LWT - Food Science and Technology 158 (2022) 113156

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of fortification with eggshell powder on injera quality

Tigist Fekadu, Angela Cassano, Ignacio Angós, Juan Ignacio Maté *

Department of Agronomy, Biotechnology and Food Sciences Engineering, ISFOOD - Institute for Sustainability & Food Chain Innovation, Universidad Pública de Navarra, 31006, Pamplona, Spain

ARTICLE INFO

Keywords:
Calcium
Eggshell powder
Bioavailability
Injera
Quality parameters

ABSTRACT

In Ethiopia there is a severe calcium nutrient deficiency problem. Cereals are appropriate vehicles for food fortification along with eggshell powder (EP), a waste product, which contains a high amount of Ca. The objective of the current study was to enrich injera (Ethiopian flat bread from cereal teff) with EP to improve Ca content and to assess its effect on quality parameters. Injera was prepared by adding 4.5% and 9% EP to the teff flour (injera I4.5 and I9.0, respectively). The fortification resulted in significantly increased bioavailable Ca from 16.1 mg/100 g (control) to 742.7 mg/100 g (I4.5) and 1743.1 mg/100 g (I9.0). With the consumption of 200 g of injera, an adult could meet 60% of the recommended dietary allowance (RDA) of Ca from I4.5 and 140% of Ca RDA from I9.0. The addition of eggshell powder affected pH, titratable acidity, color and texture. However, there was no significant effect on moisture content, aw, viscosity, microbial quality, and eye characteristics. Current research revealed that fortification of injera with EP could be a good source of total and bioavailable Ca without significant deleterious effects on the key physico-chemical quality parameters of injera.

1. Introduction

Calcium is one of the important micronutrients being that it is necessary for the normal functioning of the human body. This nutrient is a major component of bones and teeth and participates in many physiological processes of human body (Gharibzahedi & Jafari, 2017). In addition, Ca plays an important role in the prevention of chronic diseases such as high blood pressure (Hofmeyr, Duley, & Atallah, 2007). The Ca deficiency causes osteoporosis in adults, and rickets in children (Pettifor, 2004).

Milk and milk products are excellent sources of Ca. However, fulfilling Ca requirements from food sources is very difficult in areas where there is famine and drought which affect the production of livestock and their products in countries like Ethiopia. As a consequence, the level of Ca intake by the children has been below the recommended level (Tezera, Whiting, & Gebremedhin, 2017). Similarly, other researchers showed that there was an inadequate intake of Ca by women and men (Amare et al., 2012; Asayehu, Lachat, Henauw, & Gebreyesus, 2017).

In Ethiopia, the Great Rift Valley areas are commonly affected by fluorosis due to the high level of fluorine in the water. People affected by fluorosis have weak bones and weak and stained teeth. Studies have shown that the consumption of an adequate amount of Ca would help decrease fluorosis (Patel et al., 2017).

Food fortification has been proven to be effective to increase Ca intake. Cereal-based foods are usually the target food to be used to fortify Ca since they are consumed in great quantities; thus a higher level of Ca can be added (Cilla, López-García, & Barberá, 2018) to the diet. $CaCO_3$ is a common Ca source for food fortification in cereals-based foods because its addition usually does not have a significant effect on the taste and appearance of the final food product (Rafferty, Walters, & Heaney, 2007).

Injera (flat bread) is a staple food consumed equally by all age groups and by two-third of the Ethiopian population. The cereal teff, *Eragrostis tef* (Zucc.) Trotter, is a grass-type cereal that is used to cook injera. Teff has a nutrient composition similar to other cereals although it has higher iron and Ca contents (Shumoy & Raes, 2017). In addition, since teff is consumed as a whole grain, injera contains high amounts of fiber which reduce the absorption capability of minerals.

Teff injera quality is judged by consumers considering appearance and taste. Color, texture, and eyes (a honeycomb structure on top of injera) are among the quality parameters which are critical for their acceptance (Yasin, 2021; Yetneberk, De Kock, Rooney, & Taylor, 2004). Sour, soft, cohesive, and light colored injera is highly demanded.

Eggshell contains high amounts of bioavailable Ca in the form of $CaCO_3$ (Brun, Lupo, Delorenzi, Di Loreto, Loreto, & Rigalli 2013). It was shown that EP contained 97% solids, out of which 98% is $CaCO_3$

* Corresponding author.
E-mail address: juan.mate@unavarra.es (J.I. Maté).

https://doi.org/10.1016/j.lwt.2022.113156
Received 30 September 2021; Received in revised form 20 January 2022; Accepted 24 January 2022
Available online 1 February 2022

(Daengprok Daengproka, Garnjanagoonchorna, & Mineb, 2002). One gram of EP contains 360–400 mg of Ca (Schaafsma et al., 2000). Trials suggested that bioavailable Ca content in EP was estimated to be 45% (Brun, Lupo, Delorenzi, Di Loreto, Loreto, & Rigalli 2013). Animal studies showed that EP has a higher content of absorbable Ca than commercial $CaCO_3$ (Omi & Ezawa, 1998).

Research findings showed that food products like bread, sausage, pizza, spaghetti, or stew were fortified with eggshell powder thus improving the Ca content of these products (Brun, Lupo, Delorenzi, Di Loreto, Loreto, & 2013). In addition, the sensory evaluation presented an overall acceptability of the products, even though texture quality was affected. Eggshell, a waste product, is easily accessible by most Ethiopian households; and thus, it can be used as a low-cost Ca source with also contributes to a circular economy.

Even though micronutrient deficiencies are among the most important nutritional problems in Ethiopia, so far there is no mandatory food fortification program designed to be a solution to tackle the problem. Fortifying injera, with eggshell powder (EP), could be used to address the Ca deficiency problem and contribute to improving the health of Ethiopians.

The objective of this research was to fortify injera with eggshell powder and to assess its effect on some injera quality parameters, including physicochemical, physical, microbiological, and nutritional quality. As far as we know, there are no studies previously conducted related to the development of fortified injera with EP and its effect on injera quality.

2. Materials and methods

2.1. Materials

White teff flour was provided by Blonk Quality Ingredients (Spain). Free samples of eggshell powder (EP) were kindly provided by Eggnovo S.L. (Spain). The starter cultures *Saccharomyces cerevisiae* and *Lactobacillus plantarum* subsp. *plantarum* were purchased from Valencia University CECT (Spanish Type Culture Collection). Enzymes and bile for *in vitro* digestibility study were bought from Sigma-Aldrich (St Louis, MO, USA). Analytic standard reagents were obtained from Panreac, Spain (HCl, KCl, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, and 65% HNO_3); Sigma-Aldrich, Japan ($(NH_4)_2CO_3$, $CaCl_2(H_2O)_2$, $LaCl_3$)); Merck, Spain (NaOH, and 0.1 N NaOH); and J. T. Baker, Spain (KH_2PO_4). Medias and chemicals used for microbiology were purchased from OXID, France (MRS broth, MRS agar, and dehydrated culture media for microbiology); Panreac, Spain (agar technical ingredient for microbiology, and Glucose D (+), cycloheximide and chloramphenicol; VWR life science (Bacterial ultra-pure yeast extract, and tryptone peptone (peptone from casein) for microbiology; and VWR chemicals, from Belgium (Buffered peptone water, and PCA.

2.2. Injera processing

YEPD agar and broth were used to culture S. *cerevisiae* strain while MRS agar and broth for *Lactobacillus plantarum.* Culturing of the strains was first made on their respective agar medias and incubated at 30 °C for 48 h. The strains were then cultured in their respective broth at 30 °C for 24 h and then for 48 h in a new broth. Pellets of each strain were collected by centrifugation at 10,000×*g* for 15 min at a temperature of 20 °C using a SIGMA 3K30 Laborzentrifugen GmbH (Germany) centrifuge machine. Sterilized water was added to the pellets before the centrifugation was performed again to eliminate the broth. Finally, before use, the starter cultures were solubilized in small amount of sterilized water and then placed in refrigerator at 5 °C for two days. After refrigeration, the concentration was determined by making ten-fold dilutions and culturing them in their respective agar media.

Injera formulation was based on three different components: flour, water, and starter culture. Before starting the processing, teff flour and EP were sterilized in a hot dry air by an auto cooking machine RATIONAL SCC 61/06 (Germany) at 190 °C for 6 min following the procedure described by Baye et al., (2014) and cooled to room temperature.

Three different types of injera were prepared based on teff flour fortified with EP at three different levels: i) Control Injera (I0) based on 100% teff flour with no EP, ii) Injera I4.5 based on flour with 95.5% (w:w) teff flour and 4.5% EP and iii) Injera I9.0 based on flour with 91% (w:w) teff flour and 9% EP. The experiment was performed two times for each formulation. The level of EP added to the flour was selected to achieve the RDA of an adult person by consuming 200 g of injera, assuming that EP contained 50% (I4.5) or 25% (I9.0) of bioavailable Ca (FAO/WHO, 2005).

Injera was prepared following the procedure shown in Fig. 1. Firstly, to make the batter, 1100 g of the corresponding flour (teff flour and EP) and starter cultures (to reach 10^7 CFU/ml in the final batter) along with sterilized ultrapure water to reach a final weight of 2360 g were placed into a Thermomix TM-5 (Vorwerk, Germany) to be mixed and kneaded at 3 rpm for 5 min at room temperature. Once the batter was obtained, it was placed in metal pot and the top was covered with 400 ml sterilized ultra-pure water to prevent mold growth.

Fermentation was performed in a convective oven at 25 °C (room temperature in Ethiopia) for 48 h. After that, to prepare the *absit* (starter used as leaven) 150 g of fermented batter were taken and mixed well for 2 min then cooked in 450 ml of boiling water for 10 min by continual steering with a spoon. The *absit*, at approximately 60 °C, was added back to the fermenting batter and mixed well with a spoon. After that, the mixture was kept at 25 °C for about 2 h to be fermented further before cooking. Approximately 100 ml of the fermented batter was poured in a spiral manner onto an injera WASS Electronics Digital Mitad Grill, covered, and baked for 2 min at a cooking temperature of 121 °C. The baked injera was then removed and kept in an airtight container at room temperature.

The samples used for quality parameters analysis were taken immediately after preparing the batter (B0), at 24 h (B24), 48 h (B48) and 50 h (B50) of fermentation of the batter. Sampling times were chosen based on the work described by Misci et al. (2021). At each sample time, 200 g of the batter were taken and used for analysis. Finally, a cooked injera (INJ) analysis was performed. Sample collection is indicated in Fig. 1.

2.3. Physicochemical parameters

2.3.1. pH and titratable acidity

pH of batter was determined using a Crison Basic 20 pH meter by directly inserting the electrode into the batter. Titratable acidity of batter was determined based on lactic acid following the method by Harth, Van Kerrebroeck, & De Vuyst (2016). The pH of injera was measured according to AOAC Official Method 945.42 (AOAC, 2005b). The solution, prepared to determine the pH of injera, was also used to determine its titratable acidity.

2.3.2. Moisture content and water activity

Moisture content of batter and injera was determined gravimetrically. Water activity of batter and injera was measured by LabMaster-Aw (Novasina AG; Lachen, Switzerland) at 25 °C.

2.4. Physical parameters

2.4.1. Rheological properties of batter

The rheological properties of batter were analyzed using a rotatory viscosimeter Haake RV1 by means of a flow curve consisting of a ramp-up step from 0 to 500 s^{-1} during 90 s, a holding time of 30 s at 500 s^{-1} and a ramp-down step from 500 to 0 s^{-1} in 90 s. The Ostwald de Waele rheological model was applied to the ascending ramp flow curve, obtaining the consistency index (K), the flow behavior index (n) and

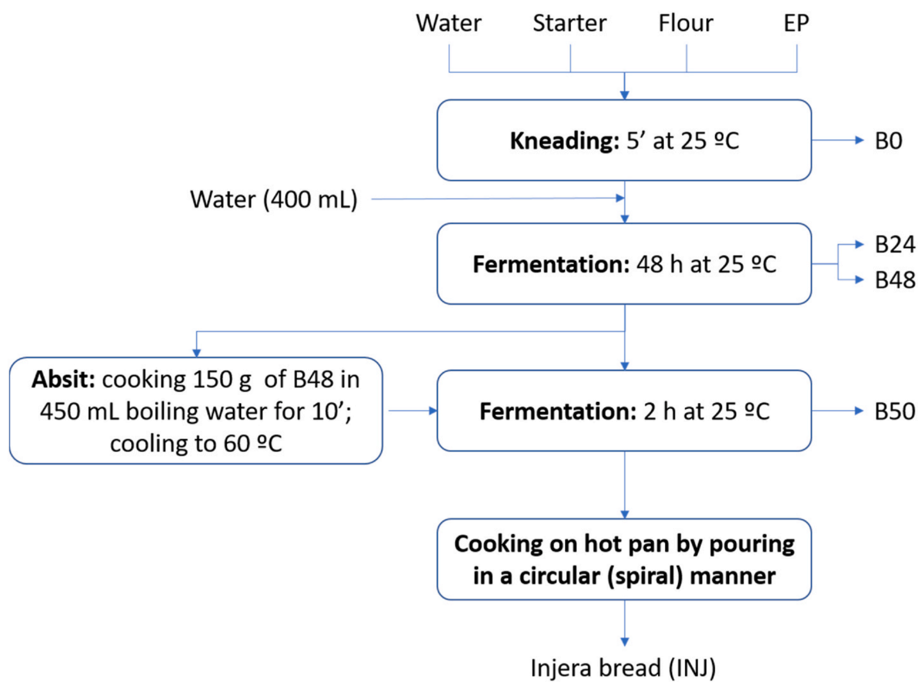

Fig. 1. Injera cooking flowchart. EP refers to eggshell powder; B0, B24, B48, B50 refer to batter samples at 0, 24, 48, & 50 h of fermentation.

dynamic viscosity (η) of the batter at a 100 s^{-1} shear gradient (Eq. (1) and Eq. (2)). Measurements were done in five repetitions.

$$\tau = K \cdot \dot{\gamma}^{n} \text{ (Ostwald de Waele model of flow curve)} \quad \text{Equation 1}$$

$$\eta = K \cdot \dot{\gamma}^{n-1} \text{ (Ostwald de Waele model of viscosity curve)} \quad \text{Equation 2}$$

where: τ is the shearing stress (Pa); K is the consistency index (Pa·s^n); $\dot{\gamma}$ is the shear gradient (s^{-1}); n is the flow behavior index (dimensionless); and η is the dynamic viscosity (Pa·s).

2.4.2. Texture profile analysis (TPA) of injera

TA.XTi plus texture analyzer (Stable Micro Systems; Surrey, UK) was used to measure the mechanical properties of injera through a TPA. Five circular samples (ø 5 cm) were placed together one on top of the other under a flat probe (P/75 compression platen) attached to the testing machine (Kim, Kwak, & Jeong, 2017). The injera samples were compressed until reaching 75% of its initial height, using a 0.049 N trigger force, and a test speed of 2 mm s^{-1}. Samples were left to rest for 5 s before the second compression. After testing, the hardness, springiness, and cohesiveness were calculated by using the software Exponent v6.1.16. Measurements were done in five repetitions.

2.4.3. Color

Color was assessed using the DigiEye Imaging System equipment (VeriVide Ltd.; UK). Five repetitions were done for both batter and injera samples, and CIE L*, a*, and b* coordinates were obtained as described by Etxabide, Kilmartin, and Maté (2021). The color difference (ΔE*) values for fortified injera (I4.5 and I9.0) with respect to control (I0) were calculated according to Eq. (3) as described by Garzon, Skendi, Lazo-Velez, Papageorgiou, & Rosell (2021).

$$\Delta E^{*} = \sqrt{(\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}} \quad \text{Equation 3}$$

2.4.4. Eye formation

Eye qualities of injera were assessed using the softwares DigiEye v.2.8.0.2 (VeriVide Ltd.; UK) and ImageJ v.1.52a (National Institutes of Health, USA) on injera pictures previously taken. DigiEye was used to calculate the proportion between the dark brown color which represent the craters (eyes) and the light color proportion which represent the remaining part (surface). Mean size (area) and eye diameters were obtained by means of ImageJ after image calibration, color to gray transformation and thresholding using the "MaxEntropy" algorithm. Binary function "Fill Holes" was also used to avoid artifacts derived from nested eyes. Five repetitions of the measurements were carried out.

2.5. Microbial analysis

Yeast, lactic acid bacteria, and total mesophilic counts were assessed to study the microbial properties of batter, and injera. Samples were analyzed in duplicate using 10 g following the procedure described by Harth, Van Kerrebroeck, and De Vuyst (2016). To assess the growth of the microorganisms, 0.1 ml of properly diluted sample of batter or injera was spread on pre-dried agar medias. MRS agar with 0.1 g/L of cycloheximide or YEPD with 0.1 g/L of chloramphenicol was used to evaluate the growth of lactic acid bacteria or yeast and molds respectively. The growth of total aerobic mesophilic bacteria was determined on PCA at 30 °C for 72 h (Harth, Van Kerrebroeck, & De Vuyst, 2018). The analysis was done two times and triplicate plates were used.

2.6. Nutritional quality

2.6.1. Total Ca

Total Ca of injera I0, I4.5, and I9.0 was analyzed following the AOAC official method 985.35 (AOAC, 2005a). Dry ashing method was first used to destroy organic matrix in a muffle furnace at 525 °C for 10 h. 1 M HNO_3 was used to aid in the ashing process and to solubilize white ash and to make dilutions. Total Ca was analyzed by inductively coupled plasma atomic emission spectroscopy (ICP - AES) at Universidad de Zaragoza, Spain. The amount of Ca was calculated and expressed as mg Ca/100 g of dried injera. The analysis of total Ca was carried out in duplicate with two samples of injeras processed independently.

2.6.2. Bioavailable Ca

A static *in vitro* simulation of gastrointestinal food digestion nature protocol developed by Brodkorb et al. (2019) was used to perform *in vitro* digestion of injera and thus to measure bioavailable Ca. Simulated stock solutions were prepared and stored at −20 °C until use (Annex Table 1). *In vitro* digestion of the injera at oral (2′), gastric (2 h), and intestinal phase (2 h) was made by incubating in Mini Shaker (VWR, USA) at 200 rpm and at 37 °C, all detail is shown in Annex Table 2.

After the digestion process, centrifugation of the sample was carried out using SIGMA 3K30 Laborzentrifugen GmbH (Germany) centrifuge machine at 3500×*g* at 4 °C for 1 h as described by Cámara, Amaro, Barberá, and Clemente (2005). The supernatant was collected and placed into a 15 ml Falcon and sent to Universidad de Zaragoza for bioavailable Ca analysis. The analysis of total Ca was carried out in duplicate with two samples of injeras processed independently.

2.7. Statistical analysis

All experiments were carried out following a completely randomized design and Analysis of variance (ANOVA) was performed, and multiple mean comparison was done by Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$ using SPSS version 27 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Physicochemical parameters

The pH of batter at different processing stages and pH of injera are shown in Fig. 2. Results indicate that the addition of EP had a significant effect on the pH of both batter and injera. The higher the amount of EP added, the higher the pH. In addition, and was expected for all studied formulations, pH of batter decreased significantly with increased fermentation time. However, there was no significant difference on pH of batter at fermentation times of 48 h and of 50 h. For all formulations, the maximum pH was found in the B0 (batter at 0 h fermentation time) samples. pH of injera was 4.1 in I0 but increased to 5.7 and 6.3 for I4.5 and I9.0 respectively.

The pH of I0 batter after 48 h of fermentation (B3) was found to be 4.1 a value comparable with pH reported by other researchers which was ranged from 3.4 to 4.1 as for similar products (Fischer, Egli, Aeberli, Hurrell, & Meile, 2014). However, the pH of the I4.5 and I9.0 batters after fermenting for 48 h (5.0 and 5.4 respectively) were significantly higher than pH of I0 batter due to the presence of $CaCO_3$. The pH of Control Injera was 4.1 which was similar to the pH reported in previous research wherein the pH value fell between 3.4 and 4.5 (Ashagrie & Abate, 2012). The pH of I4.5 and of I9.0 were 5.7 and 6.3 respectively, which was significantly higher than pH of I0. The increased pH of enriched injera will likely affect its characteristic sour taste and may increase the sweetness due to the presence of eggshell powder. In addition, the increased pH of enriched injera may have an implication on its shelf life since it would allow the growth of higher number of microorganisms.

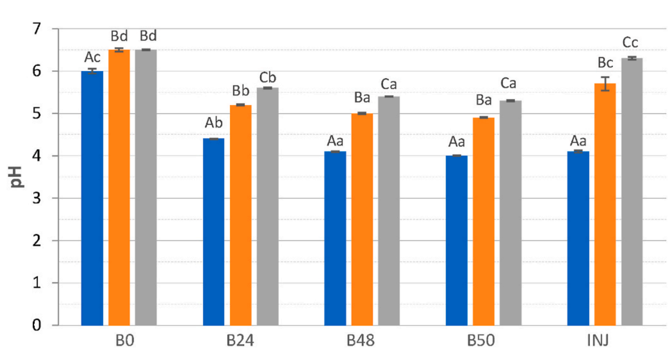

Fig. 2. Effect of eggshell powder on pH of batter (B) after 0, 24, 48 and 50 h of fermentation and injera (INJ). I0: teff flour without eggshell powder (Control); I4.5: teff flour with 4.5% eggshell powder ; I9.0: teff flour with 9.0% eggshell powder . Mean and standard error bars (n = 6). Capital letter shows difference based on formulation and small letter based on fermentation time.

The effect of EP addition on the TTA of batter and injera is shown in Fig. 3. As the fermentation time increased there was an increase in the TTA of the three types of batter. However, no significant effect was observed between samples of B48 and B50 in any batter. The TTA of control batter was the highest at all stages ranging from 3% at the beginning of the fermentation to 11.4% after fermenting for 50 h. Results also indicated that the higher the content of EP, the lower the TTA because of the neutralizing effect of $CaCO_3$. As observed for the decrease in the TTA of injera as the level of the eggshell powder increased also may have an implication on the shelf life of injera because an increase in acidity prevents the growth of microorganisms that cause spoilage.

The moisture content and water activity of batter or injera was not affected significantly due to the addition of EP. The maximum moisture content of batter was found to be 71.5 ± 0.2% (B50). Average moisture content of injera was 60.5 ± 0.8%, similar to injera moisture contents found by Cherie, Ziegler, Fekadu Gemede, Zewdu Woldegiorgis, and Yildiz (2018).

Water activity of batter was very high in all formulations and at all stages, even for injera. This fact is critical for the potential shelf life of this product. In all cases, water activity was above 0.95 allowing the growth of most microorganisms.

3.2. Rheological properties of batter

The viscosity (η_{100}), consistency index (K), and flow behavior index (n) of batter are shown in Fig. 4.

The addition of eggshell powder significantly reduced the viscosity of batter at the beginning of fermentation process (Fig. 4A). As a result of fermentation and addition of water in the process, viscosity dropped noticeably after 24–48 h, and again, after the addition of *absit* and the secondary fermentation step (B50). The reduction of viscosity due to the fermentation was so influential that the effect of the addition of EP was unnoticeable.

A similar pattern was observed in the evolution of the consistency index in time (Fig. 4B). The presence of EP only had a significant effect on K at time 0 h. After that there was a drastic decrease of K due to the fermentation which reduced differences among treatments. The consistency index for the batter at 0 h (B0) were in the range of cake batter (17–40 Pa s^n) previously reported (Noorlaila, Hasanah, Asmeda, & Yusoff, 2020).

The evolution of the flow behavior index (n) of the batter is shown in

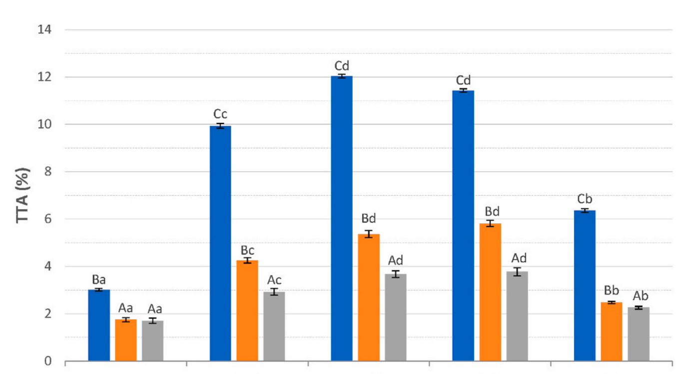

Fig. 3. Effect of eggshell powder on total titratable acidity of batter (B) after 0, 24, 48 and 50 h of fermentation and injera (INJ). I0: teff flour without eggshell powder (Control); I4.5: teff flour with 4.5% eggshell powder ; I9.0: teff flour with 9.0% eggshell powder . Mean and standard error bars (n = 6). Capital letter shows difference based on formulation and small letter based on fermentation time.

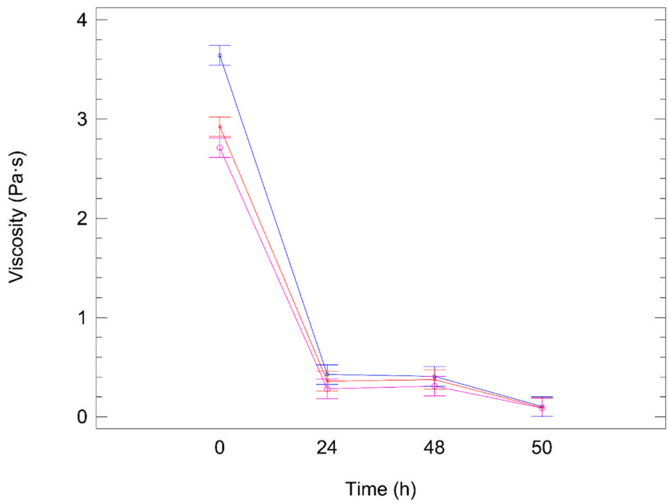

Fig. 4a. Effect of eggshell powder addition on batter viscosity ($\eta_{100}; Pa \cdot s$) during the fermentation process. I0: teff flour without eggshell powder (Control -□-); I4.5 teff flour with 4.5% eggshell powder (-x-); I9.0: teff flour with 9.0% eggshell powder (-o-).

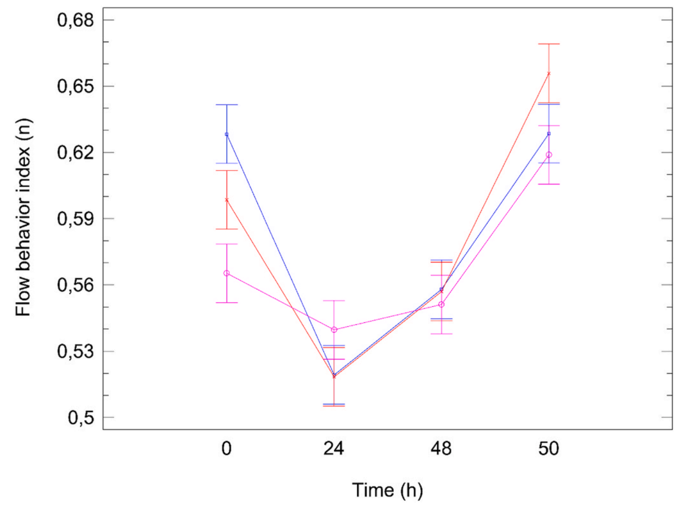

Fig. 4c. Effect of eggshell powder addition on injera batter flow behavior index (n; dimensionless) during the fermentation process. I0: teff flour without eggshell powder (Control -□-); I4.5 teff flour with 4.5% eggshell powder (-x-); I9.0: teff flour with 9.0% eggshell powder (-o-).

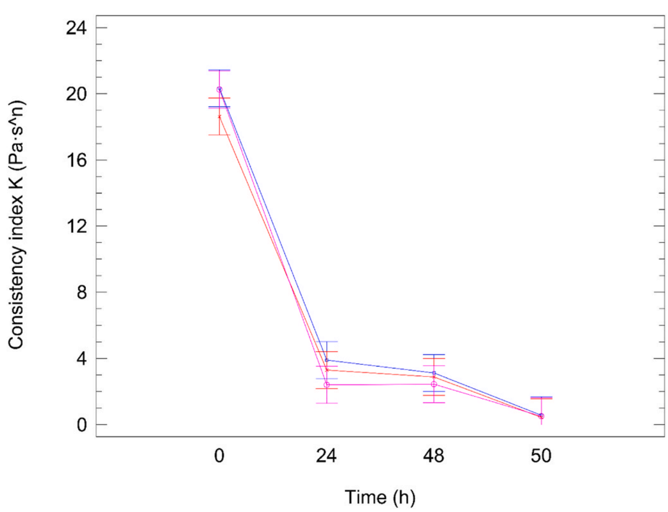

Fig. 4b. Effect of eggshell powder addition on injera batter consistency index (K; Pa·sn) during the fermentation process. I0: teff flour without eggshell powder (Control -□-); I4.5 teff flour with 4.5% eggshell powder (-x-); I9.0: teff flour with 9.0% eggshell powder (-o-).

Fig. 4C. Just after the addition of the eggshell powder, significant differences among the pseudoplastic ($n < 1$) character of all the treatments were observed. At 0 h the non-Newtonian behavior was clearly associated to the proportion of added eggshell powder, making the batter more sensitive to shearing. This effect seemed to disappear after the 24–48 h period of fermentation, in which this parameter significantly decreased for all formulations. Finally, after the addition of *absit*, the batter recovered its initial non-Newtonian character, no matter the level of eggshell powder addition, likely due to an internal relaxation of the structure of the starch granules (gelatinization).

3.3. Texture of injera

The texture parameters of injera obtained from TPA are shown in Table 1. The hardness of injera was affected by the addition of the eggshell powder although springiness and cohesiveness were not affected. The hardness of injera decreased as the level of eggshell powder increased from control injera (I0) to I4.5 and further to I9.0.

Table 1
Effect of eggshell powder on texture of injera. I0: Control (injera without eggshell powder); I4.5: injera with 4.5% eggshell powder; I9.0: injera with 9.0% eggshell powder.

Treatments	Hardness (N)	Springiness	Cohesiveness
I0	90.8 (4.1) C	0.73 (0.1) A	0.53 (0.0) A
I4.5	82.8 (3.5) B	0.74 (0.0) A	0.53 (0.0) A
I9.0	76.1 (5.2) A	0.72 (0.0) A	0.50 (0.0) A

Result of texture analysis as mean ± SD of n = 5 observations of batter and injera. Statistical analysis: ANOVA, significant difference at $P < 0.05$. Difference in capital letters in the same column show significant difference based on addition of eggshell powder.

Similar to our research, the hardness of bread decreased as the level of eggshell powder increased (Chilek et al., 2018). In addition, Bassett et al. (2014) found a decrease in the hardness of bread in the lower crust after Ca fortification with $CaCl_2$ and $CaCO_3$.

Recently, Yasin (2021) reported firmness of injera to be 3.13 N using a sharp blade cutting probe. The value found for hardness (a comparable term for firmness) in the current research is relatively higher due to the different method used to assess hardness of injera (shearing vs compression). Formerly, Yetneberk et al. (2004) conducted another study to evaluate flexion properties of injera using a three-point bending rig; the maximum bending force obtained was 0.14 N. Given the different experimental setup, results cannot be compared.

3.4. Color

The color of batter with different fermentation time, as well as the color of injera are shown in Table 2. Decreases in the lightness (L*) of batter was observed as the level of eggshell powder increased; however, there was no pattern in the characteristics of a* and b* parameters of the batter. There was also a decrease in the lightness of injera as the level of eggshell powder increased from I0 to I4.5 or to I9.0. The decrease in the lightness of injera was 3% for both I4.5 and I9.0 showing no significant differences.

A relatively similar L* value of control injera was found in the current research compared with another research value of 54.95 (Cherie et al., 2018). Chilek et al. (2018) studied the effect of EP on L* of wheat bread and found that lightness of bread decreased as the level of EP increased which is consistent with our study. A similar finding was

Table 2
The effect of eggshell powder on the color of batter and injera of batter (after 0, 24, 48 and 50 h of fermentation) and injera (INJ). I0: Control (injera without eggshell powder); I4.5: injera with 4.5% eggshell powder; I9.0: injera with 9.0% eggshell powder.

		I0	I4.5	I9.0
L*	B0	73.8 ± 0.3 Bb	73.6 ± 0.6 Bb	72.9 ± 0.7 Ab
	B24	78.4 ± 0.1 Cc	76.2 ± 0.9 Bc	75.0 ± 0.5 Ac
	B48	78.9 ± 0.3 Ccd	76.8 ± 0.7 Bc	75.8 ± 0.1 Ac
	B50	79.3 ± 0.3 Cd	769 ± 0.8 Bc	75.7 ± 0.4 Ac
	INJ	53.4 ± 0.1 Ba	51.5 ± 0.1 Aa	51.9 ± 0.1 Aa
a	B0	4.2 ± 0.1 Ab	4.0 ± 0.2 Ab	4.2 ± 0.2 Ab
	B24	4.7 ± 0.1 Bc	3.8 ± 0.3 A b	3.7 ± 0.3 Aa
	B48	3.9 ± 0.2 Aa	3.8 ± 0.2 Ab	3.8 ± 0.4 Aab
	B50	3.8 ± 0.2 Ba	3.2 ± 0.2 Aa	3.5 ± 0.3 Aa
	INJ	7.1 ± 0.2 Bd	7.2 ± 0.2 Bc	6.8 ± 0.2 Ac
b	B0	16.7 ± 0.1 Ca	17.3 ± 0.1 Bb	17.6 ± 0.2 Ac
	B24	17.6 ± 0.2 Cb	16.5 ± 0.3 Ba	16.0 ± 0.4 Aa
	B48	18.3 ± 0.3 Bc	17.5 ± 0.5 Ab	16.9 ± 0.8 Aab
	B50	18.1 ± 0.2 Bc	17.1 ± 0.4 Ab	17.4 ± 1 ABc
	INJ	19.1 ± 0.5 Cd	18.4 ± 0.5 Bc	16.6 ± 0.9 Aab
ΔE*	B0	–	5.2	5.9
	B24	–	2.6	3.8
	B48	–	2.3	3.2
	B50	–	2.7	3.7
	INJ	–	2.4	1.9

Result of color as mean ± SD of n = 5 observations of batter and injera. Statistical analysis: ANOVA, significant difference at $P < 0.05$. Difference in small letters in the column show difference based on fermentation time. Difference in capital letters in the same row show significant difference based on addition of eggshell powder.

observed by Bassett et al. (2014) when replacing 50% NaCl with $CaCO_3$ and $CaCl_2$ observing that the color of wheat bread was darker. The difference in the lightness of injera due to the addition of EP could be due to the difference in the particle size of teff flour and EP powder (Chilek et al., 2018).

Arufe et al. (2018) stated that if ΔE* is greater than 3, a difference in color can be detected by the naked eye. In the current research, for I4.5, and I9.0 injera no visual color difference was observed compared with I0 since ΔE*; the value was below 3.

3.5. Eye formation

The visual quality of injera is determined by the number and size of eyes on its surface. Injera eye parameters related to eye quality are shown in Table 3. Results indicated that the addition of EP only significantly affected eye circularity and did not affect the rest of the eye quality parameters. Total eye area (darker color) was not modified by eggshell powder addition, allowing normal gas bubble formation and subsequent reticulation process. The same conclusions were derived from the analysis of individual eye area and mean eye diameter. However, injera fortified with 4.5% eggshell powder showed a significant lower circularity (more irregular shape), probably related with small differences in the speed of manual spreading of the batter over the hot plate during the cooking process.

Sensory analysis has been used by different researchers to determine injera eye qualities which were expressed as number of eyes and eye size (Agza, Bekelea, & Shiferaw 2018; Yetneberk et al., 2004). Image analysis can be a good approach to sensory perception of eye properties of injera. Thus, Yasin (2021) used the software Injera Eyes, version 1.0.0.0 to determine the number of injera eyes in a fixed surface of 1550 × 1550 pixels, from which an "eye density" can be obtained. Our current research assessed the proportion of dark brown color area (number of pixels darker than a certain threshold) as an approach to the sensory perception of global "eye proportion" perceived by consumers. Complementarily, the present work aimed to obtain a deeper understanding about the morphology of the eyes studying its symmetry and size.

Table 3
Effect of eggshell powder addition on injera eye quality parameters. I0: Control (injera without eggshell powder); I4.5: injera with 4.5% eggshell powder; I9.0: injera with 9.0% eggshell powder.

	Treatment	Mean (±SD)
Total eye area (%)	I0	38.92 (2.53) A
	I4.5	40.18 (2.14) A
	I9.0	40.97 (2.08) A
Individual eye area (mm^2)	I0	5.87 (0.72) A
	I4.5	5.79 (0.77) A
	I9.0	5.61 (0.83) A
Eye diameter[a] (mm)	I0	2.84 (0.16) A
	I4.5	2.86 (0.20) A
	I9.0	2.78 (0.21) A
Eye circularity (minor/major axis ratio)	I0	0.70 (0.022) B
	I4.5	0.67 (0.028) A
	I9.0	0.69 (0.021) B

[a] Mean Feret diameter. Result of injera eye quality as mean ± SD of n = 5 observations of injera. Statistical analysis: ANOVA, significant difference at $P < 0.05$. Difference in capital letters in the same column show significant difference based on addition of eggshell powder.

3.6. Microbial quality

The evolution of the growth of lactic acid bacteria, total aerobic mesophilic bacteria and yeast is shown in Table 4. The results indicated the addition of EP did not have significant effect on the microbial quality. The differences in pH and acidity among batters of the different formulations and the presence of $CaCO_3$ did not significantly affect microbial evolution.

For all formulations, when the fermentation time increased from 0 h to 24 h a significant increase on microbial population was found. However, no significant differences were observed when the fermentation time increased from 24 h onwards.

After 24 h fermentation, lactic acid bacteria and total aerobic mesophilic bacteria counts were found to be higher than those of other research work in which natural fermentation (no inoculation) was used (Fischer et al., 2014). Hassen, Mukisa, Kurabachew, and Desalegn

Table 4
Effect of addition of eggshell powder on the microbial population of batter (after 0, 24, 48 and 50 h of fermentation) and injera. I0: Control (teff flour without eggshell powder); I4.5: teff flour with 4.5% eggshell powder; I9.0: teff flour with 9.0% eggshell powder.

		I0	I4.5	I9.0
Lactic acid bacteria (log cfu/g)	B0	7.2 (0.1) A	7.1 (0.1) A	7.2 (0.1) A
	B24	9.6 (0.1) B	9.6 (0.0) B	9.7 (0.3) B
	B48	9.6 (0.0) B	9.6 (0.1) B	9.6 (0.0) B
	B50	9.5 (0.1) B	9.5 (0.0) B	9.5 (0.0) B
	INJ	ND	ND	ND
Total aerobic mesophilic (log cfu/g)	B0	7.4 (0.1) A	7.3 (0.1) A	7.4 (0.1) A
	B24	9.6 (0.1) B	9.6 (0.1) B	9.8 (0.3) B
	B48	9.6 (0.1) B	9.6 (0.0) B	9.6 (0.0) B
	B50	9.5 (0.1) B	9.6 (0.0) B	9.5 (0.0) B
	INJ	ND	ND	ND
Yeast (log cfu/g)	B0	6.9 (0.0) A	7.0 (0.1) A	7.0 (0.2) A
	B24	7.8 (0.0) B	7.8 (0.1) B	7.7 (0.1) B
	B48	7.8 (0.0) B	7.8 (0.0) B	7.8 (0.0) B
	B50	7.8 (0.1) B	7.8 (0.1) B	7.8 (0.1) B
	INJ	ND	ND	ND

Microbial growth as mean ± SD at different fermentation times: in batter, 0 h (B0), 24 h (B24), 48 h (B48), 50 h (B50); and injera (INJ). Statistical analysis: ANOVA, significant difference at $P < 0.05$. Different in capital letters in the column show difference based on fermentation time.

(2018) also indicated that lactic acid bacteria and yeast did not grow as much when natural fermentation was used as compared with controlled fermentation. In addition, it is likely that flour sterilization performed in our research could have contributed to facilitate microbial growth during fermentation.

3.7. Nutritional quality

The effect of the addition of EP on both total and bioavailable Ca of injera is shown in Table 5. The level of total Ca in injera increased from 235.9 mg/100 g (dry basis, d.b.) in control injera to 2157.9 mg/100 g (d.b.) and 4397.4 mg/100 g (d.b.) for I4.5 and I9.0 respectively. This was expected due to the large $CaCO_3$ content in EP.

Total Ca content in injera I0 in the current research was high as compared with another research finding that presented 61 mg/100 g (d.b.) (Cherie et al., 2018). This could be due to the different processing methods of injera. In other research, after the last stage of fermentation, surface water on top of the batter was thrown. This practice may have decreased the total Ca since some of it may have been lost with the discarded water.

In addition, taking into account injera I0 formulation, total Ca in teff flour used in this study can be also considered higher than the one reported in the literature (141–188 mg/100 g d/b) (Kibatu, Chacha, & Kiende, 2017; Shumoy & Raes, 2017). These differences are likely based on varietal difference of teff grain. Anyhow, the values of Ca in teff were found to be higher as compared to other cereals such as maize (16 mg/100 g), wheat (15.2–39.5 mg/100 g), sorghum (5.0–5.8 mg/100 g) and rice (23 mg/100 g) expressed on d.b (Baye, 2014).

Thus, teff flour and injera could be a potentially better Ca source than other cereals. However, the percentage of bioavailable Ca for the control injera was found to be 6.8% which can be considered very low. The reason for the low level of bioavailable Ca in injera is likely due to the high content of phytates and fiber (Baye, 2014). Anti-nutrients bind micronutrients and make them unavailable for absorption by human body. A study conducted on the bioavailability of Ca in spinach showed it was also very low (1.7%) due to the high content of anti-nutrient oxalate which binds Ca and makes it unavailable for absorption (Cámara et al., 2005). An in-vitro study on the bioavailability of other minerals such as iron and zinc on injera showed that the high level of phytates also affected their bioavailability (Shumoy et al., 2017). In light of all these facts, it is important not only to increase Ca in the diet but also to ensure there is an increase in its bioavailability.

When EP was included in the formulation, bioavailable Ca increased from 16.1 mg/100 g (d.b.) for injera I0, to 742.7 and 1743.1 mg/100 g for injera I4.5 and I9.0 respectively (Table 5). This meant that the percentage of bioavailable Ca increased from 6.8% to almost 40% in both fortified formulations. It is possible that the amount of added Ca in the form of $CaCO_3$ was not affected by the antinutrients that are present in injera. It is also possible that the binding capacity of the phytates was saturated when Ca content was high enough. The high percentage of bioavailable Ca with respect to the total Ca is an indication that eggshell is an efficient source of Ca for fortified diets.

Table 5
Effect of eggshell powder on total and bioavailable calcium of injera. I0: Control (teff flour without eggshell powder); I4.5: teff flour with 4.5% eggshell powder; I9.0: teff flour with 9.0% eggshell powder.

	Total Ca (mg/100 g d.b.)	Bioavailable Ca (mg/100 g d.b.)	Percentage of bioavailable Ca (%)
I0	235.9 ± 1.7 A	16.1 ± 0.4 A	6.8 A
I4.5	2157.9 ± 38.3 B	742.7 ± 48.9 B	34.4 B
I9.0	4397.4 ± 313.72 C	1743.1 ± 164.0 C	39.6 B

Total, bioavailable and percent of bioavailable Ca as mean ± SD of n = 2 observations of injera. Statistical analysis: ANOVA, significant difference at $P < 0.05$. Different in letters in the column show difference based on addition of eggshell powder. d.b.: dry basis.

If it is assumed that the average amount of injera consumed by an Ethiopian adult is about 200 g (wet basis; w.b.), considering that the moisture content of injera is about 60%, the intake of bioavailable Ca with injera would be 12.9 mg/day when injera I0 is included in the diet. If injera I4.5 is consumed instead, the amount of bioavailable Ca would be 594.2 mg/day; and if injera I9.0 is used the amount would turn into 1394.5 mg/day. According to (FAO/WHO, 2005) the RDA and Upper Intake Level (UL) of Ca range from 1000 to 3000 mg/day. Thus, with the consumption of 200 g of injera, an adult will get 60% of the minimum daily requirement of Ca when consuming injera I4.5 and 140% of the total RDA of Ca when consuming injera I9.0. Extra Ca intake is known to minimize the risk of chronic diseases such as hypertension if the upper intake limit is not reached. In addition, Ethiopian Great Rift Valley areas are highly affected by fluorosis which has affected bone and teeth health. In this region, it is especially important to maintain high levels of Ca intake to reduce the health consequences related to fluorosis (Patel et al., 2017; Srivastava, Singh, Tripathi, & Mathur, 2017). Moreover, the life expectancy of Ethiopians is increasing; thus, there are more elderly people in the country. This group is highly susceptible to osteoporosis and likely to be at risk in the future also, if the diet does not change. Thus, there is a need to increase Ca in the diet of the population to reduce the risk of osteoporosis among the elderly, now and in the future. The use of eggshell powder as an additive in the process of injera could be part of the solution.

In addition, it is recommended that for Ca availability and utilization, instead of taking a large amount of Ca at a time it is better to take smaller amounts at different times throughout the day. Bioavailability of Ca has been shown to increase when it is taken with food instead of on an empty stomach (Rafferty et al., 2007). The tradition of consuming injera at least twice a day in a small size portion instead of consuming a large amount at one time is advantageous with respect to the use of Ca by the body.

4. Conclusion

Even though the total Ca in injera is high, its contribution to the nutritional Ca requirement is minimal since the level of bioavailable Ca is very low in this foodstuff. The current research revealed that the addition of eggshell powder is an excellent source of total and bioavailable Ca. Adding 4.5% or 9% of eggshell powder to the teff flour drastically increased the absolute and relative amounts of bioavailable Ca. Furthermore, even though the addition of eggshell powder significantly affected both pH and acidity of the batter and injera, it did not significantly affect microbial evolution in the batter, nor the physical quality parameters of injera including texture, eye parameters and color.

The addition of EP to injera formulation can be considered a new tool to improve the Ethiopian diet. Clinical studies are needed to confirm the actual benefit of the enrichment. In addition, sensory acceptability of injera with EP for Ethiopian population will have to be addressed in the future.

CRediT authorship contribution statement

Tigist Fekadu: Conceptualization, Formal analysis, Writing – original draft, Conception or design of the work, Data collection, Data analysis and interpretation, Drafting the article, Critical revision of the article, Final approval of the version to be published. **Angela Cassano:** Formal analysis, Data collection, Data analysis and interpretation, Final approval of the version to be published. **Ignacio Angós:** Formal analysis, Data analysis and interpretation, Critical revision of the article, Final approval of the version to be published. **Juan Ignacio Maté:** Conceptualization, Conception or design of the work, Critical revision of the article, Final approval of the version to be published.

Declaration of interests

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.

Annex Table 1
Volumes of electrolyte stock solutions of digestion fluids for a volume of 400 ml diluted with water

Solution	Stock concentrations	Volume (mL) to be taken for electrolyte solution preparation 400 ml (for each fluid)		
	(M)	SSF	SGF	SIF
KCl	0.5	15.1	6.9	6.8
KH_2PO_4	0.5	3.7	0.9	0.8
$NaHCO_3$	1.0	6.8	12.5	42.5
NaCl	2.0	–	11.8	9.6
$MgCl_2(H_2O)6$	0.15	0.5	0.4	1.1
$(NH_4)_2CO_3$	0.5	0.06	0.5	–
$CaCl_2(H_2O)_2$	0.3	0.025	0.005	0.02
pH **adjustment solutions**				
HCl	6	0.09	1.3	0.7
pH		7	3	7

Annex Table 2
In vitro digestion of injera

Digestion phase	Oral (SSF), pH 7	Gastric (SGF) pH 3	Intestinal (SIF) pH 7
Food or digest	5 g of control injera	10 mL from oral phase	20 mL from gastric phase
Electrolyte stock solutions (mL)	4	8	8
CaCl2(H2O)2 (0.3 M) (mL)	0.025	0.005	0.04
Enzymes	Salivary amylase	Pepsin	Trypsin in Bile salts pancreatin
Enzyme activity (U/mL) or bile concentration (mM) in total digesta (final volume in milliliters at each digestion phase, see row below)	75 U/mL	2000 U/mL	100U/mL 10 mM
Specific activity (U/mg), concentration (bile) mmol/g	5 U/mg	3500 U/mg	6 U/mg 0.667 mmol/g
Concentration of enzyme/bile solution (mg/mL)	200	0.019	133.3 6.25
Volume of enzyme/bile to be added (mL)	0.75	0.667	5 3
Type of injera sample	I0 I4.5 I.9	I0 I4.5 I9.0	I0 I4.5 I9.0
H_2O (mL)	0.129 0.195 0.208	1.168 0.9 0.758	3.86 3.865 3.869
HCl (5 M) for pH adj. (mL)	- - -	0.16 0.428 0.57	- - -
NaOH (5 M) for pH adj. (mL)	0.096 0.03 0.017	- - -	0.1 0.095 0.091
Final volume (mL)	10	20	40

Acknowledgements

Authors wish to mention Women for Africa Foundation and International Relations and Cooperation Office from Universidad Pública de Navarra for the financial support of Mrs. Fekadu.

References

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

Amare, B., Moges, B., Moges, F., Fantahun, B., Admassu, M., Mulu, A., et al. (2012). Nutritional status and dietary intake of urban residents in Gondar, Northwest Ethiopia. *BMC Public Health, 12*(1). https://doi.org/10.1186/1471-2458-12-752

AOAC. (2005a). Mineral in infant formula, enteral products, and pet foods. *Official Methods of Analysis of AOAC International, 514*, 1–3.

AOAC. (2005b). pH of bread. *Official Methods of Analysis of AOAC International*, 02.

Arufe, S., Della Valle, G., Chiron, H., Chenlo, F., Sineiro, J., & Moreira, R. (2018). Effect of brown seaweed powder on physical and textural properties of wheat bread. *European Food Research and Technology, 244*(1), 1–10. https://doi.org/10.1007/s00217-017-2929-8

Ashagrie, Z., & Abate, D. (2012). Improvement of injera shelf life through the use of. *African Journal of Food, Agriculture, Nutrition and Development, 12*(5), 6409–6423.

Bassett, M. N., Pérez-Palacios, T., Cipriano, I., Cardoso, P., Ferreira, I. M., Samman, N., et al. (2014). Development of bread with NaCl reduction and calcium fortification: Study of its quality characteristics. *Journal of Food Quality, 37*(2), 107–116. https://doi.org/10.1111/jfq.12079

Baye, K. (2014). *Synopsis : Teff : Nutrient composition and health benefits*. Ethiopia Strategy Support Program. October, 2013–2014.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., et al. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Brun, L. R., Lupo, M., Delorenzi, D. A., Di Loreto, V. E., & Rigalli, A. (2013). Chicken eggshell as suitable calcium source at home. *International Journal of Food Sciences & Nutrition, 64*(6), 740–743. https://doi.org/10.3109/09637486.2013.787399

Cámara, F., Amaro, M. A., Barberá, R., & Clemente, G. (2005). Bioaccessibility of minerals in school meals: Comparison between dialysis and solubility methods. *Food Chemistry, 92*(3), 481–489. https://doi.org/10.1016/j.foodchem.2004.08.009

Cherie, Z., Ziegler, G. R., Fekadu Gemede, H., Zewdu Woldegiorgis, A., & Yildiz, F. (2018). Optimization and modeling of teff-maize-rice based formulation by simplex lattice mixture design for the preparation of brighter and acceptable injera. *Cogent Food & Agriculture, 4*(1), 1443381. https://doi.org/10.1080/23311932.2018.1443381

Chilek, T. Z. T., Kairuaman, N. A., Ahmad, F., Wahab, R. A., Zamri, A. I., & Mahmood, A. (2018). Development of white bread fortified with calcium derived from Eggshell powder. *Malaysian Applied Biology, 47*(6), 29–39.

Cilla, A., López-García, G., & Barberá, R. (2018). In vitro bioavailability of iron and calcium in cereals and derivatives: A review. *Food Reviews International, 34*(1), 1–33. https://doi.org/10.1080/87559129.2016.1210631

Daengprok, W., Garnjanagoonchorn, W., & Mine, Y. (2002). Fermented pork sausage fortified with commercial or hen eggshell calcium lactate. *Meat Science, 62*(2), 199–204. https://doi.org/10.1016/S0309-1740(01)00247-9

Etxabide, A., Kilmartin, P. A., & Maté, J. I. (2021). Color stability and pH-indicator ability of curcumin, anthocyanin and betanin containing colorants under different storage conditions for intelligent packaging development. *Food Control, 121*. https://doi.org/10.1016/j.foodcont.2020.107645

FAO/WHO Expert Consultation. (2005). *Human vitamin and mineral requirements* (2nd ed., p. 341). Geneva: World Health Organization http://apps.who.int/iris/handle/10665/42716.

Fischer, M. M., Egli, I. M., Aeberli, I., Hurrell, R. F., & Meile, L. (2014). Phytic acid degrading lactic acid bacteria in tef-injera fermentation. *International Journal of Food Microbiology, 190*, 54–60. https://doi.org/10.1016/j.ijfoodmicro.2014.08.018

Garzon, R., Skendi, A., Antonio Lazo-Velez, M., Papageorgiou, M., & Rosell, C. M. (2021). Interaction of dough acidity and microalga level on bread quality and antioxidant properties. *Food Chemistry, 344*, 128710. https://doi.org/10.1016/j.foodchem.2020.128710

Gharibzahedi, S. M. T., & Jafari, S. M. (2017). The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Science & Technology, 62*, 119–132. https://doi.org/10.1016/j.tifs.2017.02.017

Harth, H., Van Kerrebroeck, S., & De Vuyst, L. (2016). Community dynamics and metabolite target analysis of spontaneous, backslopped barley sourdough fermentations under laboratory and bakery conditions. *International Journal of Food Microbiology, 228*, 22–32. https://doi.org/10.1016/j.ijfoodmicro.2016.04.011

Harth, H., Van Kerrebroeck, S., & De Vuyst, L. (2018). Impact of process conditions on the microbial community dynamics and metabolite production kinetics of teff sourdough fermentations under bakery and laboratory conditions. *Food Sciences and Nutrition, 6*(6), 1438–1455. https://doi.org/10.1002/fsn3.690

Hassen, Y., Mukisa, I. M., Kurabachew, H., & Desalegn, B. (2018). Evaluation of yeast and lactic acid bacteria starter cultures for the production of rice injera. *Journal of Food Processing & Technology*. https://doi.org/10.4172/2157-7110.1000721, 09(03).

Hofmeyr, G. J., Duley, L., & Atallah, A. (2007). Dietary calcium supplementation for prevention of pre-eclampsia and related problems: A systematic review and commentary. *BJOG: An International Journal of Obstetrics and Gynaecology, 114*(8), 933–943. https://doi.org/10.1111/j.1471-0528.2007.01389.x

Kibatu, G., Chacha, R., & Kiende, R. (2017). Determination of major, minor and trace elements in tef using portable total X-ray fluorescence (TXRF) spectrometer. *EC Nutrition, 1*, 51–59.

Kim, S., Kwak, H. S., & Jeong, Y. (2017). Effect of water roux starter (tangzhong) on texture and consumer acceptance of rice Pan bread. *Journal of Texture Studies, 48*(1), 39–46. https://doi.org/10.1111/jtxs.12204

Misci, C., Taskin, E., Dall'Asta, M., Fontanella, M. C., Bandini, F., Imathiu, S., et al. (2021). Fermentation as a tool for increasing food security and nutritional quality of indigenous African leafy vegetables: The case of Cucurbita sp. *Food Microbiology, 99*. https://doi.org/10.1016/j.fm.2021.103820

Noorlaila, A., Hasanah, H. N., Asmeda, R., & Yusoff, A. (2020). The effects of xanthan gum and hydroxypropylmethylcellulose on physical properties of sponge cakes. *Journal of the Saudi Society of Agricultural Sciences, 19*(2), 128–135. https://doi.org/10.1016/j.jssas.2018.08.001

Omi, N., & Ezawa, I. (1998). Effect of Egg-Shell Ca on Preventing after Ovariectomy and doing moderate physical activity are necessary been sufficient in Japan , the Ca intake in particular has never been sufficient (Ministry of Health and Welfare 1997). The Ca intake has nearly re. *Journal of Home Economics, 49*(3), 277–282.

Patel, P., Patel, P., Zulf, M., Yagnik, B., Kajale, N., Mandlik, R., et al. (2017). Association of dental and skeletal fluorosis with calcium intake and serum Vitamin D concentration in adolescents from a region endemic for fluorosis. *Indian Journal of Endocrinology and Metabolism, 21*(1), 190–195. https://doi.org/10.4103/2230-8210.196013

Pettifor, J. M. (2004). Nutritional rickets: Deficiency of vitamin D, calcium, or both? *The American Journal of Clinical Nutrition, 80*(6 Suppl), 1725–1729. https://doi.org/10.1093/ajcn/80.6.1725s

Rafferty, K., Walters, G., & Heaney, R. P. (2007). Calcium fortificants: Overview and strategies for improving calcium nutriture of the U.S. population. *Journal of Food Science, 72*(9). https://doi.org/10.1111/j.1750-3841.2007.00521.x

Schaafsma, A., Pakan, I., Hofstede, G. J. H., Muskiet, F. A. J., Van Der Veer, E., & De Vries, P. J. F. (2000). Mineral, amino acid, and hormonal composition of chicken eggshell powder and the evaluation of its use in human nutrition. *Poultry Science, 79*(12), 1833–1838. https://doi.org/10.1093/ps/79.12.1833

Shumoy, H., Lauwens, S., Gabaza, M., Vandevelde, J., Vanhaecke, F., & Raes, K. (2017). Traditional fermentation of tef injera: Impact on in vitro iron and zinc dialysability. *Food Research International, 102*(June), 93–100. https://doi.org/10.1016/j.foodres.2017.09.092

Shumoy, H., & Raes, K. (2017). Tef: The rising ancient cereal: What do we know about its nutritional and health benefits? *Plant Foods for Human Nutrition, 72*(4), 335–344. https://doi.org/10.1007/s11130-017-0641-2

Srivastava, A. K., Singh, A., Tripathi, P., & Mathur, A. (2017). Prevalence of dental fluorosis and the role of calcium supplementation in its prevention. *Journal of Medical Sciences, 17*(4), 156–161. https://doi.org/10.3923/jms.2017.156.161

Tezera, F., Whiting, S. J., & Gebremedhin, S. (2017). Dietary calcium intake and sunlight exposure among children aged 6-23 months in Dale Woreda, Southern Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development, 17*(3), 12427–12440. https://doi.org/10.18697/ajfand.79.16510

Yasin, U. A. (2021). Textural and eye qualities of injera prepared from quality protein maize and teff blended flours. *International Journal of Nutrition and Food Sciences, 10*(4), 78–85. https://doi.org/10.11648/j.ijnfs.20211004.11

Yetneberk, S., De Kock, H. L., Rooney, L. W., & Taylor, J. R. N. (2004). Effects of sorghum Cultivar on injera quality. *Cereal Chemistry, 81*(3), 314–321. https://doi.org/10.1094/CCHEM.2004.81.3.314