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Evaluation of Yeast and Lactic Acid Bacteria Starter Cultures for the Production of Rice *Injera*

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Abstract

Injera is a yeast-risen flat-bread with a unique, slightly spongy texture. It is a national staple in Ethiopia that is eaten daily in virtually every household. Despite the fact that *injera* is a favorite staple food, starters have not been evaluated for the production of rice-based *injera*. Combination of starter cultures of lactic acid (*Lactobacillus plantarum, Lactobacillus fermentum*) and yeast (*Saccharomyces cerevisiae*) and *irsho* (back slopping) were used to ferment rice batter during 96 h. Changes in pH, titratable acidity (TA) and microbial count were analyzed at 6 h intervals and consumer acceptability was done at 24 h and 48 h. LAB starters and their co-cultures yeast decreased pH from 6.35 to 4.5 and increased titratable acidity from 0.33% to 0.95% (lactic acid w/w) within 18-24 h while in the spontaneous fermentation with traditional *irsho* it took 48 h-54 h to attain pH and titratable acidity value of 0.38% and 0.93%, respectively. The number of lactic acid bacteria and yeast increased in all starter culture and naturally fermented rice batter with fermentation time. Rice *injera* prepared using combination of *L. plantarum* + *S. cerevisiae* starters were the most acceptable (score of 8.83=like extremely). Therefore, the *L. plantarum* +*S. cerevisiae* starter combination can be used for commercial production of acceptable rice *injera*.

Keywords: Rice *injera*; *Lactobacillus plantarum*; *Lactobacillus fermentum*; *Saccharomyces cerevisiae*

Introduction

Injera is the favorite staple food for majority of Ethiopians. It is a fermented, pancake-like, soft, sour, circular flatbread [1]. It is made from flour, water and a natural mixed starter culture called *irsho*. *Irsho* is a fluid saved from previously fermented dough. *Injera* can be produced from various cereals depending on availability and abundance of the cereals [2]. It can be made from teff (*Eragrostis tef*), wheat, barley, sorghum or maize, and combinations of some of these cereals [3]. Fermentation of batter for *injera* making relies on chance inoculation or back slopping. This results in a product of unpredictable and inconsistent quality [4]. In order to maintain and sustain African indigenous fermented foods and beverages, controlled fermentation is strongly recommended [5-9]. The use of suitable starter cultures improves the fermentation process, facilitates the control over the initial phase of fermentation and the predictability of derivative products also reduces the organoleptic variations and the microbiological instability of African fermented food [4,10]. Controlled fermentation can be achieved by use of pure or mixed starter cultures with appropriate technology [11].

Lactic acid bacteria (LAB) and yeasts have been reported to be the predominant microorganisms in most of the African indigenous fermented foods [12]. Stable co-metabolism between LAB and yeasts is common in many foods, enabling the utilization of substances that are otherwise non-fermentable (for example starch) and thus increasing the microbial adaptability to complex food ecosystems [13]. Lactic acid bacteria (LAB) and yeast are appreciated as starter cultures and for their health benefits [14].

Lactobacillus plantarum, *Lactobacillus brevis*, and *Lactobacillus fermentum* are regarded as the predominant species in the fermentation of teff, with *Lactobacillus plantarum* being the most dominant [15-17]. Moroni et al. [18] in their study on biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs found that LAB (*Lactobacillus pontis*) and yeasts (*Saccharomyces cerevisiae* and *Candida glabrata*) dominated teff sourdoughs. Askal and Kebede [19] found that LAB such as *P. pentosaceus, L. fermentum* and yeasts including *C. humilis, C. tropicalis S. cerevisiae* and *S. exiguous* involved in the fermentation of teff. Hiwot et al. [20] reported the preparation of *injera* using instant pre-fermented flour that showed the possibility of industrializing *injera* preparation. So far, no research has been done on the development of starter cultures for rice batter fermentation to prepare rice *injera,* a product which is getting huge acceptance by the Ethiopian community. Traditionally, rice batter fermentation uses *irsho* from the previous fermented batter to initiate new batches (back slopping). Homemakers, who bake *injera* every 2-3 days know the usefulness of this traditional starter culture. However, failures do occur in traditional fermentations leading to inconsistencies in quality of *injera*. Despite the importance of rice *injera* and challenges with inconsistencies in fermentations, starters cultures have previously not been evaluated for its production. Thus, there is a need to upgrade the quality of *injera* and bring desirable changes. This study was, therefore, carried out to evaluate the contribution of different LAB (*L. fermentum* and *L. plantarum*) and yeast (*S. cerevisiae*) starter culture combinations in rice *injera* batter fermentation.

Materials and Methods

Preparation of the starter cultures

Two strains of lactic acid bacteria (*L. fermentum* M64 and *L. Plantarum* K72) and yeast strain (*Saccharomyces cerevisiae* M17) isolated from teff *injera* fermentation were used in this study. These strains were kindly provided by the Department of Biology, School

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of Nutrition, Food Science and Technology, Hawassa University. The *Saccharomyces cerevisiae* strain was cultured on yeast extract dextrose peptone (YEPD) agar at 30°C for 48 h and then successively sub-cultured in YEPD broth at 30°C for 24 h. *Lactobacillus plantarum* and *L. fermentum* were cultured at 37°C for 48 h on Man-Rogosa-Sharpe (MRS) agar followed by two successive rounds of sub-culturing in MRS broth with incubation at 37°C for 24 h and 16 h, respectively. Yeast and LAB strains were each harvested by centrifugation at 4000 × g for 20 min and pellets were added into batter prior to fermentation in cell concentrations of 10^8 cfu/g.

Preparation of batter for rice *injera*

Wheat flour was bought from Awassa Flour Factory (Hawassa, Ethiopia). Rice and maize grains were bought from the open market (Hawassa, Ethiopia) cleaned of dust and other foreign matter and ground into flour using a local Hammer mill. The milled flours were sieved and tightly packed in polyethylene bags till use. Composite flour was prepared by mixing the flours in a ratio of 10:2:1.5 (rice: maize: wheat). Batter for *Injera* preparation was made by mixing the composite flour with water in the proportions of 1:2 (w/v) flour to water in 5 L plastic buckets.

Fermentation of batter for rice *injera*

For natural (spontaneous) fermentation, the batter was inoculated with 10% of previously fermented batter. In the controlled fermentation, cell concentrations of 10^8 cfu/g of each starter culture i.e., *L. plantarum, L. fermentum, S. cerevisiae* was added into the batter. Mixed fermentation by LAB and yeast was initiated by using equal proportion of each pure strain. The batter samples were fermented in triplicate at room temperature. Batter samples were withdrawn at 0 h, 6 h and 96 h of fermentation, for determination of microbial counts, pH and titratable acidity [21].

Preparation of rice *injera* from the cultured rice batter

The cultured batters fermented for 24 h and 48 h were baked using an electric *Mitad* (Electric-Clay plate, made in Ethiopia) to produce *Injera*. Prior to baking, 5 g (or one tea spoon) of baking powder (sodium bicarbonate) was added to about 500 ml of the fermented batter to make one *injera* pan cake. The batter was poured in a circular pattern on the *Mitad,* covered, and baked for 2 minutes. The baked *injera* was left to cool for 2 h at room temperature. Then after it was cut into triangular pieces and presented in triplicate for sensory evaluation.

Consumer acceptability test

The effect of fermentation time (24 h and 48 h), starter cultures (LAB and yeast vs *irsho* - traditional starter culture) on sensory acceptability were evaluated by using an untrained panel (n=30). A 9-point hedonic scale (9="Like extremely"; 8="Like very much"; 7="Like moderately"; 6="Like slightly"; 5="Neither like nor dislike"; 4="Dislike slightly"; 3="Dislike moderately"; 2="Dislike very much"; 1="Dislike extremely") was used to score the level of acceptance of the different attributes of rice *injera*. The rice *injera* samples without sauce (*wot)* were presented on identical serving trays and coded with three-digit random numbers. The order of sample presentation was randomized. Potable water held at room temperature was served for cleansing the palate before and between testing of *injera* samples. An empty covered beaker was provided for the purposes of expectoration.

Statistical analysis

Means ± standard deviations were calculated from three independent replicates. The data were subjected to analysis of variance (ANOVA) using SAS software (version 9.0, Addinsoft, Paris, France). Significance was set at $p<0.05$.

Results

Enumeration of LAB and yeast in rice *injera* batter fermentation

In controlled fermentations, the cell counts of LAB and *S. cerevisiae* increased from 5.09-8.45 log cfu g^{-1} and 4.11-7.5 log cfu g^{-1}, respectively" In naturally fermented rice batter, LAB counts increased from 5.08 log cfu g^{-1} to 8.30 log cfu g^{-1} after 48 h while the yeast counts increased from 4.1 log cfu g^{-1} to 7.20 log cfu g^{-1} in 24 h-48 h (Figures 1 and 2).

Changes in pH and titratable acidity during rice batter fermentation

The changes in pH of rice *injera* batter fermentation are shown in Figures 3 and 4. LAB starters (*L. plantarum* and *L. fermentum* and their co-cultures *S. cerevisiae* decreased pH from 6.35 to 4.5 and increased titratable acidity from 0.33% to 0.95% (lactic acid w/w) within 18 h-24 h. The natural fermentation took 48 h-54 h to attain a pH and titratable acidity of 0.38% and 0.93%, respectively.

Effects of LAB and yeast starters on consumer acceptability of rice *injera*

Table 1 shows the effect of starter culture and fermentation time

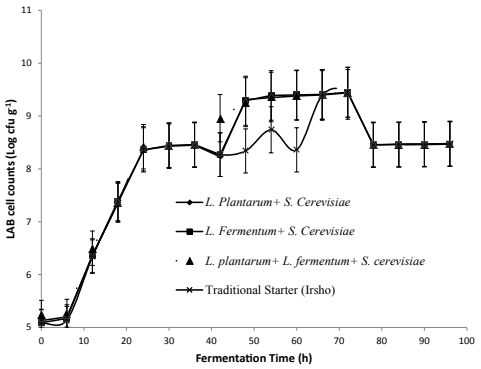

Figure 1: Changes in LAB counts during fermentation of rice batter inoculated with starters and *irsho* (back slopping). Error bars show standard deviations of three independent fermentations.

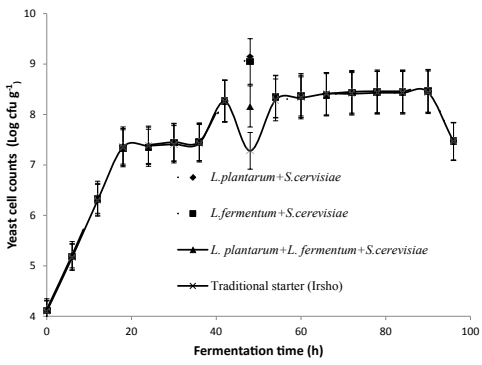

Figure 2: Yeast counts during fermentation of rice batter inoculated with starters and *irsho* (back slopping). Error bars show standard deviations of three independent fermentations.

Starter Combinations	Time	Appearance	Taste	Aroma	Mouth feel	Overall acceptability
Irsho	24	7.21 ± 0.85^{hi}	6.80 ± 1.82^{hi}	7.09 ± 1.38^{fg}	7.28 ± 1.08^{e}	7.40 ± 0.78^{c}
L. plantarum + *S. cerevisiae*	24	7.51 ± 1.24^{gh}	7.61 ± 1.22^{ef}	8.67 ± 0.97^{cde}	7.33 ± 1.47^{cd}	7.83 ± 1.20^{cb}
L. fermentum + *S. cerevisiae*	24	8.20 ± 0.91^{abcd}	7.93 ± 0.93^{d}	7.60 ± 1.36^{de}	8.00 ± 1.04^{a}	8.27 ± 0.78ab
L. plantarum + *S. cerevisiae* + *L. fermentum*	24	8.27 ± 0.82^{abcd}	8.03 ± 0.95^{bcd}	7.77 ± 1.39^{bcde}	8.10 ± 1.05^{a}	8.33 ± 0.79^{bc}
Irsho	48	7.64 ± 0.84^{fg}	7.32 ± 0.88^{fg}	7.44 ± 1.11^{ef}	7.20 ± 1.72^{f}	7.86 ± 0.92^{cb}
L. plantarum + *S. cerevisiae*	48	7.53 ± 1.26^{g}	7.62 ± 1.23^{ef}	7.67 ± 0.97^{cde}	7.34 ± 1.48^{c}	8.83 ± 1.22^{a}
L. fermentum + *S. cerevisiae*	48	8.20 ± 0.91^{abcd}	7.93 ± 0.93^{d}	7.60 ± 1.36^{de}	8.00 ± 1.04^{ab}	8.27 ± 0.78^{bcd}
L. plantarum + *S. cerevisiae* + *L. fermentum*	48	8.27 ± 0.82^{abcd}	8.03 ± 0.95^{bcd}	7.77 ± 1.39^{bcde}	8.10 ± 1.05^{a}	8.33 ± 0.79^{ab}

Values are means ± standard deviations of three independent fermentations (number of panelists = 30). Values in the same column with similar superscripts (a, b. c and d) are not significantly different at 5% level of significance. The scores are interpreted as follows: 9: Like extremely; 8: Like very much; 7: Like moderately; 6: Like slightly; 5: Neither like nor dislike; 4: Dislike slightly; 3: Dislike moderately; 2: Dislike very much; 1: Dislike extremely.

Table 1: Sensory acceptability scores of rice *injera* produced using different starter combinations.

on the acceptability of rice *injera*. Overall acceptability scores ranged from 7.40 (like moderately) to 8.83 (like extremely). Starter cultures and fermentation time significantly ($p<0.05$) affected consumer acceptability. Rice *injera* produced using a combination of *L. plantarum* + *S. cerevisiae* received the highest overall acceptability score after 48 h of fermentation although this was not significantly different for the other LAB/and yeast starter culture combinations. Rice *injera* prepared from *L. plantarum* + *S. cerevisiae* starters that was fermented for 48 h scored the highest value 8.83 (like extremely) while *injera* prepared with *Irsho* (control) scored 7.40 (like moderately) ($p<0.05$). LAB and yeast starter culture combinations also produced rice *injera* with better quality. Rice *injera* produced using traditional starter *Irsho* received significantly ($p<0.05$) lower acceptability scores than rice *injera* produced with all combinations of LAB and yeast and baked after 24 and 48 h of fermentation (Table 1).

Discussion

Changes in LAB and yeast cell counts during rice *injera* batter fermentation

All microbial genera are not of equal importance in fermentation [22], therefore candidate isolates for starter culture development have to be evaluated for their contribution during fermentation. In the present study, the numbers of LAB and yeast increased because they are predominant microorganism in the rice batter. Increment in counts of LAB (5.08 to 8.3 log cfu g^{-1}) and yeast (4.1 to 7.2 log cfu g^{-1}) were recorded in spontaneously fermented rice batter in 48 h. This is in line with Senait et al. [23] who reported yeast count of 8.49 log g^{-1} within 48 h-96 h of fermentation of *kocho* with barely for *injera* preparation. In the same study, it was also found that yeast was the most dominant organism followed by lactic acid bacteria. In another study on yeast fermentation of teff, an average yeast count of 30 log cfu g^{-1} of dough after 22 h-24 h of fermentation was observed [3].

Higher cell numbers of LAB (5-8.5 log cfu g^{-1}) and yeast (4.11-7.5 log cfu g^{-1}) were observed during rice *injera* batter fermentation within 24 h (Figures 1 and 2) comparing to the control (spontaneous fermentation). This could be due to inoculating the batter with a higher initial load of actively growing starter cultures, which are also well adapted to rice batter fermentation. The increased number of LAB and yeasts with intensive exponential phase results in shortening the duration of rice batter fermentation. The rapid growth of LAB lowers pH and favors yeast growth during the subsequent batter fermentation stage [13].

The results obtained in the present study agree with results reported by Holzapfel [10] who indicated that fast growth of starter cultures results in fast acidification and flavor development thus contributing towards ensuring product safety.

Changes in pH and titratable acidity during rice batter fermentation

The reduction in pH and increment in acidity in the current study followed the same trend as the traditionally fermented foods such as *atmit* [24], *borde* [25] and *Kunun-Zaki* [6]. Furthermore, Mukisa [26] found that LAB and the LAB/*S. cerevisiae* co-culture decreased the pH of *Obushera* to less than 4 (3.5-3.8) within 12 h although *L. fermentum* only decreased it to 4.13. While, the spontaneously fermented *Obushera* attained pH of 3.81 after 24 h. In a similar study on the preparation of *togwa*, a Tanzanian fermented food Mugula et al. [27] reported that LAB lowered the pH from 5.87 to 3.24-3.49 and increased the titratable acidity from 0.08% to 0.30%-0.44% (w/w, lactic acid) in 24 h which is in agreement with the present study. In the present study, the reduction of pH and increment of titratable acidity of the fermented rice batter

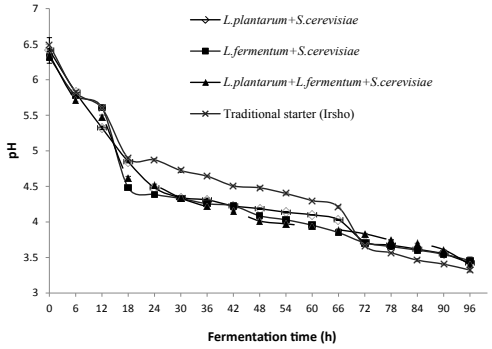

Figure 3: Changes in pH during fermentation of rice batter inoculated with starters and *irsho* (back slopping). Error bars show standard deviations of three independent fermentations.

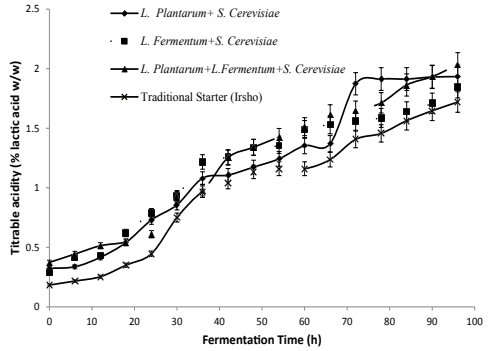

Figure 4: Changes in titratable acidity during fermentation of rice batter inoculated with starters and *irsho* (back slopping). Error bars show standard deviations of three independent fermentations.

may be attributed to the production of organic acids by the microflora. Rapid drops in pH with corresponding increase in titratable acidity have been also reported in lactic acid fermentation of corn [28]. Similar accelerated acidification by LAB starters have also been reported in related studies [27-29]. Fast acidification to pH below 4.0 is desirable because low pH together with the un-dissociated acid inhibits spoilage bacteria, enteropathogens and *Bacillus* spp. thus contributing to preservation and safety [12,22,30].

Consumer acceptability of rice *injera*

The combinations of LAB and yeast starter culture produced better quality rice *injera* compared to *Injera* produced by the traditional starter (*irsho)* with respect to appearance, taste, aroma, mouthful and overall acceptability after 24 h and 48 h of fermentation. Fast growth results in fast acidification and flavor development thus contributing towards shortening of processing time as well as ensuring product safety. The findings of the present study are in line with those of Glover et al. [11] who reported that starter culture combinations of LAB and yeasts produced better quality fermented products with more aroma compounds which improve flavor than single starters. The reason for the wide spread use of these organisms in food fermentation have been documented. The reasons include: their ability to produce desired flavor; formation of organic acids which reduce pH thus preventing growth of undesirable microorganisms; contributing to the development of the desired sensory qualities in the final product. Lactic acid bacteria are also used as 'natural' or 'selected' starters in food fermentations and exert antimicrobial effect as a result of different metabolic processes. Particularly, they have a key function in the development of the sensory and safety features of fermented food products.

The high overall acceptability scores of rice *injera* fermented with the LAB and *S. cerevisiae* starter cultures in the current study was due to co-metabolism of LAB and yeast which contributes towards development of characteristic flavor profiles of the products in which these two groups are involved. During cereal fermentations several volatile compounds are formed, which contribute to a complex blend of flavors in products. The presence of aromas such as: diacetyl, acetic acid and butyric acid make fermented cereal-based products more appetizing. Association of lactic acid bacteria and yeast during fermentation may also contribute metabolites, which could impart taste and flavor to fermented food.

The quality of rice *injera* depends on the type of raw materials, processing methods and processor practices and the starter culture used. Among the listed factors above, variety of the cereal used, and starter culture have huge impact on the sensory quality of rice *injera*. The flavor of food depends on the balance of volatile compounds that are inherently present in food or those produced during processing. A vast number of volatile compounds are synthesized and modulated by yeasts during fermentation. They significantly impart the overall quality of the product. These compounds include acids, higher alcohols, carbonyls, and esters. Previous studies on fermented cereal-mix substrate from white and red sorghum, pearl millet and wheat have showed an increase in volatile compounds due to fermentation by yeasts and lactic acid bacteria. Similar to the current study it has been reported that yeast *Saccharomyces cerevisiae* contributes to flavour development while fermenting sorghum for gowe production.

Conclusion

In conclusion, the potential of LAB and yeast starter cultures to ferment rice *injera* batter and their effect on sensory acceptability was evaluated. Accelerated acidification of rice batter was achieved by using LAB starters of *L. plantarum* and *L. fermentum* co-cultured with *S. cerevisiae*. However, rice batter fermented by *irsho* showed slow activity. Accordingly, a combination of *L. plantarum* + *S. cerevisiae* can be used to produce acceptable rice *injera*. The information obtained from this study will serve as a basis for further investigations on process optimization and eventually to establish small scale rice *injera* production. Implementation of this culture in industrial production should improve the quality and uniformity of the final product as well as food safety by preserving typical sensory quality of the traditional fermented product and inhibiting the growth of undesirable microorganisms.

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