agza, B., Bekele, R., & Shiferaw, L. (2018). Quinoa (Chenopodium quinoa, Wild.): As a potential ingredient of injera in Ethiopia. *Journal of Cereal Science, 82*, 170–174. https://doi.org/10.1016/j.jcs.2018.06.009

Ahmed, M. I., Xu, X., Sulieman, A. A., Na, Y., & Mahdi, A. A. (2020). The effect of fermentation time on in vitro bioavailability of iron, zinc, and calcium of kisra bread produced from koreeb (Dactyloctenium aegyptium) seeds flour. *Microchemical Journal, 154*, Article 104644. https://doi.org/10.1016/j.microc.2020.104644

Amankwah, E. A., Barimah, J., Acheampong, R., Addai, L. O., & Nnaji, C. O. (2009). Effect of fermentation and malting on the viscosity of maize-soyabean weaning blends. *Pakistan Journal of Nutrition, 8*(10), 1671–1675. https://doi.org/10.3923/pjn.2009.1671.1675

Anberbir, S. M., Satheesh, N., Abera, A. A., Tenagashaw, M. W., Asres, D. T., Tiruneh, A. T., Habtu, T. A., Awol, S. J., & Wudineh, T. A. (2023). Effect of blending ratio and fermentation time on the physicochemical, microbiological, and sensory qualities of injera from teff, pearl millet, and buckwheat flours. *CyTA - Journal of Food, 21*(1), 217–236. https://doi.org/10.1080/19476337.2023.2188058

Azeez, S. O., Chinma, C. E., Bassey, S. O., Eze, U. R., Makinde, A. F., Sakariyah, A. A., Okubanjo, S. S., Danbaba, N., & Adebo, O. A. (2022). Impact of germination alone or in combination with solid-state fermentation on the physicochemical, antioxidant, in vitro digestibility, functional and thermal properties of brown finger millet flours. *LWT, 154*, Article 112734. https://doi.org/10.1016/j.lwt.2021.112734

Balli, D., Cecchi, L., Pieraccini, G., Venturi, M., Galli, V., Reggio, M., Di Gioia, D., Furlanetto, S., Orlandini, S., Innocenti, M., & Mulinacci, N. (2023). Millet fermented by different combinations of yeasts and lactobacilli: Effects on phenolic composition, starch, mineral content and prebiotic activity. *Foods, 12*(4), Article 748. https://doi.org/10.3390/foods12040748

Baye, K., Guyot, J.-P., Icard-Vernière, C., Rochette, I., & Mouquet-Rivier, C. (2015). Enzymatic degradation of phytate, polyphenols and dietary fibers in Ethiopian injera flours: Effect on iron bioaccessibility. *Food Chemistry, 174*, 60–67. https://doi.org/10.1016/j.foodchem.2014.11.012

Baye, K., Guyot, J.-P., Icard-Vernière, C., Rochette, I., Mouquet-Rivier, C., Icard-Vernière, C., Picq, C., & Guyot, J.-P. (2014). Changes in mineral absorption inhibitors consequent to fermentation of Ethiopian injera: Implications for predicted iron bioavailability and bioaccessibility. *International Journal of Food Science & Technology, 49*(1), 174–180. https://doi.org/10.1111/ijfs.12295

Chandra, D., Chandra, S., Pallavi, & Sharma, A. K. (2016). Review of Finger millet (Eleusine coracana (L.) Gaertn): A power house of health benefiting nutrients. *Food Science and Human Wellness, 5*(3), 149–155. https://doi.org/10.1016/j.fshw.2016.05.004

Chinma, C. E., Azeez, S. O., Sulayman, H. T., Alhassan, K., Alozie, S. N., Gbadamosi, H. D., Danbaba, N., Oboh, H. A., Anuonye, J. C., & Adebo, O. A. (2020). Evaluation of fermented African yam bean flour composition and influence of substitution levels on properties of wheat bread. *Journal of Food Science, 85*(12), 4281–4289. https://doi.org/10.1111/1750-3841.15527

CSA. (2021). *Report on area and production of major crops*.

Devisetti, R., Yadahally, S. N., & Bhattacharya, S. (2014). Nutrients and antinutrients in foxtail and proso millet milled fractions: Evaluation of their flour functionality. *LWT - Food Science and Technology, 59*(2), 889–895. https://doi.org/10.1016/j.lwt.2014.07.003

Dykes, L. (2019). Tannin analysis in sorghum grains. In Z. Y. Zhao (Ed.), *Sorghum* (pp. 109–120). Humana Press. https://doi.org/10.1007/978-1-4939-9039-9_8.

Elyas, S. H. A., El Tinay, A. H., Yousif, N. E., & Elsheikh, E. A. E. (2002). Effect of natural fermentation on nutritive value and in vitro protein digestibility of pearl millet. *Food Chemistry, 78*(1), 75–79. https://doi.org/10.1016/S0308-8146(01)00386-7

Ethiopian Public Health Institute (EPHI) and UNICEF. (2016). National Micronutrient Survey Report. Addis Ababa, Ethiopia.

Fredlund, K., Isaksson, M., Rossander-Hulthén, L., Almgren, A., & Sandberg, A.-S. (2006). Absorption of zinc and retention of calcium: Dose-dependent inhibition by phytate. *Journal of Trace Elements in Medicine and Biology, 20*(1), 49–57. https://doi.org/10.1016/j.jtemb.2006.01.003

Frontela, C., Ros, G., & Martínez, C. (2011). Phytic acid content and "in vitro" iron, calcium and zinc bioavailability in bakery products: The effect of processing. *Journal of Cereal Science, 54*(1), 173–179. https://doi.org/10.1016/j.jcs.2011.02.015

Gabaza, M., Shumoy, H., Louwagie, L., Muchuweti, M., Vandamme, P., Du Laing, G., & Raes, K. (2018a). Iron and zinc bioaccessibility of fermented maize, sorghum and millets from five locations in Zimbabwe. *Journal of Food Composition and Analysis, 103*, 361–370. https://doi.org/10.1016/j.foodres.2017.10.047

Gabaza, M., Shumoy, H., Louwagie, L., Muchuweti, M., Vandamme, P., Du Laing, G., & Raes, K. (2018b). Traditional fermentation and cooking of finger millet: Implications on mineral binders and subsequent bioaccessibility. *Journal of Food Composition and Analysis, 68*, 87–94. https://doi.org/10.1016/j.jfca.2017.05.011

Gänzle, M. G., & Zheng, J. (2019). Lifestyles of sourdough lactobacilli–Do they matter for microbial ecology and bread quality? *International Journal of Food Microbiology, 302*, 15–23.

Gebre, B. A. (2019). Food and nutrition security potential of finger millet in Ethiopia. *Academic Research Journal of Agricultural Science and Research, 7*(5), 256–265. 10.14662/ARJASR2019.082.

Glahn, R. P., Lee, O. A., Yeung, A., Goldman, M. I., & Miller, D. D. (1998). Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 cell culture model. *The Journal of nutrition, 128*(9), 1555–1561.

Greffeuille, V., Polycarpe Kayodé, A. P., Icard-Vernière, C., Gnimadi, M., Rochette, I., & Mouquet-Rivier, C. (2011). Changes in iron, zinc and chelating agents during traditional African processing of maize: Effect of iron contamination on bioaccessibility. *Food Chemistry, 126*(4), 1800–1807. https://doi.org/10.1016/j.foodchem.2010.12.087

Greiner, R., & Konietzny, U. (2006). Phytase for food application. *Food Technology and Biotechnology, 44*(2), 125–140.

Grigoriou, C., Costopoulou, D., Vassiliadou, I., Karavoltsos, S., Sakellari, A., Bakeas, E., & Leondiadis, L. (2022). Polycyclic aromatic hydrocarbons and trace elements dietary intake in inhabitants of Athens, Greece, based on a duplicate portion study. *Food and Chemical Toxicology, 165*, Article 113087. https://doi.org/10.1016/j.fct.2022.113087

Hemalatha, S., Platel, K., & Srinivasan, K. (2007). Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. *Food Chemistry, 102*(4), 1328–1336. https://doi.org/10.1016/j.foodchem.2006.07.015

Herter-Aeberli, I., Fischer, M. M., Egli, I. M., Zeder, C., Zimmermann, M. B., & Hurrell, R. F. (2020). Addition of whole wheat flour during injera fermentation degrades phytic acid and triples iron absorption from fortified tef in young women. *The Journal of Nutrition, 150*(10), 2666–2672. https://doi.org/10.1093/jn/nxaa211

Ilowefah, M., Bakar, J., Ghazali, H. M., & Muhammad, K. (2017). Enhancement of nutritional and antioxidant properties of brown rice flour through solid-state yeast fermentation. *Cereal Chemistry Journal, 94*(3), 519–523. https://doi.org/10.1094/CCHEM-08-16-0204-R

Jan, S., Krishan Kumar, K. K., Yadav, A. N., Ahmed, N., Thakur, P., Chauhan, D., Hyder Rizvi, Q.-U.-E., & Dhaliwal, H. S. (2022). Effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of finger millet (Eleusine coracana L.). *Journal of Applied Biology & Biotechnology*, 46–52. https://doi.org/10.7324/JABB.2022.10s107

Karaman, K., Sagdic, O., & Durak, M. Z. (2018). Use of phytase active yeasts and lactic acid bacteria isolated from sourdough in the production of whole wheat bread. *LWT, 91*, 557–567.

Khoja, K. K., Buckley, A., Aslam, M. F., Sharp, P. A., & Latunde-Dada, G. O. (2020). In vitro bioaccessibility and bioavailability of iron from mature and microgreen fenugreek, rocket and broccoli. *Nutrients, 12*(4), 1057. https://doi.org/10.3390/nu12041057

Kruger, J., Taylor, J. R. N., Du, X., De Moura, F., Lönnerdal, B., & Oelofse, A. (2013). Effect of phytate reduction of sorghum, through genetic modification, on iron and zinc availability as assessed by an in vitro dialysability bioaccessibility assay, Caco-2 cell uptake assay, and suckling rat pup absorption model. *Food Chemistry, 141*(2), 1019–1025.

Makokha, A. O., Oniang'o, R. K., Njoroge, S. M., & Kamar, O. K. (2002). Effect of traditional fermentation and malting on phytic acid and mineral availability from Sorghum (Sorghum bicolor) and Finger millet (Eleusine coracana) grain varieties grown in Kenya. *Food and Nutrition Bulletin, 23*(3_suppl1), 241–245. https://doi.org/10.1177/15648265020233S147

Mamiro, P. R. S., Van, J., Mwikya, S. M., & Huyghebaert, A. (2001). In vitro extractability of calcium, iron, and zinc in finger millet and kidney beans during processing. *Journal of Food Science, 66*(9), 1271–1275. https://doi.org/10.1111/j.1365-2621.2001.tb15200.x

McKie, V. A., & McCleary, B. V. (2016). A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. *Journal of AOAC International, 99*(3), 738–743. https://doi.org/10.5740/jaoacint.16-0029

Mihret, Y., & Bultosa, G. (2017). The effect of blending ratio of tef [Eragrostis Tef (Zucc) Trotter], Sorghum (Sorghum bicolor (L.) Moench) and faba bean (Vicia faba) and fermentation time on chemical composition of Injera. *Journal of Nutrition & Food Sciences, 07*(02). https://doi.org/10.4172/2155-9600.1000583

Monro, J., & Burlingame, B. (1996). Carbohydrates and related food components: Infoods tagnames, meanings, and uses. *Journal of Food Composition and Analysis, 9*(2), 100–118. https://doi.org/10.1006/jfca.1996.0018

Mudau, M., Ramashia, S. E., & Mashau, M. E. (2022). Mineral content, functional, thermo-pasting, and microstructural properties of spontaneously fermented finger millet flours. *Foods, 11*(16), Article 2474. https://doi.org/10.3390/foods11162474

Mutshinyani, M., Mashau, M. E., & Jideani, A. I. O. (2020). Bioactive compounds, antioxidant activity and consumer acceptability of porridges of finger millet (Eleusine coracana) flours: Effects of spontaneous fermentation. *International Journal of Food Properties, 23*(1), 1692–1710. https://doi.org/10.1080/10942912.2020.1825485

Nguyen, T. T. T., Loiseau, G., Icard-Vernière, C., Rochette, I., Trèche, S., & Guyot, J.-P. (2007). Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: A new method for preparing high energy density complementary foods for young children. *Food Chemistry, 100*(2), 623–631. https://doi.org/10.1016/j.foodchem.2005.09.080

Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J.-B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition, 6*(8), 2446–2458. https://doi.org/10.1002/fsn3.846

Ogodo, A. C., Chinyere Ugbogu, O., Onyeagba, R. A., & Okereke, H. C. (2018). Proximate composition and in-vitro starch/protein digestibility of bambara groundnut flour fermented with lactic acid bacteria (LAB)-consortium isolated from cereals. *Fermentation Technology, 07*(01), 35–43. https://doi.org/10.4172/2167-7972.1000148

Ojokoh, A., & Bello, B. (2014). Effect of fermentation on nutrient and anti-nutrient composition of Millet (Pennisetum glaucum) and Soyabean (Glycine max) blend flours. *Journal of Life Sciences, 8*(8).

Ojokoh, A., Fayemi, O., Ocloo, F. C., & Nwokolo, F. (2015). Effect of fermentation on proximate composition, physicochemical and microbial characteristics of pearl millet (Pennisetum glaucum (L.) R. Br.) and Acha (Digitaria exilis (Kippist) Stapf) flour blends. *Journal of Agricultural Biotechnology and Sustainable Development, 7*(1), 1–8. https://doi.org/10.5897/JABSD2014.0236

Olukomaiya, O. O., Adiamo, O. Q., Fernando, W. C., Mereddy, R., Li, X., & Sultanbawa, Y. (2020). Effect of solid-state fermentation on proximate composition, anti-nutritional factor, microbiological and functional properties of lupin flour. *Food Chemistry, 315*, Article 126238. https://doi.org/10.1016/j.foodchem.2020.126238

Onweluzo, J. C., & Nwabugwu, C. C. (2009). Fermentation of Millet (Pennisetum americanum) and Pigeon pea (Cajanus cajan) seeds for flour production: Effects on composition and selected functional properties. *Pakistan Journal of Nutrition, 8*(6), 737–744. https://doi.org/10.3923/pjn.2009.737.744

Onyango, C. A., Ochanda, S. O., Mwasaru, M. A., Ochieng, J. K., Mathooko, F. M., & Kinyuru, J. N. (2013). Effects of malting and fermentation on anti-nutrient reduction and protein digestibility of red sorghum, white sorghum and pearl millet. *Journal of Food Research, 2*(1), Article 41. https://doi.org/10.5539/jfr.v2n1p41

Osman, M. A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Sciences, 10*(1), 1–6. https://doi.org/10.1016/j.jssas.2010.06.001

Parul, B. (2014). Anti-nutritional factors in foods and their effects. *Journal of Academia and Industrial Research (JAIR), 3*(6), 2278–2313.

Pires, S. M. G., Reis, R. S., Cardoso, S. M., Pezzani, R., Paredes-Osses, E., Seilkhan, A., Ydyrys, A., Martorell, M., Sönmez Gürer, E., Setzer, W. N., Abdull Razis, A. F., Modu, B., Calina, D., & Sharifi-Rad, J. (2023). Phytates as a natural source for health promotion: A critical evaluation of clinical trials. *Frontiers in Chemistry, 11*. https://doi.org/10.3389/fchem.2023.1174109

Pragya, S. (2012). Finger millet for food and nutritional security. *African Journal of Food Science, 6*(4). https://doi.org/10.5897/AJFSX10.010

Pranoto, Y., Anggrahini, S., & Efendi, Z. (2013). Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. *Food Bioscience, 2*, 46–52. https://doi.org/10.1016/j.fbio.2013.04.001

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*(5), 1214–1218. https://doi.org/10.1021/jf60219a031

Proulx, A. K., & Reddy, M. B. (2007). Fermentation and lactic acid addition enhance iron bioavailability of maize. *Journal of Agricultural and Food Chemistry, 55*(7), 2749–2754. https://doi.org/10.1021/jf0630015

Ramashia, S. E., Anyasi, T. A., Gwata, E. T., Meddows-Taylor, S., & Jideani, A. I. O. (2019). Processing, nutritional composition and health benefits of finger millet in sub-saharan Africa. *Food Science and Technology, 39*(2), 253–266. https://doi.org/10.1590/fst.25017

Ramashia, S. E., Gwata, E. T., Meddows-Taylor, S., Anyasi, T. A., & Jideani, A. I. O. (2018). Some physical and functional properties of finger millet (Eleusine coracana) obtained in sub-Saharan Africa. *Food Research International, 104*, 110–118. https://doi.org/10.1016/j.foodres.2017.09.065

Sade, F. O. (2009). Proximate, antinutritional factors and functional properties of processed pearl millet (Pennisetum glaucum). *Journal of Food Technology, 7*(3), 92–97.

Sakandar, H. A., Kubow, S., Azadi, B., Faryal, R., Ali, B., Ghazanfar, S., & Imran, M. (2019). Wheat fermentation with Enterococcus mundtii QAUSD01 and Wickerhamomyces anomalus QAUWA03 consortia induces concurrent gliadin and phytic acid degradation and inhibits gliadin toxicity in Caco-2 monolayers. *Frontiers in Microbiology, 9*, 3312.

Saleh, A., Zhang, Q., Chen, J., & Shen, Q. (2013). Millet grains: Nutritional quality, processing, and potential health benifits. *Comprehensive Reviews in Food Science and Food Safety, 12*, 281–295.

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: An overview. *Food Production, Processing and Nutrition, 2* (1), 6. https://doi.org/10.1186/s43014-020-0020-5

Séye, S., Momar, T. G., Fallou, S., Younoussa, D., Mamadou, S. S., Aliou, F., Doohong, M., & Mohamed, L. G. (2018). Parboiling effect on phytate contains of two senegalese pearl millet varieties (Pennisetum glaucum [L.] R.Br.). *Journal of Nutrition Health Food Science, 6*(6), 1–6.

Sharma, B., Gujral, H. S., & Solah, V. (2017). Effect of incorporating finger millet in wheat flour on mixolab behavior, chapatti quality and starch digestibility. *Food Chemistry, 231*, 156–164. https://doi.org/10.1016/j.foodchem.2017.03.118

Sharma, R., & Sharma, S. (2022). Anti-nutrient & bioactive profile, in vitro nutrient digestibility, techno-functionality, molecular and structural interactions of foxtail millet (Setaria italica L.) as influenced by biological processing techniques. *Food Chemistry, 368*, Article 130815. https://doi.org/10.1016/j.foodchem.2021.130815

Simwaka, J. E., Chamba, M. V. M., Huiming, Z., Masamba, K. G., Luo, Y., Simwaka, J., Madalitso, C., Zhuo, H., Masamba, K., & Luo. (2017). Effect of fermentation on physicochemical and antinutritional factors of complementary foods from millet, sorghum, pumpkin and amaranth seed flours. *International Food Research Journal, 25*, 1869–1879.

Singh, C. E., & Sarita, S. (2018). Effects of processing (germination and popping) on the nutritional and anti-nutritional properties of finger millet (Eleusine coracana). *Current Research in Nutrition and Food Science Journal, 6*(2), 566–572. 10.12944/CRNFSJ.6.2.30.

Sokrab, A. M., Mohamed Ahmed, I. A., & Babiker, E. E. (2014). Effect of fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes. *Journal of Food Science and Technology, 51*(10), 2608–2615. https://doi.org/10.1007/s13197-012-0787-8

Sood, S., Kumar, A., Babu, B. K., Gaur, V. S., Pandey, D., Kant, L., & Pattnayak, A. (2016). Gene discovery and advances in finger millet [Eleusine coracana (L.) Gaertn.] genomics—An important nutri-cereal of future. *Frontiers in Plant Science, 7*. https://doi.org/10.3389/fpls.2016.01634

Srivastava, U., Saini, P., & Singh, A. (2020). Effect of natural fermentation on antioxidant activity of pearl millet (Pennisetum glaucum). *Current Nutrition & Food Science, 16* (3), 306–313. https://doi.org/10.2174/1573401314666181115103328

Taylor, J. R., & Duodu, K. G. (2015). Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. *Journal of the Science of Food and Agriculture, 95*(2), 225–237. https://doi.org/10.1002/jsfa.6713

Teshome, G., Bosha, T., & Gebremedhin, S. (2019). Calcium intake and its determinants among children and women of reproductive age in Ethiopia: Further analysis of the 2016 national micronutrient survey. *Nutrients, 11*(5), Article 1144.

USEPA. (1997). *Guidelines establishing test procedures for the analysis of pollutants (App. B, Part 136, definition and procedures for the determination of the method detection limit)*.

Woldemariam, F., Mohammed, A., Fikre Teferra, T., & Gebremedhin, H. (2019). Optimization of amaranths–teff–barley flour blending ratios for better nutritional and sensory acceptability of injera. *Cogent Food & Agriculture, 5*(1), Article 1565079. https://doi.org/10.1080/23311932.2019.1565079

Yegrem, L., Abera, S., & Temesgen, M. (2021). Nutritional composition and sensory quality of injera prepared from tef (Eragrostis tef (Zucc.) Trotter) complemented with lupine (Lupinus spp.). *Cogent Food & Agriculture, 7*(1). https://doi.org/10.1080/23311932.2020.1862469