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Research article

# Folate content of a staple food increased by fermentation of a cereal using selected folate-producing microorganisms

Aynadis Tamene [a,*], Kaleab Baye [a], Christèle Humblot [b,c]

[a] Center for Food Science and Nutrition, Addis Ababa University, P.O. Box 150201, Addis Ababa, Ethiopia
[b] IRD, Montpellier, France
[c] UMR QualiSud, Université de Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de la Réunion, Montpellier, France

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ABSTRACT

Folate deficiencies are widespread in Africa due to predominantly cereal-based diets. The objective of this work was to test the feasibility of using folate-producing microorganisms to increase folate content of tef *injera*, a traditional Ethiopian fermented staple food. To this end, a strain of *Lactobacillus plantarum* previously isolated from fermented tef batter and a commercial *Saccharomyces cerevisiae* were used alone and in combination to prepare *injera*. Ten successive fermentations using backslopping from the fermented batter prepared with *L. plantarum* inoculation were performed to mimic the traditional backslopping. The highest folate content was obtained with *S. cerevisiae* (53.5 μg/100 g fresh material). All the combinations were efficient and could cover up to 22 % of the recommended nutrient intakes. All *injera* prepared with selected inoculums were preferred by sensory panelists to the traditional one. This work demonstrates the possibility to increase folate intake using folate-producing microorganisms in the conditions normally encountered in households.

## 1. Introduction

Unlike plants, fungi and many microorganisms, humans cannot synthesize folate and consequently depend entirely on dietary sources including legumes, fruits, green leafy vegetables and dairy products to prevent nutritional deficiencies (Eitenmiller et al., 2016). The recommended nutrient intake (RNI) of folate for an adult is 200–400 μg whereas for pregnant women, it is 400–600 μg (FAO/WHO, 2004). Suboptimal folate intake leads to deficiencies with potentially serious health consequences such as neural tube defects in offspring or megaloblastic anemia (Bailey and Gregory, 2006; Moore et al., 2003).

Folic acid supplementation and fortification have been proposed as ways to increase folate intake and some countries have established mandatory fortification of cereal flours with synthetic folic acid (Burgess et al., 2009). But despite the observed beneficial effects of folic acid supplementation in preventing pathologies associated with folate deficiency, there are concerns over the possible adverse effects of large-scale fortification on subpopulations, as it can mask vitamin B12 deficiency (Morris et al., 2010). Although the results have been inconsistent, some studies have linked excess folic acid intake with increased risk of cancer (Cuskelly et al., 2007). In contrast, such concerns have not been reported for natural forms of folate found in foods or produced by microorganisms during processes like fermentation (Field and Stover, 2018).

Certain lactic acid bacteria (LAB) and yeasts have the ability to synthesize folate in culture medium as well as during food fermentation (Levit et al., 2020). Most previous studies on enhancing folate level using food grade microorganisms have focused on dairy products. But recently, interest in increasing the folate content of cereal-based fermented food using microorganisms has increased (Saubade et al., 2017).

Cereal-based staple foods are widely consumed in many African countries where in most cases it undergoes a fermentation step (Guyot, 2012). For example, *injera* is a fermented staple food consumed by the wider population in Ethiopia (Baye et al., 2013). *Injera* is usually prepared from tef (*Eragrostis tef*), a cereal crop native to Ethiopia (Yetneberk et al., 2004). It has been shown that both LAB and yeasts are involved in tef fermentation in the preparation of *injera* (Fischer et al., 2014). Among yeasts, *Saccharomyces cerevisiae* has been shown to be among the dominant groups in tef fermentation (Koricha et al., 2020).

We recently showed that *Lactobacillus plantarum* P2R3FA isolated from tef dough was able to synthesize significant amounts of folate in folate-free culture medium. In addition, administration of the lyophilized cells of the *L. plantarum* P2R3FA to folic acid-deficient rats significantly

* Corresponding author.
*E-mail address:* aynadis.tamene@aau.edu.et (A. Tamene).

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increased their serum folate concentration (Tamene et al., 2019a). But as indicated by different authors, studies in culture media do not necessarily accurately predict the folate synthesis capabilities of microorganisms in food (Saubade et al., 2017). Many yeasts like *Saccharomyces* spp., some representatives of *Candida*, *Debaryomyces*, *Kodamea*, *Metchnikowia* and *Wickerhamiella* are also known to efficiently synthesize folate, and a few studies have shown that co-culturing with folate-producing LAB effectively increases the folate concentration of fermented cereals, but organoleptic quality was not always assessed (Greppi et al., 2017a; Kariluoto et al., 2014; Korhola et al., 2014). *S. cerevisiae* could be used as an alternative starter of tef fermentation if proven to increase folate production without negatively impacting the sensory acceptability of *injera* when used alone or co-cultured with *L. plantarum* P2R3FA.

*Injera* preparation include a 3-4-day fermentation step that usually includes backslopping (inoculation with a leftover from a previous successful spontaneous fermentation, called *ersho*). Experience showed that backslopping accelerated the initial fermentation phase and controlled desirable changes (Holzapfel, 1997). High folate content variability has been observed in *injera* (Tamene et al., 2019b), partly due to the folate consuming or synthesizing ability of actors of fermentation. In that specific study we have showed the possibility of increasing the average folate content of tef flour (58.7 μg/100 g DM) by the action of fermentation (Tamene et al., 2019b). The use of selected folate-producing microorganisms could help maximize the folate content of the food, but may not be possible in all contexts. The application of selected starters followed by periodic backslopping is consequently an option that can be adapted for use in different contexts, including in the home (Siragusa et al., 2009). The number of cycles required to maintain a high concentration of folate resulting from the inoculation of selected LAB thus needs to be tested.

Fermentation can drastically modify the organoleptic properties of foods (Charalampopoulos et al., 2002). Any modification in the preparation of *injera*, especially the choice of the starter cultures, could thus influence the organoleptic properties of the end product (Holzapfel, 1997). The acceptability of folate-enriched *injera* fermented with folate-producing microorganisms also needs to be investigated.

The objective of this work was thus to test the feasibility of increasing total folate concentrations in tef *injera* by using folate-producing *L. plantarum* P2R3FA previously isolated from tef dough and commercial *S. cerevisiae* as starter cultures of fermentation. The two strains were tested alone and in combination to mimic traditional fermentation. Folate content was measured and compared to *injera* prepared using the traditional method. The ability of *L. plantarum* P2R3FA to maintain continual production of folate was tested using ten successive cycles of backslopping from the dough initially fermented with *L. plantarum* P2R3FA. The sensory profiles of *injera* prepared using the different inoculums were also evaluated.

## 2. Material and methods

### 2.1. Chemicals and raw materials

Unless something else specified, all the chemicals utilized for this study were obtained from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). The raw material (tef grain) was obtained from Debre Zeit Agricultural Research Center (Debrezeit, Ethiopia).

### 2.2. Preparation of injera using the different inoculums

#### 2.2.1. Preparation of the inoculums

Five different inoculums were prepared. A leftover from a previous successful spontaneous fermentation batch *(ersho)* was collected in a household and used as inoculum to prepare traditional *injera* as control.

The folate producing *L. plantarum* P2R3FA, previously isolated from fermented tef batter (Tamene et al., 2019a) was cultivated by streaking the strain conserved at -80 °C in De Man, Rogosa, and Sharpe (MRS) broth and glycerol (40%) on MRS agar and incubated at 30 °C for 48 h. A colony was collected from each pure culture plate, grown in MRS broth (24 h, 30 °C), and centrifuged (14,000 x g, 7 min). The pellets were washed twice with the same volume (9 mL) of sterile saline solution (0.9 % NaCl) and re-suspended in the same volume of solution. The final suspension contained around $10^9$ colony-forming units (cfu)/mL. The inoculum for *injera* fermented with the folate-producing LAB was prepared by mixing this suspension with tef flour (1:1) (v/w).

A sample of dough was taken from the above fermentation (cycle 1) and used for the 2nd fermentation and another sample of dough was also taken from the 2nd fermentation and used for the 3rd fermentation and the procedure went up to ten successive batches. The samples are referred to by their cycle number (cycle 1, cycle 2, etc. up to cycle 10).

The inoculum using commercial *S. cerevisiae* was prepared by mixing sterile tap water and tef flour (1:1) (v/w) to which commercial *S. cerevisiae* powder (manufactured in France by S.I. Lesaffre with the strain 'saf-instant') was added as per the manufacturer's instructions (5 g powder to 2 Kg of flour).

The combination of *L. plantarum* P2R3FA and *S. cerevisiae* inoculum was prepared by mixing saline solution containing *S. cerevisiae* and *L. plantarum* P2R3FA (equal volume) and tef flour (1:1) (v/w). Both *L. plantarum* and commercial *S. cerevisiae* are generally recognized as safe microorganisms as identified by the United states food and drug administration.

#### 2.2.2. Preparation of injera

The traditional flow chart for preparing *injera* was adapted and is shown in Figure 1. Briefly, first whole tef grains were processed into flour, then dough was made by mixing tef flour, sterile tap water and inoculums (4:5:1) (w/v/v). Next, sterile tap water was added to cover the surface of the batter, and the mixture was left to ferment for 4 days at room temperature (1st stage fermentation). After the 1st stage of fermentation, the supernatant on the surface of the batter was disposed and supplanted with equal volume of fresh sterile tap water. Then 1/11th of the fermented batter was mixed with sterile tap water (1:3) (v/v), boiled for about 10 min and cooled to a temperature around 45 °C. The resulting product (*absit*) was added back to the remaining fermented batter to enhance proper fermentation. Together with the *absit*, sterile tap water was added to the dough (1:8) (v/v) for thinning purpose. The batter was allowed to ferment for 2 h at 25 °C until gas production was visible (2nd phase fermentation). At long last, the fermented liquid batter (450 mL) was draw off onto a hot clay, enclosed and baked for 2 min. The final flat pancake-like product is known as *injera*. Three bakings were performed for each inoculum mentioned under section 2.2.1.

#### 2.2.3. Sampling

During the preparation of *injera*, the pH of the batter was measured before and after the first and second fermentation stages. Fermented batter was sampled to measure dry matter and folate contents before the addition of *absit*, and the *injera* was also sampled to measure dry matter and folate contents and subjected to sensory analysis. Dry matter was analyzed by oven drying at 105 °C. pH was measured using an aliquot of dough immediately after diluting it with deionized water (1:1) (v/v).

### 2.3. Folate analysis

The total folate contents of tef dough and *injera* were analyzed in triplicate using the reference microbiological assay, as described previously (Kariluoto et al., 2004; Tamene et al., 2019b). Total folate content was analyzed using the growth of *Lactobacillus rhamnosus* ATCC 7469 as a folate-dependent test organism and (6S)-5-formyltetrahydrofolate (5-HCO-$H_4$ folate) as the calibrant. Method performance was affirmed by testing a blank sample and certified reference material (BCR-121 wholemeal flour) in each set of samples. Only folate contents in the range of certified value (500 ± 70 ng/g dry matter) were accepted. In addition, for triplicate samples, folate content variations ˃10 % were not accepted.

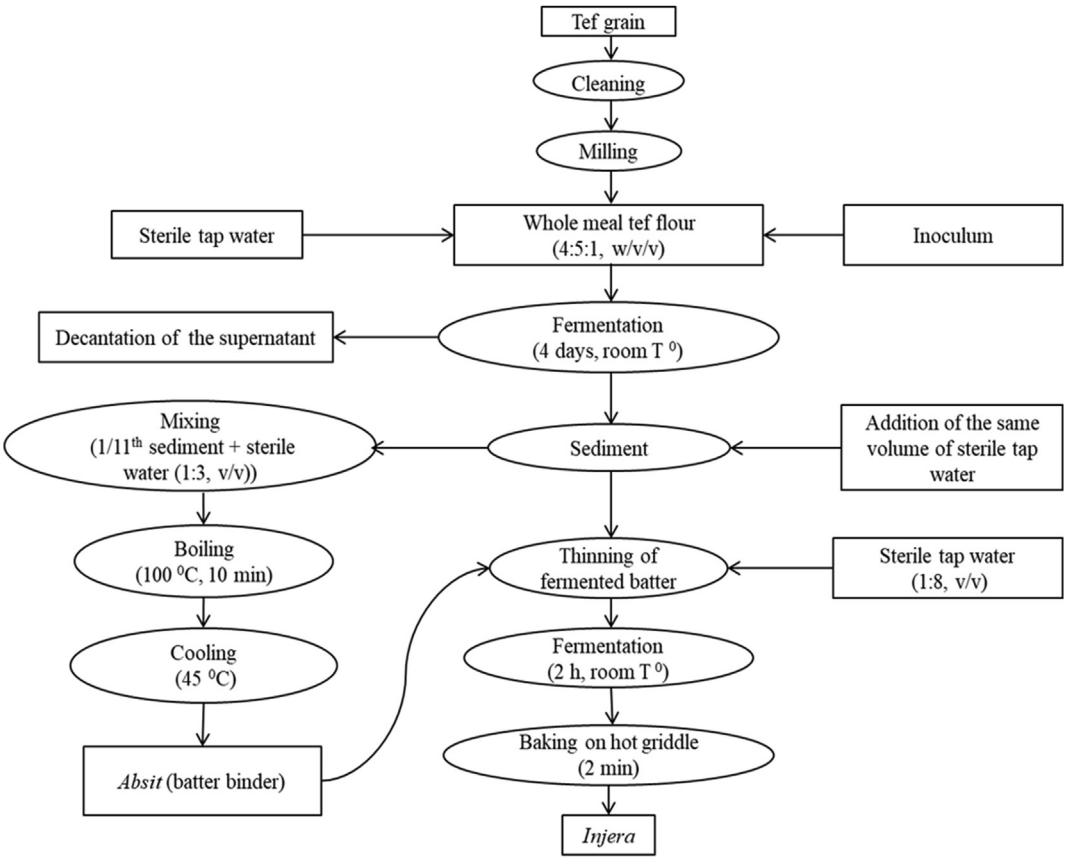


**Figure 1.** Flow chart of processing of tef into *injera* using different inoculums. Inoculums:
- *Ersho* (traditional backslopping)
- Folate producing *L. plantarum* P2R3FA
- Backslopping from the fermented batter inoculated with *L. plantarum* P2R3FA (1–10 cycles)
- Commercial *S. cerevisiae*
- Combination of *L. plantarum* P2R3FA and *S. cerevisiae.*

### 2.4. Contribution of consumption of tef injera made with different inoculums to the recommended nutrient intake (RNI) of folate

Results of Ethiopian National Food Consumption survey (EPHI, 2013) was referred to estimate the contribution of consumption of tef *injera* made with different inoculums to RNI of folate for children aged 1–3, and women of reproductive age. These population groups are prioritized as they are more vulnerable to risk of folate deficiency.

### 2.5. Acceptability studies

The acceptability and sensory profile of the prepared *injera* was judged by 30 adult healthy volunteers (women of reproductive age). The volunteer panelists were selected at random. They were instructed to evaluate all types of *injera* on the basis of appearance, color, flavor, taste, texture, and overall acceptability using a nine-point hedonic scale where 1 = liked extremely and 9 = disliked extremely. They were also instructed to rinse their mouth with water between each sample. Informed consent was obtained from all participants in the study and ethical approval was obtained from the Institutional Review Board of the College of Natural and Computational Sciences of Addis Ababa University.

### 2.6. Statistical analysis

Statistical analysis of folate and sensory acceptability of *injera* were computed using SPSS Version 20. Differences between means of folate values and sensory attribute scores were assessed using one way-analysis of variance (ANOVA) and Tukey's post hoc test. Significant mean differences were considered with a $P$ value $\leq 0.05$.

## 3. Results

### 3.1. pH of dough

The pH of the initial batter was within the extend of 5.7–6.2 with an average of 5.9 ± 0.2. In batter obtained after 1st stage fermentation, the pH ranged from 3.5 to 4.4 with an average of 3.8 ± 0.3. In batter obtained after 2nd stage fermentation, the pH ranged from 3.4 to 4.1 with an average of 3.6 ± 0.2. There was no significant difference between pH of the dough as result of inoculant differences.

### 3.2. Folate content of tef dough fermented with different inoculums

The average total folate content of dough fermented with different inoculums ranged from 52 ± 12 to 169 ± 11 μg/100 g DM (Figure 2). All the samples prepared with the selected inoculums contained higher concentrations of folate than the samples prepared using the traditional process with *ersho*. Inoculation with *L. plantarum* P2R3FA produced dough with an average folate concentration of 113 ± 5 μg/100 g DM. The different cycles of backslopping from the fermented batter inoculated with *L. plantarum* P2R3FA led to dough with high concentrations of folate, ranging from 107.5 ± 3–135.4 μg/100 g DM. The highest concentration of folate was measured in dough fermented with *S. cerevisiae*

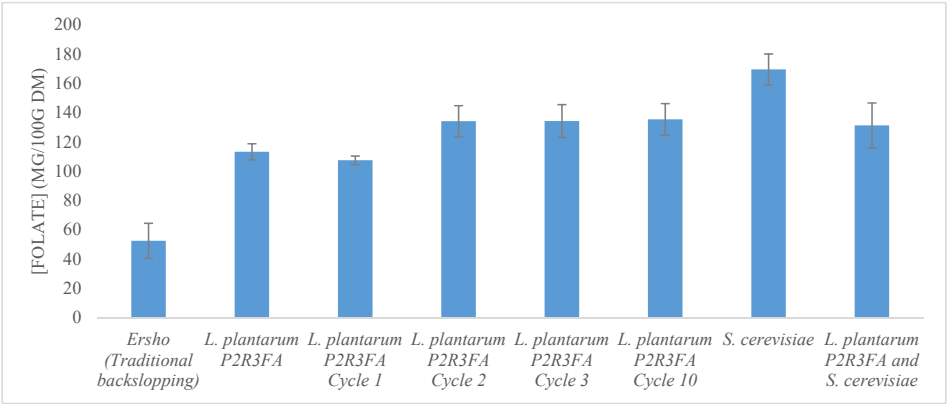


**Figure 2.** Total folate content of tef dough in μg/100 g DM, after 4 days of fermentation. Values are means and standard deviations of triplicate determinations. Bars represent the standard deviation among replicates. Significant differences were determined at $p<0.05$.

alone, with an average folate concentration of 169 ± 11 μg/100 g DM. The concentration of folate in dough made by inoculating a combination of *L. plantarum* P2R3FA and *S. cerevisiae,* was in the same range as that with the different cycles of backslopping from the batter fermented with *L. plantarum* (131 ± 15 μg/100 g DM).

### 3.3. *Folate content of tef* injera *fermented with different inoculums and their contribution to RNI*

The mean total folate contents of tef *injera* made using different inoculums and its contribution to folate RNI were calculated based on fresh weight of *injera* and are listed in Table 1. The folate content of *injera* prepared using the different inoculums followed the same trend as that observed in the fermented batter. The average total folate content of tef *injera* produced with different inoculums ranged from 14.3 to 53.5 μg/100 g fresh matter (FM). The lowest folate concentration was measured in *injera* prepared using the traditional process and the highest average total folate concentration was measured in *injera* made using *S. cerevisiae* alone. Inoculation with *L. plantarum* P2R3FA or with the different cycles of backslopping resulted in intermediate concentrations of folate ranging from 33.5 ± 3.1 to 40.2 ± 5.4 μg/100 g FM. The concentration of folate in *injera* made with the combination of *L. plantarum* P2R3FA and *S. cerevisiae* was 45.3 ± 1.7 μg/100 g FM.

The average tef *injera* consumption (66 g/d for children and 202 g/d for women of reproductive age) were used to estimate its contribution to folate requirements. The RNI of folate was 150 μg/d for children and 400 μg/d for women of reproductive age. The lowest folate contribution to RNI was found in *injera* fermented using the traditional process with *ersho* and contributed only 6% of the RNI for children and 7% of the RNI for women of reproductive age. Folate from *injera* fermented with *L. plantarum* P2R3FA alone contributed around 15% of the RNI for children and 17% of the RNI for women of reproductive age. The successive cycles of backslopping using the batter fermented with *L. plantarum* P2R3FA were even more efficient, since they contributed up to 17% of the RNI for children and 20% of the RNI for women of reproductive age. The use of the combination of microorganisms produced intermediary results i.e., 20% and 23%, while the highest contribution was noted from tef *injera* fermented with *S. cerevisiae* alone, which contributed 23% of the RNI for children and 27% of the RNI for women of reproductive age.

### 3.4. *Acceptability of* injera *made using different starters*

According to the panelists (Table 2), all the samples prepared using the selected inoculums were in the acceptable range. The overall acceptability result showed that *injera* fermented with *S. cerevisiae* was the least acceptable product whereas *injera* fermented with *L. plantarum* P2R3FA, different cycles of backslopping of *L. plantarum* P2R3FA and combination of *L. plantarum* P2R3FA and *S. cerevisiae* were the most acceptable products. The study also found out that *injera* with *ersho* (traditional backslopping) showed lower acceptability than *injera* made with *L. plantarum*. This was true for all the parameters tested, color, taste, texture, odor, and appearance.

## 4. Discussion

The present study was conducted to evaluate the feasibility of using folate-producing microorganisms to produce *injera* with high folate content in non-sterile conditions. The ability of the folate-producing *L. plantarum* P2R3FA in maintaining folate production from batch to batch fermentation was also assessed. Our study showed that *injera* produced with all folate-producing microorganisms in non-sterile

**Table 1.** Folate content of tef *injera* fermented with different starters and contribution to the RNI for folate.

| Inoculum used to prepare *injera* | Folate (*μg*/100 g FM) | Contribution to RNI (%)* | |
|---|---|---|---|
| | | Children (1–3 years old) | Women (>19 years old) |
| *Ersho* (traditional backslopping) | 14.3 ± 2.3[a] | 6.3 | 7.2 |
| *L. plantarum* P2R3FA | 33.5 ± 3.1[b] | 14.7 | 16.9 |
| *L. plantarum* P2R3FA cycle 1 | 38.0 ± 4.9[c] | 16.7 | 19.2 |
| *L. plantarum* P2R3FA cycle 2 | 39.5 ± 5.8[c] | 17.4 | 19.9 |
| *L. plantarum* P2R3FA cycle 3 | 39.3 ± 6.2[c] | 17.3 | 19.8 |
| *L. plantarum* P2R3FA cycle 10 | 40.2 ± 5.4[c] | 17.7 | 20.2 |
| *S. cerevisiae* | 53.5 ± 2.6[e] | 23.5 | 27.0 |
| *L. plantarum* P2R3FA and *S. cerevisiae* | 45.3 ± 1.7[d] | 19.9 | 22.9 |

Values of folate are means ± standard deviation. Means followed by different letters in the same column differed significantly at $P < 0.05$.
* Average *injera* consumption by children = 66 g/day, and by women of reproductive age = 202 g/day (EPHI, 2013).

**Table 2.** Sensory acceptability test for *injer*a made using different inoculums.

| *Injera* fermented with: | Color | Taste | Texture | Odor | Appearance | Overall acceptability |
|---|---|---|---|---|---|---|
| *Ersho* (traditional backslopping) | $2.8 \pm 0.9^{b}$ | $2.5 \pm 0.7^{a}$ | $2.5 \pm 0.9^{b}$ | $2.6 \pm 1.1^{ab}$ | $2.7 \pm 1.3^{b}$ | $2.5 \pm 0.8^{b}$ |
| *L. plantarum* P2R3FA | $1.5 \pm 0.7^{a}$ | $2.3 \pm 0.6^{a}$ | $1.8 \pm 1.1^{a}$ | $1.9 \pm 0.8^{a}$ | $1.5 \pm 0.6^{a}$ | $1.7 \pm 0.7^{a}$ |
| *L. plantarum* P2R3FA cycle 1 | $1.5 \pm 0.7^{a}$ | $2.2 \pm 0.9^{a}$ | $1.6 \pm 0.6^{a}$ | $1.9 \pm 1.29^{a}$ | $1.5 \pm 0.6^{a}$ | $1.6 \pm 0.7^{a}$ |
| *L. plantarum* P2R3FA cycle 2 | $1.4 \pm 0.8^{a}$ | $2.4 \pm 0.8^{a}$ | $1.6 \pm 0.9^{a}$ | $1.7 \pm 0.7^{a}$ | $1.3 \pm 0.5^{a}$ | $1.7 \pm 0.6^{a}$ |
| *L. plantarum* P2R3FA cycle 3 | $1.4 \pm 0.8^{a}$ | $2.3 \pm 0.6^{a}$ | $1.5 \pm 0.4^{a}$ | $1.6 \pm 0.5^{a}$ | $1.5 \pm 0.4^{a}$ | $1.6 \pm 0.4^{a}$ |
| *L. plantarum* P2R3FA cycle 10 | $1.5 \pm 0.7^{a}$ | $2.4 \pm 0.6^{a}$ | $1.5 \pm 0.5^{a}$ | $1.8 \pm 0.8^{a}$ | $1.4 \pm 0.5^{a}$ | $1.6 \pm 0.5^{a}$ |
| *S. cerevisiae* | $3.6 \pm 0.7^{c}$ | $3.8 \pm 1.1^{b}$ | $3.4 \pm 0.9^{c}$ | $3.5 \pm 1.3^{b}$ | $3.5 \pm 0.9^{c}$ | $3.6 \pm 1.0^{c}$ |
| *L. plantarum* P2R3FA and *S. cerevisiae* | $1.8 \pm 0.6^{a}$ | $2.4 \pm 1.4^{a}$ | $1.8 \pm 0.7^{a}$ | $2.9 \pm 2.1^{b}$ | $1.7 \pm 0.7^{a}$ | $2.1 \pm 0.9^{ab}$ |

Values are the mean of 30 measurements ±standard deviation. Means followed by different letters in the same column differed significantly at $P < 0.05$.
Range is from 1 = extremely liked to 9 = extremely disliked.

conditions had high folate content and better sensorial attributes than their traditional counterpart.

A few studies have isolated folate producing microorganisms from cereal and non-cereal fermented products, estimated their folate production and bioavailability (Greppi et al., 2017b; Korhola et al., 2014; Laiño et al., 2015). However, the effect of the strains on the sensory quality of the fermented products was not taken into consideration. To the best of our knowledge, this study presents the results of the first trial to study the effect of using different starters in cereal fermentation both on the amount of total folate and on the sensory profiles of the product.

In the present study, *injera* prepared using the traditional process had the lowest folate content. Nevertheless, it contributed up to 6% and 7% RNI of folate for children 1–3 years of age and for women of reproductive age, respectively. This is in agreement with our previous findings, in which the folate content of samples collected from different households ranged from 7.1 to 20.1 μg/100 g FM and contributed up to a maximum of 10 % of the RNI of folate of the two population groups (Tamene et al., 2019b). From these data, it can be considered that *injera* made from tef is a good source of folate intake for the Ethiopian population. Considering the frequent consumption of this staple food (daily), any improvement in folate content should have a direct positive impact on the folate intakes of the population.

The use of folate producing *L. plantarum* P2R3FA was very efficient since *injera* produced with this strain could contribute up to 15% and 17% of the RNI for children 1–3 years of age and women of reproductive age, respectively. The efficiency of increasing folate content of other cereals using selected folate-producing LAB has already been reported earlier (Greppi et al., 2017a; Kariluoto et al., 2014). Traditional family preparation of *injera* includes backslopping from a previous successful spontaneous fermentation. Any modification of the process to increase the folate content of *injera* should be kept to the strict minimum. This is why after the first inoculation with a pure LAB, we checked the efficiency of backslopping from the batter originally prepared with *L. plantarum* P2R3FA inoculum. The conserved and even increased production of folate in the 10 successive cycles demonstrates the efficiency of this strategy, despite the lack of sterile conditions. A longer duration should now be tested to assess the maximum number of backslopping cycles that can be used to obtain the highest folate content of *injera*.

Measuring the concentration of the inoculate strain from one batter to another would also enable strain survival to be monitored throughout backslopping. However, this is a technical challenge since the work needs to be done in non-sterile conditions, and endogenous microbiota containing other LAB would limit the identification of our strain among the complex microbiota. Considering the efficiency of this bacterium, the use of the combination of regular inoculation and backslopping should allow application in all contexts. Indeed, the only sterilized compound was water. In Ethiopia tap water is drinkable, but the recommended use of previously boiled water could increase the safety of the end product, while limiting contamination by exogenous microorganisms.

Our study also showed that the tef batter fermented with *S. cerevisiae* alone had the highest total concentration of folate. This result is in line with previously reported findings that the bakers' yeast *S. cerevisiae* was also the best producer of folate in other cereal fermentations (Kariluoto et al., 2006; Korhola et al., 2014). *S. cerevisiae* was found to be the most efficient inoculum and *injera* fermented with *S. cerevisiae* could contribute around 23% and 27% of the RNI for children (1–3 years) and women of reproductive age, respectively. Considering that natural fermentation of tef batter is obtained with a combination of LAB and yeast, we also tested the combination the folate-producing *L. plantarum* P2R3FA and baker's yeast. In our case, the combination resulted in significantly higher folate content than *L. plantarum* P2R3FA but lower than the folate content obtained when baker's yeast was used alone. This is in line with other work in which the combination of *L. rhamnosus* LC-705 and *S. cerevisiae* ABM5131 produced 6-fold more folate than LAB alone and a similar amount of folate to yeast alone (Korhola et al., 2014).

Many studies have dealt with nutritional improvement of fermented foods, but the organoleptic quality of the final product has rarely been assessed, even if it has been shown that the sensory properties of fermented foods mainly depend on the microorganism used for fermentation (Papastoyiannidis et al., 2006). In our study, *injera* prepared using the selected *L. plantarum* P2R3FA strain resulted in the highest scores for sensory attributes. The sensory score of each attributes (color, taste, texture, odor and appearance) of *injera* fermented with *L. plantarum* P2R3FA was in the range between $1.5 \pm 0.7$ to $2.3 \pm 0.6$ (all attributes were very much liked). Although *S. cerevisiae* was the most efficient inoculum in increasing the folate content of *injera*, it was the least acceptable product to the members of the sensory panel. *Injera* produced with the combination was preferred to the *injera* produced with yeast alone.

## 5. Conclusion

We have shown the feasibility of producing folate rich traditional cereal-based fermented food using folate producing microorganisms. Indeed, up to 27% of RNI was obtained with a single staple food, and accounts for the dietary habits of the population. The use of folate producing LAB or bakers' yeast can thus increase the folate intake of Ethiopians. The use of a selected *L. plantarum* strain previously isolated from the same food allowed the production of folate that remained stable at least over 10 cycles of backslopping. In combination with commercial *S. cerevisiae, L. plantarum* could be used to enhance the folate content of *Injera* with a better acceptability by the potential consumers. The local production and distribution of the inoculum would be a sustainable alternative to food fortification or supplementation to increase the folate intake of the Ethiopian population. Future studies should replicate our study using tef and other cereals that can be used to prepare *injera* for the purpose of strengthening the conclusion made out of this particular study.

## Declarations

### Author contribution statement

Aynadis Tamene: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Kaleab Baye: Analyzed and interpreted the data.

Christèle Humblot: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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### Data availability statement

The authors do not have permission to share data.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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