

CO-FERMENTATION OF *KOCHO* WITH BARLEY FOR AN IMPROVED *INJERA*

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ABSTRACT: *Injera*, a pancake type sour bread, was prepared by co-fermenting *kocho* with barley to determine its nutrient composition and the microorganisms involved in the fermentation process. The predominant organisms identified were *Lactobacillus*, *Bacillus* and yeasts. The fermentation process was characterized by the fall in pH from 5.0 to 4.2 and rise in the titratable acidity from 0.20% to 0.50% during 96 hrs of fermentation. The co-fermentation of *kocho* with barley increased the protein content of the fermented *injera* by 2.6–fold. The *injera* prepared from co-fermented dough of *kocho* and barley was found to be acceptable to Ethiopian consumers and had very good keeping qualities. A fermentation scheme was therefore developed for the production of *injera* with improved protein content in which *kocho* and barley flour were fermented for 96 hrs with barley flour.

Key words/phrases: Barley, cereals, co-fermentation, dough, *injera*, *kocho*

INTRODUCTION

Fermented products of *ensete* (*Ensete ventricosum*) constitute a major part of the daily diets for about 10 million people in Southern and South western parts of Ethiopia (Tsedeke Abate *et al.*, 1996). Solid state fermentation (without soaking) is an important processing method of *ensete*. The most popular products of the *ensete* fermentation process are *kocho* and *bullá*.

Lactic acid bacteria are common microflora involved in the *ensete* fermentation process. Berhanu Abegaz Gashe (1987) reported that lactic acid bacteria and yeasts were responsible for the acid production and characteristic flavour development in *kocho* and *bullá*. The involvement of more than one species of lactic acid bacteria has also been reported in the fermentation process of *kocho*

and *bull*. The roles of lactic acid bacteria in fermented foods include improvement in flavour, texture, aroma, nutrient bioavailability and microbiological safety against pathogenic bacteria. These properties have been recently documented (Ayele Nigatu *et al.*, 1996; Kelbessa Urga, 1996). The important roles of lactic acid bacteria in fermented foods have been reviewed by Gibbs (1987).

Although *ensete* is one of the country's major root crops and an important carbohydrate source, the fermented product, *kocho*, has two major draw-backs: a rapid post-harvest deterioration and low protein content of about 1% (Agren and Gibson, 1968). Increasing the protein content of *ensete* would thus help reduce some of the limitations related to its utilization.

Injera, a pancake-type sour bread which is a common traditional food in Ethiopia, is prepared by natural lactic acid fermentation from cereals such as tef, barley, wheat, sorghum or maize or their mixtures. In his study on the microbiology of tef fermentation, Berhanu Abegaz Gashe (1985) identified *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Streptococcus* and some yeasts. Other studies have also confirmed the relatedness of these lactic organisms found in fermented tef and fermented *kocho* (Ayele Nigatu *et al.*, 1994). Interestingly, natural lactic fermentation has been found to increase the nutritive value of cereals, liberation of amino acids, synthesis of certain vitamins and the availability of trace minerals (Fields and Zamora, 1979).

On the other hand, production and consumption of *kocho* and related products as a basic diet is quite common in the southern parts of the country where cereals are less consumed. Cereals, such as barley, are good sources of protein though not as rich as legumes. As cereals are more convenient for *injera* production than legumes, one possible way of improving the nutrient content of *kocho* is by mixing it with cereals for *injera* production which would possibly give an acceptable product.

In areas where *ensete* is the main staple diet, consumption of *injera* either from cereals or *kocho/bulla* is limited. The development of *injera* by co-fermentation of *kocho/bulla* with cereals would therefore decrease the loss of *kocho/bulla* due to deterioration and would increase the nutritive value of *injera* prepared from

these starchy food items. This report, therefore, describes a processing method involving co-fermentation of *kocho* and barley flour for the production of nutritionally improved fermented *ensete*-based *injera*.

MATERIALS AND METHODS

Preparation of ensete and cereal flour

Kocho and cereals (wheat, barley, sorghum, maize and tef) were purchased from an open market in Addis Ababa. The *kocho* was sun dried and milled using a laboratory Cyclotec sample mill (Tector-AB, Sweden) to fine powder and sieved through a 0.05 mm mesh and had 10% moisture content (dry weight basis). The cereals were also milled to fine powder and sieved as above.

Fermentation process

Kocho and cereal flours were mixed in varying proportions. Starter, left-over of the previous fermentation, was added in a 1:1.6 (w/v) ratio. Twenty nine combinations of *kocho* and cereals with different proportions were tried. Although in this study five combinations were studied we report results of these combinations with an emphasis on *kocho*-barley (1:1, w/w) co-fermentation process. The mixtures were fermented at room temperature for 96 hrs. At the end of the fermentation process the liquid layer over the dough was poured-off and about 10% of the fermented dough was boiled for 5 min, cooled to 45° C and mixed with the rest of the fermenting mass which was important for the secondary stage of the fermentation process.

Sampling and isolation of microorganisms

Periodic samples of the fermenting mass were serially diluted using sterile peptone water (0.1% peptone and 0.9% NaCl) and pour-plated on to different agar media. Plates were incubated at 30–32° C for *Bacillus* spp, lactic acid bacteria and yeasts for 24–72 hrs. Plates for *Enterobacteriaceae* were incubated at 37° C for 24–48 hrs.

The media employed included MRS agar for *Lactobacillus* spp., Sucrose Gelatin Agar (SGA) composed of [g(l)⁻¹] distilled water, 10; Sucrose, 20; Gelatin, 13;

Nutrient Broth, 15; Agar for *Leuconostoc* spp. Dextrose Tryptone Agar (DTA) for *Pediococcus* spp., Slanetz and Bartley medium for *Streptococci* and MacConkey agar for members of the *Enterobacteriaceae*. Yeast were counted using Potato Dextrose Agar (PDA), and *Bacillus* spp. were isolated from appropriate diluent on Trypton Soy Agar (TSA) after incubating at appropriate temperature. For identification of suspected colonies, selective enrichment media and biochemical conformation were used.

Identification of the isolates

The identification of isolates were made using Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, 1984; Noel and John, 1984).

Determination of pH and titratable acidity

The change in pH of fermented dough was determined every 24 hrs using a pH meter (Radiometer, Copenhagen).

Total titratable acidity (TTA) expressed as percent lactic acid was determined by titrating 10 gm of co-fermented sample against 0.1 M NaOH to 8.3 pH end point with an automatic titrater (Bergman and Beving, Sweden).

Total nitrogen was determined by the rapid micro-Kjeldahl method of Concon and Soltess (1973). Total protein was estimated as 6.25xN%. Crude fibre, fat, ash and moisture contents were determined according to the methods in AOAC (1984). Carbohydrates were estimated by difference. Energy values were estimated using physiological fuel value of carbohydrates and protein as 4 Kcal(gm)⁻¹ and fat as 9 Kcal(gm)⁻¹, respectively.

Sensory evaluation of co-fermented baked Products

A ten-man panel group drawn from employees of the Ethiopian Health and Nutrition Research Institute was used in assessing the appearance, flavour, taste and textural characteristics of *injera* following the method of Newell and MacFarlane (1987). Data were analyzed using analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$. A multi comparison scoring difference test was used to determine if there were any perceived differences among the *kocho*-cereal *injera*. *Injera* prepared from tef was used as a control.

RESULTS AND DISCUSSION

The test showed that there were less significant differences in colour, test and odour between the tested *injeras* (Table 1). There were no significant differences ($p < 0.05$) in the general acceptability of *injera* made with *kocho*-barley and other different types of cereals and the control. However, the acceptability score for *kocho*-barley-sorghum mixture was significantly ($p < 0.05$) lower compared to the control.

Table 1. Quality characteristics of *injera* from *kocho*-cereal mixture**.

Cereal mixture	pH*	Proportion	Colour	Odour	Texture	Taste	Overall acceptability
<i>Kocho</i> -barley	3.9	1:1	1	1	1	1	1
<i>Kocho</i> -barley	4.0	2:3	2	2	2	2	2
<i>Kocho</i> -wheat-barley	4.2	3:1:1	2	2	2	2	2
<i>Kocho</i> -barley-maize	4.1	3:1:1	3	3	3	3	3
<i>Kocho</i> -barley-sorghum	4.1	3:1:1	4	4	4	4	4
<i>Kocho</i> -tef-barley	3.8	3:1:1	1	1	1	1	1
Tef <i>injera</i> (control)	4.0	-	1	1	1	1	1

*, pH of fermented dough just before baking; **, Sample scores are based on a 4-point scale with 1, excellent; 2, very good; 3, good; 4, fair.

Addition of barley at 50% (w/w) yielded *injera* in which the colour and odour were similar to that of the traditionally prepared and accepted tef *injera*. Therefore, this mixture was used subsequently to evaluate the microbiological and biochemical characteristics of the fermented *injera* (Table 1).

The protein and fat contents of *injera* increased with increasing cereal supplementation. *Kocho*:barley mixture 1:1 (w/w) yielded the overall higher value. The protein content of traditionally prepared *kocho* was 1.9% but, when

co-fermented with 50% of barley increased to 4.9%. The fat content was increased 3-folds (Table 2).

Table 2. Nutritional value of *kocho*-barley 1:1 w/w flour mixture per 100 gm.

Item	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fibre (%)	Carbohydrates (%)	Energy (Kcal)
<i>Kocho</i>	10.1	1.5	0.4	1.9	2.0	84.1	348.0
Barley	8.1	1.7	2.0	7.9	1.9	78.4	363.0
<i>Kocho</i> -Barley	9.1	1.6	1.2	4.9	1.9	81.25	355.0

The present method improved the protein content of *injera* made from *kocho*:barley mixture by 2.6 fold. This procedure does not need any microbial culture handling or maintenance, characteristics of microbial protein fortification as reported by Azoulay *et al.* (1980) for *cassava*. Similar studies were also reported in the use of legumes for increasing the protein content of fermented maize, *ogi* (Akinrele *et al.*, 1969) and *cassava* (Oyewole and Airbor, 1992).

Titrateable acidity expressed as percent lactic acid content of the mash increased from 0.20 in fresh mash to 0.50 at the end of 96 hrs fermentation period (Table 3). As a result, a concomitant reduction in pH which declined to 4.2 during 96 hrs fermentation period was observed.

In an earlier study of *kocho* fermentation, *Leuconostoc mesenteroids*, *Streptococcus faecalis*, *Lactobacillus coryneforms*, *Lactobacillus plantarum* and *Pediococcus* spp., yeasts and spore-formers were responsible for acid production and flavour development (Berhanu Abegaz Gashe, 1987). In our study, it was possible to isolate and enumerate substantial number of organisms such as *Lactobacillus* spp., *Bacillus* spp and yeasts (Table 3). Originally, *Bacillus* species were the only organisms present in *kocho* flour prior to co-fermentation (Table 4). The other organisms like yeasts and *lactobacillus* might have been destroyed by the drying temperature during the preparation of *kocho* flour. The spore-formers, which were *Bacillus* spp. reached colony number greater than 10^6g^{-1} within 72 hrs and declined to 10^4g^{-1} at 96 hrs of fermenta-

tion. The acidic pH of the fermenting *kocho*-barley mixture is not conducive for the growth of *Bacillus* species since they grow well at the pH range of 4.9–9.3 (Jay, 1978). However, *Bacillus* spp are known to produce amylolytic enzymes in starchy foods such as *kocho* where they supply fermentable sugars for use by fermentative microflora such as lactic acid bacteria. *Bacillus* spp have also been reported to play similar role in tef dough fermentation (Berhanu Abegaz Gashe, 1985). Nevertheless as their viable cells are affected at lower pH values (below 4.2) yeasts might be predominantly responsible for flavour development in the co-fermented *kocho*-barley dough.

Table 3. pH, titratable acidity and population of microorganisms in co-fermented sample.

Fermentation time	pH	TTA (%)	Asp [cfu(ml) ⁻¹]	LAB [cfu(ml) ⁻¹]	Yeast [cfu(ml) ⁻¹]	Total AC [cfu(ml) ⁻¹]
0	5.0	0.20	3.0x10 ²	9.6x10 ⁴	4.0x10 ⁴	5.0x10 ⁴
24	4.7	0.30	6.0x10 ³	16.0x10 ⁴	4.8x10 ⁴	6.8x10 ⁴
48	4.6	0.38	6.7x10 ⁴	1.1x10 ⁷	2.0x10 ⁶	3.4x10 ⁶
72	4.4	0.40	1.0x10 ⁶	5.4x10 ⁷	5.0x10 ⁷	6.9x10 ⁷
96	4.2	0.50	2.0x10 ⁴	4.0x10 ⁷	6.0x10 ⁸	8.1x10 ⁸

Asp, Aerobic spore-formers; LAB, Lactic acid bacteria; Total AC, Total aerobic count.

Table 4. Sources of microorganisms in co-fermented sample.

Organisms	Population of cfu(ml) ⁻¹		
	Barley dough	Starter	Kocho flour
<i>Bacillus</i> spp.	4x10 ¹	-	2x10 ¹
Yeast (<i>Saccharomyces</i> spp.)	5x10 ¹	1x10 ¹	-
<i>Enterobacteriaceae</i> spp.	1x10 ¹	-	-
<i>Lactobacillus</i> spp.	3x10 ¹	4x10 ¹	-

As shown in Table 3 the lactic acid bacteria attained higher population with increase in fermentation time as the fermentation of the combination is a typical lactic acid fermentation characterized by the drop in pH and concomitant rise in titratable acidity (Fields and Zamora, 1979). Yeasts reached colonies of $3.1 \times 10^8 \text{g}^{-1}$ within 96 hrs of fermentation and were the most dominant organisms followed by lactic acid bacteria when fermentation was terminated. It can be, however, suggested that these organisms could be responsible for the observed changes in acidity and flavour. The characteristic flavour and aroma resembling that of a mixture of diacetyl and acetic acid was concentrated on the surface of the fermented dough.

A butyric acid aroma was also present. The formation of diacetyl in yeast fermentation has long been known and is formed from α -acetolactic acid synthesized inside the cell and transferred into the fermenting medium (Rose and Harrison, 1971). This is a sign of good *injera* dough. The production of acid in the dough follows closely the growth of the organisms.

The scheme reported here involves a simple food processing unit operation similar to most traditional food processing schemes which could easily be adapted to the household level. There is, however, the need to determine the economic aspect of the process. The scheme will yield optimum benefits in seasons or regions when and where the price of the cereals is relatively cheaper than that of *kocho*.

There is also the need to investigate the nutritional implications of the cereal-carbohydrate interactions in the final product. This will help to determine the net availability of the nutrients to the consumer. In conclusion, an acceptable *injera* can be made from *kocho*-cereal combination by traditional methods.

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